THE USE OF HIBISCUS SABDARIFFA INFUSION FOR THE PREVENTION AND TREATMENT OF HIGH BLOOD LIPIDS COMPARED WITH STATIN TREATMENT

Article · January 2020

CITATION
1

READS
274

3 authors, including:

Afaf Abdullah
mysain university
4 PUBLICATIONS **5** CITATIONS
SEE PROFILE

Taha Hasan Alyasiri
University of Misan
7 PUBLICATIONS **18** CITATIONS
SEE PROFILE

Plant Archives Vol. 20, Supplement 2, 2020 pp. 812-817 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE USE OF *HIBISCUS SABDARIFFA* INFUSION FOR THE PREVENTION AND TREATMENT OF HIGH BLOOD LIPIDS COMPARED WITH STATIN TREATMENT

Afaf. A. Tarmooz[1], Taha H. Al-Yasiri[2] and Yahya Abdali[3]
[1]Department of pharmacognosy, College of Pharmacy, University of Misan, Iraq.
[2]Department of Clinical Laboratory Sciences College of Pharmacy, University of Misan, Iraq.
[3] College of Dentistry University of Misan,Iraq.
E-mails: Dr.Afaf@uomisan.edu.iq, taha_hassan@uomisan.edu.iq

Abstract

The purpose of the experiment is to study the effect of infusion of *hibiscus sabdariffa* in experimental mice that induced with hyperlipidemia. Fifty mice was divided randomly for five groups. The first groups C1(-ve control) is left normal, the second group C2 (+ve control) was induced by hyperlipidemia and not treated, the third group PH (Prophylaxis group) was treated with cold extract *hibiscus sabdariffa* before and after induced by hyperlipidemia, the fourth group SH (induction hyperlipidemia and treated with statin), The fifth group HH (induction hyperlipidemia and treated with *hibiscus sabdariffa.* The result showed that the fourth group that treated by statin high significant effect compared than others all groups. The third (PH) group was showed significant effected in cholesterol, triglyceride level and decrease in body weight compared with fifth group (HH) and control groups.
***Keywords*:** *Hibiscus sabdariffa*, Hyperlipidemia, Statin, Mic, Prophylaxis.

Introduction

The major causes of death are the cardiovascular diseases in all world which involve a broad range of ailment, including hyperlipidaemia, hypertension, coronary heart sickness thromboembolism and heart impairment. (Eisenberg *et al*., 1998).

Hyperlipidemia is a primary factor for many diseases that can cause complications like atherosclerosis, hypertension that can lead to stroke and fatty liver also it is the considerable element in the expansion to cardiovascular disease and death (Tajfard *et al.,* 2014; Asgary, *et al.,* 2014). For hyperlipidemia treated and controlled there are many drug develops as well as changing in live style (Hendrani *et al*., 2016). Drugs that used as generality to decrease total cholesterol synthesis are statins, fibrates, and bile acid sequestrant niacin and ezetimibe. The mechanism of action of Statins via suppress the HMG-co a reductase enzyme, that lead to impairment in the function of a specified bodily organ or system like liver dysfunction and myopathy (Aranda *et al*., 2013)

Since thousands of years ago, herbaceous drugs represented the prime combination as a traditional medication. They have made significant contribution to human health through their health care, therapeutic and medicinal characteristic and in the prevention of ailment (Calixto, 2000).

Under the developing of drug, many of the modern medicines that are used conventionally are plant sources, digoxin, morphine, and colchicine. Used of herbal remedies as a traditional and from practice passed on from people to other has depended by the people on herbaceous treated. In all world, the using of medicinal plants increasing until these times (WHO, 2010).

Plant of Roselle (*Hibiscus sabdariffa)* is a flowering plant; the genus belongs to Malvaceae; family, number of species more than 300 grown in all over the world. This plant believed to be native to the old world tropics, used as herbal tea like an infusion. (Mohamed *et al*., 2012).

Hibiscus sabdariffa L., (Malvaceae), the common name of it was "roselle", is an important medicinal plant local grow in India and Malaysia, although it grow and establish widely in the tropics and subtropics of both hemispheres and in many areas in Central America. (Morton and Dowling, 1987) Have a medicinal property, calyx is the most extremely used portion of the plant, the leaves and seeds are often eat, curries and potherbs (Ezejindu and Iro, 2017). Hibiscus sadariffa has common names tea. (Olaleye, 2007) It have different names like 'Karkade' in Egypt, 'Asam Paya' in Malaysia, "Luoshen Hua' in Chinese, 'Lamanda' in Zambia and "Zobo' in Nigeria (Wang *et al*., 2000) acidify tea (sour tea) that come from containing acidity which reach to 15%-30% plant acids, including citric acid, malic acid, tartaric acids and allo-hydroxycitric acid lactone. Hibiscus plant acid give the specialty and identified to this plant, this plant have many chemical constituents like Beta-carotene, Beta-sitosterol, alkaloids, anthocyanin L-ascorbic acid, polysaccharides, arabins and arabinogalactans, quercetin**,** gossypetin also contain sugars components galactose, arabinose, mannose, glucose, xylose, and rhamnose. (Tori *et al*., 2011).

Plant has fibrous stem, short branches, shining red with acidity test- calyces. In public remedy, soaking the herb in liquid is used as a diuretic and to treated of gut disturbance, diseases of liver, hypertension and high body temperature. (Monroy *et al* 2007). It also have effect as anticancer (Olvera-García *et al.,* 2008; Lin *et al*., 2005) as well as antioxidant (Farombi and Fakoya, 2005).

Hibiscus sabdariffa Calyces have been discussed, described by a numeral of search that it has contain Flavonoids, polyphenolic acids, also contain Cyanidin-3-O-Sambubioside, Anthocyanins, like Delphinidin-3-O-glucoside, and Delphinidin-3-O-Sambubioside (Ali *et al*., 2005). Also it contain protocatechuic acid (Tseng *et al*., 1997; Lee *et al*., 2002) or that reasons numerous research confirming the supposition that antioxidant play a preventative role in human health when used as nutrients or medicines. the plants are utilized throughout the world as

afood and curative objectives and as it has a capacity for therapeutic uses (Ames *et al*., 1993).

The juice/concoction prepared from the medical plants that consumed for health benefits are taken as prophylactic, healing against diabetes and hypertension. several studies have been proved that uses of Hibiscus sabdariffa lead to health benefits like antihypertensive as well as pharmacologic particular such as anti-oxidant, antimicrobial , hepato, nephron-prophylactic. Hibiscus sabdariffa is safety when used at dosage 5000 mg/kg according to many research. Thus, its pharmacological and nutritional advantage could be exploited in various pathological conditions like cancer cardiovascular disease and neurologic (Owoade *et al.,* 2006).

Materials and Methods:

Plant Material

Dried calyces of Hibiscus sabdariffia obtained from Hibiscus flowers were purchased from local market in the Misan Iraq.

Hibiscus drink Extraction preparation

The dried calyces of Hibiscus (20g) were to (1000 mL) of distilled water and soak for 12 hours (steeping) and keep cool then filtrate the extraction to obtain a solution without any particles then it can be utilized (cold infusion).

The Laboratory Animals

Laboratory Albino BALB/c mice 50 were used at this study. The mice randomly divided to 5 groups include control positive, control negative, Prophylaxis, Hibiscus, and Statin as the general divisions. At the beginning of the experiment a hyperlipidemia diet where prepared by adding of 1% cholesterol in the diet and H_2O_2 in the drinking water for 1 month of the experiment for two groups (hibiscus and statin). Then this was stopped and given to the prophylaxis group except for the H_2O_2 in the drinking water and continue for a month as well. While the hibiscus and statin taking the treatment for the dyslipidemia. The statin group received atorvastatin (1 tablet of 20 mg) for 1 month. While the same amount the prophylaxis and hibiscus groups received the hibiscus extraction for 1 month (Sunnemark *et al*., 2000).

Parameters measured in the experiment

1. Lipid profile
    a. Total Cholesterol (TC)
    b. Triglycerides (TG).
2. Weight gain

Blood Sampling

For the measuring of the lipid profile the blood sample were collected at the end of every month. Blood was drawn via cardiac puncture from anesthetic mice. Anesthesia was induced by intramuscular injection of xylazine (20m/ml) and the animals were operated under aseptic conditions. The collected was centrifuged at 2500 rpm for ten minutes then the serum was collected for the lipid profile measurement. (Friedewald *et al*., 1972)

Lipid profile Determination:

Lipid profile tests included triglycerides, cholesterol, were calculated according to formula by (Friedewald *et al*., 1972)

Determination of Total Cholesterol (TC):

The enzymatic method provided by Linear Chemicals Company relies on ESP to determine cholesterol in the serum.

Determination of Total Triacylglycerol (TG):

The enzymatic method provided by Linear Chemicals Company relies on ESP to determine triacylglycerol in the serum.

Statistical Analysis:

In this study a one-way ANOVA analysis was performed using at level in ($P < 0.05$) significantly. The differences between groups were determined depending on the least important differences (LSD). (Morgan et al 2012)

Results and Discussion

Weekly measure of body weight

Body weight data are showed in Table (2).Initial body weights were similar for all groups. Diverse weight changes were observed in the treated groups Compared to the changes in the control group (-ve) ((29.34±1.77) and control group (+ve)(27.41±1.68), in the table (2) observed there was not significant ($p < 0.05$) changes in body weight in groups (controls –ve, +ve) observed that there was a statistically - significant ($p < 0.05$) in body weight in group P , S and H which was decreased in body weight compared with group control (+ve C and group control –ve), at the end week of the treatment ,Besides, a significant ($P<0.05$) reduction in the mean values of P,S and H treated groups .The changes in the body weight gave an evidence of a correlation between the induce hyperlipidemia and the treatment with plants that there was a significant ($p < 0.05$) decrease in the body weight of P,S and H. however these mice remained active and did not show any sign of trouble .The treated groups assign weight losses, while the control and induce hyperlipidemia groups posted weight gain.

The ability of HS polyphenols to support reduce body weight through repression of lipid gathering through fat absorption-excretion while also enhances normalize the glycemic indicator and glucose tolerance in obese mice models. (Herranz-López *et al.,* 2017).

Most new drugs produce from nature versus pharmacological goal like human immunodeficiency virus, acquired immune deficiency syndrome, cancer, al zheimer's, malaria fever, and soreness. Medicinal plants have the effective action which conflict obesity by increase the level of metabolism and suppress relish like Synephrine, xanthine and caffeine or by using high doses like fiber (Yuliana *et al*., 2017). Carvajal *et al*., 2009 and Hansawasdi *et al*., 2013 noted there were Lowering in body weight by using hibiscus plant, which help to excreted numbers of fatty acid like (linoleic, palmitic, and oleic, and lower stearic acids), and inhibit pancreatic amylase.

Determination of lipid profile effect Total Serum Cholesterol (TC) Concentration (mg/dl) and Serum Triacylglycerol Concentration (mg/dl)) of hibiscus

The effect of hibiscus on mean values of serum total cholesterol and Triacylglycerol levels of mice are shown in table (1).The results showed that after 2 months of treatment there was a significant ($p < 0.05$) differences in serum TC

concentration were recorded between the treated groups and compared with (control +ve and group control –ve).

The results showed a significantly ($p < 0.05$) decreased in TC of treated groups S, H and P with the mean values (63.83±5.16, 79.36±4.68 and 76.01±7.15) respectively compared with CO (+ve control) group with mean value (168.01±9.87) There were a clear reduction in TC concentrations in all treated groups of S, P and H as compared to control group (untreated group). In the table(1) also illustrates the mean values of serum TG concentration in the treated groups, untreated and control groups, there was a significant decrease ($P<0.05$) in S,P, and H groups after two months of treatment with a mean values (52.60±2.67, 77.80±12.83 and 74.60±5.71) respectively.

The protective role of hibiscus against triacylglycerolidemia was clarified TAG values in groups S, P and H compared with Co (+ve control) untreated group.

A significant decrease ($P<0.05$) in TAG was observed in group S (52.60±2.67) comparing with the group P (77.80±12.83), group H(74.60±5.71) and group CO(+ve control) (94.60 ±2.48). The prominent significant elevation was observed in group CO(-ve control) with the mean value of(201.18±5.31).

Depending on the result clarified in the table (1), there were significant ($P<0.05$) differences in serum mean values of TAG -C were observed in treated groups P, H and CO (-ve control) comparing to the group CO (+ve control).

Our result are in agreement with Emmanuel Nnamonu *et al.* (2013) as well as - Hajifaraji, (2018) where they noticed that treated with hibiscus significantly lowered total cholesterol (TC) and triglyceride levels . Several ways, the dried red calyces of *hibiscus sabdariffa* are used as habitual medicine for diuretic, hypocholesterolemia, antihypertensive and mucolytic result may explain changes in lipid profile of hypercholesterolemia animals treated by the hibiscus. This hypocholesterolemia effect has been attributed to its abundant antioxidant composition.

The herbal that contain Hydroxycitric acid (HCA) can reduce body weight gain and fat accumulation in obese and enhance metabolic state. HCA preventing acetyl-CoA forming and hence triacylglyceride and cholesterol biosynthesis by inhibitory action on citrate lyase. Extract constituents enhancing triacylglyceride disintegration thruogh lingual and gastric lipases, and excretion of fatty acids through of enhancing inter esterification from the sn-2 site of glycerol to sn-1 and sn-3 (Gaya *et al.,* 2009; Tomar *et al.,* 2019).

After makes experience in vivo the Yang *et al.* (2019) and fellow workers confirmed the action of aqueous *Hibiscus sabdariffa* extract, as anti-hyperlipidemia and showed the polyphenols act to lowering in hepatocyte lipid content bring from dose-dependent fatty acid synthase and HMG-CoA reductase, through adenosine monophosphate-activated protein kinase activation and sterol regulatory element binding protein (SREBP-1) reduction. Also Adegunloye *et al.* (1996), had initial advertised that the dried red calyces of Hibiscus sabdariffa was used as traditional medicine for diuretic, hypocholesterolemia, antihypertensive and mucolytic effects. The pigments (anthocyanin) which are accountable foremost for red colour were delphinidin-3-glucoside and cyanidin-3-glucoside which informed by Kalt *et al.,* 1996), Anthocyanin was also point to have many times more antioxidant activity than ascorbate.

The pigments (anthocyanin) phenolic natural found in the dried flower of *H. sabdariffa* has been reported severally to have cardio protective, hypocholesterolemia, anti-oxidative and hepatoprotective effects in human and animals (Carvajal *et al*., 2012).

Organic acids (citric and malic acids) found in flowers of *H. sabdariffa* as a main compounds, fundamentally, anthocyanins, glycosides, fiber, and a myriad of flavonoids (Gruenwald *et al.,* 2007; Al-Yasiri and Mohammed, 2019) Organic acid and anthocyanin present in calyax, also flavonoids and glycosides are least present (Segura‐Carretero *et al.,* 2008; Ali, 2005).

Anthocyanins, especially dulfedine-3 sambopioside and cyanidine 3 sambopioside, have been confirmed as the active ingredients responsible for antioxidants and the effects of low cholesterol in the HS, possibly because they are present in a relatively high amount in aqueous extracts. (Gruenwald *et al*., 2007; Segura‐Carretero *et al*., 2008)

However, Yang (36) studied the differences between clouds extract, polyphenols extract (74%) including protactic acid (24.2%), catechin (2.7%), galocatechin (2.4%), caffeine acid (19.9%), and gallocatechin cases. 30.0%) with aqueous extract of HS containing anthocyanins (2.5%), polyphenol acid (1.7%) and flavonoids (1.4%), and confirmed that high-pheno extract levels noted a higher ability to lower whole cholesterol and LDL-C cholesterol and raise HDL-C cholesterol, Which indicates that polyphenols in causing anti-cholesterol effects. (Carvajal *et al*., 2005; Hopkins, 2008).

HS may handicap atherosclerosis formation and enhance vasoreactivity by preventing of the creating of macrophage-obtain foam cells and/or decreased of LDL-C oxidation due to antioxidant effects of the plant, which useful for improving cardiovascular risk factors. (Farombi *et al.,* 2007).

Table 1: The lipid profiles test parameters of total serum cholesterol concentration and serum triacylglycerol (TAG) concentration and living body weight of different groups (control ,treated with hibiscus after 2 months compared with one drug.

Groups	Parameters	TC mg/dl	TAG mg/dl	Body weight (g)
Co (-ve control) normal		71.88±4.14^{b}	94.60 ±2.48^{b}	29.34±1.77^{a}
Co (+ve control) induce hyperlipidemia and not treated		168.01±9.87^{a}	201.18±5.31^{a}	27.41±1.68^{a}
P(Prophylaxis induce hyperlipidemia and treated orally with hibiscus)		76.01±7.15^{b}	77.80±12.83^{b}	21.37±1.10^{b}
S (induce hyperlipidemia and treated with statin orally)		63.83±5.16^{b}	52.60±2.67^{c}	21.32±1.79^{b}
H (induce hyperlipidemia treated with hibiscus)		79.36±4.68^{b}	74.60±5.71^{b}	19.88±1.20^{b}

-n=5 for each group

-Different letters in column refer to significant ($P<0.05$) differences between them.

-The values represent Mean± SE

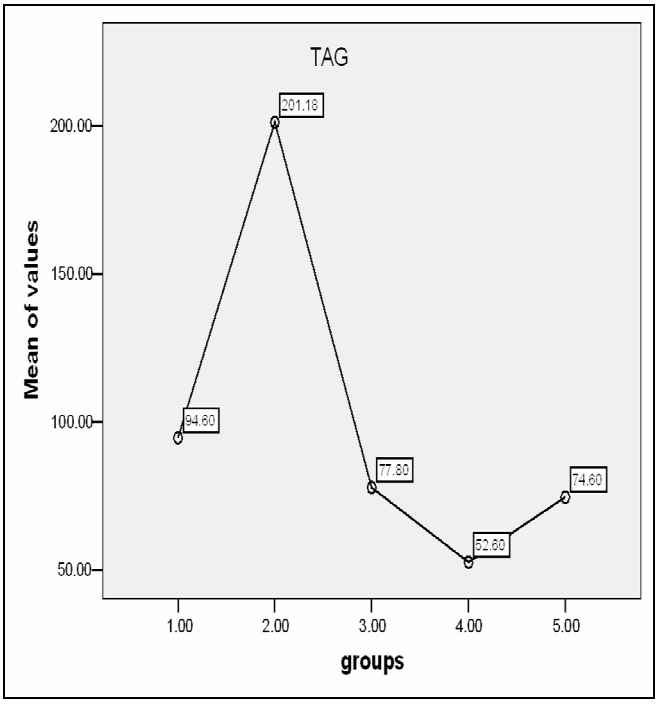

Fig. 1 : Show the mean values of total serum triacylglycerol concentration (mg/dl).

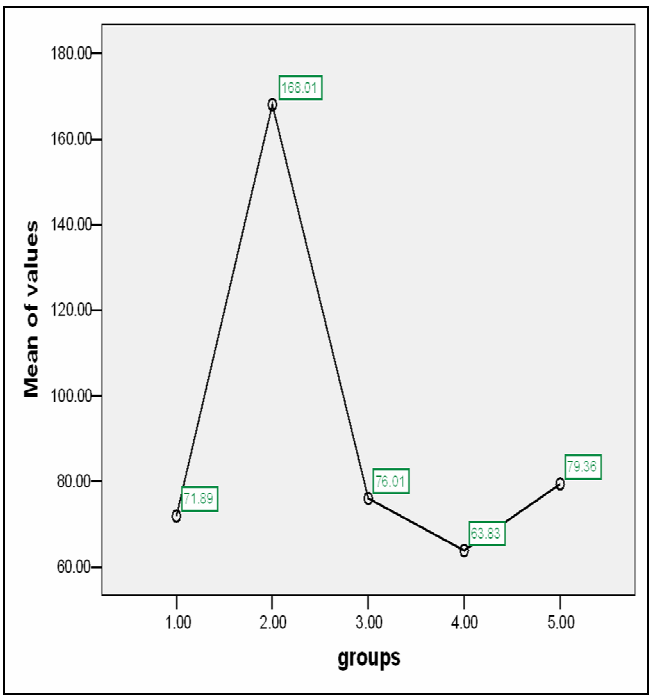

Fig. 2 : Show the mean values of total cholesterol concentration (mg/dl)

The study is about the evaluation of the cold hibiscus effect in decreasing the hyperlipidemia and body weight in the study 49-albino mice.

Hibiscus cold extraction is beneficial medical plant in addition to it has used as good consumed beverage hot or cold. As a conclusion the experiment yielded a successful results in lowering the lipid profile the hibiscus being one of the most beneficial medical plant that can be used as alternative if the anti hyperlipidemic medicine was have side effects use to the person.

Roselle, the medicinal plant content different medically compounds called (phytochemical) and nutritional, that uses in various medical issues like cancer, inflammatory diseases, diabetes and different cardiovascular diseases has been well examined by different scholars in different place and determined as a safe.

Prevention of cardiovascular disease has to be integrated into primary health care

References

Adegunloye, B.J.; Omoniyi, J.O.; Owolabi, O.A.; Ajagbonna, O.P.; Sofola, O.A. and Coker, H.A. (1996). Mechanisms of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. African Journal of Medicine and Medical Sciences, 25(3): 235-238.

Ali, B.H.; Al Wabel, N. and Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. Phytotherapy Research, 19(5): 369–375.

Ali, B.H.; Wabel, N.A. and Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 19(5): 369-375.

Al-Yasiri, T.H. and Mohammed, M.F. (2019). Influence of Organic acids (Acetic, Citric acid and Blend) In vitro on growth of *E.coli* O157: H7: in Poultry feed.Research Journal of Pharmacy and Technology, 12(5): 2468-2472.

Ames, B.N.; Shigenaga, M.K. and Hagen, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging.Proceedings of the National Academy of Sciences of the United States of America, 90(17): 7915-7922.

Aranda, J.F.; Madrigal-Matute, J.; Rotllan, N.; & Fernández-Hernando, C. (2013). Micro RNA modulation of lipid metabolism and oxidative stress in cardiometabolic diseases.Free Radical Biology and Medicine, 64: 31-39.

Asgary, S.; Rafieian-Kopaei, M.; Shamsi, F.; Najafi, S.; and Sahebkar, A. (2014). Biochemical and histopathological study of the anti-hyperglycemic and anti-hyperlipidemic effects of cornelian cherry (*Cornus mas* L.) in alloxan-induced diabetic rats.Journal of Complementary and Integrative Medicine, 11(2): 63-69.

Calixto, J.B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of medical and Biological research, 33(2): 179-189.

Carvajal-Zarrabal, O.; Barradas-Dermitz, D.M.; Orta-Flores, Z.; Hayward-Jones, P.M.; Nolasco-Hipólito, C.; Aguilar-Uscanga, M.G. and Bujang, K.B. (2012). *Hibiscus sabdariffa* L.; roselle calyx, from ethnobotany to pharmacology. Journal of experimental pharmacology, 4.

Carvajal-Zarrabal, O.; Hayward-Jones, P.M.; Orta-Flores, Z.; Nolasco-Hipólito, C.; Barradas-Dermitz, D.M.; Aguilar-Uscanga, M.G. and Pedroza-Hernández, M.F. (2009). Effect of *Hibiscus sabdariffa* L. dried calyx ethanol extract on fat absorption-excretion, and body weight implication in rats. BioMed Research International, 2009.

Eisenberg, D.M.; Davis, R.B.; Ettner, S.L.; Appel, S.; Wilkey, S.; Van Rompay, M.; & Kessler, R.C. (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. Jama, 280(18): 1569-1575.

Ezejindu, C.N. and Iro, O.K. (2017). Nutritional and Chemical Evaluation of Carbonated and Naturally Flavoured Zobo (*Hibicus Sabdariffa*) Drinks. Direct Research Journal of Public Health and Environmental Technology, 2(5): 49-53.

Farombi, E.O. and Fakoya, A. (2005). Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. Molecular nutrition & food research, 49(12): 1120-1128.

Farombi, E.O. and Ige, O.O.(2007). Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of *Hibiscus sabdariffa* in alloxan‐induced diabetic rats.Fundamental & clinical pharmacology, 21(6): 601-609.

Friedewald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.Clinical chemistry, 18(6): 499-502.

Gaya, I.B.; Mohammad, O.M.A.; Suleiman, A.M.; Maje, M.I. and Adekunle, A.B. (2009). Toxicological and lactogenic studies on the seeds of *Hibiscus sabdariffa* Linn (Malvaceae) extract on serum prolactin levels of albino wistar rats. The Internet Journal of Endocrinology, 5(2): 1-6.

Gruenwald, J.; Brendler, T. and Jaenicke, C. (2007). PDR for herbal medicines. Thomson, Reuters.

Hajifaraji, M.; Matlabi, M.; Ahmadzadeh-Sani, F.; Mehrabi, Y.; Rezaee, M.S.; Hajimehdipour, H. and Roghani, K. (2018). Effects of aqueous extracts of dried calyx of sour tea (*Hibiscus sabdariffa* L.) on polygenic dyslipidemia: A randomized clinical trial. Avicenna journal of phytomedicine, 8(1): 24.

Harikrishnan, V.S. (2017). "A comparison of various methods of blood sampling mice and rats: Effects on animal welfare." Lab Anim: in Extraction techniques of Medicinal plants.

Hendrani, A.D.; Adesiyun, T.; Quispe, R.; Jones, S.R.; Stone, N.J.; Blumenthal, R.S.; and Martin, S.S. (2016). Dyslipidemia management in primary prevention of cardiovascular disease: Current guidelines and strategies. World journal of cardiology, 8(2): 201.

Herranz-López, M.; Olivares-Vicente, M.; Encinar, J.A.; Barrajón-Catalán, E.; Segura-Carretero, A.; Joven, J. and Micol, V. (2017). Multi-targeted molecular effects of Hibiscus sabdariffa polyphenols: An opportunity for a global approach to obesity. Nutrients, 9(8): 907.

Hopkins, A.L.; Lamm, M.G.; Funk, J.L. and Ritenbaugh, C. (2013). *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies. Fitoterapia, 85: 84-94.

Kalt, W.; Prange, R.K. and Lidster, P.D. (1993). Post-harvest color development of strawberries: Influence of maturity, temperature and light. Canadian journal of plant Science, 73(2): 541-548.

Lee, M.J.; Chou, F.P.; Tseng, T.H.; Hsieh, M.H.; Lin, M.C. and Wang, C.J. (2002). Hibiscus protocatechuic acid or esculetin can inhibit oxidative LDL induced by either copper ion or nitric oxide donor.Journal of Agricultural and Food Chemistry, 50(7): 2130–2136.

Lin, H.H.; Huang, H.P.; Huang, C.C.; Chen, J.H. and Wang, C.J. (2005).Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. Molecular Carcinogenesis, 43(2): 86–99.

Mohamed, B.B.; Sulaiman, A.A. and Dahab, A.A. (2012). Roselle (*Hibiscus sabdariffa* L.) in Sudan, cultivation and their uses. Bull. Environ. Pharmacol. Life Sci, 1(6): 48-54.

Monro y-Orti Z.C. and Castillo -Espana, P. (2007). Plantas Medicinales Utilizadas en el Estado de Morelos.2nd ed.México: Publisher CCNABIO.405 p.

Morgan, G.A.; Leech, N.L.; Gloeckner, G.W. and Barrett, K.C. (2012). IBM SPSS for introductory statistics: Use and interpretation. Routledge.

Morton, J.F. and Dowling, C.F. (1987). Fruits of warm climates (Vol.20534). Miami, FL: JF Morton.

Nnamonu, E.I.; Ejere, V.C.; Ejim, A.O.; Echi, P.C.; Egbuji, J.V.; Eze, T.R. and Eyo, J.E. (2013). Effects of *Hibiscus Sabdariffa* calyces aqueous extract on serum cholesterol, body weight and liver biomarkers of *Rattus Novergicus*. International Journal of Indigenous Medicinal Plants, 46(4): 1405-1411.

Olaleye, M.T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. Journal of Medicinal Plants Research, 1(1): 9-13.

Olvera‐García, V.; Castaño‐Tostado, E.; Rezendiz‐Lopez, R.I.; Reynoso‐Camacho, R.; González de Mejía, E.; Elizondo, G.; & Loarca‐Piña, G. (2008). *Hibiscus sabdariffa* L. extracts inhibit the mutagenicity in microsuspension assay and the proliferation of HeLa cells.Journal of Food Science, 73(5): T75-T81.

Owoade, A.O.; Adetutu, A. and Olorunnisola, O.S. (2016). Influence of Polyphenolic Contents on the Antioxidant Properties of *Hibiscus sabdariffa* Extract (HSE), Aged Garlic Extract (AGE) and Garlic Tablet (GT) in Vitro. Food and Nutrition Sciences, 7(6): 417-428.

Segura‐Carretero, A.; Puertas‐Mejía, M.A.; Cortacero‐Ramírez, S.; Beltrán, R.; Alonso‐Villaverde, C.; Joven, J. and Fernández‐Gutiérrez, A. (2008). Selective extraction, separation, and identification of anthocyanins from *Hibiscus sabdariffa* L. using solid phase extraction‐capillary electrophoresis‐mass spectrometry (time‐of‐flight/ion trap).Electrophoresis, 29(13): 2852-2861.

Sunnemark, D.; Harris, R.A.; Frostegård, J. and Örn, A. (2000). Induction of early atherosclerosis in CBA/J mice by combination of *Trypanosoma cruzi* infection and a high cholesterol diet. Atherosclerosis, 153(2): 273-282.

Tajfard, M.; Mobarhan, M.G.; Rahimi, H.R.; Mouhebati, M.; Esmaeily, H.; Ferns, G.A & Abdul-Aziz, A.F.(2014). Anxiety, depression, coronary artery disease and diabetes mellitus; an association study in Ghaem Hospital, Iran. Iranian Red Crescent Medical Journal, 16(9).

Tomar, M.; Rao, R.P.; Dorairaj, P.; Koshta, A.; Suresh, S.; Rafiq, M. and Venkatesh, K.V. (2019). A clinical and

computational study on anti-obesity effects of hydroxycitric acid. RSC advances, 9(32), 18578-18588.

Tori Hudson, N.D. Hibiscus Sabdariffa: A Research Review of Its Uses and Safety [Online]. [cited 2011].

Tseng, T.H.; Kao, E.S.; Chu, C.Y.; Chou, F.P.; Lin Wu, H.W. and Wang, C.J. (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes.Food and Chemical Toxicology, 35(12): 1159–1164.

Villalpando-Arteaga, E.V.; Mendieta-Condado, E.; Esquivel-Solís, H.; Canales-Aguirre, A.A.; Gálvez-Gastélum, F.J.; Mateos-Díaz, J.C. and Márquez-Aguirre, A.L. (2013). *Hibiscus sabdariffa* L. aqueous extract attenuates hepatic steatosis through down-regulation of PPAR-γ and SREBP-1c in diet-induced obese mice. Food & function, 4(4): 618-626.

Wang, C.J.; Wang, J.M.; Lin, W.L.; Chu, C.Y.; Chou, F.P. and Tseng, T.H. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats.Food and chemical toxicology, 38(5): 411-416.

World Health Organization (2010). Traditional herbal remedies for primary health care.

Yang, M.Y.; Peng, C.H.; Chan, K.C.; Yang, Y.S.; Huang, C.N. and Wang, C.J. (2009). The hypolipidemic effect of *Hibiscus sabdariffa* polyphenols via inhibiting lipogenesis and promoting hepatic lipid clearance. Journal of agricultural and food chemistry, 58(2): 850-859.

Yuliana, N.D.; Jahangir, M.; Korthout, H.; Choi, Y.H.; Kim, H.K. and Verpoorte, R. (2017). Herbal medicine for obesity treatment: a review.