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Study The Histological and Hematological Changes associate with administration of Eucalyptus oil by Oral and Inhalation in Laboratory Mice (*Mus musculus*)

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Submitted to the Council of the College of Science/University of Misan as Partial Fulfillment of the Requirements for the Master Degree in Biology

By

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﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ
وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صَدَقَ اللهُ الْعَلِيُّ الْعَظِيمُ

سورة المجادلة الآية (١١)

Supervisor Certificate

I certify that this thesis entitled " Study The Histological and Hematological Changes associate with administration of Eucalyptus oil of Orally and Inhalation in Laboratory Mice (*Mus musculus*) "

Submitted by (Zainab Muhsan Jaseb)has been prepared under my supervision at the College of Science,
University of Misan; as partial fulfillment of the requirements for the Degree of Master in Biology.

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Date: / /2021

Recommendation of Head of Biology Department
Because of the available recommendations; I forward this thesis to debate by the examining committee.

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College of Science/ University of Misan
Date: / /2021

Dedication

To the owner of the first and last credited,

to the guide, the right way....

God Almighty

To the man of struggle,

to the one who planted values and principles,

to the one who spent the flower of his youth in raising his children...
my beloved father

To the sweetness of milk,

whose milk did not mix with the sugar of interests....

To the tender chest,

which was a cold shadow for me in the expatriation of life....

To the one who missed the warmth of her applause with joy at my achievement at this moment.....

To whose sincere prayers were the secret of my success….

My dear aunt (may God have mercy on her)

ZAINAB 

Acknowledgment

Praise be to God, to whom belongs all that is in the heavens and the earth, and to Him be praise in the hereafter, and He is the Wise, the All-Knowing.

Praise be to God, praise as He deserves it, and prayers and peace be upon His Prophet, whose name is derived from His Mahmoud name, and upon the God of the pure and the pure, and upon his righteous Companions.

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SUMMARY

Summary

The study was conducted to extract Eucalyptus oil, identify its chemical components, identify the average lethal dose (LD_{50}) in mice, determine histological, hematological, weight, and behavioral changes in each method of administration, as well as reveal the safe and non-toxic method (orally, inhalation, mixing). Eucalyptus essential oil was extracted from the leaves of *Eucalyptus camaldulensis* by hydro-distillation method, Clevenger- type apparatus, for 3-4 hours.

In the experiment, (170) male and female mice were used, (50) mice were used to calculate the mean lethal dose (LD_{50}) when eucalyptus oil was administered orally for 14 days. The mice were divided into 5 groups, in each group (10) mice each a control group, received a normal saline, and 4 groups were treated with Eucalyptus oil at a doses of 1200,1600, 2000, 2400 mg/kg. The karber method was used to calculate LD_{50} in mice and its average was 1820 mg/kg.

Then (120) male and female mice were used to determine the histological, hematological changes due to Eucalyptus oil. The mice were divided into 4 groups, a control, orally, an inhalation, and mixing group, (20) mice each group, the orally group was given Eucalyptus essential oil orally by gavage at a dose of 1000mg/kg for 4 weeks. The inhalation group was exposed to eucalyptus oil inhalation for 15 minutes in a closed cage, and the mixing group give orally and inhaled Eucalyptus oil at the same dose.

During the study period, weights were measured every week, clinical symptoms were recorded in each group, then euthanasia of mice, blood withdrawal every week, and excision of the trachea, lung, esophagus, stomach, small intestine, liver, kidney, and heart. Two types of dyes were used for histological studies hematoxylin and eosin and periodic acid Schiff (PAS).

The results of the current study showed that eucalyptus oil extracted from the leaves of *Eucalyptus camaldulensis* has 98 chemical compounds, and the most important of these compounds are Ledene, β-Eudesmol, Aromandendrene, and Cineole.

Clinical signs appeared, including dizziness, loss of appetite, lethargy, and slow movement in the orally and mixing group, but in the inhalation group, no clinical signs appeared when compared with the control group, and there is a significant decrease ($P<0.05$) in body weight for the orally and mixing group, while in the inhalation group, it continued to grow during the experimental period. The results indicate changes in hematological parameters, there was a significant increase ($P<0.05$) in WBCs and a significant decrease ($P<0.05$) in RBCs, HGB, HCT, PLT in the orally and mixing group, but in the inhalation group, no changes in hematological parameters occurred.

Histological changes occurred in the esophagus of mice in the orally and mixing group, such as the congestion in sub mucosa, erosion and sloughing in mucosa, as for the inhalation group, no histological changes occurred. There was a decrease in the thickness of the mucosa and an increase in the thickness of the sub mucosa in the orally and mixing group. Also, the mucosa interacted strongly with PAS in the orally and mixing group and the inhalation group, the results were similar to the control group.

Histological changes occurred in the stomach of mice in the orally and mixing group, such as hemorrhage in the mucosa layer, errosin and sever sloughing of epithelial layer, edema in the mucosa between gastric glands and in the muscularis propria, while inhalation group, no histological changes occurred. There was a decrease in the thickness of the mucosa and an increase in the thickness of the sub mucosa in the orally and mixing group. Also, the mucosa interacted strongly with PAS, in the orally and mixing group, and the inhalation group, the results were similar to the control group.

Histological changes occurred in the small intestine of mice in the orally and mixing group, such as hyperemia in the villi, dilation of blood vessels in the sub mucosa, infiltration of inflammatory cells, sloughing of the villi, as for the inhalation group, no histological changes were occurred. There was a decrease in the thickness of the mucosa and an increase in the thickness of the sub mucosa in the orally and mixing group. Also, the mucosa and the sub mucosa interacted strongly with PAS, in the orally and mixing group and the inhalation group, the results were similar to the control group.

Histological changes occurred in the Trachea of mice in the orally and mixing groups, such as congestion in the mucous, erosion and removal of epithelial cells and loss cilia of the mucosa, expansion sub mucosa, inflammatory cell infiltration, shatter trachealis muscle, while in the inhalation group, no histological changes were seen. There was an increase in the lumen of the trachea in the orally and mixing, inhalation group, decrease in the thickness of the mucosa in the orally and mixing group. Also, the mucosa, submucosa interacted strongly with PAS in the orally and mixing group. In the inhalation group, the results were similar to the control group.

Histological changes occurred in the lung of mice in the orally and mixing groups, such as hyperemia in the bronchioles, severe hyperemia in the alveoli wall, deformities of the epithelium bronchioles, increased thickness of the epithelium of the bronchioles and alveoli, accumulation of inflammatory cells, rupture wall alveoli. As for the inhalation group, no histological changes occurred. There was an increase in the lumen of the alveoli in the orally and mixing, inhalation group, and decrease in the lumen of the orally and mixing group. Also, bronchioles had a strong reaction with PAS, interalveolar septum had a strong reaction with PAS in the orally and mixing group. In the inhalation group, the results were similar to the control group.

Histological changes occurred in the liver of mice in the orally and mixing group, such as hyperemia central vein, severe fatty degeneration, sinusoid spaces, partical degradation nuclei, disfiguration hepatocytes. While in the inhalation group, no histological changes occurred.

Histological changes occurred in the kidney of mice in the orally and mixing group, such as hyperemia in the glomerular, atrophy of Bowman's capsule, destruct in the renal tubules blood vessels, decrease in the size and number of epithelial cells in the renal tubules, renal tubular cast. While in the inhalation group, no histological changes occurred.

Histological changes occurred in the heart of mice in the orally and mixing group, such as hyperemia in the muscle fibers, roughness in the heart muscle. While in the inhalation group, no histological changes occurred.

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List of Abbreviations

Eucalyptus oil	EuO
World Health Organization	WHO
central nervous system	CNS
Respiratory Syncytial Virus	RSV
United States	US
Generally Recognized As Safe	GRAS
Periodic acid Schiff	PAS
Haematoxylin & Eosin	H&E
Food and Drug Administration	FDA
Median Lethal Dose	LD_{50}
White blood cell count	WBC
Red blood cell count	RBC
Hemoglobin concentration	HGB
Blood platelet	PLT
lymphocytes	LYM
monocytes	MID
neutrophils	GRAN
Hematocrit	HCT
mean corpuscular volume	MCV
mean corpuscular hemoglobin	MCH
mean corpuscular hemoglobin concentration	MCHC
red blood cell distribution width	RDW
mean platelet volume	MPV
platelet crit	PCT
platelet distribution width	PDW
grams	g
Micrometer	µm
Figure	Fig.
kilogram	kg
milligram	mg
millilitre	ml
formalin acetic acid alcohol	FAA
Gas chromatography-mass spectrometry	GCMS
Standard Error	SE
salt sodium chloride	NaCl
litre	L
Social Package of Social Sciences	SPSS
Chronic Obstructive Pulmonary Disease	COPD
Retention Time	RT

CHAPTER ONE

INTRODUCTION

1- Introduction

Well before the discovery of germs and microbes, the idea is that most herbs have healing potential and what we now call antimicrobials (McMahon *et al*., 2010), in the old testament, humans used plants to treat infectious diseases, and some of those traditional medicines continue to be part of local medicine and disease treatment (Heinrich *et al*., 2012).

For at least 6000 years, several ancient civilizations such as Egypt, China, and India have employed essential oils as a complementary and alternative therapy (Alok *et al*., 2000). Because of the importance of medicinal plants since the early ages of history, many types of herbs have been discovered in the tombs of the pharaohs and the history of herbal medicine in Iraq goes back more than 4,000 years to Sumerian civilization (Al-Alousy, 2018), Iraq has the biodiversity of many herbal medicines, where Eucalyptus has been used by local populations in traditional medicine for a long time (Shahbaz, 2010).

Iraq, like every other Middle East countries, has two societies, rural and urban. All of these societies depend to a large extent on the rich traditional heritage of medicinal plants to treat a variety of ailments. The doctor's name was "Hakeem" and he employed medicinal herbs to treat a variety of ailments, in both industrialized and developing countries, ensuring the safety, quality, and effectiveness of herbal medicines and herbal products has become a major problem (Al–Douri, 2014).

Essential oils are usually regarded harmless, with a few negative effects, and several have been authorized as food additives by the US Food and Drug Administration (FDA), falling under the category of generally regarded as safe (Ali *et al*., 2015).

Researchers are interested in medical treatments derived from natural or herbal sources (Wang *et al*., 2008), According to the World Health Organization (WHO), traditional medicine is used by 80% of the population in underdeveloped nations (Ekor, 2014), Due to allergies and side effects of synthetic medicine were attention to the herbs available in nature and their use in medicine (Wang *et al*., 2008).

Many people think that essential oils are safe for human usage since they have maintained as natural, are risk-free, and have no side effects (Vostinaru *et al*., 2020).

Eucalyptus consists of approximately 700 species, over 90% is *Eucalyptus camaldulensis* (Da Cruz *et al*., 2001), It belongs to the Myrtaceae family (Akin *et al*., 2010). Eucalyptus oil is produced from different parts of the plant like leaves, flowers, fruits, roots, and bark. Eucalyptus oil is extracted using many methods and techniques such as steam distillation, solvents, and critical fluid extraction (Ribeiro-Santos *et al*., 2018).

Because of its, anti-inflammatory, and antiseptic characteristics; Eucalyptus oil has been employed in a variety of daily applications (Sabo and Knezevic, 2019), anti-oxidant (Barra *et al*., 2010), in the treatment of bacterial infections for several diseases (Knezevic *et al*., 2016), anti-fungal (Gakuubi *et al*., 2017), anti-viral (El-Baz *et al*., 2015), and anti-parasitic effect (Nosratabadi *et al*., 2015), In the treatment of cystitis, diabetes (Dawoud and Shayoub, 2015), Insecticide, pain reliever, and rheumatism therapy (Maruyama *et al*., 2005).

It can be used to treat respiratory disorders, runny nose, sore throats, asthma, nasal congestion, and bronchitis by helping to release sputum, relieve cough, and relax respiratory muscles (Vigo *et al*., 2004).

The widespread misconception that all herbal treatments and associated natural products are not only useful, but also fully safe to use has the potential to harm consumers' health (Radulović *et al*., 2013a).

1-1- Aims of the study

1- Extraction of Eucalyptus essential oil from *Eucalyptus camaldulensis* and determined the chemical structures of Eucalyptus oil.

2- Determine the LD_{50} of Eucalyptus essential oil in the mice and the safety of Eucalyptus oil.

3- Determining the safety and harmful method of administration (orally, inhalation, mixing) of eucalyptus essential oil.

4- Observe the clinical signs after using eucalyptus oil (orally, inhalation, mixing) in mice.

5-Determine the hematological and histological changes in the esophagus, trachea, lung, stomach, intestine, heart, liver, and kidney.

CHAPTER TWO
LITERATURE REVIEW

2- Review of Literature

2-1- Classification of Eucalyptus Plant

The plant kingdom comprises many beneficial herbs, they consider the plant as a primary source for numerous medications, that used in the treatment of a variety of harmful human diseases, safer plant products were used by researchers and scientists (Elsayed, 2012).

Eucalyptus is a fast-growing tree that resists salinity, water scarcity, and dryness, The habitat of this tree is Australia and it is native all over the world and has many names including red gum, river chewing gum and red chewing gum (McMahon *et al* ., 2010).

Eucalyptus is an odorous evergreen tree, grown in every tropical and sub-tropical region and the Middle East, including Iraq (Hur *et al* ., 2000).

This is a relatively large perennial tree, reaching lengths of 20-50 meters, and maybe longer than 1000 years old, Eucalyptus is a derivative from the two Greek words Eu meaning good and Kalypso meaning hidden **(**Ghasemian *et al* ., 2019).

It has a vast natural distribution and has a large group of species which includes more than 800 species worldwide (Neelam *et al*., 2014), One of these is a species *Eucalyptus camaldulensis* and the scientific classification is as follows (Sonker *et al* ., 2017).

Kingdom : Plantae

Class : Magnoliapsida

Order : Myrtales

Family : Myrtaceae

Genus : Eucalyptus

Species : *Eucalyptus camaldulensis*

Eucalyptus camaldulensis is also known as *Eucalyptus rostrata*.

2-2- Methods of Extraction Eucalyptus Essential Oil

Essential oils are secondary metabolites produced by plants to respond to stress, infections, and parasitic attacks. That essential oils are volatile organic compounds (Ahmad *et al*., 2005). Extraction methods include the following.

I- Hydrodistillation:

In this method, plant materials and water are placed in a flask and the heat is in the bottom, after which the aromatic vapor is released and condensed into the liquid, and the oil increases as the distillation time increase, This method can also be used for the extraction of oils from solid parts such as wood and roots. Essential oils contain substances with boiling points up to 200°C or higher temperatures (Barazandeh, 2005).

II- Steam distillation:

In this method, the plant material is put in a flask and the water in another flask, and steam passes through the plant material to evaporate the oils, which is almost similar to the method of hydrodistillation. But this method characterized by high oil production (Sefidkon *et al*., 2008).

III- Vacuum distillation:

A lot of eucalyptus oils are extracted by vacuum distilling, and distillation occurs at a lower temperature and this process is performed under the pressure of less than 1 atm (Zhang *et al*., 2010).

IV- Supercritical liquid extraction (SFE):

In this method, certain gases such as CO2 are used to form non-polar solvents which remove the essential oil from plant materials at an appropriate temperature and pressure; This method is used to segregate oil from heat-sensitive materials (Rao and Pandey., 2007).

V- Subcritical water extraction (SWE):

This method uses hot water (100-374℃) as an extractant and high pressure, it is faster and more efficient than hydrodistillation, this method is characterized by shorter duration, high quality, and low costs, environmentally friendly (Herrero *et al*., 2006).

VI- Solvent extraction:

In this method, a hydrocarbon solvent is added to the plant material, which helps dissolve the essential oil, and pure alcohol is used to extract the oil when the alcohol evaporates, the oil leaves behind, and this method is not considered the best as it may leave a small number of solvents behind and may cause allergies and affect the immune system (Zhang *et al*., 2012).

VII- Maceration:

In this method, plant material is soaked with vegetable oil, heated, and filtered (Rao and Pandey, 2007).

2-3- Eucalyptus Essential Oil

Eucalyptus oil (EuO) serves as the most important highly concentrated therapeutic agent, is a mixture of aldehydes, ethers, saturated and unsaturated hydrocarbons, esters, alcohols, phenolic oxides, terpenes, and ketones, which are responsible for generating the characteristic odor in the oil (Dunning, 2013). EuO is a yellowish or pale liquid Figure (2-1) with a pleasant aromatic fragrance (Horváth and Ács, 2015).

It is an essential oil obtained from eucalyptus and the oil represents about 5% of the mass of the leaf and this percentage varies according to the different types (Batish *et al* ., 2008). Essential oils are responsible for generating the smell in the plant (Rao and Pandey, 2007). Essential oils are easily extracted and can be easily degraded in the environment (Hu *et al*., 2014).

Eucalyptus oil contains many individual components which have biological activity are secreted into special cells, secreting channels, cavities, or glandular hairs (Buchbauer and Baser, 2009).

It is well known that EuO production depends on many factors, including environmental conditions, soil type and composts (Stefanello *et al*., 2011), and the climate, the harvest time, the method of extraction, the duration of extraction, and the part of the plant used (Alok *et al*., 2000), it also depends on age, geographic region and genetic markers (Isman, 2000).

Essential oils are produced in every part of the plant (buds, flowers, leaves, stalks, twigs, seeds, fruits, roots, wood, and bark) (Silva *et al* ., 2012).

Figure (2-1) : Eucalyptus Oil (Quispe -Flores, 2017).

2-3-1- Chemical Components of the Essential oil

The essential oil are composed of different mixtures of more than 200 ingredients, including oleoptene and stearoptene (Mohammad, 2015). There are only some chemical and compositional distinctions among the compounds, which can be classified into two parts:

1- Volatile fraction: It contains about 90-95% oil and contains hydrocarbons as terpenes including sesquiterpene, monoterpene, and diterpenes, as well as other substances such as alcohols, esters, and aldehydes, ketones (Prats and Jiménez, 2004 ; Başer and Demirci, 2007).

2- Nonvolatile fraction: It represents approximately 1-10% of the aromatic oil and comprises fatty acids, sterols, waxes, hydrocarbons, carotenoids, and flavonoids, lactones (Prats and Jiménez, 2004).

2-3-1-1-Hydrocarbons

Essential oils are composed of chemical compounds which contain hydrogen and carbon as basic components. Isoprene is a type of hydrocarbon that occurs in essential oils. (Turek and Stintzing, 2013).

2-3-1-2-Terpenes

The term ene appears at the end of the names of these compounds, including monoterpene, sesquiterpene, and diterpenes.

Terpenen is considered an antiseptic, bactericidal, anti-inflammatory, antiviral, and antiseptic and is divided into monoterpenes, sesquiterpenes, and diterpenes (Roslan, 2014).

A- Monoterpenes

The chemical formulation is $C_{10}H_{16}$, and these molecules consist of two units of isoprene, Unsaturated hydrocarbons constitute the majority of these substances C_{10}, However, some molecules, such as alcohol, carboxylic acid, and ketones are oxygenated derivatives are called monoterpenoids (Soares-Castro *et al*., 2021), These chemicals are used as analgesics, expectorants, stimulants, and bactericidal agents (Hamad *et al*., 2016), Examples of these compounds are Menthol, Monocyclic, Camphor, α or β Pinenes, Carvone and Limonene (Pourmortazavi *et al*., 2003).

B-Sesquiterpenes

$C_{15}H_{24}$ is the chemical formula of those compounds. It includes more than 500 compounds and consists of three isoprene units (Varutbangkul *et al*., 2006), These chemicals have anti-cancer and anti-leukemia properties, along with cytotoxic and antibacterial properties. They can also cause skin sensitivities in humans and can act as insect repellants, such as xanthanolides, guaianolides, etc (Chizzola, 2013).

C- Diterpenes

These molecules have the chemical formula $C_{20}H_{32}$ and consist of 4 units of isoprene, which have medicinal importance, antitussives, antifungals, and expectorations include them. They have a function in hormonal balance and stress, for instance, phytane (Hussein and El-Anssary, 2019).

2-3-1-3-Alcohols

These chemicals have a hydroxyl group (OH) in their structure and have antifungal, antiseptic, germicidal, and bactericidal activities, Alcohols, on the other hand, have a very low or no harmful impact on the body or the skin, thus they're regarded safe to use, such as nerol, geraniol and linalool (Aziz *et al*., 2018).

2-3-1-4-Aldehydes

The chemical formula of these compounds are R_CHO, Essential oils consist of aldehyde compounds, which are beneficial in the treatment of Candida and other fungal infections, therefore classified as antifungal, antiviral, anti-inflammatory, antiseptic, sedative, and antiseptic. Examples are citronellal and lemon balm (Rungqu, 2015).

2-3-1-5-Esters

When alcohol combines with acid, an ester is created. Since ester-containing essential oils are widely employed as sedatives and in maintaining equilibrium and owing to the presence of alcohol, they are thought to be active antimicrobials. While physicians stressed that esters are antifungal, they may also be used to analgesics and to balance the nervous system. They are also devoid of toxins, except for methyl salicylate, which is poisonous. Examples of esters are linlyl acetate, Geranyl, etc (Dhifi *et al*., 2016).

2-3-1-6-Ketones

Many essential oils are used to treat respiratory problems because they encourage the movement of mucus and relieve congestion. Essential oils containing ketone have a beneficial effect on wound healing Essential oils and encouraging the formation of scar tissue. Ketones by nature non-toxic, except for more toxic thujone and one of the largest of these compounds Fenchone, Menthone (Ali *et al*., 2015).

2-3-1-7-Acids

The acids in the oils regulate the acidity of the oil. These organic acids are found free in oil, but only in very small quantities. Examples are lactic, cinnamic citric, and benzoin (Clarke, 2009).

2-3-1-8-Lactones

These compounds are anti-inflammatory, febrifuge, expectorant, have a greater expectorant impact than ketones. They also play a role in the synthesis of prostaglandins (Hanif *et al*., 2019).

2-3-2- *Eucalyptus camaldulensis* oil components

Cineole is the major component of eucalyptus oil which is derived from the fresh leaves of the plant, which has a high proportion ranging from 50 to 90%, but substantial changes that proportion can occur with different seasons throughout the year, and leaves often have a large proportion of cineole (Al-Snafi, 2017).

Cineole has many synonyms are eucalyptol, 1,8-cineole, trepan, and 1,8-oxido-p-menthane, It is azeotrope in water at a temperature of 99.55°C, but it is more soluble in cold water, and it is the component that gives the oil its strong smell (Geof, 2007)

In addition to 1,8-cineole, the oil contains many other components, including α-Pinene, Terpinene,α-Iinene epoxide, Isoamyl isovalerate, Fenchyl, Camphene, β-Pinene, Eucalyptol, ϒ-Terpinene, δ- alcohol, α-Camphdenic Aldehyde, t-Pinocarveol, Myrtenal, Z-Carveol, d-Carvone, Cymene 5-ol, Benzyl valerate, α-Gurjunene, b-gurjunene, Aromadendrene, Alloaromadendrene, Phenethyl Isovalerate, Ledene, Epiglobulol, Ledol Viridiflorol, Eremophilene, and g-Cadinene.

(Ashraf *et al*., 2010 ; Daroui-Mokaddem *et al*., 2010)

2-3-3-Factors affecting the chemical components of Eucalyptus oil

The following factors have a significant impact on the proportion of chemicals in eucalyptus oil (Tsiri *et al*., 2003).

1_ Plant gene variation due to hybridization and generation, which may result in a wide range of volatile oils concerning different ecosystems.

2_ Variable soil nutrients and their concentration in the leaves may cause variable plant metabolism, as a result, different essential product output.

3_ Various climatic conditions and environmental patterns across the world, as well as different seasons of the year, all play an influence on the chemical components of the oil.

4_ The plant part used to extract the essential oil and its maturity may have a significant impact on the oil components.

2-4- Application of Eucalyptus Essential Oil

Eucalyptus is known around the world for its various medicinal applications. Shown as Figure (2-2)

1- Effect Respiratory system:

Because eucalyptus reduces mucus release (Yadav and Chandra, 2017). Eucalyptus oil is used to treat coughs, colds, bronchitis to relieve cold and flu symptoms, also treat fever, nasal congestion, and headaches, to treat asthma and tuberculosis.

2- Effect on the mouth and teeth:

The anti-microbial properties of eucalyptus oil have been verified against streptococci, lactobacilli, and fungi like *Candida albicans* (Al-Mizraqchi, 2009).

3- Dermatological effects:

The use of eucalyptus extract on the scalp and hair health has been checked, as it helps to improve hair luster and hair growth as well as being an anti-dandruff and is also used in treating skin ulcers and acne. (Mamada *et al*., 2008).

4- Healing wounds:

Eucalyptus oil contains antibacterial qualities is also an antiseptic, making it suitable for the treatment of wounds, burns, and ulcers. It may be used as an ointment for bites or injuries. (Dhakad *et al*., 2018).

5- Regular and activate organs:

It is used to regulate and activate various systems such as strengthening its immunity against measles and cystitis also treats and treats skin conditions such as injuries, pigmentations, burns, herpes treatment,

rheumatoid arthritis treatment, muscle and joint pain (Mulyaningsih *et al*., 2011).

6- Anti-bacterial:

Eucalyptus extract is effective against Gram-positive and Gram-negative strains of bacteria, The most prominent are *Staphyloccocus aureus, Bacillus subtilis, Escherichia coli, Haemophilus influenza*, and other species (Elaissi *et al*., 2010).

7- Antifungal:

It is used to treat fungal infections in the skin, mouth, vagina, and urinary tract as eucalyptus oil can inhibit the growth of fungi such as *Candida albicans* and other fungi (Damjanović-Vratnica *et al*., 2011).

8- Anti-parasites:

Eucalyptus oil acts against pests, ticks, mites, and lice and may cause 100% or 50% mortality in larvae or adults (Clemente *et al*., 2010).

9- Anti-virus:

Eucalyptus oil possesses antiviral properties, in particular herpes and other viruses. It was recently summarized that Eucalyptus may have therapeutic potentialities that act as an inhibitor of Covid-19 (Sharma and Kaur, 2020).

10- Anti-tumor effect:

Eucalyptus oil has an anti-tumor property in delaying the growth of cells M14 WT and M14 adriamycin resistant cells (Carnesecchi *et al*., 2002).

11- Anti-Diabetes:

Eucalyptus extract is used to combat low blood glucose levels bosses in blood sugar because it stimulates beta cells to insulin secretion (Dawoud and Shayoub, 2015).

12- Antioxidant effect:

Eucalyptus oil is a potent antioxidant that can rid the body of formed free radicals (Ghaffar *et al*., 2015).

13- Analgesic and Relaxation effect:

It is used in relaxation, anesthesia, sleep, improve mood, behavioral improvement and treatment of epilepsy, treatment of Alzheimer's disease, paralysis, stroke, and tremors, Eucalyptus is used to treat depression and anxiety as eucalyptus oil has increased in popularity as an alternative to the treatment of central nervous system disorders (Anusha *et al*., 2012), and It also provides sedation and pain relief by affecting the central nervous system (CNS) or the peripheral nervous system (Dobetsberger and Buchbauer, 2011).

14- insecticide:

Insects cause damage to crops and the transmission of numerous diseases. Eucalyptus oil was used to combat insects, When the oil was used to remove insects, positive results were found for various insects, It is used as a natural repellent for insects and mosquitoes (Aarthi *et al*., 2010).

15- cosmetic using:

It is used in the perfume industry and cosmetics (cleansers, soaps, creams, and lotions), It is used as a flavor in the food industry (Rajeswara *et al*., 2003).

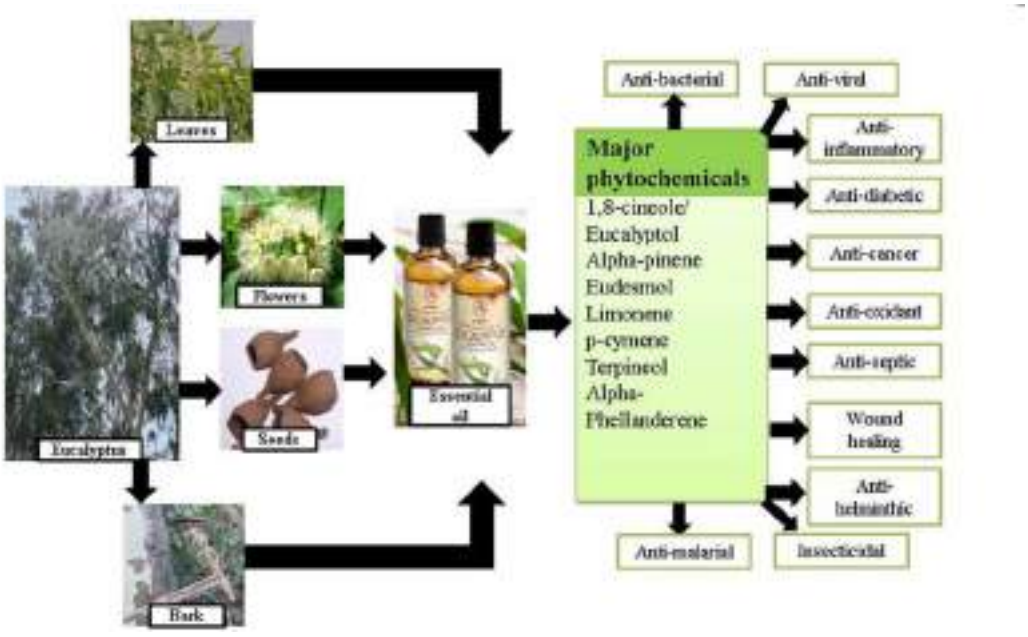


Figure (2-2): Uses of Eucalyptus Oil (Kumar, 2021)

2-5- Administration Methods of Eucalyptus Oil

Eucalyptus oil has many applications and more important is its use in natural therapeutic medicine, There are three traditional ways of using oil and other methods as shown below.

1-Inhalation (Aromatically)

Historical use, The steam respiration method is used on a bowl of warm water containing eucalyptus leaves with a towel on the head, The second way of breathing is by using a pocket inhaler (Sadlon and Lamson, 2010).

There are two pathways are involved in mediating the effects of inhaled eucalyptus oil constituents, the nerve path that acts on the central nervous system through the olfactory nerve, and the drug route that acts through the bloodstream (Christen-Zaech *et al*., 2003).

Inhalation is the method by which toxicities are minimal and it is effective in eliminating respiratory diseases, treating sleeplessness and other mental illnesses (Zhong *et al*., 2019).

Kamyar (2009) which explained the importance of inhalation Eucalyptus oil to a 3-year-old patient with Respiratory Syncytial Virus (RSV) was treated with eucalyptus oil, in which 3 drops were placed on a piece of fibrous gauze and inserted into a fan base in a closed room and was continually inhaled by the patient.

As well as explain Rao and Pandey (2007) put 12 drops of oil per 150 ml of boiling water or 1 teaspoon per liter of canceled hot water (Horváth and Ács, 2015), Inhalation is often effective for mood change effects thus the oil components directly affect the limbic system.

The action of the oil can be summarised by inhaling it as follows, involves the integration of essential oil into a biological signal to the receptor cells in the nose by inhaling, The signal is transmitted to the limbic system and hypothalamus of the brain by the olfactory bulb. These signals cause the brain to release neuro messengers like serotonin, endorphin and noradrenalin. among others, which exhale a feeling of comfort and well-being (Alok *et al*., 2000).

The volatilization or evaporation of the oil makes it ideal for pulmonary management in treating respiratory diseases, and a small portion of inhaled compounds may be rapidly absorbed at the alveolar level and through the lining of the airways. Pulmonary absorption of oil compounds has been confirmed and the rate of absorption is dependent on nature, the concentration of inhaled volatile substances and local physiological factors such as respiratory mechanics (Kohlert *et al*., 2000).

Except for some studies and research that have summarized that eucalyptus oil is dangerous, there are no well-defined studies that support and prove that eucalyptus oil is harmful. However, most studies found no evidence of oil toxicity while inhaled (Oyedeji *et al*., 2009).

2- Topically application:

This is the preferred way to use a lot of essential oils, however, oils require significant dilution because they can cause skin irritation because the oil can penetrate the skin easily, because it is lipotropic and the molecular structure is quite small and the oils can be readily absorbed in the blood and surrounding tissues (Esmaeili, *et al*., 2012).

The oil is used as a pomade for adults and children over 2 years old, Eucalyptus oil should not be used on the faces of children and infants, as well as during pregnancy and infants (Tisser and Young, 2013).

EuO has been used as a dermal ointment on mammals because of its antibacterial, fungal, and viral properties (Yadav and Chandra, 2017), and applies to different animals to remove parasites from the skin (Toloza *et al*., 2008). EuO can be used locally for relieving muscular and joint pain (Ahmad *et al*., 2005).

As well as the skin may absorb it when topically applied and transport it throughout the body through the blood cycle and reach all internal organs (Bradley *et al*., 2007).

The ability of this oil to penetrate subcutaneous tissues is one of the most important characteristics of this treatment, It also has complex effects because of its complex structure and chemical properties (Alok *et al*., 2000).

The contact of the oil with the skin during massage with oil may lead to systemic absorption of chemical components in oil depending on the contact time, the size of the radiance of the exposed skin surface, and the concentration of the compound, The oil can penetrate skin barriers and facilitate the absorption of other topically applied drugs by inducing a conformational modification of intercellular proteins in the corneal layer and by increasing the drug partitioning (Herman and Herman, 2015).

Transdermal absorption of many monoterpenes, such as α-pinene, camphor or limonene, and other compounds that can pass through the glandular barrier and produce systemic effects, It has been used in the treatment of anxiety with the application of drops to the skin and massage and direct inhalation or respiration the smell of oil (Rao and Pandey, 2007).

3- Orally Administration

Certain essential oils are ingested, typically diluted with water or in capsules and this method is rarely used, Oral absorption of oil is the most toxic way (Rao and Pandey, 2007).

When administered orally, absorption occurs along the gastrointestinal tract. The drug passes through the intestinal wall and moves to the liver before being transported into the bloodstream. The liver chemically metabolizes eucalyptus oil, When administered orally, food in the gastrointestinal tract may affect the extent and speed of absorption of the drug. Therefore, the drug should be taken on an empty stomach (Verma *et al*., 2010).

After an orally dose of EuO, the systemic absorption of many compounds contained in their chemical composition might be substantial, A study in rats demonstrated that following oral administration of radiolabelled transanethole, over 90% of the substance has been absorbed by the gastrointestinal tract in the bloodstream being then metabolized and excreted into feces and urine (Vostinaru *et al*., 2020).

The solubility of the individual active substances has the biggest influence on absorption in the organism. The EO chemicals tend to interact with food in the gastrointestinal system. Consequently, the active compounds may escape the solubilization and absorption of the stomach.

Moreover, the kinetics rate depends on the activity of digestive enzymes to release the EuOs components from the fatty acid bonds (Rubio *et al*., 2014).

Steroids and terpenoids (monoterpenes, carotenoids, triterpenoids, and phylloxanthins) show lipophilic. Lipophilic molecules of EuO tend to form micelles, and they are absorbed in the small intestinal tract with other lipids. Furthermore, their lipophilic nature allows for simple penetration across the epithelial cell membrane. Thus, the molecular forms are delivered to the small intestine where are liberated and hydrolyzed with lipids (Rodriguez-Concepcion *et al*., 2018) (Papada *et al*., 2018).

4- Intraperitoneal injection:

The efficacy of Euo has been tested to relieve pain or analgesia by intraperitoneal injection of a group of rats at a concentration of 10 mg/kg, mice were injected intraperitoneally with eucalyptus extract at a dose of 100 mg/kg after these mice were infected with cancer cells, where the results demonstrated that the oil is effective against cancer and has antioxidant activity in the body (Zein *et al*., 2016).

5- Subcutaneous injection:

100 mg/kg of mice were injected subcutaneously for oil efficacy as an anti-inflammatory and considerably reduces migration of neutrophils and inflammation cells (Zein *et al*., 2016).

2-6- Side Effects and Toxicity Eucalyptus Oil

The recent growing interest in medicinal plants to prevention and treatment of numerous human and animal diseases, There is increasing concern about the medical safety of plants, Scientists have documented serious toxic reactions when using herbs and plants for medical purposes, because of the toxicity of certain chemicals of plants (Oduola *et al*., 2010). The use of traditional remedies has resulted in adverse reactions that sometimes put people at risk (Saad *et al*., 2006).

Eucalyptus is considered Generally Recognized As Safe (GRAS) by the FDA and has presumed that 0.1 mg/kg of oil is safe for drinks, 5mg/kg for food, 15 mg/kg for sweets, and 50 mg/kg for alcoholic drinks (Kfoury *et al*., 2019).

When the intraperitoneal injection of a group of mice with eucalyptus oil and another group which inhaled eucalyptus oil was used, and side effects like drowsiness or dizziness occurred after the drug was injected (Lee *et al*., 2019).

The cineol has been approved for safe use by the United States Food and Drug Administration (FDA) and it has complications like mild skin irritation - mucosa membrane because cineole has minor side effects that do not cause significant toxic effects on the skin structure (Aqil *et al*., 2007).

Eucalyptus varieties contain high levels of phenolic and combined compounds that may be toxic as koalas who feed on Eucalyptus have ways of removing toxic compounds from the liver, numerous compounds within EuO have side effects which include 1-8 - Cineol, Ritin, Tannins, Cyanogenic glycosides (Ghasemian *et al*., 2019).

Mortality was observed in humans who consume Eucalyptus oil from 4 to 5 ml, symptoms that have appeared including vomiting up to 4 hours after ingestion, breathing issues, polypnea, bronchospasm, and tachypnoea, along with effects on the nervous system, Decrease or loss of reflexes and depression that may affect coma and convulsions, The acute toxicity of the oil is attributable to the ingestion of large amounts of undiluted oil (Flaman *et al*., 2001 ; Tisser and Young, 2014).

Another toxicity for a 22-year-old Indian man who ingested roughly 15 ml of Eucalyptus oil while suffering from stomach discomfort and displaying evident poisoning signs. The patient underwent a gastric lavage and was able to restore his health (Ittyachen *et al*., 2019).

Many symptoms appeared in humans and animals including internal irritation, abdominal pain, vomiting, seizures, breathlessness, convulsions, and may lead to death (Kumar *et al*., 2015).

It is believed that eucalyptus compounds are not cancer-causing, determining the toxicity and side effects of eucalyptus can be difficult because the type and quantity of compounds depend on the type of plant, the age of the leaves, and the method of oil extraction (Ghasemian *et al*., 2019).

The median lethal dose of eucalyptus was estimated in mice who administered the oil orally at a different dose, and the LD_{50} was 2334.4 mg/kg and EuO was found to be present is moderately toxic following the World Health Organisation classification (Shalaby *et* al., 2011).

Dermatologically toxic, When applying the oil directly to the skin, negative reactions occur, including irritation and sensitiveness (Michalak, 2018).

An aqueous emulsion containing 5% eucalyptus oil was administered to mice at different oral doses of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 ml/kg. The LD_{50} of the eucalyptus oil emulsion was determined and was found to be between 3 and 3.5 ml/kg (Gebremickael, 2017).

The severity of the skin reaction varies depending on how it is applied, dilution, exposure location, skin integrity, age, environmental conditions, etc, and all these factors can play a significant role in the skin toxicity, along with the presence of sunlight and also temperature and humidity (Burfield, 2000).

The inflammation of the skin occurs rapidly during the first use of the oil as a red streak or burn, and the most dangerous oil contains phenolic compounds, Symptoms include a red rash and can be painful for some persons (Buckle, 2014).

Neurotoxicity of the oil, the oil can easily be carried through the blood-brain barrier and reaches the central nervous system after systemic absorption, convulsions occur, indicating neurotoxicity and the components responsible for spasticity are cineol (Vostinaru *et al*., 2020).

Weight loss, muscular stiffness, trembling, total or absence of movement, and loss of balance (Stojanovic *et al*., 2019).

Children are more susceptible to being poisoned when they consuming oil (Sonker *et al*., 2017).

2-6-1- Factors that increase the toxicity of Eucalyptus oil

This oil is not free from oxidation reactions with the age at which oil is stored (Henley *et al*., 2007). Because oils are sensitive to destruction by oxidation, eucalyptus oil should be stored carefully to maintain its activity and reduce the risk of negative reactions, Therefore, the oil has to be stored in a refrigerator or a cool, dark place (dark bottles) (Sivakumar and Bautista-Banos, 2014).

Sunlight exposure of eucalyptus oil (ultraviolet rays) is one of the most prevalent causes for oil's chemical components to be changed, as a result of which it loses its effectiveness (Jia *et al*., 2019).

2-7- Effect of Eucalyptus oil on body weight

Despite their widespread use, the safety of essential oils has not yet been fully determined, The negative effects in experimental animals are due to the complexity of the chemical components of the oil (Lis-Balchin. 2005). The results of recent animal research involving the effects of Eucalyptus oil on body weight are summarized.

A study Hu *et al*., (2014), showed significant changes in body weight of rats in the groups that orally administered Eucalyptus oil in high doses compared to the control group, and now indicated that the high dose of Euo can reduce weight and not grow in a normal way.

(Gebremickael, 2017) stated that after administering the mice orally with Eucalyptus oil emulsion at a dose of 1.5, 2 ml/kg, there were no significant differences in body weight between the treated groups and the

control group. This indicates that mice are healthy by ingesting an aqueous Eucalyptus oil emulsion.

Gbenou *et al*., (2013) the weight loss of rats after oral administration of *Eucalyptus citriodora* oil indicated a decrease in body weight in the treated group to more than half the weight of the mice in the control group after 4 days of administration of the oil at a dose of 400-600 mg/kg.

Bababaalian *et al*., (2020) showed that Eucalyptus oil improves the growth of rainbow trout after consuming eucalyptus oil with high doses of food for 8 weeks, where a significant increase in fish weight was observed.

2-8- Effect of Eucalyptus Oil on Hematological Parameters

Potential toxicity of essential oils and hematological and histopathological changes and their components were analyzed in laboratory animals, mostly rats, and LD_{50} was used to determine toxicity (Tisser and Young, 2014).

Concluded Shalaby *et al*., (2011) when rats were administration pure eucalyptus oil (not emulsified) at different oral doses 1500, 2250, 3375, 5062 mg/kg for 30 days, the results showed an increase in WBC and a decrease in RBC, HB, and PLT.

Revealed Hu *et al*., (2014) found no changes in hematological parameters WBCs, RBCs, HGB, HCT, PLT when comparing the treated and control group after oral administration to rats of eucalyptus oil from the company at different doses for 30 days.

2-9- Effect of Eucalyptus oil on Body tissues

Stojanović *et al*., (2019) indicated the presence of histological changes in the stomach, duodenum, liver, and kidney of mice upon orally administration of essential oil. The changes included the occurrence of Capillary vasodilatation, Single spotted erosions, Inflammatory cells infiltration, Glandular cell vacuolization in the stomach, as for the duodenum occurrence villi necrosis, inflammatory cells infiltration, Interstitial edema, Capillary vasodilatation, Histological changes in the liver include Vascular congestion, Cytoplasmic micro vacuolization, activated kupffer cells, Parenchymatous degeneration, Eosinophilic infiltration, Sinus spaces narrowing, Karyopyknosis, Hypereosinophilic areas, Changes in glycogen content. As for the kidney occurrence glomerular hypertrophy, Peritubular infiltration, Perivascular infiltration, Tubule cell vacuolization, Cloudy swelling, Hemorrhage, Glomerular cellularity.

Fandohan *et al*., (2008) was found that there were obvious histological changes in the stomach and liver of rats that were administered orally to essential oil, where mucosa atrophy occurred, as well as an increase in lymphocytes.

In the liver and kidney sections, there were minor histological changes such as localized necrosis, vacuolar degeneration of the liver, hyaline casts, and lymphatic infiltration of the kidney. When administered orally, Eucalyptus oil emulsion was found to have no apparent adverse effects (Gebremickael, 2017).

Histological changes appeared upon microscopic examination, including congestion in the central vein, inflammatory cell infiltration, and the appearance of small vacuoles in the liver, and in the kidneys, lobulated renal corpuscles, and desquamation of epithelial cells of the renal tubules. Hemorrhage in the interstitium (Shalaby *et al*., 2011).

Bababaalian *et al*., (2020) found were no histological changes in the intestines and kidneys of rainbow trout when consuming Eucalyptus oil with the food.

A separate study was conducted by Hu *et al*., (2014), The oil was administered orally and diluted in different concentrations in rats for 14

days at dosages of 2772, 3267, 3960, 4750, and 5742 mg/kg, where eucalyptus oil from Xinran Company was utilized, and the histological results showed that there were changes in the liver, including congestion in the central vein and vesicular degeneration, as well as the occurrence of tissue changes in the kidneys, including hyperemia of the glomerulus, as well as the appearance of granulomatous degeneration in the renal tubular epithelial cells, no histological changes occurred in lungs, stomach, intestine, heart when compared with the control group.

In an experiment carried out by the researcher Gbenou *et al*., (2013) where the oil was administered orally in rats at doses of 400,600 mg/kg and it was noted that the weight of the rats decreased by more than half the weight of the rats in the control group and the occurrence of histological changes in the stomach and liver of rats, including the occurrence of erosion or tearing Stomach mucosa, the emergence of a clear change in the stomach when compared with the control group, as well as changes in the liver of the group dosed with eucalyptus oil, including the structural architecture of the liver.

In a study, Sheikhzadeh *et al*., (2011) about the effect of eucalyptus oil on the blood parameters and the activity of respiratory explosions in carp, where the carp were administered orally with eucalyptus oil in different doses, and the results indicated a significant increase in WBC, as well as in the rate of hematocrit, a large increase in respiratory burst activity of blood neutrophils ($P< 0.05$).

In another experiment that performed by Abou Nazel *et al*., (2014), The mice were administered orally with essential oil for 15 days, observed histological changes in the liver, including cytoplasmic vacuoles, an increase in kupffer cells, deterioration of hepatocytes with pyknotic nuclei and infiltration of neutrophils and lymphocytes. In the kidneys, renal tubule cell deterioration, focal necrosis, and infiltration were shown. Cellular and large capsule space and degeneration of the cells lining the renal tubules.

The effects of Eucalyptus oil and cineole on rat tracheal rings were studied, and the results revealed that eucalyptus oil had a considerable influence on rat tracheal ring enlargement. The researchers observed that eucalyptus oil and cineol had biological effects on the trachea, such as

bronchial muscle relaxation and bronchodilation (Nascimento *et al*., 2009).

A group of rats was administered eucalyptus oil orally via gavage at a dose of 8 or 32 mg/kg, 6 days per week for 8 weeks. The results showed no toxicity to rats and no change in body weight during the trial period. Furthermore, food and water consumption were normal, and in the histological study, no changes were observed in the tissues of the lungs, liver, spleen, kidneys, and adrenal glands (Bhowal and Gopal, 2015).

CHAPTER THREE

MATERIALS AND METHOD

3-Materials and Methods

3-1-Apparatus, Instruments, Chemicals used

3-1-1-Laboratory Apparatuses

Some apparatuses were used in this study, as shown in the following Table (3-1).

Table (3-1) The Table shows the name of the apparatus, the and the origin

No	Name Apparatus	Manufacture Company	Country
1	Light Microscope	Olympus	Japan
2	Microtome	Leitz	Germany
3	Electric Oven	Binder	Germany
4	Refrigerator	LG	Korea
5	Water bath	Tafesa Hannover	Germany
6	Hot plate	Tglassco	India
7	Incubator	Biocotek	China
8	Distillation device	WB2800	Germany
9	Sensitive balance	Kerm	Germany
10	Digital Camera	Sony	Japan
11	Magnetic Stirrer	Daihan. Lab .tech	Korea
12	Heating Mantle	MTOPs	Korea
13	Electrical Balance	Kerm	Germany
14	Automated Hematological Analyzer	Mindray	China
15	paraffin dispenser	Premiere	China

3-1-2- Instruments

Some instruments were used in this study, as shown in the following Table (3-2).

Table (3-2) The Table shows the name of the instruments and their origin.

No	Name instruments	Manufacture Company	Country
1	Surgical Set	Hebson	India
2	Micro pipetes	DRAGON	Germany
3	knife of microtome	LG	Korea
4	Oral gavage		China
5	Cotton	Citioglas	China
6	Cover slip	Klempa	USA
7	Plastic cups	Shangai Blopak	China
8	Slide	Citioglas	China
9	Syringe	Citioglas	China
10	Glove	Broche	China
11	Filter paper	Whatman	China
12	Metal embedding template	Local made	Local made
13	Plastic cage	Kajeen	Iran
14	EDTA tube	Vacuette	China
15	Condenser	ISOLAB	Germany
16	Round flask	ISOLAB	Germany
17	Separator Funnel	StonyLab	New York
18	Adapter	StonyLab	New York

3-1-3- Chemicals

Some chemicals were used in this study, as shown in the following Table (3-3).

Table (3-3) The Table shows the name of their chemicals and the origin

No	Name chemicals	Company	Country
1	Ethanol (absolute 100%)	BDH	England
2	Formalin	BDH	England
3	xylene	BDH	England
4	Paraffin wax	Merck	Germany
5	Haematoxylin & Eosin	BDH	England
6	Canada Balsam	Roth	Germany
7	Chloroform	Sigma	Switzerland

8	Fuchsin Basic	Dakocytomation	Denmark
9	Peridic acid	Dakocytomation	Denmark
10	Normal Saline Solution	Fresenius Kabi	Germany
11	Hydrochloric Acid	Sigma	Switzerland
12	Charcol Activated	BDH	England
13	Glacial acetic acid	BDH	England
14	Potassium alum	Hi media	India
15	Glycerin	RPI	USA
16	Sodium phosphate, monobasic, monohydrate	Hi media	India
17	Sodium phosphate, dibasic, anhydrous	BDH	England
18	n-Hexane	Pure Chemicals Co	India

3-2-Plant material collection

The leaves of *Eucalyptus camaldulensis* Figure (3-1) were collected from September to december 2020 from the governorate of Misan / awash garden. The taxonomic identity of the plant was confirmed by Assist.Prof.Dr. Sahar A.A.malik in University of Basrah College of Science and M. sadeq sabeeh in University of Misan College of Science.

3-3-Extraction of Eucalyptus Essential Oil

Freshly collected leaves of *Eucalyptus camaldulensis* were cleaned by using distilled water to get rid of the mud and dirt over its surface and air-dried at room temperature under shade (Abed and Naife, 2018). Then, chopped into smaller pieces and the essential oil extraction was carried out by using hydro-distillation technique Clevenger-type apparatus, consists of a heating mantle and a round flask (1000 ml) connected to a condenser through an adapter, the weight of 100 grams of leaves were separately mixed with 500 ml of distilled water in a rounded flask (1L) Figure (3-2), and allow the steam to pass through the condenser for cooling, and the mixture of water and oil is collected in a round (500 ml).

The purpose of cooling in the condenser is to separate the oil from the water because most of the essential oils do not combine well with the water due to the difference in density (Shalaby *et al*., 2011), and subjected to hydrodistillation for three hours.

The essential oil were separated by n-hexane, The extraction process was repeated several times to collect about 20 ml of oil, and the obtained oil was stored in a refrigerator away from light, after which the chemical compounds of oil were analyzed by Gas Chromatography Mass Spectrometry (GC-MS) technology in the laboratories of Nahran Omar central affiliated to the Basra Oil Company.

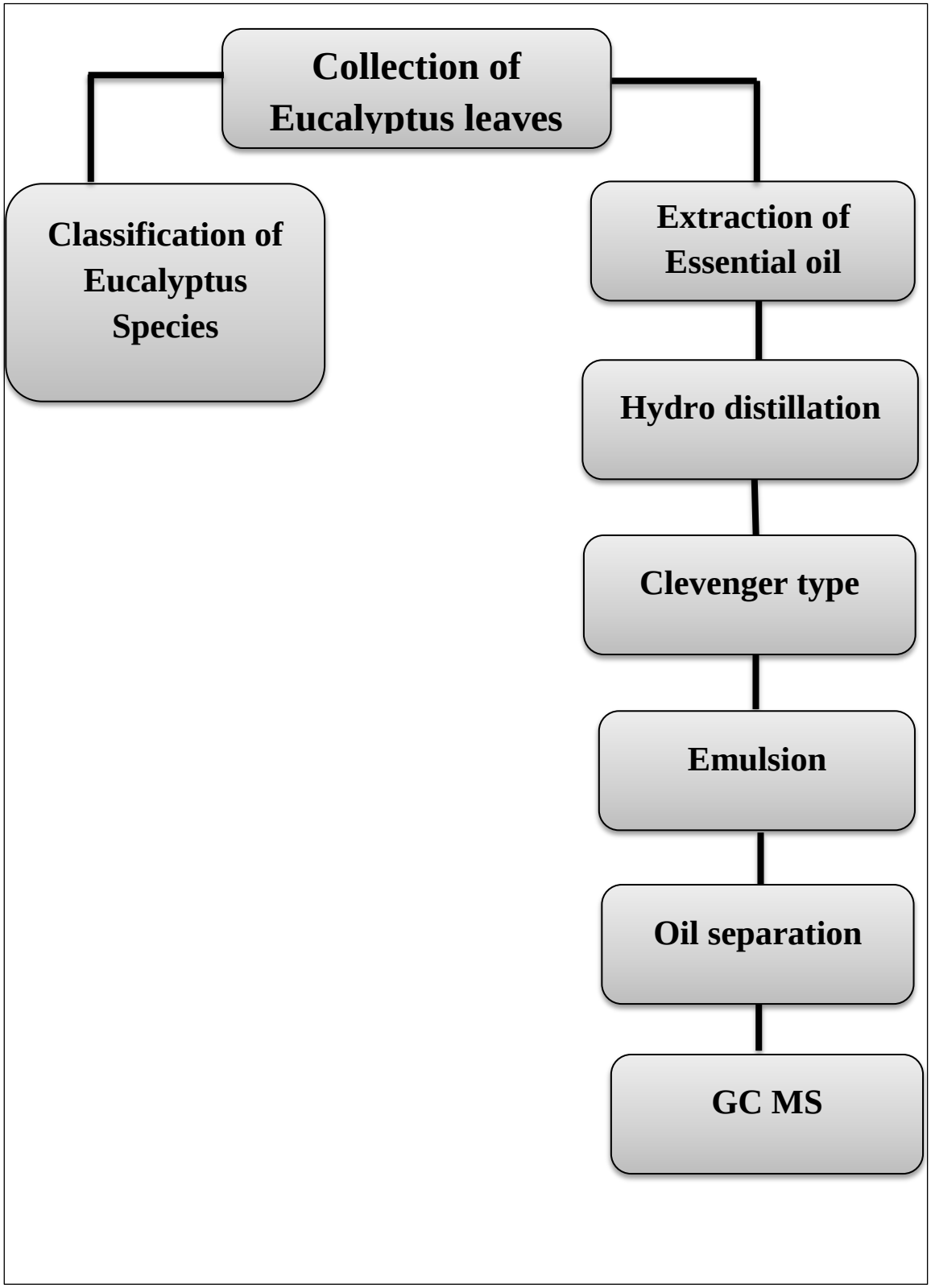


Diagram (3-1) Eucalyptus Study Design

Figure (3-1) *Eucalyptus camaldulensis*

Figure (3-2) hydro distillation technique Clevenger-type apparatus

3-4-Experimental Animals

Male and female BALB/c mice are aged 8–12 weeks their average weight was 25 gm, pathogen free mice, were obtained from the animal house the department of biology, of the college of sciences university misan. The experimental animals were transported to the animal house located in the department of biology of the university of misan, where they were placed in plastic cages covered with a metal mesh sheet, the animal cages were spread with sawdust and the mulch was replaced twice a week, with the continuation of cleaning and sterilization of the cages, the animals were left for two weeks for conditioning in the animal house before treating with eucalyptus oil.

The study included 170 mice and housed 5 mice per cage in a 12/12 h light/dark cycle with food and tap water, The animal food consists of 50% wheat, 30% fish, greens 13%, salt sodium chloride (NaCl) 2% and raw fat 5% (Al-Attar and Jihad, 2013).

All experimental procedures were approved by the local animal ethics committee and met german and international guidelines. The handling and use of animals were following the institutional guidelines.

3-5- Calculation of Median Lethal Dose (LD_{50}) of Eucalyptus Oil

The experiment was conducted to determine the median lethal dose (LD_{50}), in five groups 10 mice each (n=10/group) of male mice, like the following, the control group was administered normal saline, The animals were administered eucalyptus oil once each time/ daily. If the animal survived from the first dose, the next dose of the mice would be increased. and if the animal died from the first dose, the next dose of animal is reduced, all animals have fasted for three hours before the administration of eucalyptus oil, and one hour after the eucalyptus oil administration , The four groups were administration by oral gavage using eucalyptus oil at doses of, 1200, 1600, 2000, 2400 mg/kg of body weight, and depending on the weights of mice, the dose was calculated by µl and the average of weights was 25g, mice administered was continued for 14 days. Clinical signs and behavior effects were observed in mice for 24 hours and registration of deaths and survival. The toxicological effect

was evaluated according to the mortality rate expressed by LD_{50} (Karber, 1931) expired animals were counted for the calculation of LD_{50} . The arithmetic method of Karber was used for the determination of LD_{50} according to the following equation:

$$LD_{50} = LD_{100} - \sum \frac{(a \times b)}{N}$$

LD_{100} = Lethal dose causing 100% death.

N = total number of animals in a group.

a = the difference between two successive doses administered.

b = the average number of dead animals in two successive doses

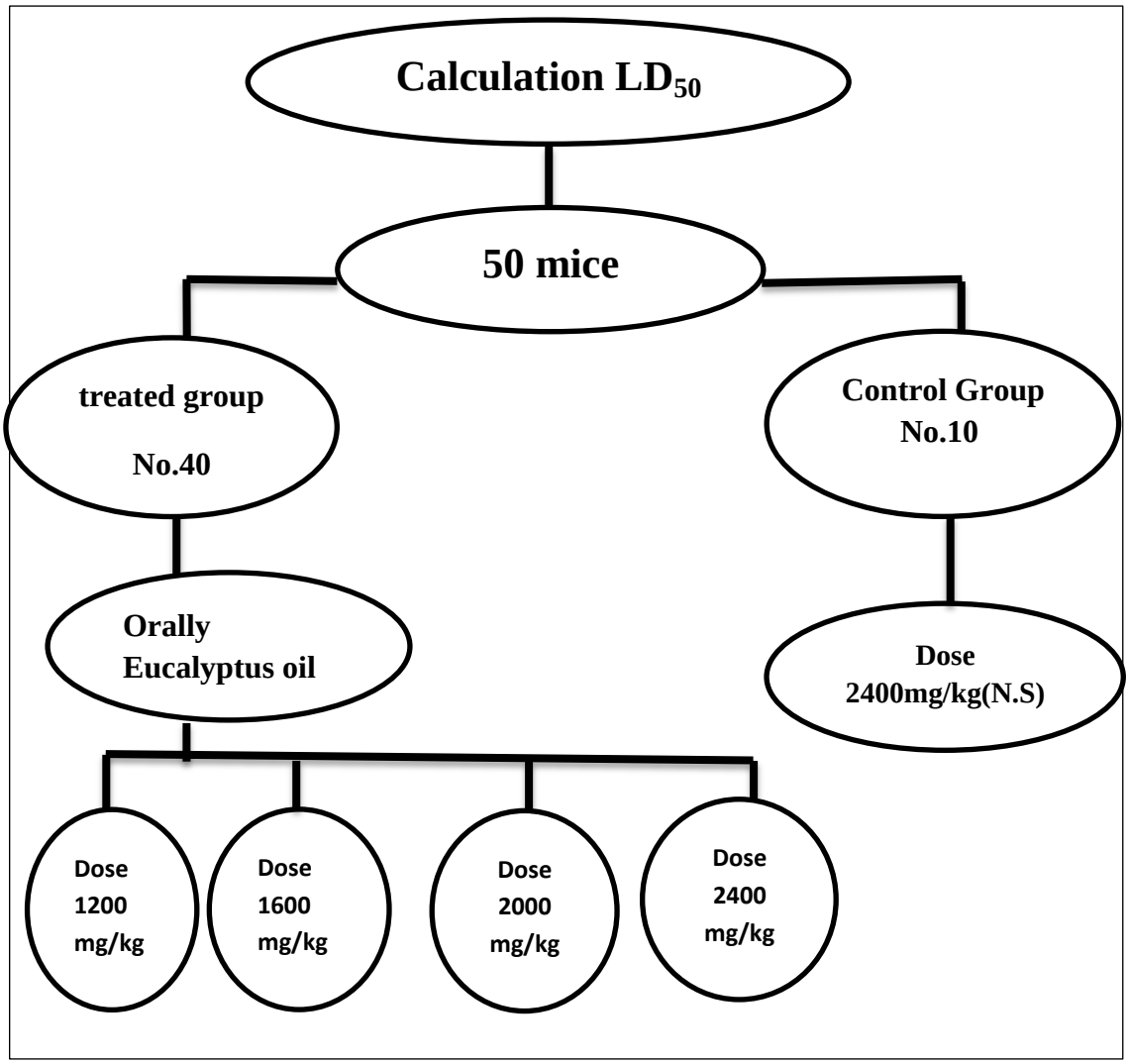


Diagram (3-2) Calculation LD_{50} Method

3-6- Histological and Hematological Changes Study

This study was conducted using 120 male and female adult mice, and the animals were divided into four groups as follows:

Group I (control): This group included 60 mice divided into three groups (orally, inhalation, mixing) and administered 1000 mg/kg of normal saline for four weeks (28 days) in orally and mixing group, in inhalation group was exposed to inhaling by placing 1000 mg/kg of normal saline on cotton in a closed cage for 15 minutes a day.

Group II (orally): included 20 mice that were administered eucalyptus oil orally by oral gavage dose 1000 mg/kg for four weeks.

Group III (Inhalation): included 20 mice this group was exposed to inhaling eucalyptus oil by placing 1000 mg/kg of oil on cotton in a closed cage for 15 minutes/ daily.

Group IV (Mixing): included 20 mice this group administered Eucalyptus oil oral dose 1000 mg/kg and also inhaled eucalyptus oil for 15 minutes/ daily.

With measuring the body weights of mice by the electrical balance in all groups in the four weeks, and recording clinical signs and behavior effects on mice, all animals have fasted for three hours before the administration of eucalyptus oil, and one hour after the eucalyptus oil was administered (Gebremickael, 2017).

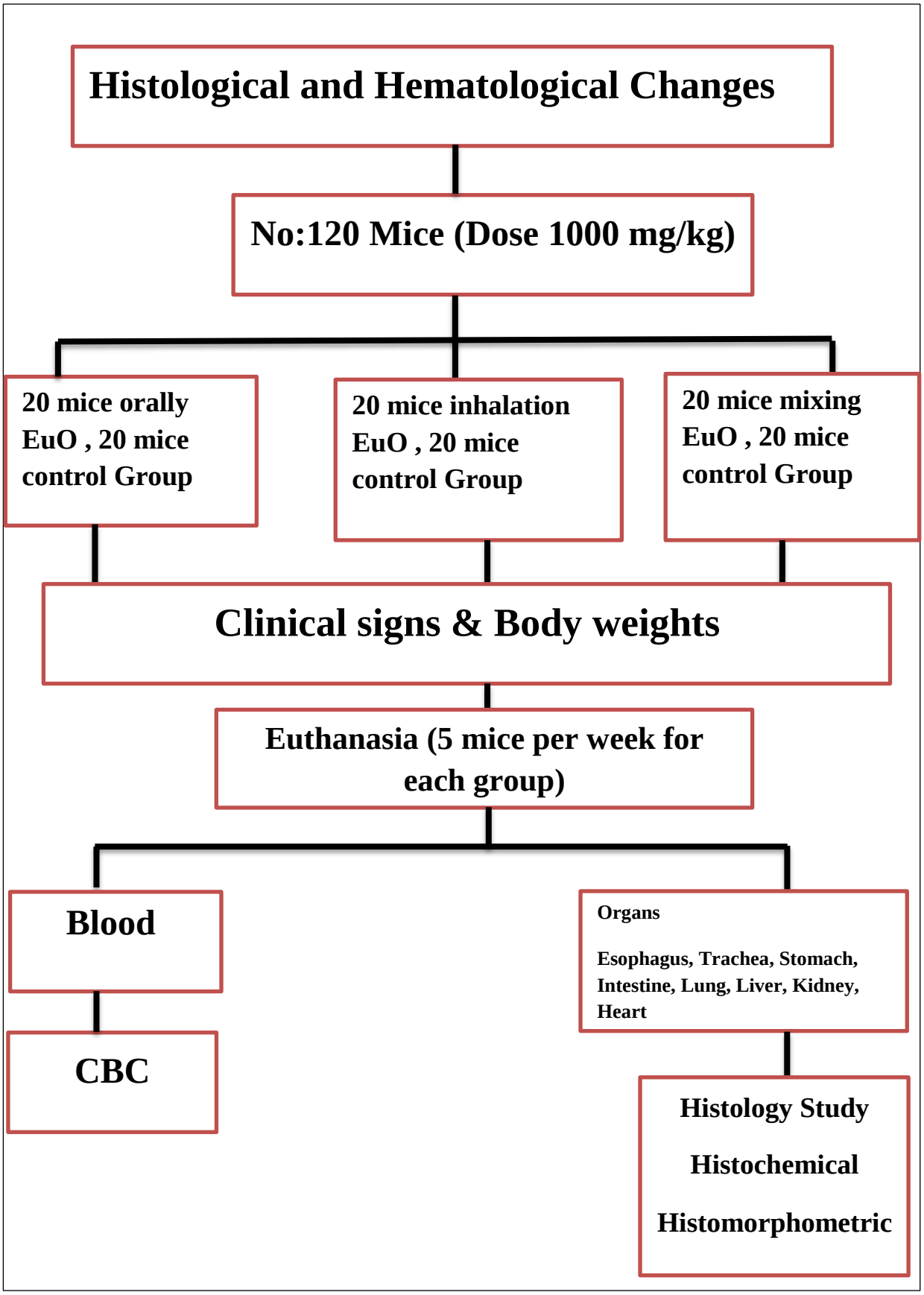


Diagram (3-3) Histological and Hematological Changes

3-7- Collection of Blood Samples

Euthanasia to mice by placed 1 ml of chloroform (CHCl3) on cotton and then placed it in a closed cage containing mice. (Blackshaw *et al*., 1988). The blood was collected every week from the heart using a syringe (3ml). The blood samples were taken from the heart (1ml) (Parasuraman *et al*., 2010), preferably from the ventricle slowly to avoid collapsing of heart and the blood was placed into a tube containing EDTA, The complete blood count was calculated using Automated Hematological Analyzer (mindary BC- 30S CBC). The hematological parameters measured including White blood cell count (WBC), lymphocytes (LYM), monocytes (MID), neutrophils (GRAN), Red blood cell count (RBC), Hemoglobin concentration (HGB), Blood platelet (PLT), Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet crit (PCT), platelet distribution width (PDW).

3-8- Collection of Organs Specimens

The 5 mice were killed every week from each group , mice were dissected as follows.

1-After euthanizing the mice and collecting blood, the animals were dissected and placed on the dorsal surfers in the dissection plate and the anterior and posterior limbs were fixed.

2- A longitudinal incision was made in the midline in the form of a T-shape of the skin and it starts at the urogenital opening up to the lower jaw.

3- Two transverse slits were made, passing the anterior and posterior limbs.
4- Separation of the skin from the muscular layer.
5- Incise transversely under the thoracic cavity and diaphragm.
6- The lungs, trachea, esophagus, stomach, small intestine, heart, liver, and kidneys were removed and washed with normal saline (Basim, 2019)

3-9-Histological Sections Preparation

Depending on the method of Luna (1968) The preparation of tissues slides were as follows.

1-fixation:

The current tissue study samples were fixed in neutral buffered 10% formalin solution for 48 hours.

2-Washing:

Samples were washed after fixation with formalin solution with running tap water for 3-4 hours to remove fixative residue from samples.

3- Dehydration :

The samples were passed after washing with a series of ethyl alcohol, in ascending concentration (70%, 2h), (80%, 1 h), (96%, three changes, 2h each), (100% overnight to remove the water in the samples), then (100% 2 hour).

4- Clearing:

Transfer of samples to xylene in two stages, for each stage for two hours.

5- Infiltration and Embedding:

The samples were transferred from xylene to a solution of xylene and paraffin wax and at a ratio of 1: 1 inside an electric oven at 60 °C for one hour. Then the samples were transferred to paraffin wax and in two stages for two hours each, then the samples were placed in molds filled with paraffin wax.

6- Trimming and Sectioning:

The wax molds containing the samples were trimmed with a sharp scalpel and fixed in the microtome, and they were cut with a thickness of 5 microns in the form of longitudinal and transverse sections. A drop of the egg albumin was placed on glass slides. Then the strip containing the samples was transferred to the water bath and the strip was carried on the slides and transferred to a hot plate with a 37 ° C to brushing the tapes on the slide.

7- Staining:

7-1- Hematoxylin & Eosin staining

The staining process was carried out according to the method of (Luna,1968) according to the following steps

1- Remove the wax from the tissue sections by placing it in xylene in two stages for 10 minutes for each.

2- Passing the tissue sections with a series of ethyl alcohol, in descending concentration (100%, 95%, 70%) for 3 min for each concentration.

3- Tissue sections were transferred to distilled water for 5 min.

4- The tissue sections were transferred to the hematoxylin Ehrlich stain for 15 minutes.

5- The tissue sections were washed with tap water for 10 minutes until the color turned blue.

6- Histological sections were placed in eosin stain for 5 minutes.

7- Sections were washed with tap water for 2 minutes.

8- Passing the sections with a series of ethyl alcohol, ascending the concentration (70%,95%,100%) and for 5 seconds for each concentration.

9- Slides were placed in xylene and two stages for 3 minutes for each stage.

9- The tissue sections were mounted with Canada balsam by placing a drop on the cover slide, then the slides were transferred to a hot plate at 37 ° C.

3-10-Histochemical Study

3-10-1- Periodic Acid Schiff (PAS) Staining

The staining process was carried out according to the method of Bancroft and Stevens, (2012) according to the following steps.

1- Remove the wax from the tissue sections by placing it in xylene in two stages for 10 minutes for each.

2- Transfer slides to a series of ethyl alcohol, lowering the concentration (100%, 95%, 70%) for 3 min for each concentration.

3- Tissue sections were transferred to distilled water for 5 min.

4-The sections were transferred to a periodic acid solution for 5 minutes.

5-The sections were washed with tap water for 5 minutes and then slides were placed in distilled water for 2 minutes.

6- Slides were transferred to Schiff's reagent for 30 minutes and then transferred to distilled water for 2 minutes.

7- The slides were washed with tap water for 5-10 minutes.

8- The slides were transferred to a chain of ethyl alcohol ascending concentrations (70,95,100%), for each concentration of 5 seconds.

9- Slides were placed in xylene and two stages for 3 minutes for each stage.

10- The tissue sections were mounted with Canada balsam by placing a drop on the cover slide, then the slides were transferred to a hot plate at 40 ° C.

3-11- Examining and Photoimaging of Histological Sections

The tissue sections were examined using a light microscope installed with different magnification powers (100X, 400X) and the sections were photographed with a digital Camera, in the Animal Tissue Laboratory at the College of Sciences/University Misan.

3-12- Histomorphometric

Tracheal lumen and alveoli were measured and mucosa, sub mucosa, muscularis, and serosa thicknesses were measured in the control, inhalation, orally, and mixing groups using light microscopy with an ocular micrometer after matching the ocular micrometer with Stage micrometer using the magnification force (Galigher and kozloff , 1964).

3-13- Statistical Analysis

Extract the mean and standard error of weight body, hematological parameters, lumen of trachea and alveoli and thickness of mucosa, sub mucosa, muscularis and serosa in the control, inhalation, orally and mixing groups, and the data were analyzed using the statistical program Social Package of Social Sciences (SPSS) , using independent samples T-test to calculate the statistical differences (Griffith, 2007).

CHAPTER FOUR

RESULTS

4- Results

4-1-Results of Gas Chromatography-Mass Spectrometry Analysis

The amount of Eucalyptus oil extracted by hydrodistillation is 2 ml per 500 grams (Figure 4-1) from the leaves of a plant *Eucalyptus camaldulensis*, The eucalyptus oil was fractionated and the bioactive chemical components in the oil were detected using the GC-MS technique. The chemical name of each compound, Peak, Retention time, and Area%, were obtained, 98 compounds were detected, the main chemical components as shown in Table(4-1), Figure (4-2), where the area of Eucalyptol was (4.606) and Peak 5, β-Pinene (1.396) and Peak 12, Isolpulegol (1.454) and Peak 16, α-terpineol (3.374) and Peak 17, cis-p-mentha-1(7),8-dien-2-ol (1.274) and Peak 20, Thymol (2.072) and Peak 25, Aromandendrene (8.018) and Peak 37, Ledene (22.68) and Peak 48, γ-Eudesmol (3.874) and Peak 50, β-Eudesmol (12.921) and Peak 51, 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol (2.042) and Peak 56, Dioctyl terephthalate (2.686) and Peak 87.

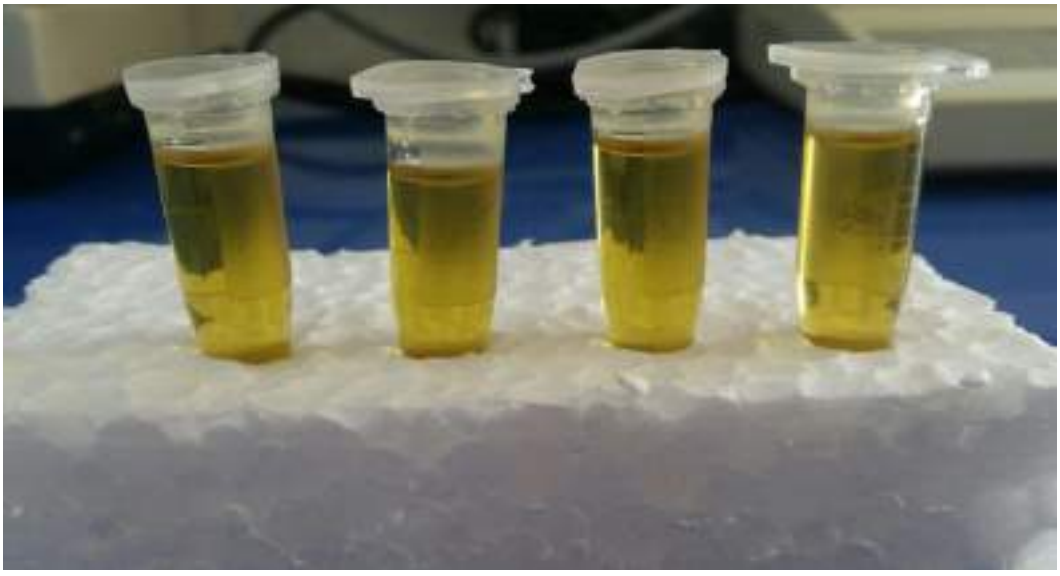
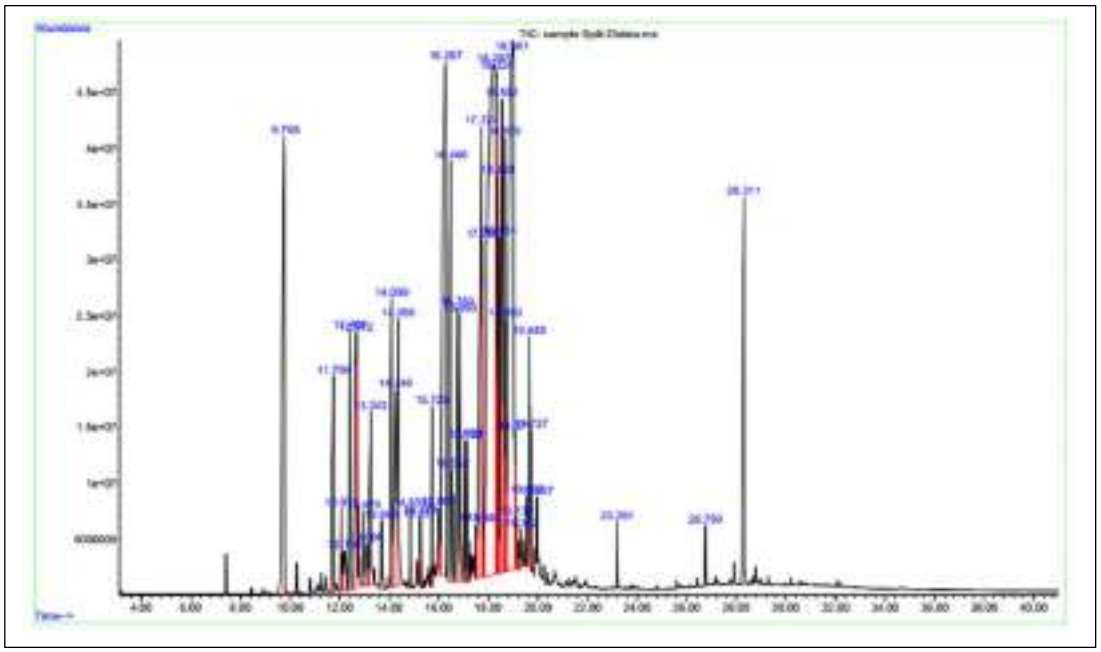


Figure (4-1): Eucalyptus oil extracted using Hydro distillation by Clevenger-type apparatus



Figure(4-2): Chromatogram showed the identity of the main compounds of oil of *Eucalyptus camaldulensis.*

Table (4-1): The Main Chemical Composition of Eucalyptus Oil

No	Composite Name	Peak	RT	Area%	Chemical Formula
1	Eucalyptol	5	9.765	4.606	$C_{10}H_{18}O$
2	β-Pinene	12	11.744	1.396	$C_{10}H_{16}O$
3	Isolpulegol	16	12.404	1.454	$C_{10}H_{18}O$
4	α-terpineol	17	12.667	3.374	$C_{10}H_{18}O$
5	cis-p-mentha-1(7),8-dien-2-ol	20	13.238	1.274	$C_{10}H_{16}O$
6	Thymol	25	14.357	2.072	$C_{10}H_{14}O$
7	Aromandendrene	37	16.254	8.018	$C_{15}H_{24}$
8	Ledene	48	18.215	22.68	$C_{15}H_{26}O$
9	γ-Eudesmol	50	18.632	3.874	$C_{15}H_{26}O$
10	β-Eudesmol	51	18.978	12.921	$C_{15}H_{26}O$
11	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8 ,8a-octahydronaphthalene-2,3-diol	56	19.655	2.042	$C_{15}H_{24}O_2$
12	Dioctyl terephthalate	87	28.304	2.686	$C_{15}H_{24}O_2$

4-2- Calculation of Median lethal Dose (LD_{50}) of Eucalyptus Oil

The LD_{50} the value was 1820 mg/kg was as showed in (Table 4-3). Mortality was recorded only at 1600, 2000 and 2400 mg/kg, three mice out of ten animals have died overnight following the administration in the group (2) that received 1600 mg/kg, and seven mice out of ten animals in group(3) died within 24 hours that received 2000 mg/kg, and nine mice out of ten animals in group (4) died within 24 hours that received 2400 mg/kg, calculated as showed in (Table 4- 2).

Table (4-2): The results of oral acute toxicity test of eucalyptus essential oil in mice

Group	No. mice	dose mg/kg	No. of death	No. of Survived	Mortality %	Livability%
Control	10	2400	0	10	0	100
Group 1	10	1200	0	10	0	100
Group 2	10	1600	3	7	40	70
Group 3	10	2000	7	3	70	30
Group 4	10	2400	9	1	90	10

Table (4-3): Calculation of LD_{50} by the method of karber

Group	Dose mg/kg	No. of animals dead	Dose difference(a)	Mean mortality(b)	Probit (a× b)
Group 1	1200	0	400	0	0
Group 2	1600	3	400	1.5	600
Group 3	2000	7	400	5	2000
Group 4	2400	9	400	8	3200

Sum of the product = 5800

$$LD_{50} = LD_{100} - \sum \frac{(a \times b)}{N}$$

$$= 2400 - 580$$

$$= 1820 \text{ mg /kg}$$

4-3- Results of Clinical Signs

Results from this study in mice that administered eucalyptus oil orally and mixing groups with 1000 mg/kg and for 4 weeks (28 days) demonstrated some clinical signs after 5 minutes of administering the dose including dizziness and loss of appetite, lethargy, and slow movement. These signs were noticed from the first week and that the severity of these signs increased with 28 days of administration compared to a control group, However, in the inhalation group, these clinical signs do not appear when eucalyptus oil is inhaled during the experiment period as shown in Table (4-4).

4-4- Results of the Body of Weights

The results showed there were no significant differences ($P>0.05$) in the inhalation group in the average weights of the animals, as (24.8±0.44) in the first week, second week (25.8±0.30), third week(27.8±0.38), and (29.7±0.29) weights were recorded in the fourth week compared with weights in fourth weeks of the control group that continued to grow and gains weight within four weeks, and weights were recorded a (24.9±0.22) in the first week, second week (26.0±0.22), third week (28.2±0.20), and (29.9±0.17) in the fourth week.

A significant decrease in the mice body weight ($P<0.05$) when comparing with a control group in the mean weights of the mice in orally and mixing groups after the four weeks of eucalyptus essential oil administration where the weights were in the first week of the orally group (23.3 ± 0.23), second week (21.0±0.26), third week (18.2±0.24) and the fourth week the weights of (15.8±0.10). The mice in the mixing group were in the first week (23.0±0.16), second week (21.5±0.63), third week (18.3±0.17), and decreased in the fourth week (16.2±0.19), as shown in Table (4-5).

Table (4-4): shows clinical signs in mice in the first and fourth weeks.

Clinical signs	Group	1^{st} week	period min	4^{th} week	period min
dizziness, loss of appetite, lethargy, slow movement	control	negative	-	negative	-
	orally	positive	5	positive	10
	inhalation	negative	-	negative	-
	mixing	positive	5	positive	10

Table (4-5): shows the changes in the body weights of mice over the four week

Group	body weights (gram)			
	1^{st} week	2^{nd} week	3^{rd} week	4^{th} week
Control	24.9 [a] ± 0.22	26.0 [a] ± 0.22	28.2 [a] ± 0.20	29.9 [a] ±0.17
Inhalation	24.8 [a] ± 0.44	25.8 [a] ± 0.30	27.8 [a] ± 0.38	29.7 [a] ±0.24
Orally	23.3 [b] ± 0.23	21.0 [b] ± 0.26	18.2[b] ± 0.24	15.8[b] ± 0.10
Mixing	23.0 [b] ± 0.16	21.5 [b] ± 0.63	18.3[b] ± 0.17	16.2[b] ± 0.19

*Note: the value represents (mean± SE), Vertically similar letters indicate that there are no significant differences ($p>0.05$), different letters vertically between the values indicate that there are significant differences ($p<0.05$).

4-5- Results of Hematological Study

The results showed no significant differences (P> 0.05) between the hematological parameters in the first week in the inhalation, orally, and mixing group when comparing the control group.

The results indicated significant differences (P <0.05) in white blood cells (WBCs) and their types in the orally and mixing group in the fourth week. while the inhalation group, there is no significant difference in (P> 0.05) in the WBCs, compared with the control group.

The results showed significant differences (P< 0.05) in red blood cells orally and mixing group, where RBCs in the fourth week. As for the inhalation group, there were no significant differences (P >0.05) compared with the control group.

The results showed significant differences (P< 0.05) hemoglobin in both orally and mixing groups in the fourth week, while in the inhalation group, there were no significant differences (P >0.05) in hemoglobin concentration compared with the control group.

The results showed significant differences (P< 0.05) hematocrit in both orally and mixing groups in the fourth week, while in the inhalation group, no significant differences (P >0.05) in HCT.

Also, the results indicated that there were significant differences (P< 0.05) in the numbers of blood platelets in both the orally and mixing group, and in the inhalation group, there were no significant differences (P> 0.05) in the rate of PLT compared with the control group.

The results indicated other hematological parameters MCH, MCV, MCT, HCT, MCHC, RDW, MP V, PCT, PDW. There were no significant differences (P>0.05) in the four weeks for the three groups: orally, mixing, inhalation compared to the control group as shown in Table (4-6)

Table (4-6) Observed Hematological parameters changes after administration eucalyptus essential oil to the mice in the control, orally, inhalation, mixing.

Hematological Parameters	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
WBC ($x10^9$/L)	5.4 ^{a} ±0.70	5.5 ^{a} ± 0.15	5.8 ^{a} ±0.26	5.6 ^{a} ±0.28	5.6 ^{a} ±0.06	5.4 ^{a} ± 0.13	9.08 ^{b} ±0.18	9.2 ^{b} ±0.22
LYM ($x10^9$/L)	4.8 ^{a} ±0.13	4.8 ^{a} ± 0.22	5.1 ^{a} ±0.22	4.9 ^{a} ±0.30	4.9 ^{a} ±0.30	4.8 ^{a} ± 0.22	8.0 ^{b} ±0.20	8.2^b ±0.25
MID ($x10^9$/L)	0.3 ^{a} ±0.03	0.4 ^{a} ± 0.04	0.4 ^{a} ±0.04	0.4 ^{a} ±0.03	0.4 ^{a} ±0.05	0.3 ^{a} ± 0.03	0.4 ^{a} ±0.04	0.4 ^{a} ±0.03
GRAN ($x10^9$/L)	0.3^a ±0.03	0.3^a ± 0.03	0.3^a ±0.03	0.3^a ±0.03	0.3^a ±0.03	0.3^a ± 0.03	0.6^b ±0.03	0.6^b ±0.04
RBC ($x10^{12}$/L)	9.7 ^{a} ± 0.10	9.9 ^{a} ± 0.16	9.0 ^{a} ±0.35	9.2 ^{a} ± 0.20	9.6 ^{a} ± 0.06	9.4 ^{a} ± 0.05	5.5 ^{b} ±0.15	5.8 ^{b} ± 0.21
HGB (g/dL)	13.4 ^{a} ± 0.22	14.0 ^{a} ± 0.63	13.4 ^{a} ± 0.25	13.1 ^{a} ± 0.33	13.5 ^{a} ± 0.22	13.2 ^{a} ± 0.45	9.3 ^{b} ± 0.14	9.4 ^{b} ± 0.21
HCT (%)	39.8^a ±1.23	38.5 ^{a} ±1.93	40.0 ^{a} ±1.78	38.8 ^{a} ±1.72	40.4^a ±1.13	37.9 ^{a} ±1.82	22.5 ^{b} ±0.49	21.1^b ±0.55
MCV (fL)	46.3^a ±3.10	44.0^a ±2.18	43.2^a ±1.78	43.28^a ±1.78	46.1^a ±2.59	45.6^a ±2.19	43.0^a ±1.88	41.1^a ±1.88
MCH (pg)	15.0 ^{a} ±0.70	15.3 ^{a} ±0.57	14.5 ^{a} ±0.35	15.2 ^{a} ±0.37	15.2 ^{a} ±0.43	15.5 ^{a} ±0.64	14.6 ^{a} ±0.55	15.6 ^{a} ±0.29
MCHC (g/dL)	36.7 ^{a} ±1.96	35.1 ^{a} ±0.65	36.7 ^{a} ±2.3	39.9 ^{a} ±0.99	37.3 ^{a} ±1.63	36.4 ^{a} ±0.10	36.1 ^{a} ±2 .28	38.9 ^{a} ±1.35
RDW %	20.3 ^{a} ±0.85	21.6 ^{a} ±0.45	20.4 ^{a} ±0.67	22.2 ^{a} ±0.51	20.6 ^{a} ±0.77	21.9 ^{a} ±0.28	20.7 ^{a} ±0.69	22.5 ^{a} ±0.53
PLT ($x10^9$/L)	974 ^{a} ± 8.2	940 ^{a} ± 20.0	940 ^{a} ± 4.83	946 ^{a} ± 16.9	984 ^{a} ± 9.1	922 ^{a} ± 16.2	568 ^{b} ± 14.7	535 ^{b} ± 9.38
MPV (fL)	5.1^a ±0.26	4.8 ^{a} ±0.31	5.4 ^{a} ±0.21	5.84 ^{a} ±0.29	5.3^a ±0.16	5.0 ^{a} ±0.28	5.2 ^{a} ±0.24	5.8 ^{a} ±0.08
PCT (mL/ L)	1.4 ^{a} ± 0.14	1.28 ^{a} ± 0.10	1.6 ^{a} ± 0.21	1.5 ^{a} ± 0.15	1.54 ^{a} ± 0.10	1.38 ^{a} ± 0.09	1.6 ^{a} ± 0.22	1.4 ^{a} ± 0.14
PDW %	16.7 ^{a} ± 1.14	17.1 ^{a} ± 0.68	16.9 ^{a} ± 0.41	15.8 ^{a} ± 0.32	17.1 ^{a} ± 1.85	17.2 ^{a} ± 0.70	16.8 ^{a} ± 0.40	16.3 ^{a} ± 0.45

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences ($p>0.05$), different letters horizontally between the values indicate that there are significant differences ($p<0.05$).

4-6- Results of Esophagus

4-6-1- Histological Changes

Microscopic examination of the esophagus tissue sections in mice of the control group showed that in mice it consists of four layers, Mucosa, Submucosa, muscularis, and adventitia from the inside to the outside of the esophagus, Mucosa which consists of (epithelium, lamina propria, muscularis mucosa), The type of epithelium of mucosa is keratinized stratified squamous epithelium, lamina propria consists of loose connective tissue, capillaries, lymphatic vessels, and inflammatory cells, as muscularis mucosa consists of smooth muscle fibers. The submucosa consists of connective tissue and contains blood vessels, lymph vessels, and nerves. The muscularis layer consists of skeletal muscles and between these muscles, there is a nerve plexus. Adventitia consists of loose connective tissue (Figure 4-3).

Histological sections of the esophagus of mice treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the esophagus in the control group and that no histological change was apparent (Figure 4-4) during the experiment.

Histological sections of the esophagus of mice that were administered orally, showed the presence of tissue damage, including the emergence of erosion and sloughing in the mucosa and slight congestion in the sub mucosa (Figure 4-5), in the first week and these changes increased in the second and third week, as for the fourth week, hypertrophy also appeared in the muscularis mucosa, as well as an increase of erosion and sloughing in the mucosa, became severe congestion in the sub mucosa compared to the first week (Figure 4-6).

As for the histological sections of the mixing group, there was also the emergence of erosion and sloughing in the mucosa and slight congestion in the sub mucosa in the first week (Figure 4-7). These changes increased in the second, third, and fourth week (Figure 4-8), and the results of the changes in the mixing group were similar to the results of the orally group.

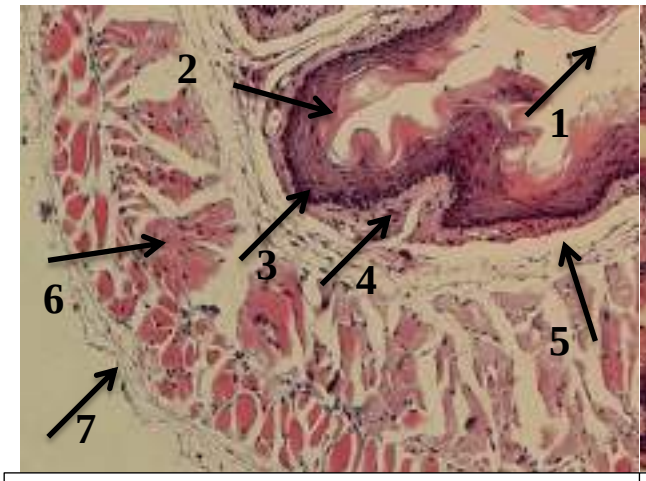


Fig (4-3)section of control group of esophagus mice showing the normal structure of esophagus ,Lumen of esophagus (1),keratinized stratified squamous epithelium(2), lamina propria (3),muscularis mucosa(4) Sub mucosa(5), muscularis (6),adventitia (7) H & E

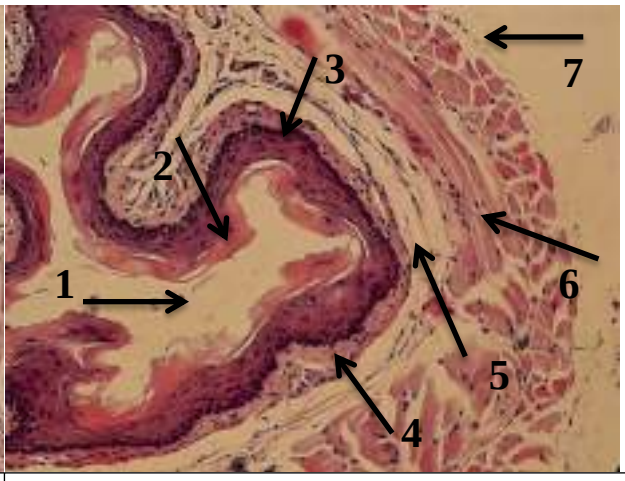


Fig (4-4)section of inhalation group 4^{th} week of esophagus mice showing,Lumen of esophagus (1), keratinized stratified squamous epithelium(2), lamina propria (3), muscularis mucosa(4),Sub mucosa (5),muscularis (6),adventitia (7) , (H & E stain, 100X).

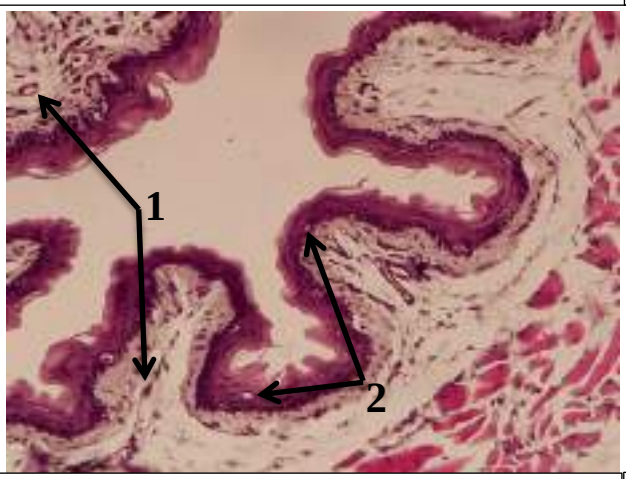


Fig (4- 5) section of esophagus mice of orally group 1^{st} week showing, Little congestion in sub mucosa(1), erosion and sloughing in mucosa(2) , (H & E stain 100X)

Fig (4-6) section of esophagus mice of orally group 4^{th} week showing, Congestion in sub mucosa(1)erosion and sloughing in mucosa(2), hypertrophy muscularis mucosa(3)H & E stain 100X)

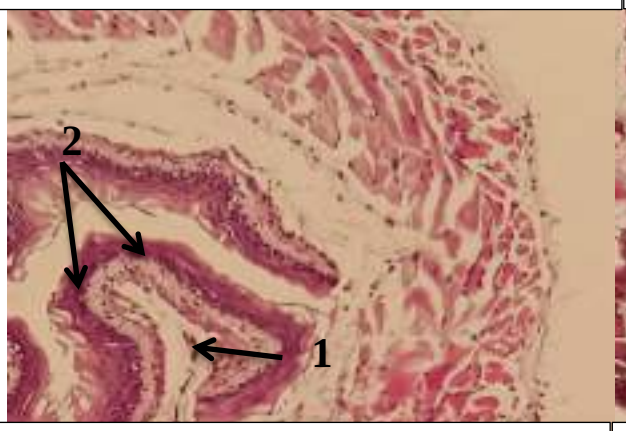
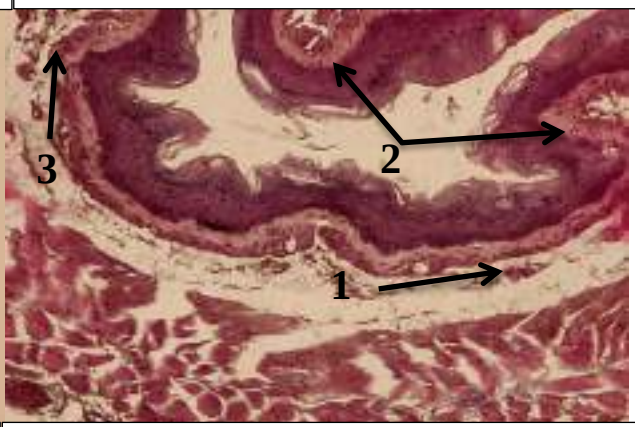


Fig (4-7) section of esophagus mice of mixing group 1^{st} week showing ,Little congestion in sub mucosa(1), erosion and sloughing in mucosa(2) , (H & E stain 100X)



Fig(4-8) section of esophagus mice of mixing group 4^{th} week showing, Congestion in sub mucosa(1),) erosion and sloughing in mucosa(2), hypertrophy muscularis mucosa(3)H & E stain 100X)

4-6-2-Histomorphometric Study:

The results of the histomorphometry of the esophagus tissue showed no significant differences (P> 0.05) in thickness of mucosa, sub mucosa, Muscularis, and adventitia in the first week in the inhalation, orally, and mixing group when compared with the control group. The results indicated significant differences (P <0.05) in thickness of mucosa in the orally and mixing group, which recorded a mean of (6.5±0.19) and (7.0±0.22) respectively in the fourth week as shown in Table (4-7).

The results indicated significant differences (P <0.05) in thickness of sub mucosa in the orally and mixing group, which recorded a mean of (10.6±0.32) and (10.7±0.17) respectively in the fourth week, there are also no significant differences (P >0.05) in thickness of Mascularias and adventitia in orally, mixing, and inhalation group, as shown in Table (4-7).

Table (4-7): Mean thickness of mucosa, submucosa, muscularis, and adventitia in the esophagus of mice in the first and fourth weeks

Thickness of Layers	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
Mucosa	11.6 [a] ±0.16	12.0[a] ±0.21	10.8 [a] ±0.49	11.4 [a] ±0.18	11.9 [a] ±0.17	11.7 [a] ±0.22	6.5 [b] ±0.19	7.0 [b] ±0.20
Sub mucosa	8.6 [a] ±0.17	8.4 [a] ± 0.22	8.0 [a] ±0.42	8.8 [a] ±0.32	8.7 [a] ±0.13	8.5 [a] ± 0.28	10.6 [b] ±0.32	10.7 [b] ±0.17
Mascularias	27.4 [a] ±0.56	27.7 [a] ± 0.76	25.2 [a] ±0.80	28.5[a] ±0.55	27.6 [a] ±0.44	27.8 [a] ± 0.40	25.6 [a] ±0.93	26.3 [a] ± 0.91
Adventitia	4.82 [a] ±0.27	4.6[a] ± 0.29	4.5 [a] ±0.28	4.8 [a] ±0.14	4.6 [a] ±0.30	4.4 [a] ± 0.31	4.8 [a] ±0.20	4.0 [a] ± 0.14

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences (p>0.05), different letters horizontally between the values indicate that there are significant differences (p<0.05).

4-6-3-Histochemical Study:

The results of histochemical Study of the esophagus of mice in the control, orally, inhalation, mixing group, as shown in the following table.

Table (4-8): Reaction mucosa, submucosa, musacularis and adventitia with PAS in esophagus.

Group	Stain	Mucosa	Submucosa	Muscularis	Adventitia
Control	PAS	Moderate	Weak	Moderate	Weak
Orally	PAS	Strong	Moderate	Strong	Moderate
Inhalation	PAS	Moderate	Weak	Moderate	Weak
Mixing	PAS	Strong	Moderate	Strong	Moderate

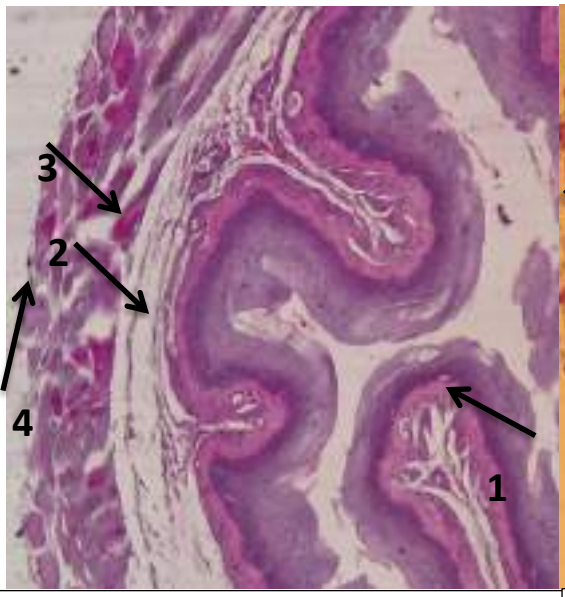


Fig (4-9) esophagus of control group showing(1) mucosa with a moderate reaction with PAS (2) sub mucosa with weak reaction with PAS (3) muscularis with a moderate reaction with PAS (4) adventitia with weak reaction with PAS.100X

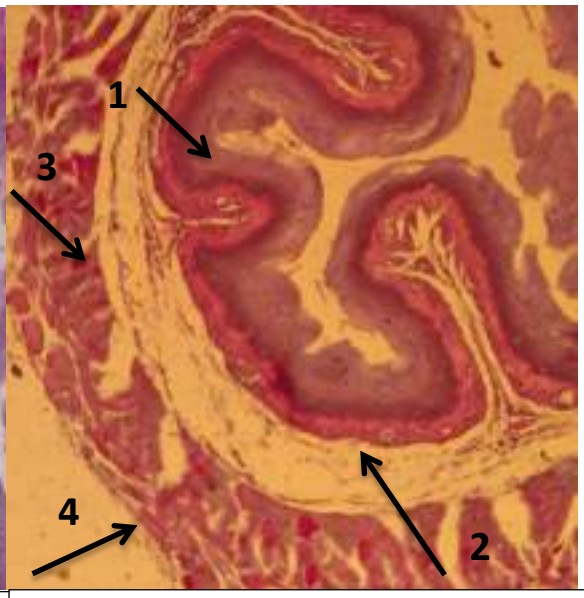


Fig (4-10) esophagus of inhalation group showing (1) mucosa with a moderate reaction with PAS . (2) sub mucosa with weak reaction with PAS .(3) muscularis with a moderate reaction with PAS .(4) adventitia with weak reaction with PAS.100X

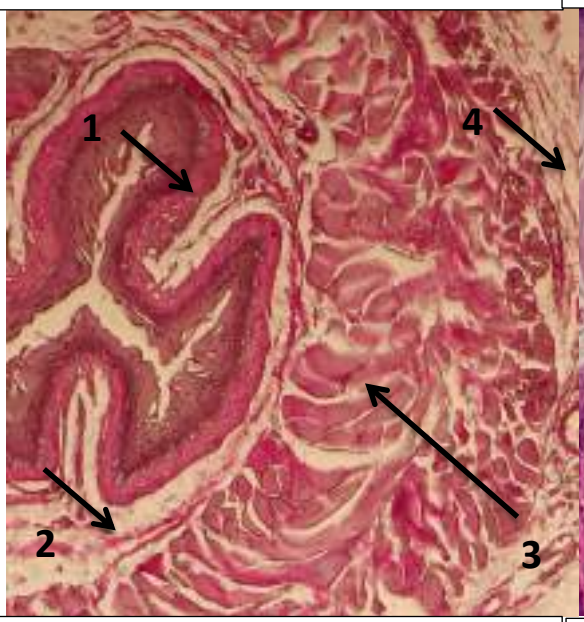


Fig (4-11) esophagus of orally group showing (1) mucosa with a strong reaction with PAS . (2) sub mucosa with moderate reaction with PAS .(3) muscularis with a stronge reaction with PAS.(4) adventitia with moderate reaction with PAS.100X

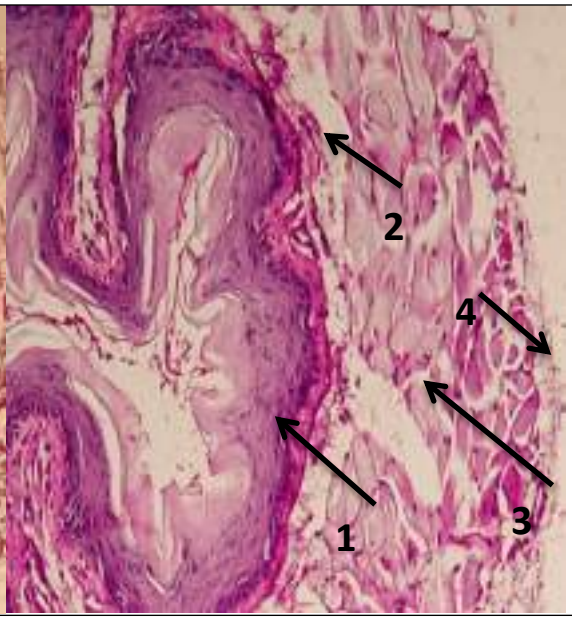


Fig (4-12) esophagus of mixing group showing (1) mucosa with a strong reaction with PAS . (2) sub mucosa with moderate reaction with PAS .(3) muscularis with a strong reaction with PAS .(4) adventitia with moderate reaction with PAS.100X

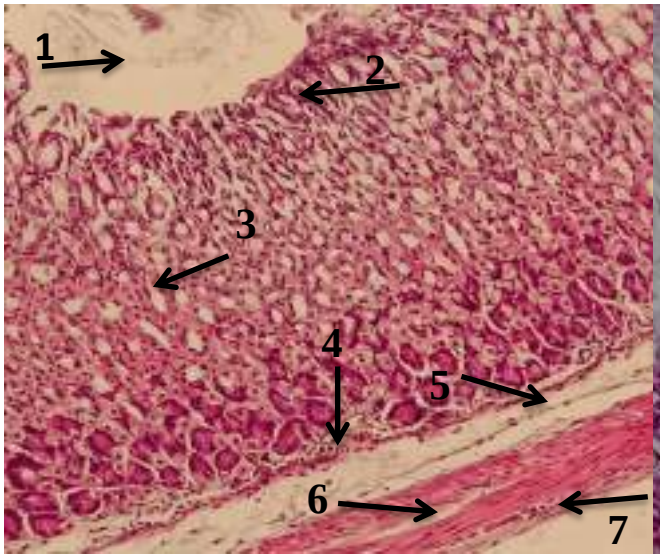
4-7- Results of Stomach

4-7-1- Histological Changes

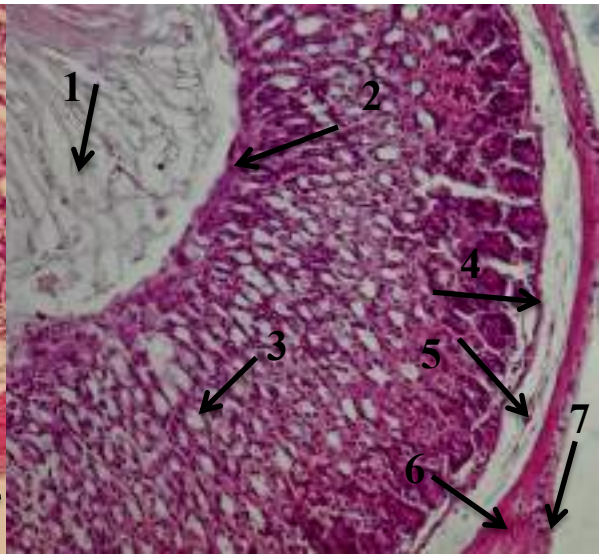
The microscopic examination of the stomach of mice in the control group showed that it consists of fourth layers from the inside to the outside which are mucosa and consists (barrier epithelium, lamina propria, muscularis mucosa), Submucosa, and muscularis propria, and serosa. The mucosa is the thickest layer and consists of a simple columnar epithelium and contains gastric glands, which are of the simple or branched tubular type, and also contain peptic or central cells and oxyntic cells. The submucosa layer consists of connective tissue in which extends blood vessels and nerves, and the muscle layer consists of two layers of circular, Longitudinally fibers, between these layers is a fastening tissue. The serosa layer consists of a simple squamous epithelium Figure (4-13).

Histological sections of the stomach of experimental animals treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the stomach in the control group and that no histological change was apparent Figure(4-14).

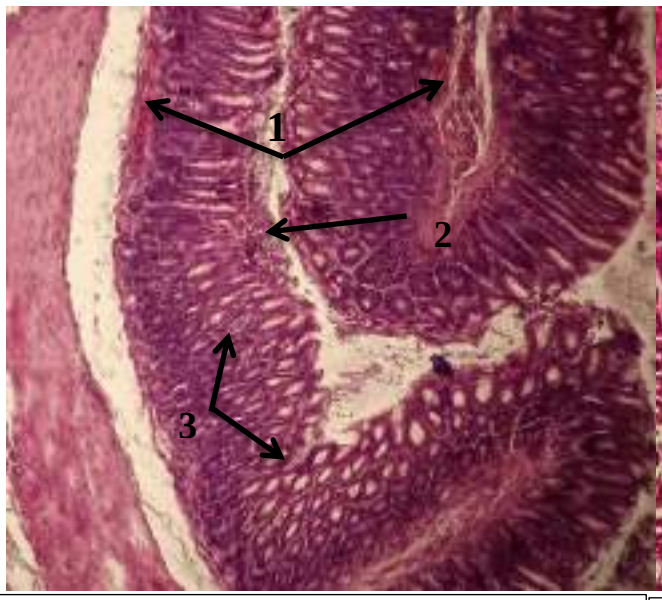
Histological sections of the stomach of mice that administered orally showed, the presence of tissue damage, including hemorrhage in the mucosa layer, Moderate superficial erosion in the gastric mucosa, and the occurrence of necrosis in some areas as well, and these changes occurred in the first-week Figure(4-15), In the fourth week, the changes included of hemorrhage in the mucosa layer, the occurrence of sever superficial erosion of epithelium layer in the mucosa, in addition to marked edema in the mucosa between gastric glands and in the muscularis propria Figure (4-16).



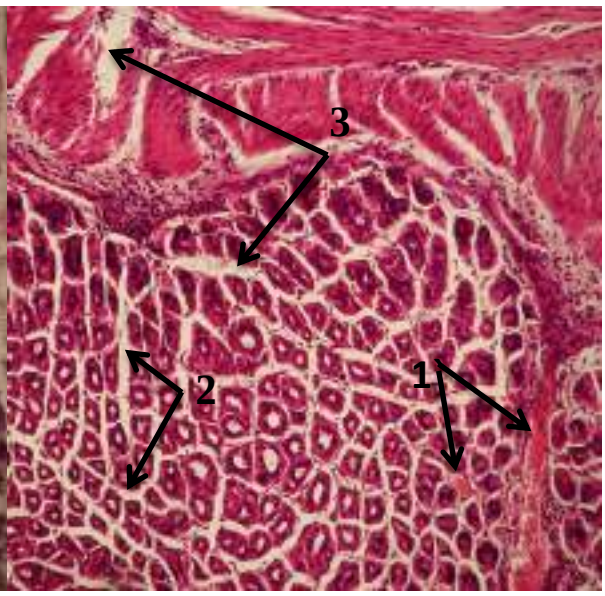
Figure(4-13)section of control group of Stomach mice showing the normal structure of stomach ,Lumen of stomach (1), simple columnar epithelium(2), gastric glands(3), musularis mucosa (4), Sub mucosa(5), muscularis (6),serosa (7) ,(H & E stain, 100X).



Figure(4-14)section of inhalation group of Stomach mice showing ,Lumen of stomach (1), simple columnar epithelium(2), gastric glands(3), musularis mucosa (4), Sub mucosa(5), muscularis (6),serosa (7) ,(H & E stain, 100X).



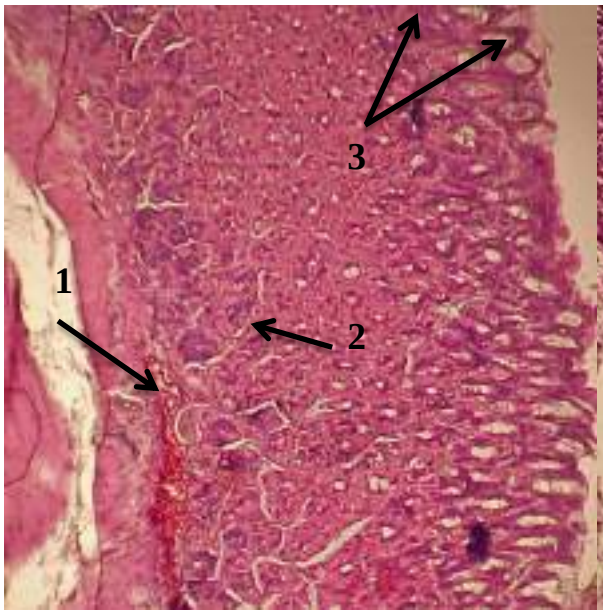
Figure(4-15) section of stomach mice orally group1^{st} week showing hemorrhage (1) in mucosa , superficial erosion in the gastric mucosa (2), necrosis (3) ,(H & E stain, 100X).



Figure(4-16)section of stomach mice orally group4^{th} week showing hemorrhage in the mucosa layer(1), errosin and sever sloughing of epithelial layer (2), edema in the mucosa between gastric glands and in the muscularis propria,(H & E stain, 100X).

As for the histological sections of the mixing group, there was also congestion in mucosa layer, Moderate superficial erosion of the epithelial layer lining the stomach, and the occurrence of necrosis in the first-week Figure (4-17).

Hemorrhage in the mucosa layer, the occurrence of sever superficial erosion of epithelial layer, congestion in sub mucosa, as well as the mild edema in the mucosa between gastric glands and in the muscularis propria in the fourth-week Figure (4-18), In the second and third week, the changes were almost identical to the changes of the first and fourth week.



Figure(4-17) section of stomach mice mixing group1st week showing hemorrhage (1) in the mucosa layer, Modrate superficial erosion of the epithelial layer (3) , necrosis (3) ,(H & E stain, 100X).

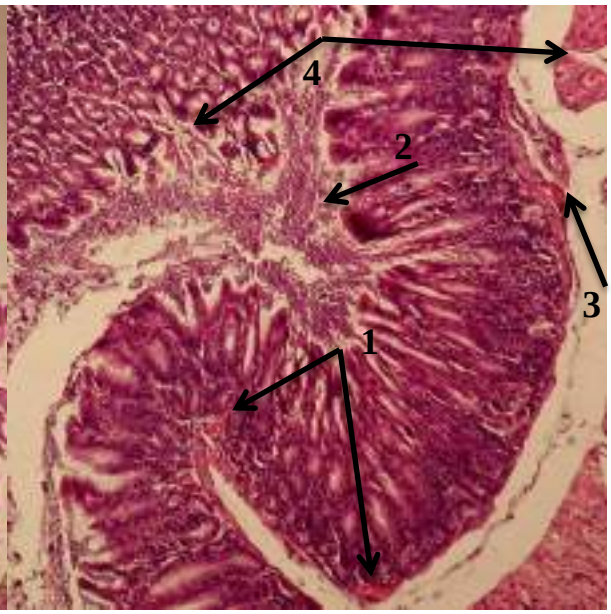


Figure (4-18) section of stomach mice mixing group 4th week showing hemorrhage (1) in the mucosa layer, sever superficial erosion of epithelial layer (2), congestion in sub mucosa(3), edema in the mucosa between gastric glands and in the muscularis propria (4), (H & E stain, 100X).

4-7-2-Histomorphometric Study:

The results of the histomorphometry of the gastric tissue showed no significant differences (P> 0.05) in thickness of mucosa, sub mucosa, Muscularis, and serosa in the first week in the inhalation, orally, and mixing group when comparing the control group, as shown in Table(4-9).

The results indicated significant differences (P <0.05) in thickness of mucosa in the orally and mixing groups, which recorded a mean of (41.6±0.01) and (40.0±1.94) respectively in the fourth week, The results indicated significant differences (P <0.05) in thickness of sub mucosa in the orally and mixing group, which recorded a mean of (6.5±0.31) and (7.0±0.28) respectively in the fourth week, compared with the control group as shown in Table (4-9). The results indicated no significant differences (P >0.05) in thickness of Mascularis and serosa in orally, mixing, and inhalation group as shown in Table (4-9).

Table (4-9): Mean thickness of mucosa, submucosa, muscularis, and serosa in the stomach of the control, Inhalation, Orally, and Mixing group.

Thickness of Layers	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
Mucosa	53.4 ^{a} ±2.14	55.0 ^{a} ±2.42	49.6 ^{a} ±1.32	50.2 ^{a} ±1.86	55.1 ^{a} ±3.07	55.6 ^{a} ±2.04	41.6 ^{b} ±1.01	40.0 ^{b} ±1.94
Sub mucosa	4.8 ^{a} ±0.26	4.5 ^{a} ± 0.39	5.0 ^{a} ±0.37	5.2 ^{a} ±0.28	4.6 ^{a} ±0.32	4.3 ^{a} ± 0.36	6.5 ^{b} ±0.31	7.0 ^{b} ± 0.28
Mascularias	8.2 ^{a} ±0.23	8.4 ^{a} ± 0.49	7.8 ^{a} ±0.49	8.4 ^{a} ±0.29	8.3 ^{a} ±0.25	8.6 ^{a} ± 0.33	7.8 ^{a} ±0.34	8.5 ^{a} ± 0.29
Serosa	6.0 ^{a} ±0.63	6.0^{a} ± 0.25	6.2 ^{a} ±0.40	5.7 ^{a} ±0.22	6.3 ^{a} ±0.37	6.1 ^{a} ± 0.36	6.2 ^{a} ±0.28	6.5 ^{a} ± 0.38

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences (p>0.05), different letters horizontally between the values indicate that there are significant differences (p<0.05).

4-7-3-Histochemical Study:

The results of histochemical Study of the stomach of mice in the control, orally, inhalation, mixing group, as shown in the following table.

Table (4-10): Reaction mucosa, submucosa, musacularis and serosa with PAS in stomach

Group	Stain	Mucosa	Submucosa	Muscularis	Serosa
Control	PAS	Moderate	Weak	Moderate	Weak
Orally	PAS	Strong	Moderate	Strong	Moderate
Inhalation	PAS	Moderate	Weak	Moderate	Weak
Mixing	PAS	Strong	Moderate	Strong	Moderate

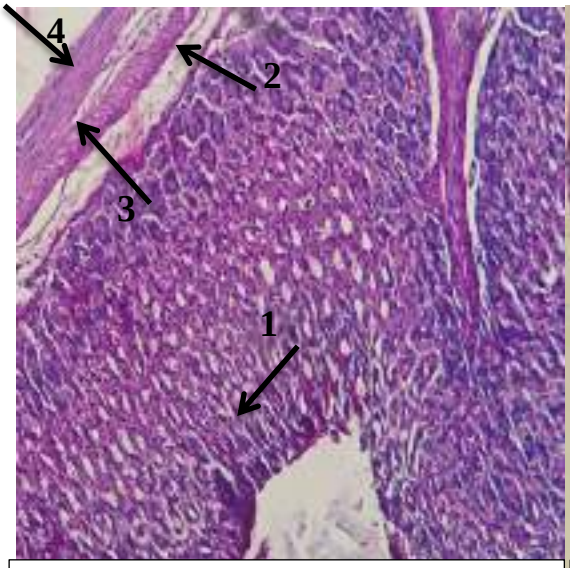


Fig (4-19) stomach of control group showing(1) mucosa with a moderate reaction with PAS (2) sub mucosa with weak reaction with PAS (3) muscularis with a moderate reaction with PAS (4) serosa with weak reaction with PAS.100X

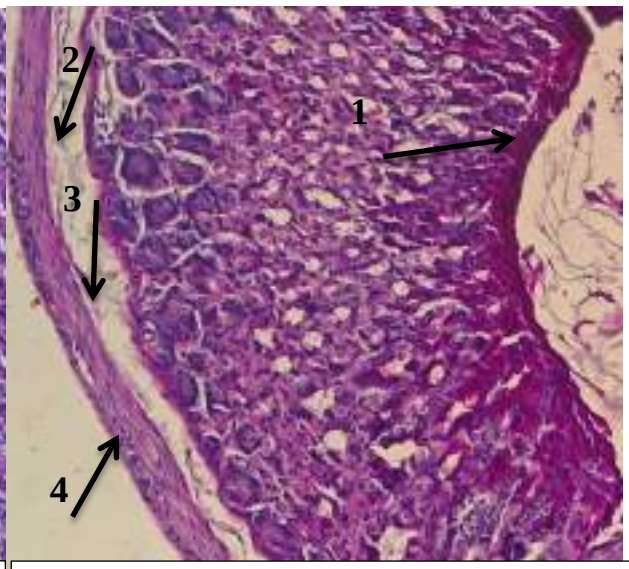


Fig (4-20) stomach of inhalation group showing(1) mucosa with a moderate reaction with PAS (2) sub mucosa with weak reaction with PAS (3) muscularis with a moderate reaction with PAS (4) serosa with weak reaction with PAS.100X

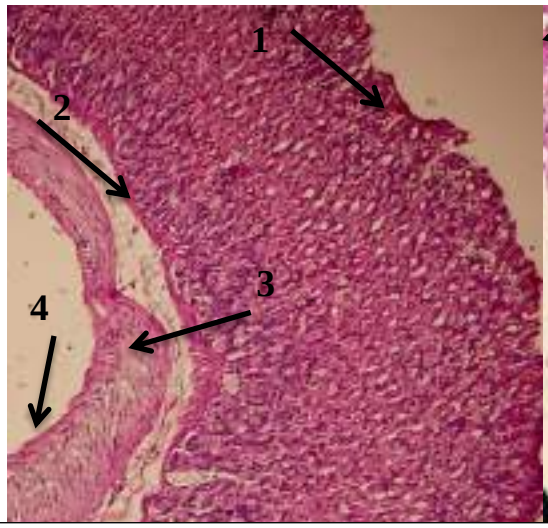


Fig (4-21) stomach of orally group showing(1) mucosa with a strong reaction with PAS (2) sub mucosa with moderate reaction with PAS (3) muscularis with a strong reaction with PAS (4) serosa with moderate reaction with PAS.100X

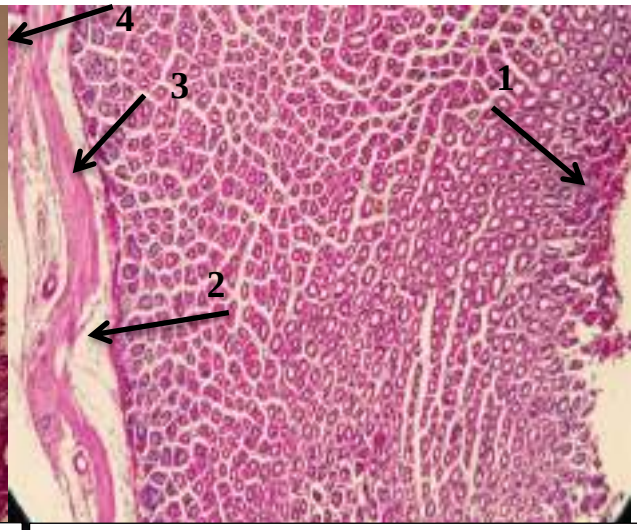


Fig (4-22) stomach of mixing group showing(1) mucosa with a strong reaction with PAS (2) sub mucosa with moderate reaction with PAS (3) muscularis with a strong reaction with PAS (4) serosa with moderate reaction with PAS.100X

4-8- Small Intestine (duodenum)

4-8-1- Histological Changes

The results of the microscopic examination of the duodenum of mice in the control group showed that it consists of four layers of inside to the outside, which is the mucosa, which consists of long finger-shaped, leaflike, protrusions called villi, and at the base of the villi there are crypts lieberkuhn and contain goblet cells and also contain stem cells and Baneth cells. The submucosa layer consists of connective tissue and extends blood vessels and nerves, Brunner glands, the muscularis consists of two layers of fibers, long outward and circular inward, and the serosal layer consists of a simple squamous epithelium Figure (4-23).

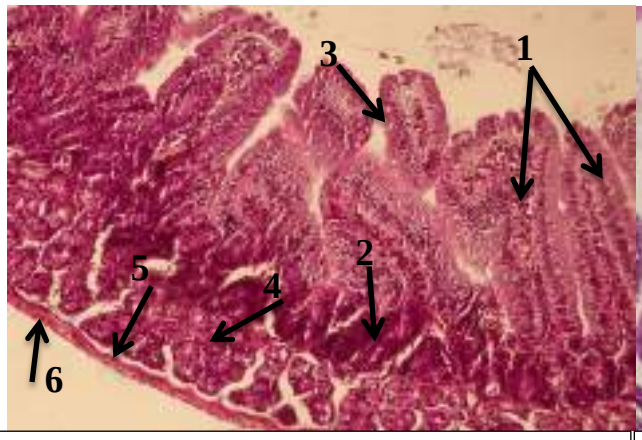
Histological sections of the duodenum of experimental animals treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the duodenum in the control group and that no histological change was apparent Figure(4-24).

Histological sections of the duodenum of mice that were administered orally showed, the presence of tissue damage, including hyperemia in the mucosa layer(villi), slight deformation and sloughing of the villi, dilation of blood vessels in the sub mucosa and these changes occurred in the first-week Figure(4-25).

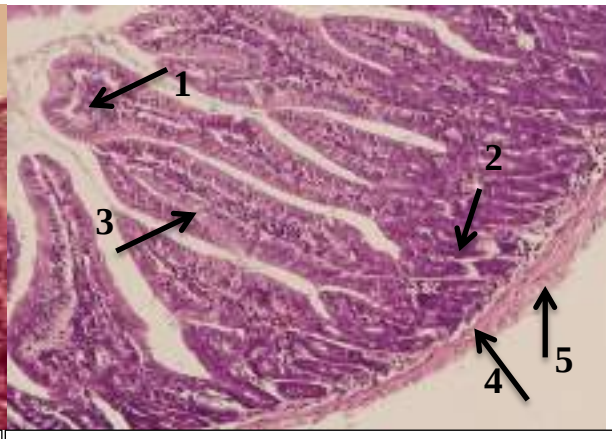
In the fourth week, increased hyperemia in the mucous (villi), strong deformation and sloughing of the villi, dilation of blood vessels with oedema in the sub mucosa, Figure(4-26).

As for the histological sections of the mixing group, there was also hyperemia in the mucosa layer (villi), hyperemia in the sub mucosa, sloughing of the epithelial cells in the mucosa in the first week Figure (4-27).

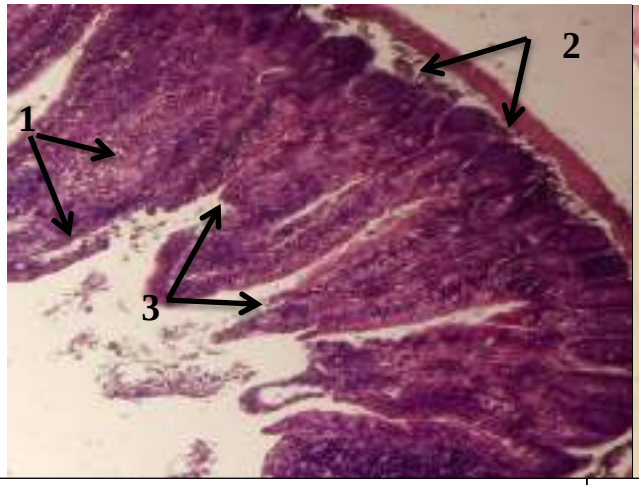
In the fourth week increased hyperemia in the villi, hyperemia in the sub mucosa, strong deformation and sloughing of the villi, Figure (4-28), In the second and third weeks, the changes were almost identical to the changes of the first and fourth week.



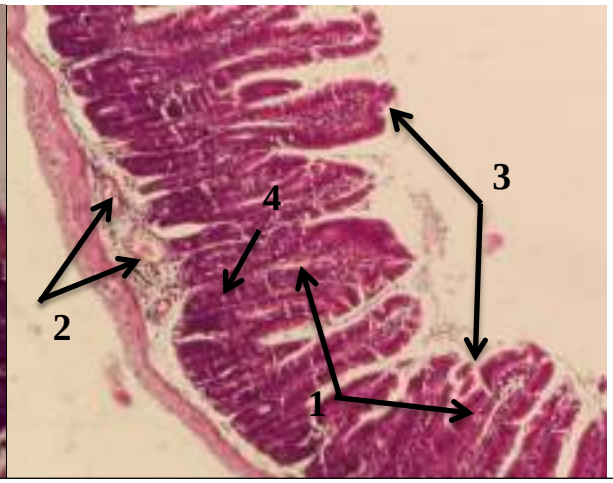
Fig(4-23)section of control group of duodenum mice showing villi(1), crypts lieberkuhn(2) , goblet cells (3), Brunner glands(4), muscularis (5),serosa (6) ,(H & E stain, 100X).



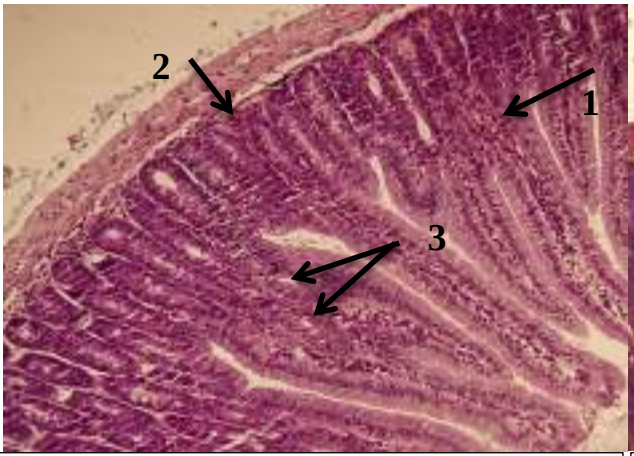
Fig(4-24) section of Inhalation group of duodenum mice showing villi(1), crypts lieberkuhn(2) , goblet cells (3) , muscularis (4),serosa (5) ,(H & E stain, 100X).



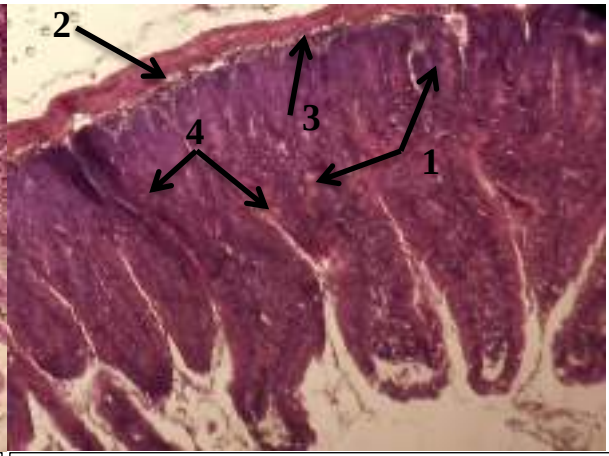
Fig(4-25) section of duodenum mice orally group1st week showing hyperemia in the villi(1), hyperemia in the sub mucosa(2), slight sloughing of the villi (3) ,(H & E stain, 100X).



Fig(4-26) section of duodenum orally group4th week showing hyperemia in the villi(1), dilation of blood vessels in the sub mucosa(2), infiltration of inflammatory cells (3), sloughing of the villi (4),(H & E stain, 100X).



Fig(4-27) section of duodenum mice mixing group1st week showing hyperemia in the villi(1), hyperemia in the sub mucosa(2), sloughing of the epithelial cells(3) ,(H & E stain, 100X).



Fig(4-28) section of duodenum mice mixing group 4th week showing hyperemia in the villi(1), dilation of blood vessels in the sub mucosa(2), infiltration of inflammatory cells (3), sloughing of the villi (4),(H & E stain, 100X).

4-8-2-Histomorphometric Study:

The results of the histomorphometry of the small intestinal (duodenum) tissue showed no significant differences (P> 0.05) in thickness of mucosa, sub mucosa, Muscularis, and serosa in the first week in the inhalation, orally, and mixing group when comparing the control group, as shown in Table(4-11).

The results indicated significant differences (P <0.05) in thickness of mucosa in the orally and mixing group, which recorded a mean of (42.5±2.21) and (54.5±2.25) respectively in the fourth week, there are also significant differences (P <0.05) in thickness of sub mucosa in the orally and mixing group, which recorded a mean of (5.2±0.34) and (5.5±0.26) respectively in the fourth week, compared with the control group as shown in Table (4-11). The results indicated no significant differences (P >0.05) in thickness of Mascularis and serosa in orally, mixing, and inhalation group in the fourth week as shown in Table (4-11).

Table (4-11): Mean thickness of mucosa, submucosa, muscularis, and serosa in the intestine of the control group, Inhalation, Orally, and Mixing group.

Thickness of Layers	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
Mucosa	68.4 ^{a} ±2.44	67.4 ^{a} ±3.56	64.4 ^{a} ±1.76	64.7 ^{a} ±2.03	70.1 ^{a} ±2.04	68.8 ^{a} ±3.07	42.5 ^{b} ±2.21	54.5 ^{b} ±2.25
Sub mucosa	3.24 ^{a} ±0.39	3.4 ^{a} ± 0.38	3.1 ^{a} ±0.23	3.6 ^{a} ±0.35	3.4 ^{a} ±0.23	3.2 ^{a} ± 0.17	5.2 ^{b} ±0.34	5.5 ^{b} ± 0.26
Mascularis	3.28 ^{a} ±0.20	3.2 ^{a} ± 0.36	3.34 ^{a} ±0.27	3.6 ^{a} ±0.32	3.6 ^{a} ±0.23	3.2 ^{a} ± 0.23	4.0 ^{a} ±0.17	3.8 ^{a} ± 0.29
Serosa	2.2 ^{a} ±0.28	2.4 ^{a} ± 0.31	2.1 ^{a} ±0.28	2.4 ^{a} ±0.27	2.6 ^{a} ±0.22	2.1 ^{a} ± 0.24	2.4 ^{a} ±0.18	2.2^{a} ± 0.18

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences (p>0.05), different letters horizontally between the values indicate that there are significant differences (p<0.05).

4-8-3-Histochemical Study:

The results of histochemical Study of the small intestine of mice in the control, orally, inhalation, mixing group, as shown in the following table.

Table (4-12): Reaction mucosa, submucosa, musacularis and serosa with PAS in small intestine.

Group	Stain	Mucosa	Submucosa	Muscularis	Serosa
Control	PAS	Strong	Moderate	Weak	Weak
Orally	PAS	Strong	Strong	Moderate	Weak
Inhalation	PAS	Strong	Moderate	Weak	Weak
Mixing	PAS	Strong	Strong	Moderate	Weak

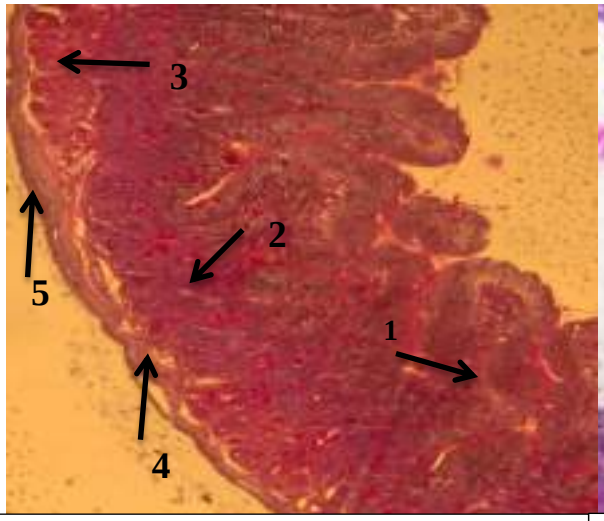


Fig (4-29) duodenum of control group showing goblet cells(1) ,crypts lieberkuhn (2) with a strong reaction with PAS,burner gland (3) in sub mucosa with moderate reaction with PAS, muscularis(4) with a weak reaction with PAS serosa(5) with weak reaction with PAS.100X

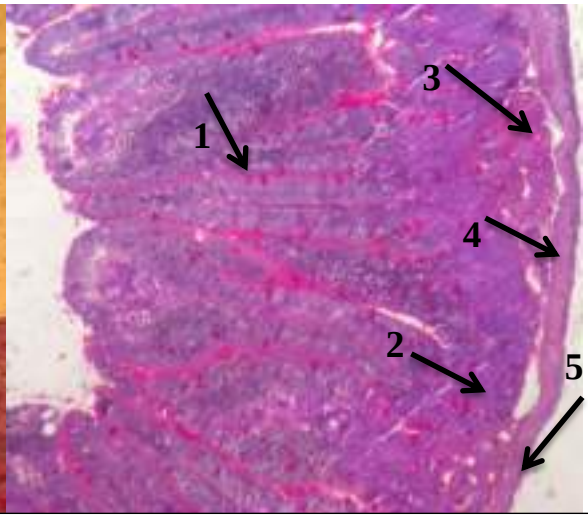


Fig (4-30) duodenum of inhalation group showing goblet cells(1) ,crypts lieberkuhn (2) with a strong reaction with PAS,burner gland (3) in sub mucosa with moderate reaction with PAS, muscularis(4) with a weak reaction with PAS serosa(5) with weak reaction with PAS.100X

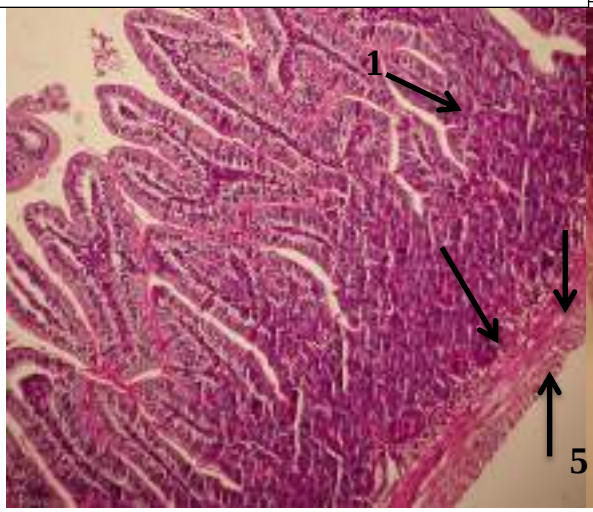


Fig (4-31) duodenum of orally group showing goblet cells(1) ,crypts lieberkuhn (2) with a strong reaction with PAS,burner gland (3) in sub mucosa with strong reaction with PAS, muscularis(4) with a moderate reaction with PAS serosa(5) with weak reaction with PAS.100X

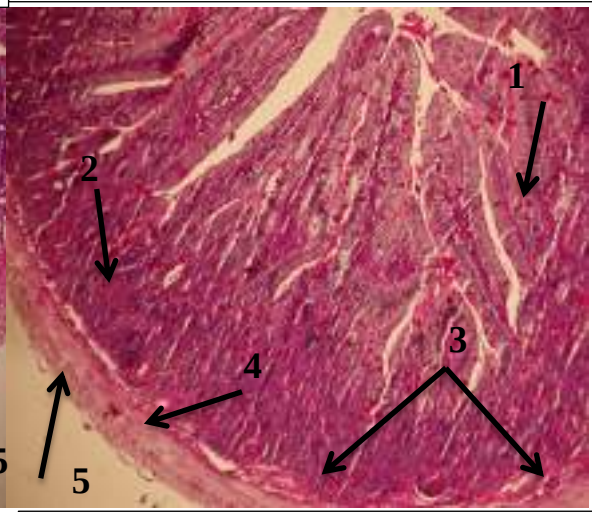


Fig (4-32) duodenum of mixing group showing goblet cells(1) ,crypts lieberkuhn (2) with a strong reaction with PAS,burner gland (3) in sub mucosa with strong reaction with PAS, muscularis(4) with a moderate reaction with PAS serosa(5) with weak reaction with PAS.100X

4-9- Results of Trachea

4-9-1- Histological Changes

The results of the microscopic examination of the trachea of mice in the control group showed that it consists of layers of inside to the outside, which is the mucosa, which is lined with a ciliated pseudostratified columnar epithelium and contains glands that produce mucus, and in the submucosa layer is contains of soft connective tissue and contains the submucosa with C-shaped hyaline cartilage rings. The ends of the cartilage rings are open, and the trachealis muscle forms a bridge between these ends, and the outer layer surrounding the trachea is the adventitia and which consists of a loose connective tissue, Figure (4-33).

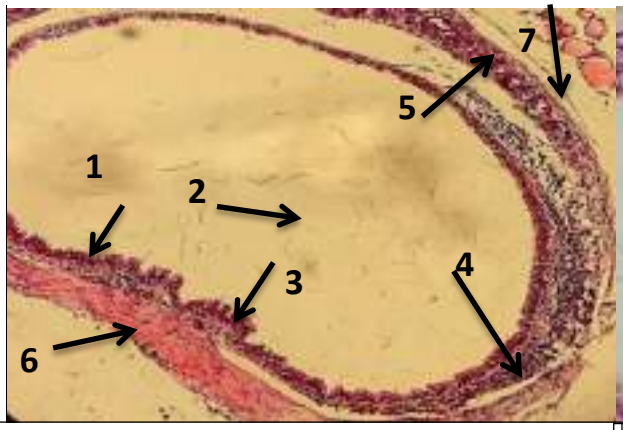
Sections of trachea in the inhalation group that it was similar to the histological composition of the trachea in the control group and that no histological change was apparent, Figure(4-34).

Histological sections of the trachea of mice that administered orally showed, the presence of tissue damage, including slight congestion in the mucous layer, as well as erosion and removal of epithelial cells and loss cilia of the mucosa and congestion in the sub mucosa layer and these changes occurred in the first week, Figure(4-35).

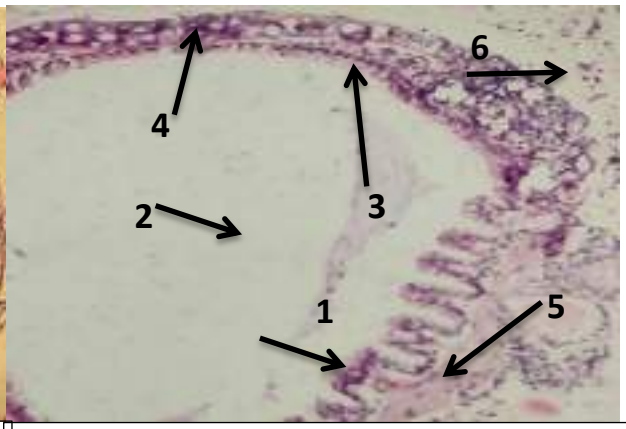
In the fourth week, Slight congestion in the mucosa layer, as well as the presence of inflammatory cell infiltration (an increase in inflammatory cells), destruction of the surface of the epithelial layer in the mucosa, and loss of cilia, shatter of trachealis muscle with the expansion of the submucosa layer compared to the first-week, Figure (4-36).

Histological sections of the trachea in the mixing group were shown Slight congestion in the mucous layer, as well as erosion and removal of epithelial cells and loss cilia of the mucosa, and congestion in the sub mucosa layer, and these changes occurred in the first week Figure (4-37).

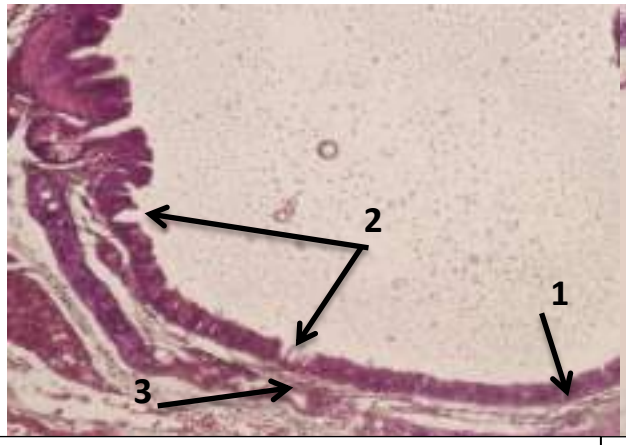
In the fourth week, Slight congestion in the mucosa layer, as well as the presence of inflammatory cell infiltration, destruction of the surface of the epithelial layer in the mucosa, and loss of cilia shatter of trachealis muscle with the expansion of the sub mucosa layer compared to the first week, Figure (4-38).



Fig(4-33)section of control group of trachea in mice showing cili(1), lumen(2), ciliated pseudostratified columnar epithelium(3),lamina propria(4) cartilage(5), trachealis muscle(6) adventitia (7),(H & E stain, 100X).

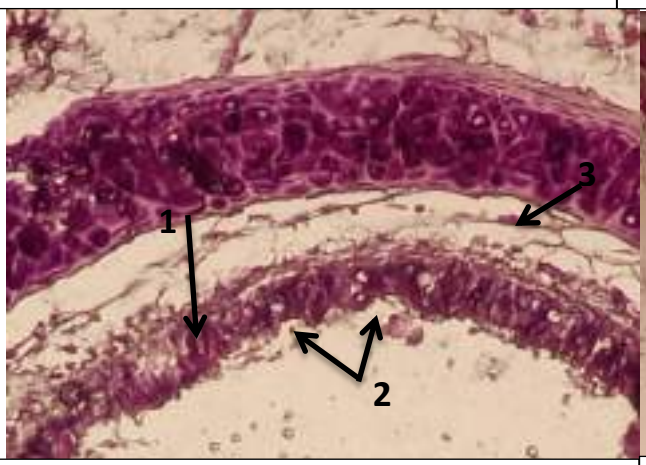


Fig(4-34)section of inhalation group of trachea in mice showing cili(1), lumen(2), ciliated pseudostratified columnar epithelium(3), cartilage(4), trachealis muscle(5) adventitia (6),(H & E stain, 100X).

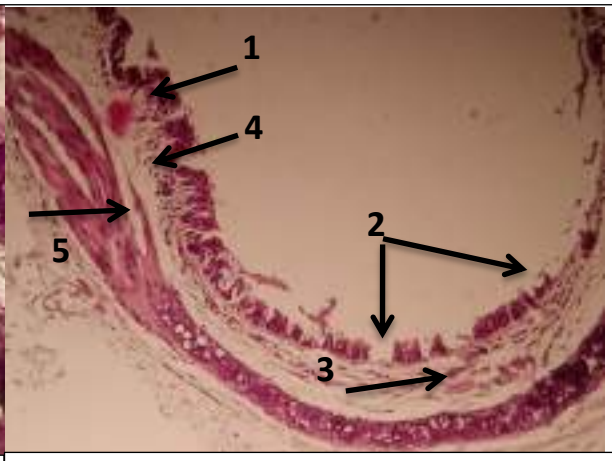


Fig(4-35)section of orally group 1^{st} week of trachea in mice showing slight congestion in the mucous layer(1), erosion and removal of epithelial cells and loss cilia of the mucosa (2), congestion in the submucosa (3),(H&E stain 100X)

Fig(4-36)section of orally group 4^{th} week of trachea in mice showing slight congestion in the mucous(1), erosion and removal of epithelial cells and loss cilia of the mucosa (2), expansion sub mucosa(3), inflammatory cell infiltration(4),shatter trachealis muscle(5) ,(H&E stain 100X).



Fig(4-37) section of mixing group 1^{st} week of trachea in mice showing slight congestion in the mucous layer(1), erosion and removal of epithelial cells and loss cilia of the mucosa (2), congestion in the submucosa (3),(H&E stain 100X)



Fig(4-38) section of mixing group 4^{th} week of trachea showing slight congestion in the mucous(1), erosion and of epithelial cells and loss cilia (2), expansion submucosa (3), inflammatory cell infiltration (4),shatter trachealis muscle(5), H&E stain 100X).

4-9-2-Histomorphometric Study:

The results of the histomorphometry of the trachea tissue showed no significant differences (P> 0.05) in the lumen of trachea, the thickness of mucosa, sub mucosa, trachealis muscle and in the first week in the inhalation, orally, and mixing group when comparing the control group, as shown in Table(4-13).The results indicated significant differences (P <0.05) in the lumen of the trachea in inhalation, orally, and mixing group, which recorded a mean of (69.2±3.06),(82.2±2.43) and (68.1±2.41) respectively in the fourth week, there are also significant differences (P <0.05) in thickness of mucosa in the orally and mixing group, which recorded a mean of (4.2±0.22) and (4.0±0.33) respectively in the fourth week as shown in Table (4-13). The results indicated no significant differences (P >0.05) in thickness of sub mucosa and trachealis muscle in orally, mixing, and inhalation group in the fourth week as shown in Table (4-13).

Table (4-13): Mean thickness in Trachea of Lumen of the trachea, mucosa, submucosa, and trahealis muscle of the control, Inhalation, Orally, and Mixing group.

Thickness of Layers	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
Lumen	48.8 [a] ±3.81	53.1 [a] ±3.31	58.1 [a] ±3.29	55.3 [a] ±3.98	53.2 [a] ±3.30	69.2 [b] ±3.06	82.2 [b] ±2.43	68.1 [b] ±2.41
Mucosa	6.18 [a] ±0.40	6.0 [a] ± 0.35	5.8 [a] ±0.23	5.6 [a] ±0.39	5.8 [a] ±0.32	5.6 [a] ± 0.26	4.2 [b] ±0.22	4.0 [b] ± 0.33
Sub mucosa	25.3 [a] ±1.84	24.8 [a] ± 1.89	23.7 [a] ± 1.47	26.5 [a] ± 1.83	24.6 [a] ± 1.64	26.3 [a] ± 2.44	27.0 [a] ±1.44	28.7 [a] ± 2.09
trahealis muscle	7.2 [a] ±0.38	7.2 [a] ± 0.20	6.5 [a] ± 0.35	7.3 [a] ± 0.30	7.8 [a] ± 0.49	7.2 [a] ± 0.24	6.9 [a] ±0.32	7.2[a] ± 0.20

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences (p>0.05), different letters horizontally between the values indicate that there are significant differences (p<0.05).

4-9-3-Histochemical Study:

The results of histochemical Study of the Trachea of mice in the control, orally, inhalation, mixing group, as shown in the following table.

Table (4-14): Reaction mucosa, cartilage, Trachealis muscle and adventitia with PAS in Trachea.

Group	Stain	Mucosa	Cartilage	Trachealis muscle	Adventitia
Control	PAS	Moderate	Strong	Moderate	Weak
Orally	PAS	Strong	Strong	Moderate	Moderate
Inhalation	PAS	Weak	Strong	Moderate	Weak
Mixing	PAS	Strong	Strong	Moderate	Moderate

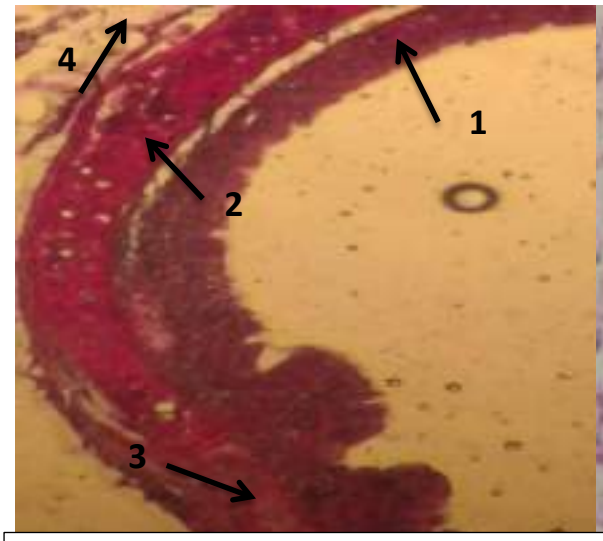


Fig (4-39)Trachea of control group showing Mucosa(1) a moderate reaction with PAS, cartilage (2) with strong reaction with PAS, trachealis muscle (3) with a moderate reaction with PAS, adventitia (4) with weak reaction with PAS.100X

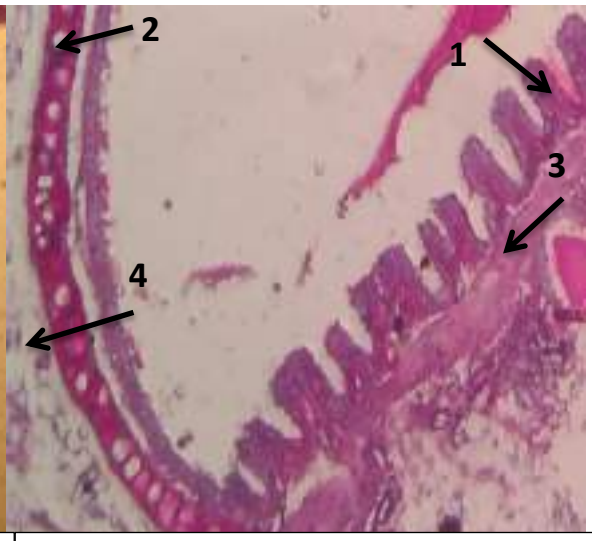


Fig (4-40)Trachea of inhalation group showing Mucosa(1) a weak reaction with PAS, cartilage (2) with strong reaction with PAS, trachealis muscle (3) with a moderate reaction with PAS, adventitia (4) with weak reaction with PAS.100X

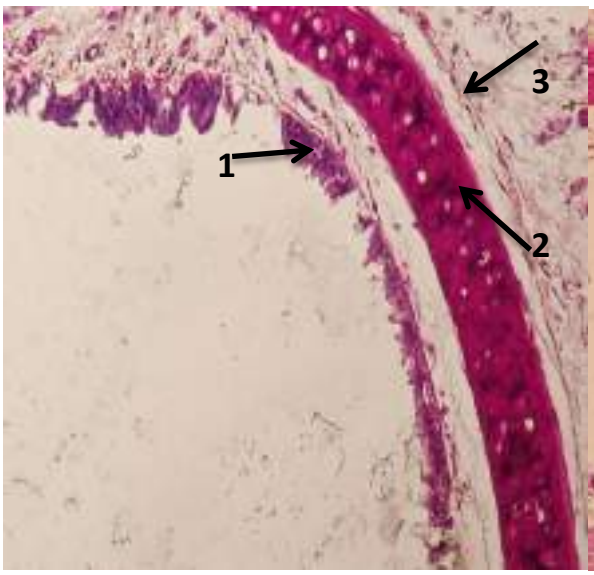


Fig (4-41)Trachea of orally group showing Mucosa(1) a strong reaction with PAS, cartilage (2) with strong reaction with PAS, adventitia (3) with moderate reaction with PAS.100X

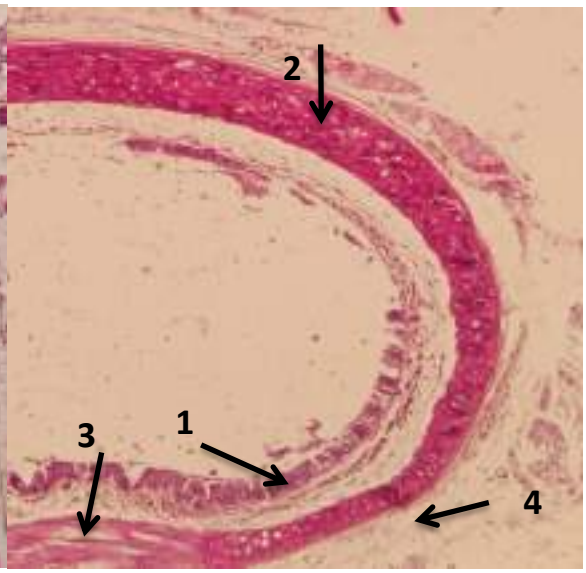


Fig (4-42)Trachea of inhalation group showing Mucosa(1) a strong reaction with PAS, cartilage (2) with strong reaction with PAS, trachealis muscle (3) with a moderate reaction with PAS, adventitia (4) with moderate reaction with PAS.100X

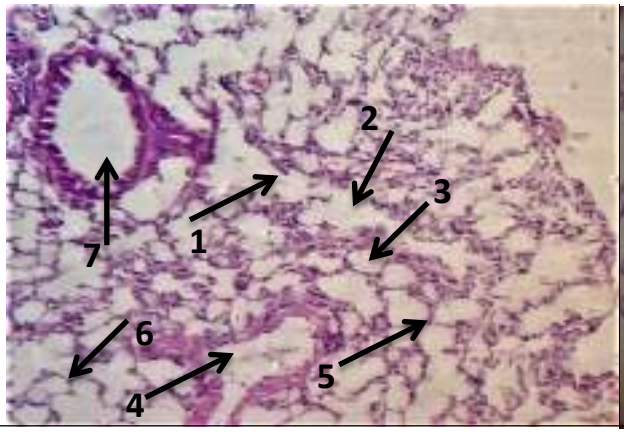
4-10- Results of Lung

4-10-1- Histological Changes

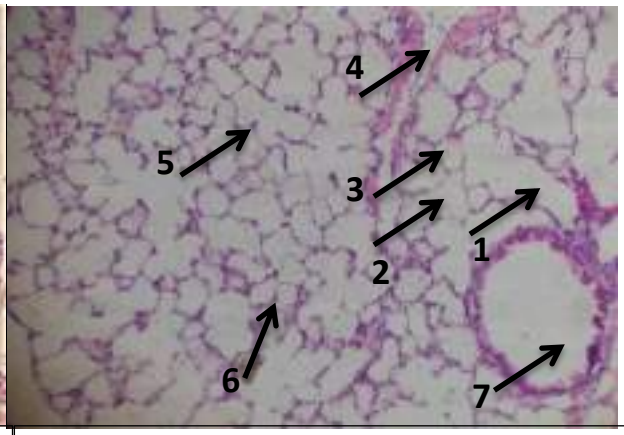
The microscopic examination of the parenchyma of the lung of mice in the control group showed that the respiratory part called the acinus consists of respiratory bronchiole, alveolar ducts, alveolar sacs, and alveoli, and also consists of pulmonary veins, arteries, and alveolar sacs connected with each other by pores of Cohn. The alveoli are lined with an epithelial layer of squamous cells, and bronchioles are lined with a simple ciliated epithelium containing cuboidal or columnar cells called Clara or club cells, Figure (4- 43).

Histological sections of the lung of mice treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the lung in the control group and that no histological change was apparent, Figure (4- 44) throughout the experimental.

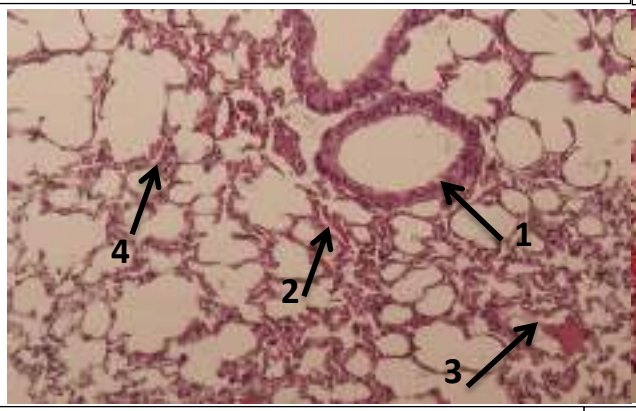
Histological sections of the lung of mice that were administered orally, showed the presence of tissue damage, including the slight hyperemia in the epithelium of the bronchioles, in the wall of the alveoli sac and inside the alveolar sac, and an increase in the thickness of the epithelium of the alveoli with irregular changes in the alveoli in the first week, Figure (4- 45). While in the fourth week, slight hyperemia in the epithelium of the bronchioles, severe hyperemia in the alveoli wall, deformities of the epithelium lining the bronchioles, increased thickness of the epithelium of with destruction of the bronchioles and alveoli, accumulation of inflammatory cells in the peri-bronchioles and alveoli, and rupture of the wall of the alveoli, Figure (4- 46). As for the histological sections of the lung of the mixing group, showed slight hyperemia in the epithelium of the bronchioles, in the wall of the alveoli sac and inside the alveolar sac, and an increase in the thickness of the epithelium with irregular in shape of the alveoli in the first week, Figure (4- 47). While in the fourth week, slight hyperemia in the epithelium of the bronchioles, severe hyperemia in the alveoli wall, deformities of the epithelium lining the bronchioles, increased thickness of the epithelium of the bronchioles and alveoli, accumulation of inflammatory cells in the peri-bronchioles and alveoli, and rupture of the wall of the alveoli, Figure (4- 48).



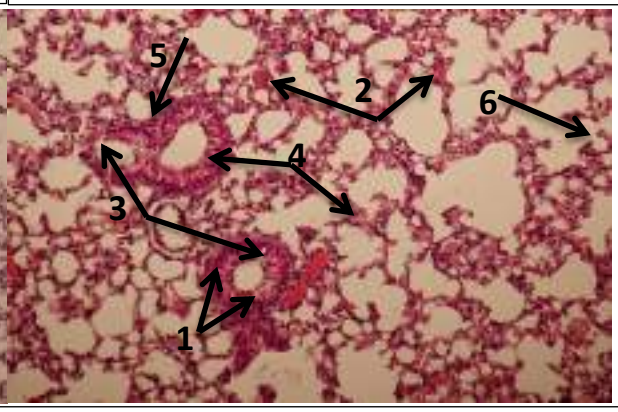
Fig(4-43)section of control group of lung mice showing alveolar ducts(1), alveolar sac (2) alveoli(3), pulmonary vein(4), pores of Cohn (5) ,squamous cells (6), bronchioles (7) (H & E stain, 100X).



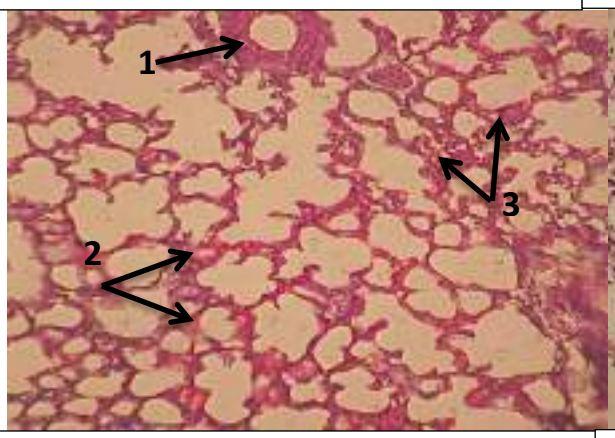
Fig(4-44)section of inhalation group of lung mice showing alveolar ducts(1), alveolar sac (2) alveoli(3), pulmonary vein(4), pores of Cohn (5) ,squamous cells (6), bronchioles (H & E stain, 100X).



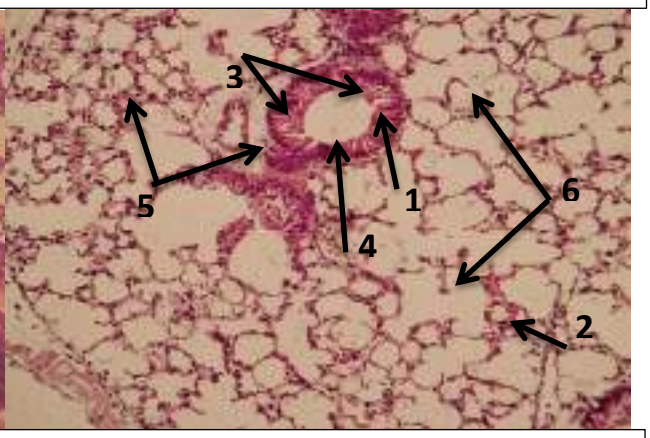
Fig(4-45)section of orally group 1^{st} week of lung mice showing slight hyperemia in the epithelium of the bronchioles (1), in the wall of the alveoli (2) and inside the alveolar sac(3), increase in the thickness of the epithelium with destruction of bronchioles and the alveoli (4). H&E stain 100X)



Fig(4-46)section of orally group 4^{th} week of lung mice showing slight hyperemia in the bronchioles(1), severe hyperemia in the alveoli wall(2), deformities of the epithelium bronchioles (3), increased thickness of the epithelium of the bronchioles and alveoli(4), accumulation of inflammatory cells (5), rupture wall alveoli (6) H&E stain 100X)



Fig(4-47)section of mixing group 1^{st} week of lung mice showing slight hyperemia in the epithelium of the bronchioles (1), hyperemia in the wall of the alveolar sacs (2), increase in the thickness of the epithelium with irregular shape of the alveolar sacs (3). H&E stain 100X)



Fig(4-48)section of orally group 4^{th} week of lung mice showing slight hyperemia in the bronchioles(1), severe hyperemia in the alveoli wall(2), deformities of the epithelium bronchioles(3), increased thickness of the epithelium of the bronchioles (4), accumulation of inflammatory cells(5), rupture wall alveolar sacs (6)H&E stain 100X)

4-10-2-Histomorphometric study:

The results of the histomorphometry of the lung showed significant differences (P <0.05) in the lumen of bronchioles in orally, mixing group, which recorded a mean of (15.3±0.40) and (15.9±0.41) respectively in the first week, as well significant differences (P <0.05) in the lumen of bronchioles in inhalation, orally, and mixing group, which recorded a mean of (21.2±1.29), (10.9±0.37) and (10.5±0.41) respectively in the fourth week in the fourth week as shown in Table (4-15). There are also significant differences (P <0.05) in the lumen of alveoli in inhalation, orally, and mixing group, which recorded a mean of (4.5±0.38),(4.6±0.32) and (4.7±0.38) respectively in the fourth week., compared with the control group as shown in Table (4-15).

Table (4-15): Mean of Lumen of alveoli and bronchioles in Lung of the control, Inhalation, Orally, and Mixing group.

Lumen	1^{st} week				4^{th} week			
	control	inhalation	orally	mixing	control	inhalation	orally	mixing
Bronchioles	18.0 [a] ±0.50	19.0 [a] ± 0.48	15.3 [b] ±0.73	15.9 [b] ±0.41	16.8 [a] ±0.62	21.2 [b] ± 1.29	10.9 [b] ±0.37	10.5 [b] ±0.41
Alveoli	3.8 [a] ±0.40	4.0 [a] ±0.38	4.1 [a] ±0.40	3.7 [a] ±0.12	3.4 [a] ±0.29	4.5 [b] ±0.38	4.6 [b] ±0.32	4.7 [b] ±0.38

*Note: the value represents (mean± SE), Horizontally similar letters indicate that there are no significant differences (p>0.05), different letters horizontally between the values indicate that there are significant differences (p<0.05).

4-10-3-Histochemical Study:

The results of histochemical Study of the Lung of mice in the control, orally, inhalation, mixing group, as shown in the following table.

Table (4-16): Reaction Epithelium bronchioles, Muscularis bronchioles, Interalveolar septum with PAS in lung.

Group	Stain	Epithelium bronchioles	Muscularis bronchioles	Interalveolar septum
Control	PAS	Moderate	Moderate	Weak
Orally	PAS	Moderate	Strong	Strong
Inhalation	PAS	Weak	Strong	Weak
Mixing	PAS	Moderate	Strong	Strong

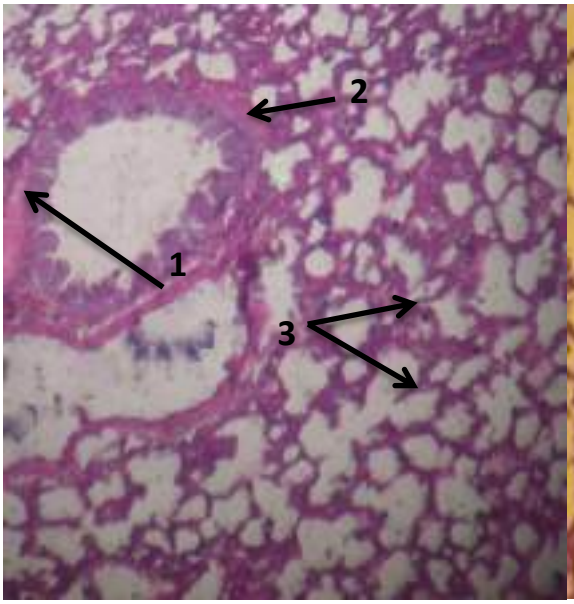


Fig (4-49) lung of control group showing, epithelium bronchioles (1) with a moderate reaction with PAS , muscularis of the bronchioles (2)had a moderate reaction with PAS, interalveolar septum (3) had a weak reaction with PAS 100X

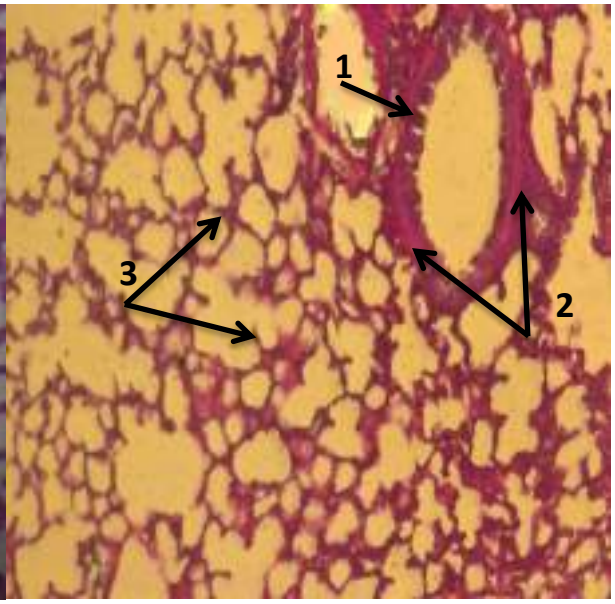


Fig (4-50) lung of inhalation group showing, epithelium bronchioles (1) with a weak reaction with PAS , muscularis of the bronchioles (2)had a strong reaction with PAS, interalveolar septum (3) had a weak reaction with PAS 100X

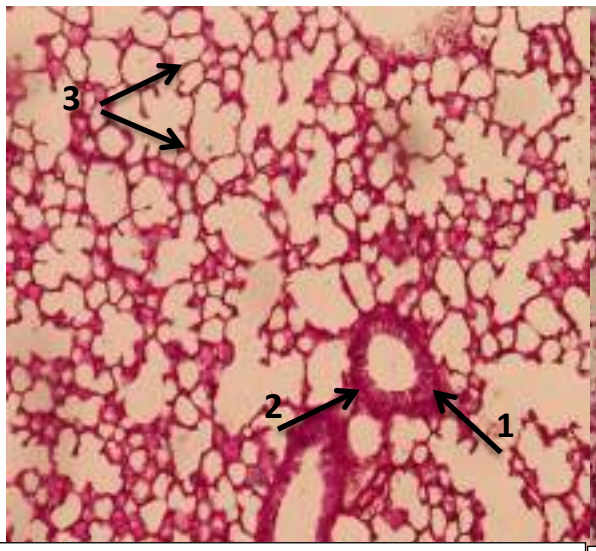


Fig (4-51) lung of orally group showing, epithelium bronchioles (1) with a moderate reaction with PAS , muscularis of the bronchioles (2)had a strong reaction with PAS, interalveolar septum (3) had a strong reaction with PAS 100X

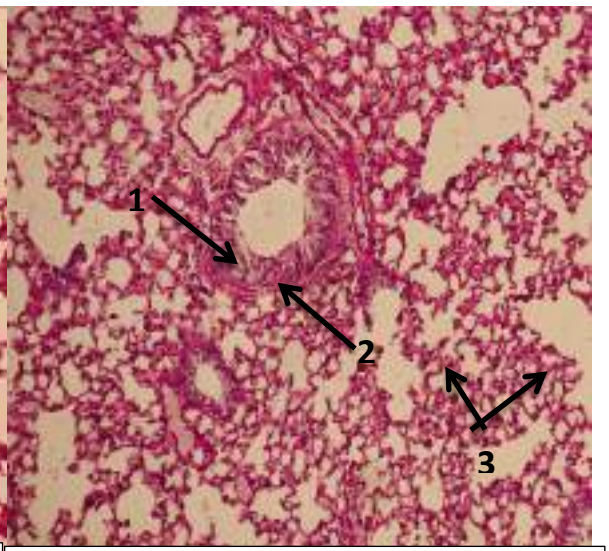


Fig (4-52) lung of mixing group showing, epithelium bronchioles (1) with a moderate reaction with PAS , muscularis of the bronchioles (2)had a strong reaction with PAS, interalveolar septum (3) had a strong reaction with PAS 100X

4-11- Results of Liver

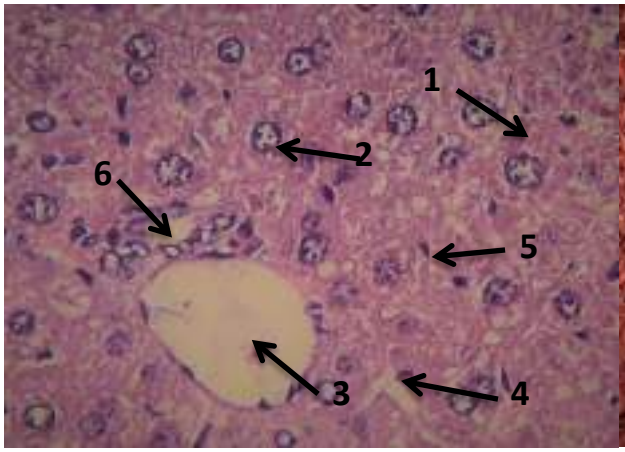
4-11-1- Histological Changes

The microscopic examination of the liver of mice in the control group showed that the hepatocytes are the main cell in the liver It is polygonal and arranged in hepatic cords or classic hepatic lobule that bifurcate from the central vein towards the periphery of the lobule these cells containing one or more round central nuclei. The sinusoids appear as pale white areas and contain kupffer cells and stellate cells called ito cells. The sinusoids are bifurcated and determined with each other in the center of the lobule, forming a central vein The bile ductules are lined with a simple cuboidal epithelium, Figure (4-53).

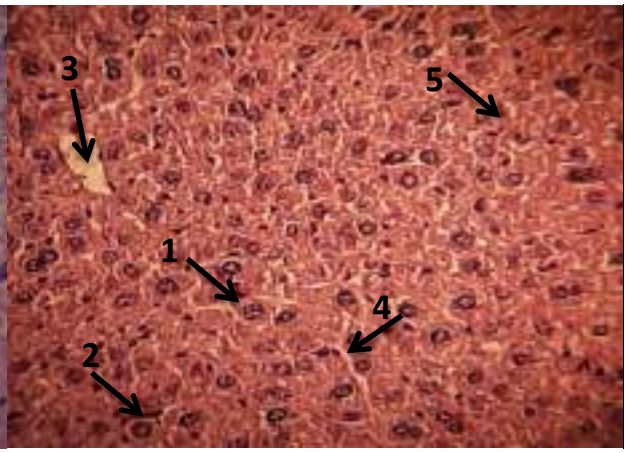
Histological sections of the liver of mice treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the liver in the control group and that no histological change was apparent (Figure 4-54) for experimental.

Histological sections of the liver of mice that were administered orally showed the presence of tissue damage, including the hyperemia in the central vein with clotting, slight fatty degeneration in the cytoplasm of hepatic cells found in the liver of mice, as well as the appearance of sinusoidal spaces in the first week, Figure (4-55), as for the fourth week, hyperemia also in the central vein, sever fatty degeneration in hepatic cells, disfiguration of the hepatocyte, partial degradation of the nucleus of the hepatic cell, as well as the appearance of sinusoidal spaces, Figure (4-56).

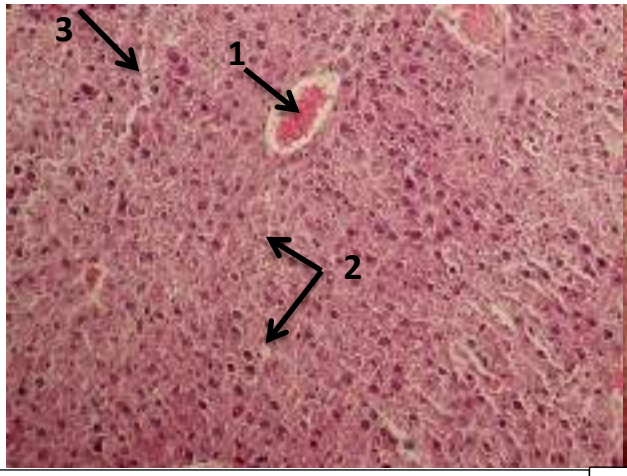
As for the histological sections of the mixing group, there was also the hyperemia in the central vein, slight fatty degeneration in the cytoplasm of hepatic cells found in the liver of mice, as well as the appearance of sinusoidal spaces in the first week, Figure (4-57), as for the fourth week, hyperemia in the central vein, sever fatty degeneration in hepatic cells, partial degradation of the nucleus of the hepatic cell, disfiguration of the hepatocyte, as well as the appearance of sinusoidal spaces, Figure (4-58).



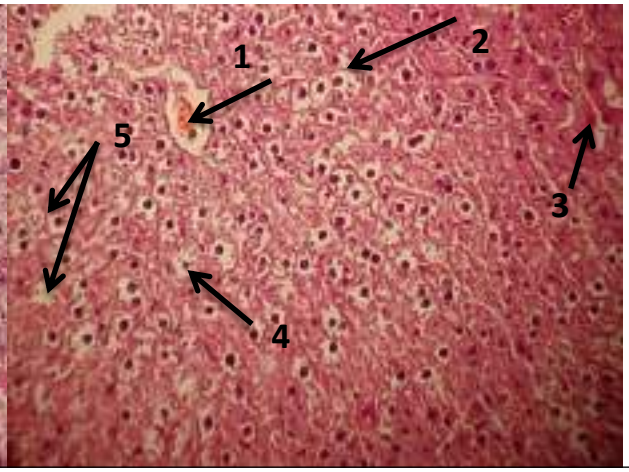
Fig(4-53) section of control group of liver mice showing Hepatocytes (1) , nucleus hepatic cell (2) , central vein (3) , sinusoids (4),Kupffer cells (5), bile ductules (6) (H & E stain, 100X).



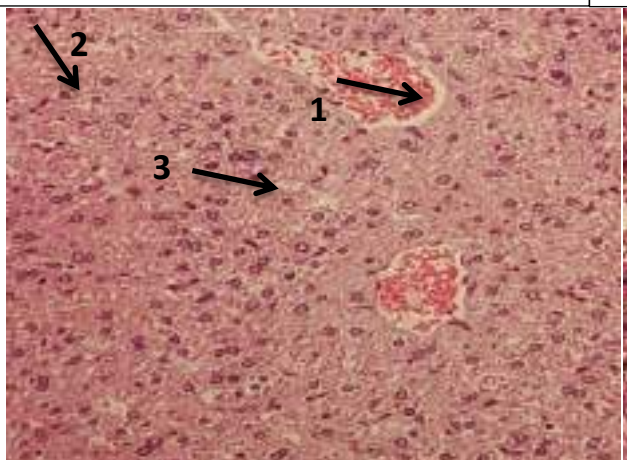
Fig(4-54) section of inhalation group of liver mice showing Hepatocytes (1) , nucleus hepatic cell (2) , central vein (3) , sinusoids (4),Kupffer cells (5), (H & E stain, 100X).



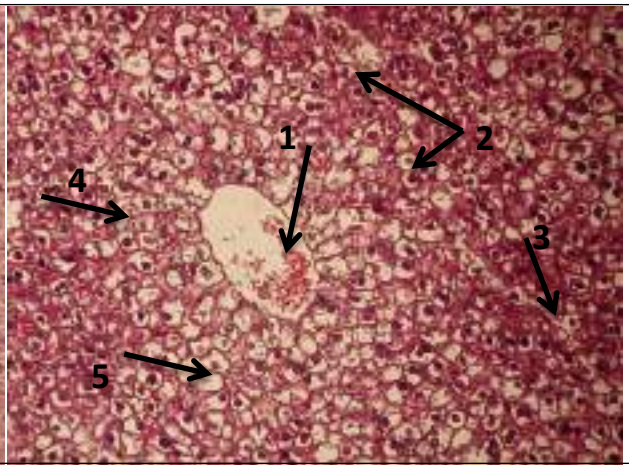
Fig(4-55) section of orally group 1^{st} week of liver mice showing hyperemia central vein (1) , slight fatty degeneration (2) ,sinusoid spaces (3) (H & E stain, 100X).



Fig(4-56) section of orally group 4^{th} week of liver mice showing hyperemia central vein (1),severe fatty degeneration (2), sinusoid spaces (3), partical degradation nuclei(4),disfiguration hepatocytes (5) (H &E E stain,



Fig(4-57) section of mixing group 1^{st} week of liver mice showing hyperemia central vein (1) , slight fatty degeneration (2) ,sinusoid spaces (3) (H & E stain, 100X).



Fig(4-58) section of mixing group 4^{th} week of liver mice showing hyperemia central vein(1), severe fatty degeneration(2) ,sinusoid spaces (3) partial degradation (4), disfiguration hepatocytes (5) (H & E stain, 100X).

4-12- Results of Kidney

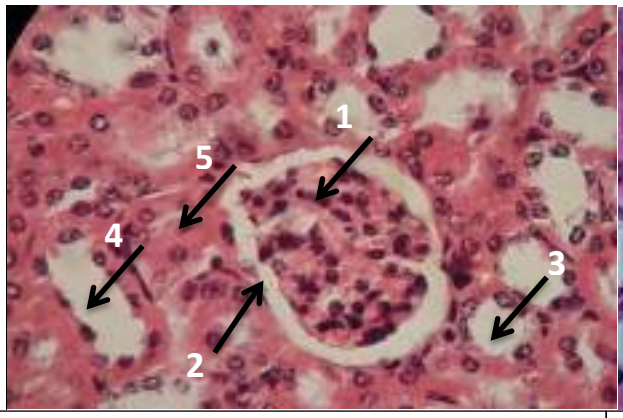
4-12-1- Histological Changes

The nephron is the functional unit in the college and it consists of the glomerulus, glomerular which is a network of capillaries surrounded by an epithelial stimulator called Bowman's capsule, then proximal convoluted tubule, then henle of the loop, the distal convoluted tubule, and then the collecting tubules, the nephron enters an afferent artery and an outgoing efferent artery. The proximal convoluted tubule is lined with a simple cuboidal epithelium containing the brush border, the loop of Henle is in the shape of a U-shape, and the distal tubule lacks the brush border, Figure (4-59).

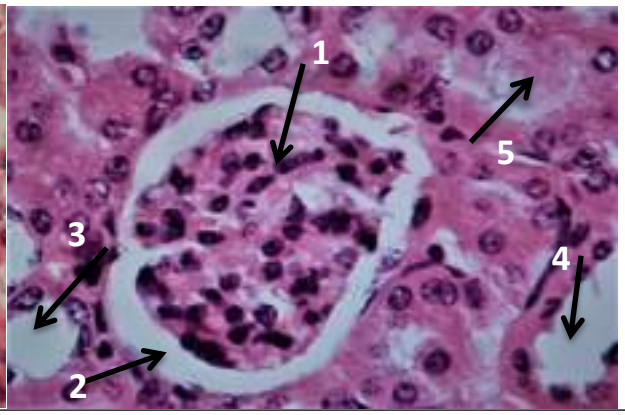
Histological sections of the kidney of mice treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the kidney in the control group and that no histological change was apparent (Figure 4-60) for experimental.

Histological sections of the kidney of mice that were administered orally, showed the presence of tissue damage, including the hyperemia in the glomeruli, atrophy of Bowman's capsule, destruction of the renal tubules blood vessels in the first week, Figure (4-61), as for the fourth week, hyperemia also in the glomerular, atrophy of Bowman's capsule, destruction of the renal tubules blood vessels, decrease in the size and number of epithelial cells in the renal tubules, renal tubular cast, Figure (4-62).

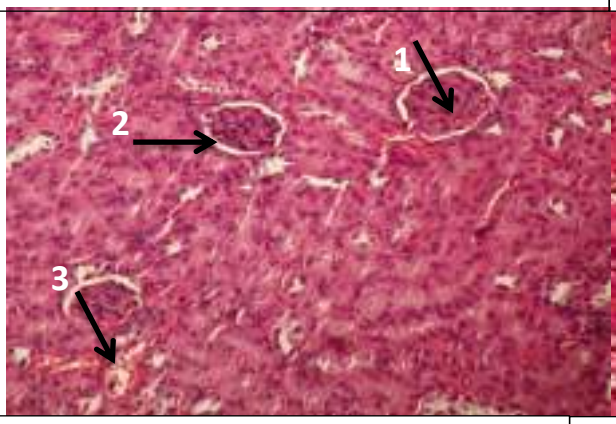
As for the histological sections of the mixing group, there were also the hyperemia in the glomeruli, atrophy of Bowman's capsule, destruction of the renal tubules blood vessels, in the first week, Figure (4-63), as for the fourth week, hyperemia in the glomerular, atrophy of Bowman's capsule, destruction of the renal tubules blood vessels, decrease in the size and number of epithelial cells in the renal tubules, renal tubules cast, Figure (4-64).



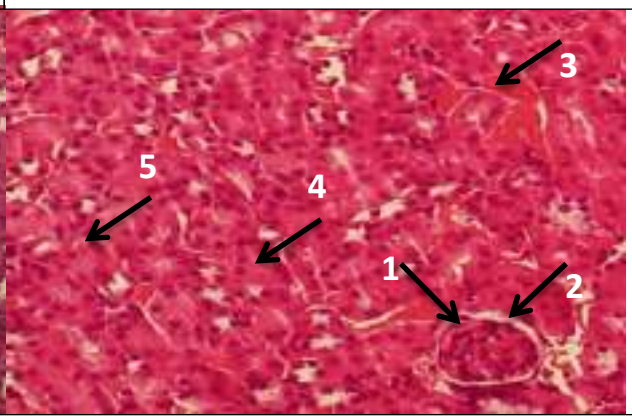
Fig(4-59)section of control group of kidney showing Glomerulus(1), Bowman's capsule(2), proximal convoluted tubule(3), distal convoluted tubule (4),simple cuboidal epithelium(5) (H & E stain, 400X).



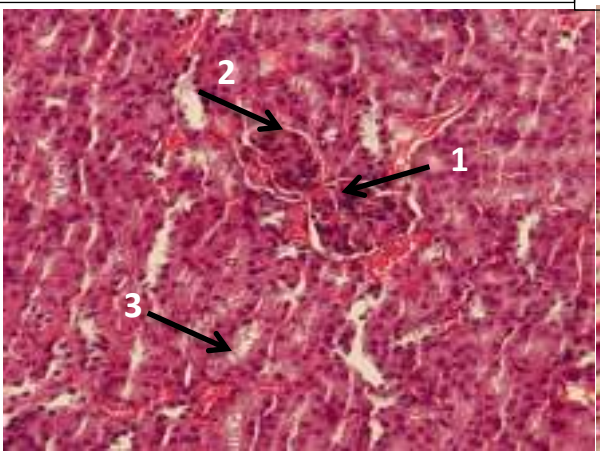
Fig(4-60)section of inhalation group of kidney showing Glomerulus(1),Bowman's capsule(2), proximal convoluted tubule(3), distal convoluted tubule(4),simple cuboidal epithelium (5) (H & E stain, 400X).



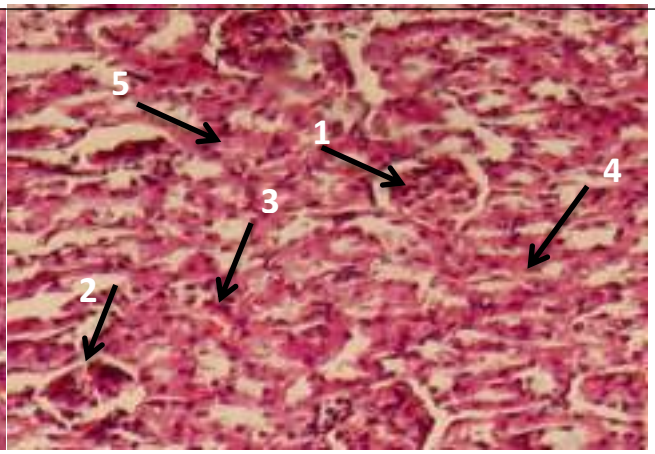
Fig(4-61)section of orally group 1^{st} week of kidney mice showing hyperemia in the glomerular (1) atrophy of Bowman's capsule(2), destruction in the renal tubules blood vessels (3) (H & E stain, 100X).



Fig(4-62)section of orally group 4^{th} week of kidney mice showing hyperemia in the glomerular (1) atrophy of Bowman's capsule(2), destruct in the renal tubules blood vessels (3) decrease in the size and number of epithelial cells in the renal tubules (4), renal tubular cast (5)(H & E stain, 100X).



Fig(4-63)section of mixing group 1^{st} week of kidney mice showing hyperemia in the glomerular (1), atrophy of Bowman's capsule(2), destruction the renal tubules blood vessels (3) (H & E stain, 100X).



Fig(4-64)section of mixing group 4^{th} week of kidney mice showing hyperemia in the glomerular (1), atrophy of Bowman's capsule(2), hemorrhage in the renal tubules (3), decrease in the size and number of epithelial cells in the renal tubules (4), renal tubular cast (5) (H & E stain, 100X).

4-13- Results of Heart

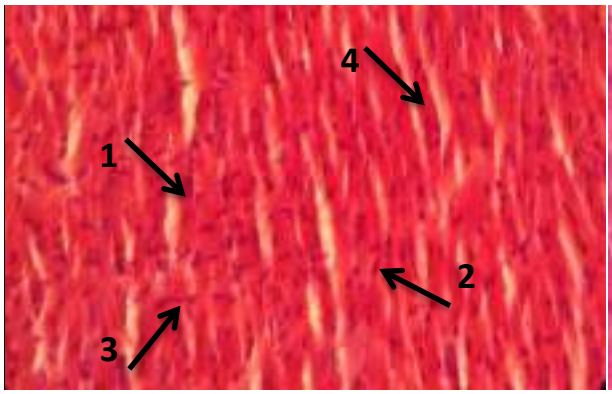
4-13-1- Histological Changes

The microscopic examination of the heart of mice in the control group showed the myocardium, which is an intermediate layer and forms the main tissue in the heart. It is composed of signal cardiomyosyte and contains one or more rectangular nuclei linked together by intercalated discs and forming collagen fibers that lead to the extracellular matrix. The heart muscle contains specialized cells that conduct electrical signals, and it also contains blood vessels, Figure (4-65)

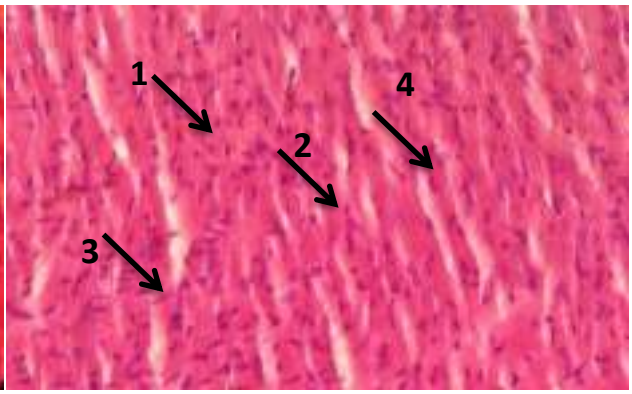
Histological sections of the heart of mice treated with eucalyptus oil by inhalation showed that it was similar to the histological composition of the heart in the control group and that no histological change was apparent (Figure 4-66) for experimental.

Histological sections of the heart of mice that were administered orally, showed the presence of tissue damage, including the hyperemia in the muscle fibers in the first week, Figure (4-67), as for the fourth week, hyperemia also in the muscle fibers, roughness in the heart muscle, Figure (4-68).

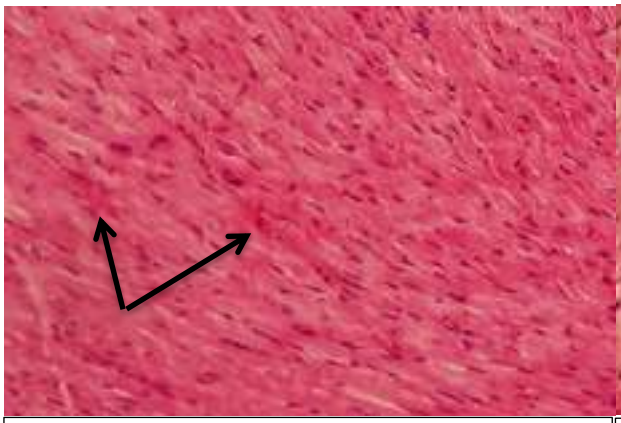
As for the histological sections of the mixing group, there was also the hyperemia in muscle fibers in the first week, Figure (4-69), as for the fourth week, hyperemia also in the muscle fibers, roughness in the heart muscle, Figure (4-70).



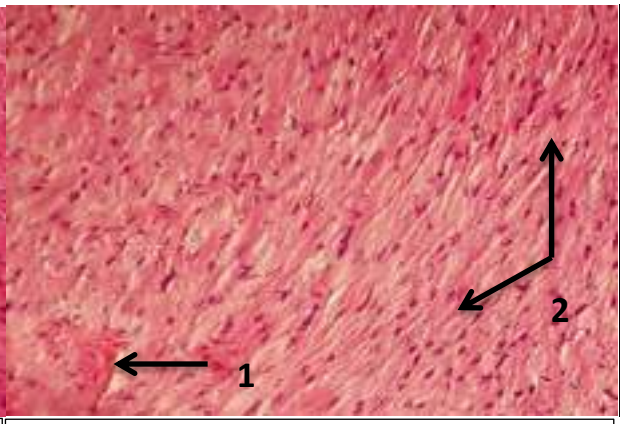
Fig(4-65)section of control group of heart showing Cardiomyocytes (1) , Cardiomyocyte nuclei (2) ,intercalated discs (3) ,myocardium fibers (4),(H & E stain, 400X).



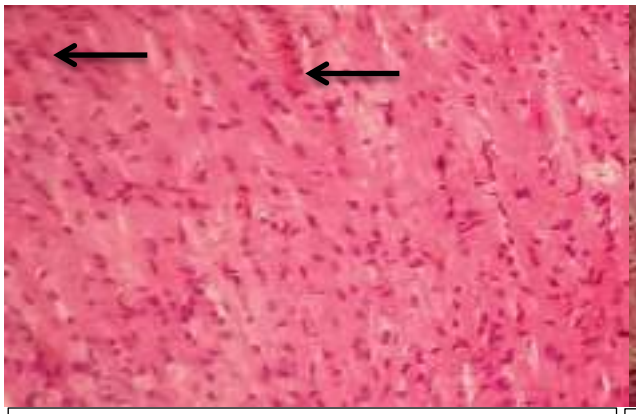
Fig(4-66)section of inhalation group of heart showing Cardiomyocytes (1) , Cardiomyocyte nuclei (2) ,intercalated discs (3) ,myocardium fibers (4),(H & E stain, 400X).



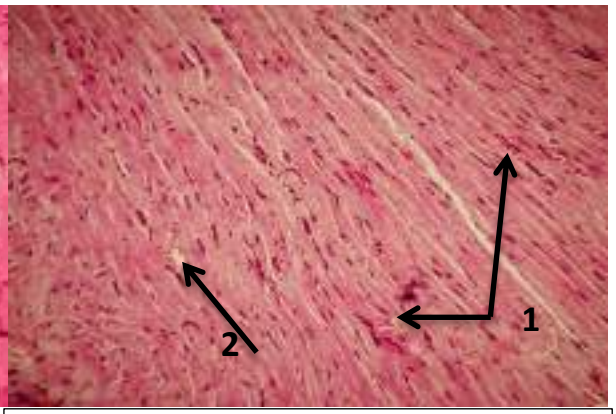
Fig(4-67)section of orally group 1^{st} week of heart mice showing hyperemia in the muscle fibers (arrow) (H & E stain, 100X).



Fig(4-68)section of orally group 4^{th} week of heart mice showing hyperemia in the muscle fibers (1), roughness in the heart muscle (2) (H & E stain, 100X).



Fig(4-69)section of mixing group 1^{st} week of heart mice showing hyperemia in the muscle fibers (arrow) (H & E stain, 100X).



Fig(4-70)section of mixing group 4^{th} week of heart mice showing hyperemia in the muscle fibers (1), roughness in the heart muscle (2) (H & E stain, 100X).

CHAPTER FIVE

DISCUSSION

5-Discussion

The results of the current study showed that oil analysis using GCMS, calculating the LD_{50} value, many clinical signs and weight changes for treated mice, and blood and histological changes, Histomorphometric and Histochemical, in the esophagus, stomach, intestines, trachea, lung, liver, kidneys and heart, which can be discussed as follows :

5-1- Gas Chromatography-Mass Spectrometry Analysis

Essential oils contain several chemical components, including terpenes, alcohols, phenols, ketones, aldehydes, and esters (Ashraf *et al*., 2010), for oil production, the leaves are the best part of the plant (Cheng *et al*., 2009).

The results of the current study obseved that the highest percentage of components in the oil of *Eucalyptus camaldulensis* was Ledene area 22.6%, β-Eudesmol 12.9%, and Aromandendrene 8.0%, while the percentage of cineole was 4.6%.

Whereas Mubarak *et al*., (2015) indicated that the highest percentage in the components of the oil of *Eucalyptus camaldulensis* was *γ*-Terpinene with an amount of 72.5%, followed *o*-Cymene 14.6%, then Terpineol with an amount of 6.7%, then cineole 0.9%.

Differences in the components of the EuO can be attributed to a variety of factors, including soil, age of plants, climate, environmental conditions, geographical location, harvest time, and plant part (Marzoug *et al*., 2011). Alternatively, as previously stated, differences in the chemical components of oil might be attributable to genotype (Zouari *et al*., 2012).

In the current studies, there were differences from those that indicated that cineole is the major component, as it is a high percentage (Su *et al*., 2006). As for our study, it showed that the active compound, which occupied a high percentage, is Ledene component.

EuO extracted by hydrodistillation produced a pleasant and refreshing odor, Previous studies have found GCMS analysis of eucalyptus oil, and cineole ratio in Brazil was high 85-90% (Yang *et al*., 2004). The average rate in Argentina is about 60% (Lucia *et al*., 2008). In Morocco, the percentage of cineole was low, about 22.4-17.2% (Karemu *et al*., 2013).

Dehydration is one of the variables that might affect oil production and chemical component since a lack of water causes oil output to decrease (Silva *et al*., 2006).

Drought has a substantial impact on chemical components because it causes an increase in terpene synthesis, particularly cineole and linalool. Young eucalyptus leaves (five-month seedlings) have a higher cineole concentration than mature tree leaves (Leicach *et al*., 2010).

5-2- Calculation of Median lethal Dose (LD_{50}) of Eucalyptus Oil

The toxicity study is very important in compounds that are used as a treatment in the body of the living organism, as well as the verification of the toxic effects of materials used in laboratory experiments. There are indications of a dose of toxicity to eucalyptus oil (Kumar *et al*., 2015).

The results of the current study showed that was LD_{50} value of pure Eucalyptus oil was 1820 mg/kg, The result was agreement with the report (Yu *et al*., 2010) where the LD_{50} value was 1824 mg/kg, While Hu *et al*., (2014) stated that the LD_{50} value of eucalypt oil is 3811mg/kg because the oil used in the experiment was purchased from the xinran company with a concentration of 70%, it has been stated Wei *et al*., (2002) that eucalyptus oil is of low toxicity.

shalaby *et al*., (2011) demonstrated that the LD_{50} for pure eucalyptus oil was 2334 mg/kg in rats, and eucalyptus oil has been proven to be moderately dangerous according to the World Health Organization.

The toxicity of eucalyptus oil has been classified as Generally Recognized As Safe (GRAS) by the United States Environmental Protection Agency (Batish *et al*., 2008). Owing to the increased demand for essential oils for medical use, EuO demands additional investigation

of its efficacy and safety due to the dangers and negative influence on health.

Although some studies have found that eucalyptus oil to be non-toxic and harmless, the current investigation was undertaken to assess the hazardous consequences of ingesting eucalyptus oil in mice Farzamfar *et al*., (2008). The toxicity of the essential oil is directly related to the chemical composition of the oil (Radulović *et al*., 2017).

5-3- Clinical Signs

From the results of the current study, some clinical symptoms appeared in the orally group and mixing, while the inhalation group did not show signs during the experiment period, including dizziness, lethargy, slow movement, and loss of appetite.

Consistent with the findings Stojanovi *et al*., (2019), which revealed that spastic movements, tremors, reduced motility, hyperventilation, loss of balance, muscular stiffness, weakness, and tumbling occurred following oral administration of essential oil at the dosage of 1-1.5 mg/kg.

Clinical signs appeared when eucalyptus oil was administered orally at a dose of 1560 mg/kg such as irregular breathing, seizure, extreme sensitivity to noise, stupor, and cyanosis, as well as death caused by respiratory failure (Bhowal and Gopal, 2015).

Hu *et al*., (2014), stated that after administering rats orally with EuO at different doses there were no clinical symptoms in the mice behavior, growth, and motor activities during the study period. because the eucalyptus oil used in an aqueous emulsion.

5-4-Body weights

The results of the current study showed a decrease in the body weights of mice in the orally and mixing group, while in the inhalation group, they continued to grow when compared with the control group.

The result was agreement Hu *et al*., (2014), and it showed significant changes in body weight in the groups that administered Eucalyptus oil in high doses compared to the control group, and indicated that the high dose of Euo can reduce weight and not grow in a normalhy, Changes in body weight have been used as an indicator of adverse effects of medications and chemicals.

As Asnaashari *et al*., (2010) have shown that administering an essential oil orally causes loss of appetite, which has a role in losing body weight.

The explanation for the weight loss might be related to an insufficient formation of antioxidants in the internal body in eliminating free radicals produced when eucalyptus oil is administered orally, which resulted in a large decrease in the body weight of mice (Ofusori and Adejuwon, 2011).

Gebremickael (2017) stated that after administering the mice orally with Eucalyptus oil emulsion at a dose of 1.5, 2 ml/kg, there were no significant differences in body weight between the treated groups and the control group. This indicates that mice are healthy by ingesting an aqueous Eucalyptus oil emulsion.

As Hu *et al*., (2014), indicated that there were no changes in the behavior of mice when consuming eucalyptus oil in different doses as eucalyptus oil was used at a concentration of 70% from xinran company.

5-5- Hematological Study

One of the most sensitive indicators for determining medication toxicity in people and animals is the blood (Rahman *et al*., 2001).

Because the blood profile normally gives vital information about the body's reaction to damage, lesion, deprivation, and stress, hematological characteristics can be used to identify the amount of a drug's harmful impact (Raza *et al*., 2002 ; Rahman *et al*., 2001).

In clinical pathology, the recommended important hematological tests include WBCs, RBCs, HGB, HCT, PLT, MCH, MCV, MCHC, and MCH, MCHC In most animals, these are the most important indicators for detecting anemia (Akpamu *et al*., 2011).

The results of the current study showed that there were significant differences in the hematological parameters of the orally and mixing group, where there was an increase in WBCs and a decrease in the number of RBCs, HGB, HCT, and PLT, while in the inhalation group, no significant differences appeared between the hematological parameters when compared with the control group.

This result is consistent with Jun *et al*., (2013) who indicated that when eucalyptus oil is inhalation, no changes in hematological parameters occur.

This result agrees with Shalaby *et al*., (2011) where eucalyptus oil was administered in rats with different doses and it showed an increase in the number of WBCs, and a decrease RBCs, HGB, PLT, RBCs, and PLT are formed in the bone marrow, The decrease in RBCs and PLT might be related to the action of eucalyptus oil on hematopoiesis in the bone marrow, as RBCs and PLT are generated in the bone marrow (Jamel Al-layl *et al*., 2004). Decrease levels of RBCs and HGB can indicate anemia in this study (Choudhari and Deshmukh, 2007).

The decrease in red blood cells may be attributed to the effect of eucalyptus oil in the kidneys, where kidney cells secrete about 85-90% of the total level of Erythropoietin, so the symptoms of chronic kidney disease are accompanied by severe anemia (Machalinska *et al*., 2002b).

Or as a result of the damage that eucalyptus oil causes when it reaches the stomach and thus causes a decrease in the absorption of some vitamins B12, which plays a major role in the emergence of red blood cells or their synthesis in the bone marrow, and therefore any damage that affects the stomach, affects the process of blood cell formation.

It was mentioned by Isa *et al*., (2007) eucalyptus increases immunity because it contains vitamin C, beta-carotene, B12, chlorophyll, and essential fatty acid, and it is also a good source of iron.

Hu *et al*., (2014) showed no changes in hematological parameters WBCs, RBCs, HGB, HCT, PLT when comparing the treated and control group after oral administration to rats of eucalyptus oil from the company at different doses for 30 days.

This result did not agree with Gebremickael *et al*., (2017) after oral administration of aqueous emulsion of eucalyptus oil to mice at doses of 1.5, 2 mg/kg, no changes in hematological parameters were observed.

Bello (2015) he confirmed in an experiment he made when using a group of quail birds that ate dry eucalyptus leaves, an increase in WBCs, RBCs, HGB, HCT, PLT, MCH, MCV, MCHC, MCH, MCHC, PCV.

These findings might imply that eucalyptus oil contains compounds that activate white blood cells, and increasing platelets, evidence that eucalyptus oil stimulates thrombocytopenia and thrombocytosis (Rogers, 2011).

The liver has a role in the secretion of the hormone Erythropoietin with a ratio of 10-15% of the total level of this hormone, which acts as a growth regulator to regulate the production and maturity of red blood cells in the bone marrow (Machalinska *et al*., 2002a).

The chlorinated component in eucalyptus has been shown to have a substantial impact on biological activities such as anti-inflammatory, antiviral, and antibacterial (Holst and Engvild, 2000 ; Kery *et al*., 2001).

5-6- Esophagus

The results of the current study of histological sections in the esophagus of mice treated with Eucalyptus oil in the orally and mixing group showed some histological changes during the experiment period, including erosion and sloughing in the mucosa and congestion in the sub mucosa and these changes increased during the four weeks compared with the control group.

These histological changes depend on the amount of the dose as well as the duration of the dose, meaning that the changes increased with the increase in the amount and duration of the dose, these changes are reactions to the effect of Eucalyptus oil.

The result is consistent with Yekeler *et al*., (2001) where he observed the appearance morphological changes in the epithelium of the esophagus. It was shown that the least toxic sign on the tissue layers depends on the dose, whether the changes are mild or moderate.

The result is consistent with Guy *et al*., (2007) where the results revealed the appearance of congestion in the esophageal mucosa, and this change is evidence of esophagitis due to Eucalyptus oil.

The results of the current study in histomorphometric showed a significant decrease in the thickness of the mucosa and a significant increase in the thickness of the submucosa in the oral and mixed group, but in the inhaled group, there were no significant differences in the tissue layers when compared with the control group.

The result is consistent with Lourenco *et al*., (2018) an increase in the thickness of sub mucosa appeared. This result is compatible with (Bertolini *et al*., 2017) a decrease in the thickness of mucosa.

Perhaps the reason for the increase in the thickness of submucosa or dilation of blood vessels, is the increase in white blood cells in abundance due to the inflammation that occurred when taking eucalyptus oil.

The results of the current study showed in histochemical, the interaction of mucosa and muscularis was strong with PAS, Submucosa, and adventitia moderate interaction with PAS in the oral and mixed group, but in the inhalation group it was similar with the control group.

The strong interaction of mucosa with PAS may be since this layer secretes neutral mucous substances, which are mucopolysaccharides, and the mucus inhibits the action of microorganisms as well as works to moisturize and protect the mucosa layer (Igbokwe, and Obinna, 2016).

5-7- Stomach

The results of the current study showed histological changes in the stomach of mice in the oral and mixed group, including the occurrence of Moderate superficial erosion in the gastric mucosa, and the edema in the mucosa between gastric glands and in the muscularis propria, in the first week and these changes increased in the period of administration. As for the inhaled group, no histological changes appeared when compared with the control group, It has been observed that an increase in the dose leads to an increase in tissue injury.

The result is agreement with Stojanović, *et al*., (2019) who observed that hemorrhage in the stomach tissue and the appearance of erosion also in the mucosa layer after oral administration with eucalyptus oil at different doses 12.3_13.7mg/kg.

The result is consistent with Fandohan *et al*., (2008) after oral administration of Essential oil in rats for 14 days at different doses of 50,500,1000,1500,2000,3000,3500mg/kg the results revealed clear changes in the morphological structure of mucosa. Visible breakdown and erosion of the stomach wall with the disappearance of the superficial epithelium and the mucosa becoming indistinct, revealing the presence of some components in the essential oil that irritate the digestive tract when using high doses.

The result is consistent with Fandohan *et* al., (2008) which revealed obvious histological changes in the stomach of rats after oral administration of eucalyptus oil at a dose of 1500 mg/kg, including rupture of the epithelium in the mucosa and the disappearance of the superficial epithrelium, he explained that the application of unrecommended doses of the oil can cause functional damage to the stomach.

The result is consistent with Ofusori and Adejuwon (2011) which indicated that erosion occurred in the surface layer of mucosa as well as

the appearance of abnormalities and irregular appearance in the mucosa stomach when administering a plant extract orally for 48 days.

The result is also compatible with Sharhan *et al*., (2020) where it was observed that gastric necrosis occurred in mice and possibly eucalyptus oil causes destruction of epithelial cells in mucosa stomachs of mice.

The result is inconsistent with Hu *et al*., (2014) after oral administration of eucalyptus oil in rats, no histological changes occurred in the stomach when compared with the control group.

The results of the current study showed that when histomorphometric of the layers of the stomach, a significant decrease in the thickness of the mucosa layer and a significant increase in the sub mucosa in the oral and mixing group, while the inhalation group did not show differences in the thickness of the tissue layers in the stomach when compared with the control group, and may be the decrease in the thickness of mucosa It is due to the erosion and tearing that occurred in the mucosa, as well as the increase in the thickness of the sub mucosa, which may be due to the increase in white blood cells due to the inflammation caused when consuming eucalyptus oil.

The results of the current study showed that in the histochemical of rat stomach, the mucosa layer had a strong interaction with PAS in the orally and mixing group, and in the inhalation group, the reaction was moderate in the mucosa.

This result is consistent with the findings of Stojanović, *et al*., (2019) where he noted that mucosa had a strong interaction with PAS after oral administration with eucalyptus oil at different doses 12.3-13.7mg/kg, increased mucus secretion in the stomach, and mucus is known to be produced throughout the digestive system and helps protect the mucosa and preserve the integrity (Corfeild and Skukla, 2003).

Increased interaction with PAS, may be due to increased mucus production as a result of eucalyptus oil stimulation, as well as the response of mice to toxic substances found in eucalyptus oil when taken orally.

5-8- Small Intestine

The results of the current study showed the occurrence of histological changes in the small intestine of mice in the oral and mixed group, including hyperemia in the mucous (villi), strong deformation and sloughing of the villi, dilation of blood vessels with oedema in the sub mucosa, but in the inhaled group, no histological changes occurred when compared with the control group.

The result is consistent with Stojanovic *et al*., (2019) the occurrence of mucosa hyperemia as well as erosion or rupture of villi after oral administration of essential oil at a dose of 12.3_13.7 mg/kg.

These changes are evident in the intestine because the small intestine is the location where toxic substances are absorbed because of its relatively large surface area and long contact time (McQueen, 2010).

The result is consistent with Boeing *et al*., (2021) the occurrence of histological changes in the mice intestine such as ruptures in the mucosa as well as the dissociation, vacuum degeneration in the mucosa.

The result was consistent with Abbasi *et al*., (2016) which revealed histological changes in the intestine of mice, including mucosa rupture or distortion.

The result is inconsistent with Hu *et al*., (2014) after oral administration of eucalyptus oil in rats, no histological changes occurred in the intestine when compared with the control group.

The mucosa inflammation in the intestine is a serious inflammation of the small intestine, and this inflammation may be used as a standard in the evaluation of treatments used (Erben *et al*., 2014).

Lipophilic molecules of EOs tend to form micelles, and they are absorbed in the small intestine together with other lipids. Moreover, their lipophilic character enables them to have easy penetration via the epithelial cell membrane. Thus, the molecule forms are delivered to the small intestine where are released and hydrolysed together with lipids (Rodriguez-concepcion *et al*., 2018 ; Papada *et al*., 2018).

The results of the current study showed that in histomorphometric of the intestines of mice, there was a significant decrease in the thickness of mucosa and a significant increase in the thickness of the sub mucosa, in the oral and mixed group, but in the inhaled group, there were no significant differences when compared with the control, and the decrease in mucosa thickness may be due to the previously mentioned histological changes.

The result was in agreement with Boeing *et al*., (2021), which revealed a decrease in mucosa thickness in mice as well as a decrease in villi length and an increase in sub mucosa thickness.

The results of the current study in the histochemical of the orally and mixing group showed a strong interaction of mucosa and sub mucosa with PAS, while the inhalation group was identical to the control group.

The result is in agreement with Stojanovic *et al*., (2019) the results revealed a strong interaction of mucosa in the intestine with PAS after oral administration of essential oil at a dose of 12.3-13.7mg/kg, as the essential oil did not change the patterns of mucus distribution but increased mucus production and therefore the result of strong interaction with PAS and increased mucus interaction with PAS is probably due to increased mucus stimulated by essential oil as well as the body's response to toxic substances present in the intestines.

The goblet cells secrete mucus and are glycoproteins that form a protective layer in the lumen of the intestine. Mucous forms a physiological barrier that prevents the invasion of pathogenic microorganisms. It also has an immune function, as mucus increases with inflammation (Zhang, 2020 ; Grondin *et al*., 2020).

It was mentioned by Isa *et al*., (2007) eucalyptus increases immunity because it contains vitamin C, beta-carotene, B12, chlorophyll, and essential fatty acid, and it is also a good source of iron.

The result is also consistent with Al-Qudah (2014) revealed increased mucus production in the mucosa layer of the intestine in mice.

5-9-Trachea

The results of the current study showed the occurrence of histological changes in the trachea of mice in the oral and mixed group, including inflammatory cell infiltration, destruction of the surface of the epithelial layer in the mucosa, and loss of cilia, as for the inhalation group, there are no histological changes when compared with the control group.

The result is consistent with Mussa *et al*., (2018) revealed histological changes in the trachea, including erosion, epithelium loss in mucosa, loss of cilia, and increased infiltration of inflammatory cells. These changes in the trachea depend on the amount of the Eucalyptus oil dose.

The result is consistent with Vaezi *et al*., (2011) which revealed changes in the trachea, including loss of cilia, changes in cilia length, and a decrease in the length of ciliary columnar cells, which results in a decrease in the number of goblet cells.

Unsaturated fat molecules may be susceptible to damage due to generated free radicals, which lead to effects on cells and lead to programmed cell death, or blocks the ability of cells to receive nutrients and cellular exchanges will lose this is accompanied by cell death (Han *et al*., 2001), Thus, it leads to a loss of the respiratory epithelium in the trachea (Carter *et al*., 2012).

The result is consistent with Cavanagh (2003) infiltration of the lymphocytes of the mucosa and hyperemia and rupture epithelium of the tracheal lumen.

The results of the present study showed that in histomorphometric of the trachea, an increase in the tracheal lumen occurred in the orally, inhalation, and mixing group, as well as a decrease in mucosa thickness in the oral and mixed group when compared with the control group.

Experiments have shown that cineole found in eucalyptus oil components has a role in expanding and relaxing the tracheal rings (Nascimento *et al*., 2009).

The results of the current study in the histochemical of mice tracheal mucosa and sub mucosa showed a strong interaction with PAS in the orally and mixing group.

The result agrees with Cavanagh (2003) revealed a strong interaction of mucosa in the trachea with PAS.

5-10- Lung

The results of the current study showed the occurrence of histological changes in the lung in the orally and mixing groups. The changes hyperemia in the epithelium of the bronchioles and in the alveoli wall, deformities of the epithelium lining the bronchioles, accumulation of inflammatory cells in the peri-bronchioles and alveoli. As for the inhalation group, there were no histological changes in the lung when compared with the control group.

The result is also consistent with Zagorski *et al*., (2003) increase in the number of inflammatory cells in the lung of mice.

The result is consistent with Topcu-Tarladacalisir *et al*., (2014) the occurrence of alveolar hemorrhage and infiltration of inflammatory cells in the lung of mice, an effect on the alveolar epithelium, and a distortion of the alveolar structure.

The result is inconsistent with Hu *et al*., (2014) after oral administration of eucalyptus oil in rats, no histological changes occurred in lungs when compared with the control group.

The loss of alveolar epithelial cells and consequent impairment of the integrity of the alveolar-capillary barrier (blood- air barrier) and lead to an impairment in the physiologic trans-epithelial fluid transport system resulting from alveolar edema (Miller *et al*., 2001).

The results of the current study showed the accumulation of inflammatory cells, Inflammatory cell infiltration is one of the indicators of pneumonia (Han *et al*., 2012).

An increase in inflammatory cells in the lung does not mean an increase in neutrophils, neutrophils represent a small part of the total cell number (Zagorski *et al*., 2003).

High doses of the substance may lead to obstruction of the respiratory tracts, causing pressure on the alveolar walls and their rupture, which is represented by connective tissue consisting of elastic fibers, or perhaps

rupture of the alveolar walls resulting from partial obstruction of the respiratory tracts as a result of the accumulation of mucus as well as weakness of the elastic tissue in the respiratory tracts (Liqaa, 2010)

The cause of hyperemia in the pulmonary blood vessels may be due to blood pressure as a result of the accumulation of blood within the pulmonary circulation, and these vessels become congested with blood (Liqaa, 2010).

As for the infiltration of inflammatory cells, this depends on the high concentration of some toxic substances, and the migration of these cells depends on the nature of the tissue damage (Kishimoto and Fujii, 2002).

The result is consistent with Benson *et al*., (2011) the occurrence of hyperemia in the lung of mice, abnormalities in the alveolar walls, and the occurrence of inflammatory cell infiltration.

The increase in inflammatory cells is mostly in lymphocytes and this increase depends on the dose (Shraideh *et al*., 2013) that inflammation in the lung indicates the toxic components of the oil.

Eucalyptus oil is used as an expectorant for upper respiratory tract infection (Wang *et al*., 2017). It improves lung function and dilates the bronchi in patients with chronic obstructive pulmonary disease (COPD) (Worth *et al*., 2009).

The results of the current study of histomorphometric of rat lung showed an increase in the alveolar lumen in the oral, inhalation, and mixing group, and a decrease in the bronchial lumen in the orally and mixing group, and in the inhalation group, an increase in the lumen of the bronchioles.

The result is consistent with Kuhn *et al*., (2000) an increase in the thickness of the walls of the bronchioles and consequently a decrease in the lumen, and also revealed an expansion of the alveolar lumen in the rat lung.

The results of the current study in the histochemical of mice lung, bronchiolar epithelium showed moderate interaction with PAS and alveolar septum strong interaction with PAS in the oral and mixing group and in the inhaled group the interaction was weak with PAS.

The result is consistent with (Jain-Vora *et al*., 1997) alveolar walls strongly interacting with PAS, which indicates the presence of neutral glycoproteins.

5-11-Liver

The results of the study showed histological changes in the liver of mice, the presence of hyperemia in the central vein, sever fatty degeneration in hepatic cells, disfiguration of the hepatocyte, partial degradation of the nucleus of the hepatic cell in the orally and mixing group, and in the inhalation group, there were no histological changes when compared with the control.

The result is consistent with Hu *et al*., (2014) after oral administration of eucalyptus oil in rats, histological changes occurred in the liver, including central vein hyperemia and vesicular degeneration of hepatocytes.

The result is consistent with Shalaby *et al*., (2011) the occurrence of histological changes in the liver of rats after administration eucalyptus oil, hyperemia occurred in the blood vessels, as well as small vacuoles appeared in the hepatocytes, and indicated that eucalyptus oil had a detrimental effect on the structure of the liver membrane and its functional integrity.

The result is consistent with Abou Nazel *et al*., (2014) after oral administration of essential oil in mice, the occurrence of histological changes in the liver, represented by distortion of hepatocytes and necrosis.

The result is consistent with Gepremickael *et al*., (2017) after orally administration of aqueous emulsion of eucalyptus oil in mice, histological changes occurred in mice liver including focal necrosis, vacuolar degeneration, hepatolobular architecture malformation, and pyknosis. These changes occur when a signal is received to trigger apoptosis (Berghe *et al*., 2010). Results Hu *et al*., (2014) The liver appears to be the organ targeted by eucalyptus oil poisoning.

Stojanovic *et* al., (2019) has shown that an increased oral dose of eucalyptus oil results in increased occurrence of histological changes in the liver of mice.

The liver is a crucial organ in vertebrate metabolism since it can biotransform, accumulate, and inactivate a wide range of harmful chemicals. The liver is a significant organ for the study of toxic compounds (De Oliveira *et al*., 2012).

Damage from toxic or immunologic insult may cause hydropic degeneration of hepatocytes in which cells take on a swollen, edematous appearance with irregularly clumped cytoplasm and vacuolations (Kumar *et al*., 2002).

In hepatocytes, the appearance of pyknosis and protoplasmic changes such as vacuolation may indicate morphologic indications of necrosis or degeneration (Berghe *et al*., 2010)

Abnormal changes in the structure of the hepatocytes also result in distortion of the general liver microscopic architecture including the eradication of some of the sinusoidal spaces (Ebaid *et al*., 2007).

Abou Nazel *et al*., (2014) hypothesized that Eugenol metabolism produces vinylogous quinone methide, which may have a role in hepatotoxicity.

The sinusoids are the major regions of the liver that are damaged by the essential oil since the blood comes from the gastrointestinal tract, which carries the potentially toxic substances, is transported to the liver through the hepatic portal vein to the portal area (McQueen, 2010).

Liver cells are also affected by the essential oil components, and these cells play a significant part in liver tissue destruction (McQueen, 2010).

Certain hepatocytes may have eosinophilic cytoplasm as a result of cell organelle enlargement and lysosome rupture, which is the first step in hepatocyte necrosis (Pandey *et al*., 2008).

The report revealed that eucalyptol gets metabolized by rat and human liver microsomes as well as by several recombinant cytochrome oxidases

to solely 2- hydroxy-eucalyptol (Miyazawa *et al*., 2001a ; Miyazawa and Shindo, 2001b).

In the liver are signs of hepatitis and might be induced by eucalyptus oil's oxidative stress (Martinez–Alfaro *et al*., 2011).

According to Junqueira and Carneiro (2008), because the blood runs in blood capillaries (sinusoids) from the periphery to the center of the hepatic lobule, liver cells might also be exposed to a gradient of blood components, Peripheral cells are the first to receive nutrients and oxygen, as well as toxic substances and, are thus the first to be affected.

The process of blood cells reaching the liver more rapidly was described as vascular congestion, and this process facilitates white blood cell (neutrophil, monocyte, and lymphocyte) migration and is involved in the inflammatory process and the removal of foreign material (De Oliveira *et al*., 2012).

5-12-Kidney

The results of the study showed histological changes in the kidneys of mice, the occurrence of hyperemia also in the glomerular, atrophy of Bowman's capsule, destruction of the renal tubules blood vessels, decrease in the size and number of epithelial cells in the renal tubules, renal tubular cast in the orally and mixing group, while in the inhalation group there were no histological changes when compared with the control group.

The result is consistent with Hu *et al*., (2014) after oral administration of eucalyptus oil to rats, histological changes occurred in the kidneys, including hyperemia in the glomerular, granulomatous degeneration, and narrowing of the renal tubules, indicated that eucalyptus oil causes damage to the kidneys of mice.

The result is consistent with Gepremickael *et al*., (2017) histological changes that appeared in the kidney of mice after oral administration of eucalyptus oil emulsion, the occurrence of pyknosis in renal tubule epithelial cells.

The result is consistent with Shalaby *et al*., (2010) the occurrence of histological changes in the kidney of rats after oral administration of

eucalyptus oil, hyperemia occurred in the renal tubules, and distortion of the epithelial cells of the renal tubules.

The result is consistent with Abou Nazel *et al*., (2014) that after oral administration of essential oil in rats, histological changes in the kidney, including renal tubular degeneration, vesiculation of tubules with pyknotic nuclei, and renal tubular cell degeneration occurred.

Stojanović *et* al., (2019) have shown that an increased oral dose of eucalyptus oil leads to increased appearance of histological changes in the kidney of mice.

These histological changes in the renal tubules might lead to a rise in the concentration of possible essential oil components in the urine filtrate (McQueen, 2010).

The result did not agree with (Bababaalian *et al*., 2020) which revealed that there were no histological changes in the kidney of trout after eating meals containing essential oils.

Koubaa-Ghorbel *et al*., (2020) According to the study, the essential oil has negative effects on the kidney and causes elevated levels of creatinine and urea in plasma, which are the main indicators for nephrotoxicity (Koubaa-Ghorbel *et al*., 2019).

It was shown by Kinhult *et al*., (2003) The synthesis of free radicals is thought to be the cause of damage to the epithelial cells of the kidney.

Due to increased blood flow, the kidney are vulnerable to drug-induced toxicity, according to the study (Evenepoel *et al*., 2010)

Kidneys are particularly vulnerable to the adverse effects of chemicals and drugs because they filter and concentrate a variety of chemicals and compounds that can accumulate to dangerous levels (Loh and Cohen, 2009).

5-13- Heart

The results of the current study showed the occurrence of histological changes in the heart of mice, including hyperemia also in the muscle fibers, necrosis in the muscle fibers, roughness in the heart muscle in the oral and mixed group, but in the inhaled group, no histological changes occurred when compared with the control group.

The result is consistent with Heidarpour *et al*., (2012) who observed histological changes in the heart of mice, including hyperemia as well as necrosis in the heart muscle.

The result is consistent with Amara *et al*., (2019) the occurrence of histological changes in the heart of mice, the occurrence of hemorrhage in the cardiac muscle, and the emptiness of cytoplasm in the cardiac muscle cells.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6-1- Conclusions

The current study reached to many important conclusions

1-The Histology of Eucalyptus leaves showed that the lamina consists of an upper and lower epidermis, and the cells are circular or elongated. The type of mesophyll is isobilateral and palisade tissue ranges (2-3) layers, as well as the presence of cavities, ducts that contain oil.

2-The components of eucalyptus oil were identified using GCMS technology, revealing 98 compounds, the most important of which are Ledene, β-Eudesmol, Aromandendrene, and Cineole.

3-The LD_{50} of Eucalyptus oil orally administered in mice is 1820 mg/kg.

4-Clinical signs appeared including dizziness, loss of appetite, lethargy, and slow movement in the orally and mixing group.

5-The results of the current study showed a decrease in body weight for the orally and mixing group.

6-The results indicate changes in hematological parameters, there were an increase in WBCs and a decrease in RBCs, HGB, HCT, PLT in the orally and mixing group.

7-Histological changes occurred in the esophagus, stomach, intestine, trachea, lung, liver, kidney, the heart of mice in the orally and mixing group, The liver, kidneys, and lungs were the most damaged organs and histopathological change.

8-In the inhaled group, there were no clinical symptoms, changes in hematological parameters, and histological changes, also, there is no decrease in body weight and the mice were in good health, similar to the control group.

9-The interaction of mucosa with PAS was strong in the oral and mixed group, in the inhaled group, it was moderate or weak, similar to the control group.

10-The occurrence of changes in the thickness of the mucosa and sub mucosa in the oral and mixed group, and in the inhaled group, an increase in the bronchial and alveolar lumen of the lung.

11-It was found that Eucalyptus oil when administered orally has significant toxic effects in mice, but when inhalation, no toxic effects occur, and it is considered safe and not dangerous or toxic.

12- From the results of the study, it was found that Eucalyptus oil works to expand the trachea and bronchi when inhalation.

6-2- Recommendations

It was found from above that eucalyptus oil has toxic effects in mice when administered orally and mixed, and non-toxic when inhaled. Therefore, the current study recommends the following:

1- Conducting other studies to determine the effect of eucalyptus oil on other organs such as the brain, skin, and reproductive system, determine the effect on fertility rate, bone marrow, thymus, thyroid, adrenal, and spleen.

2-The use of eucalyptus oil with concentrations and doses lower than those used in this experiment to determine its effects on the body's organs at a lower dose.

3- Conducting other studies to find out the effect of eucalyptus oil on biochemical parameters such as liver and renal function tests.

4- Conducting an immunohistochemical study in coloring the areas of the stomach, intestines, lung, liver, and others.

5- Conducting a histochemical study to detect lipids and nucleic acids in the stomach, intestines, lung, liver, kidney, and others.

6- Conducting a study on pregnant mice and their fetuses.

7- Conducting a study on other types of eucalyptus and knowing their components and their effect on mice.

8- Eucalyptus oil can be inhaled to treat respiratory problems such as colds, nasal congestion, and asthma, because it expands the airways and alveoli.

9- Conducting a study by other methods of administration such as subcutaneous injection or intraperitoneal injection.

10-determining the effect of eucalyptus essential oil on the oxidative stress markers such as (TBARS,H2O2,Glutathione).

11-determining the effect of eucalyptus essential oil on the oxidative enzymes activity such as (SOD, Catalase, GPR,GR and G-S-Transferase).

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APPENDIX

Appendix 1:Chhemical components of Eucalyptus oil

Compound Label	RT	Aeara %
Eucalyptol	9.765	4.61
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-	11.756	1.40
Pinocarvone	12.071	0.45
Isoborneol	12.196	0.37
Terpinen-4-ol	12.408	1.45
alpha.-Terpineol	12.672	3.37
Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate	12.979	0.44
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis	13.096	0.46
p-Mentha-1(7),8-dien-2-o	13.243	1.27
Geraniol	13.689	0.41
Thymol	14.099	1.79
Thymol	14.246	1.79
Thymol	14.356	2.07
2-Oxabicyclo[2.2.2]octan-6-ol,1,3,3-trimethyl-, acetate	14.839	0.29
(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8ahexahydroazulene	15.227	0.54
Copaene	15.271	0.05
1HCyclopropa[a]naphthalene,1a,2,3,3a,4,5,6,7boctahydro-1,1,3a,7-tetramethyl-, [1a(1a.alpha.,3a.alpha.,7b.alpha.)]-	15.725	0.78
1HCyclopropa[a]naphthalene1a,2,3,5,6,7,7a,7boctahydro1,7,7atetramethyl[1aR(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	16.003	0.73
Aromandendrene	16.267	8.02
Aromandendrene	16.486	3.00
beta.-Guaiene	16.552	0.74
1HCycloprop[e]azulene,1a,2,3,5,6,7,7a,7boctahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	16.743	1.75
1HCycloprop[e]azulene,1a,2,3,5,6,7,7a,7boctahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	16.86	1.50
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-	17.05	0.55

methylene-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)-		
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	17.123	0.72
1HCycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	17.511	0.25
1HCycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	17.724	0.15
2-((2R,4aR,8aS)-4aMethyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	17.834	6.57
1HCycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	18.207	22.68
2-Naphthalenemethanol,decahydr.alpha.,.alpha.,4atrimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	18.551	3.66
2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, (2R-cis)-	18.639	3.87
.beta.-Guaiene	18.683	12.92
2-Naphthalenemethanol,decahydr.alpha.,.alpha.,4atrimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	18.961	0.21
5-Benzofuranaceticacid, 6-ethenyl-2,4,5,6,7,7ahexahydro-3,6-dimethyl-.alpha.-methylene-2-oxo-,methyl ester	19.071	0.56
6-Isopropenyl-4,8adimethyl-1,2,3,5,6,7,8,8 aoctahydronaphthalene-2,3-diol	19.13	0.32
6-Isopropenyl-4,8adimethyl-1,2,3,5,6,7,8,8 aoctahydronaphthalene-2,3-diol	19.342	0.45
Corymbolone	19.452	2.04
Corymbolone	19.562	0.87
6-Isopropenyl-4,8adimethyl-1,2,3,5,6,7,8,8 aoctahydronaphthalene-2,3-diol	19.649	0.11
(-)-Isolongifolol,methyl ether	19.737	0.30
6-Isopropenyl-4,8adimethyl-	19.957	0.06

1,2,3,5,6,7,8,8aoctahydronaphthalene-2,3-diol		
1-Heptatriacotanol	20.653	0.30
1,4-Benzenedicarboxylic acid,bis(2-ethylhexyl) ester	28.311	2.69
8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.beta.-methoxy-4,4-dimethyl	30.2	0.02

Appendix 2: Hematoxylin stain

Hematoxylin Solution (Ehrlich's)

ethanol (100%)……..100 mL

glycerol……100 mL

glacial acetic acid…….10 mL

hematoxylin…….2g

Humotoxylin is mixed with ethanol alcohol, then added glycerol and glacial acetic acid, then added a quantity of potassium alum ,these components place in glass bottle exposed to sunlight. The vial is opened for a period, then closed and shaken. This process is repeated for several weeks until the dye matures.

Appendix 3: eosin Y stain

Eosin Y ..... 1 g

Distilled water 20ml

95% Ethanol..... 80 ml

Mix to dissolve and store in room temperature.

Appendix 4: Periodic Acid Schiff PAS technique

Solutions

Periodic Acid Solution:

Periodic acid ..... 1 g

Distilled water..... 100 ml

Schiff Reagent:

Basic fuchsin.....1.0 gm.

Sodium metabisulphite .....2 gm.

Distilled water.....100 ml

Hydrochloric acid..... 2 ml

Charcoal activated ..... 0.3 gm

Dissolve basic fuchsin in boiling water, cool at 50 ^{0}C and filter. add sodium metabisulphite and HCl.store at dark room at room temperature overnight. Add charcoal, shake for one minute and filter.

Results: Glycogen, neutral glycoprotein : magenta

الخلاصة

أجريت الدراسة لاستخلاص زيت الأوكالبتوس ، والتعرف على مكوناته الكيميائية ، وتحديد متوسط الجرعة المميتة (LD_{50}) في الفئران ، وتحديد التغيرات النسيجية والدموية والوزنية والسلوكية في كل طريقة اعطاء ، وكذلك الكشف عن الطريقة الآمنة وغير السامة.

تم استخلاص زيت الأوكالبتوس من أوراق *Eucalyptus camaldulensis* بطريقة التقطير المائي بجهاز Clevenger لمدة 3-4 ساعات.

في التجربة ، تم استخدام (170) فأرًا ذكروأنثى ، واستخدمت (50) فأرًا لحساب متوسط الجرعة المميتة (LD_{50}) عند تناول زيت الأوكالبتوس عن طريق الفم لمدة 14 يومًا. قسمت الفئران إلى 5 مجموعات ، في كل مجموعة (10) فئران ، مجموعة ضابطة ، تلقت محلول ملحي و 4 مجموعات تمت معالجتها بزيت الأوكالبتوس بجرعة 1200 ، 1600 ، 2000 ، 2400 مجم / كجم. تم حساب الجرعة المميتة المتوسطة (LD_{50}) باستخدام طريقة كاربر في الفئران وكانت 1820 مجم / كجم.

ثم استخدام (120) فأراً ذكر وأنثى لتحديد التغيرات النسيجية الدموية الناتجة عن زيت الأوكالبتوس. قسمت الحيوانات إلى 4 مجموعات ، مجموعة ضابطة ، مجموعة فموية ، مجموعة استنشاق ، مجموعة مختلطة ، وفي كل مجموعة مكونة من 20 فأرًا ، تم إعطاء المجموعة الفموية زيت الأوكالبتوس عن طريق الفم بجرعة 1000 مجم / كجم لمدة 4 أسابيع. تم تعريض مجموعة الاستنشاق لاستنشاق زيت الأوكالبتوس لمدة 15 دقيقة في قفص مغلق ، المجموعة المختلطة تناولت عن طريق الفم واستنشقت زيت الأوكالبتوس .

خلال فترة الدراسة ، تم قياس الأوزان كل أسبوع ، وتم تسجيل الأعراض السريرية في كل مجموعة ، والقتل الرحيم ، وتم سحب الدم كل أسبوع ، واستئصال القصبة الهوائية والرئة والمريء والمعدة والأمعاء الدقيقة والكبد والكلى والقلب.). تم استخدام نوعين من الأصباغ للدراسات النسيجية: هيماتوكسيلين ويوزين و حامض البريوديك شيف (PAS).

أظهرت نتائج الدراسة الحالية أن زيت الأوكالبتوس المستخرج من أوراق *Eucalyptus camaldulensis* يحتوي على 98 مركبًا كيميائيًا ، وأهم هذه المركبات هي ledene، -β Eudesmol، Aromandendrene ، و Cineole.

ظهور علامات سريرية على الفئران بما في ذلك الدوخة ، وفقدان الشهية ، والخمول ، وبطء الحركة في الفموية والمختلطة، ولكن في مجموعة الاستنشاق ، عدم ظهور اعراض سريرية عند مقارنتها بالمجموعة الضابطة. وهناك انخفاضًا معنوي ($P < 0.05$) في وزن الجسم المجموعة الفموية والمختلطة ، بينما في مجموعة الاستنشاق استمر في النمو خلال فترة التجربة ، كانت هناك زيادة معنوية ($P < 0.05$) في كرات الدم البيضاء وانخفاض معنوي ($P < 0.05$) في كرات الدم الحمراء ، HGB، HCT، PLT في المجموعة الشفوية والمختلطة ، ولكن في مجموعة الاستنشاق ، لم تحدث تغييرات في المعلمات الدموية.

الخلاصة

حدثت تغيرات نسيجية في مريء الفئران في المجموعة الفموية والمختلطة ، احتقان في الطبقة تحت المخاطية ، تآكل وانسلاخ في الغشاء المخاطي واحتقان ، اما في مجموعة الاستنشاق ، عدم حدوث تغيرات نسيجية. كان هناك انخفاض معنوي في سمك الغشاء المخاطي وزيادة في سمك الغشاء المخاطي الفرعي في مجموعة الفموية والمختلطة. كما تفاعل الغشاء المخاطي بقوة مع PAS في المجموعة الفموية والمختلطة واما في مجموعة الاستنشاق كانت النتائج مماثلة لمجموعة التحكم.

حدثت تغيرات نسيجية في معدة الفئران في المجموعة الفموية والمختلطة ، نزيف في الغشاء المخاطي ، تآكل وانسلاخ شديد للطبقة الظهارية ، وذمة في الغشاء المخاطي بين الغدد المعدية وفي البروبريا العضلية، بينما في مجموعة الاستنشاق ، عدم وجود تغييرات نسيجية. كان هناك انخفاض في سمك الغشاء المخاطي وزيادة في سمك الغشاء تحت المخاطي في مجموعة الفموية والمختلطة. كما تفاعل الغشاء المخاطي بقوة مع PAS في المجموعة الفموية والمختلطة وفي مجموعة الاستنشاق كانت النتائج مشابهة لمجموعة السيطرة.

حدثت تغيرات نسيجية في الأمعاء الدقيقة للفئران في المجموعة الفموية والمختلطة ، احتقان في الزغابات ، تمدد الأوعية الدموية في الطبقة تحت المخاطية ، تسلل الخلايا الالتهابية ، تأكل الزغب ، اما في مجموعة الاستنشاق ، عدم حدوث تغيرات نسيجية. كان هناك انخفاض في سمك الغشاء المخاطي وزيادة في سمك الغشاء تحت المخاطي في مجموعة الفموية والمختلطة. كما تفاعل الغشاء المخاطي والغشاء تحت المخاطي بقوة مع PAS ، في المجموعة الفموية والمختلطة وفي مجموعة الاستنشاق وكانت النتائج مماثلة لمجموعة التحكم.

حدثت تغيرات نسيجية في القصبة الهوائية للفئران في المجموعتين الفموية والمختلطة ، مثل احتقان الغشاء المخاطي ، تآكل وإزالة الخلايا الظهارية وفقدان أهداب الغشاء المخاطي ، تمدد الغشاء تحت المخاطي ، تسلل الخلايا الالتهابية ، تحطيم عضلات القصبة الهوائية ، في مجموعة الاستنشاق ،عدم حدوث تغيرات نسيجية. كان هناك زيادة في تجويف القصبة الهوائية في مجموعة الفموية والاستنشاق والمختلطة، وانخفاض في سمك الغشاء المخاطي في مجموعة الفموية والمختلطة. كما تفاعل الغشاء المخاطي وتحت المخاطية بقوة مع PAS في المجموعة الفموية والمختلطة وفي مجموعة الاستنشاق كانت النتائج مماثلة لمجموعة التحكم.

حدثت تغيرات نسيجية في رئة الفئران في المجموعات الفموية والمختلطة ، مثل احتقان في القصيبات ، احتقان شديد في جدار الحويصلات الهوائية ، تشوهات في ظهارة القصيبات ، زيادة سمك ظهارة القصيبات والحويصلات الهوائية ، تراكم الخلايا الالتهابية ، تمزق الحويصلات الهوائية ، اما في مجموعة الاستنشاق لم تحدث تغيرات نسيجية. كان هناك زيادة في تجويف الحويصلات الهوائية في مجموعة الفموية والمختلطة والاستنشاق ، وانخفاض في تجويف المجموعة الفموية والمختلطة. أيضا ، القصيبات لها تفاعل قوي مع PAS ، الحاجز بين الحويصلات الهوائية كان له تفاعل قوي مع PAS في مجموعة الفموية والمختلطة وفي مجموعة الاستنشاق كانت النتائج مماثلة للمجموعة الضابطة.

الخلاصة

حدثت تغيرات نسيجية في كبد الفئران في المجموعة الفموية والمختلطة ، مثل احتقان الوريد المركزي ، وتنكس دهني شديد ، وتدهور جزئي لنواة ، الفراغات الجيبانية ، بينما في المجموعة الاستنشاق لم تحدث تغيرات نسيجية.

حدثت تغيرات نسيجية في كلى الفئران في المجموعة الفموية والمختلطة ، مثل احتقان في الكبيبات ، ضمور كبسولة بومان ، تدمير الاوعية الدموية في الأنابيب الكلوية ، وانخفاض في حجم وعدد الخلايا الظهارية في الأنابيب الكلوية ، بينما في مجموعة الاستنشاق لم تحدث تغيرات نسيجية.

حدثت تغيرات نسيجية في قلب الفئران في المجموعة الفموية والمختلطة ، مثل احتقان في ألياف العضلات ، وخشونة في عضلة القلب ، بينما في مجموعة الاستنشاق ، لم تحدث تغيرات نسيجية.

وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم علوم الحياة

دراسة التغيرات النسيجية والدموية المرافقة لإعطاء زيت الاوكالبتوس بالتجريع الفموي والاستنشاق في الفئران المختبرية (*Mus musculus*)

رسالة مقدمة
الى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

زينب محسن جاسب الناجي
بكالوريوس علوم /علوم الحياة (٢٠١٨)

بأشراف

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