ANTI-OXIDANT ACTIVITY OF NOVEL AZO COMPOUND DERIVED FROM PYRIMIDINE AND SULPHONAMIDE (SPI)

Article · November 2015

CITATIONS
0

READS
33

3 authors:

Ishraq Jasim
University of Misan
10 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Mohammed Al-Diwan
University of Basrah
51 PUBLICATIONS **38** CITATIONS
SEE PROFILE

Wasfi A. Al-Masoudi
University of Basrah
74 PUBLICATIONS **170** CITATIONS
SEE PROFILE

ISSN 2347-6893

ANTI-OXIDANT ACTIVITY OF NOVEL AZO COMPOUND DERIVED FROM PYRIMIDINE AND SULPHONAMIDE (SPI)

Ishraq J. Hassan
Department of Physiology ,Pharmacolgy and Chemistry, College of Veterinary Medicine, University of Basrah, Iraq
Muhammed A. AL-Diwan
Department of Physiology ,Pharmacolgy and Chemistry, College of Veterinary Medicine, University of Basrah, Iraq
Wasfi A. Al-Masoudi
Department of Physiology ,Pharmacolgy and Chemistry, College of Veterinary Medicine, University of Basrah, Iraq

ABSTRACT

The present study aimed to investigate the potential effect of 4-[(Z)-(2,4-diamino-6-chloropyrimidin-5-yl)diazenyl]benzene-sulfonamide (SPI) in alleviating Hematological and serum biochemical alterations induced by sodium nitrite toxicity in rats. For this purpose 40 adult female rats and 40 adult male rats were divided in to 5 groups for each sex (8 rats each); Group (1): received distilled water by i.p injection and served as a control, Group (2) was daily administered Dimethyl sulphoxide (DMSO) at a dose of 3.33 ml/kg B.W. by i.p injection. Group (3) was daily administered sodium nitrite (NaNO2) at a dose of 6.33 mg/kg B.W. by i.p injection. Group (4) was daily administered of NaNO2 at a dose of 6.33 mg/kg B.W. by i.p injection and after one hour of NaNO2 administration the treated rats given the new compound SPI at a dose of 3.42 mg/kg B.W. by i.pinjection. Group (5) was daily administered of NaNO2 at a dose of 6.33 mg/kg B.W. by i.pinjection and after one hour of NaNO2 administration the treated rats given the new compound SPI at a dose of 1.71 mg/kg B.W. byi.p injection. The obtained results indicated that sodium nitrite possesses a deleterious effect on blood cytology, induce oxidative damage, hepato-renal dysfunction, increase of MDA and sexual hormone defect. The administration of SPI with sodiumnitrite minimized the hazard effects of sodium nitrite, it improved RBCs count, PCV, Hb concentration ,total and differential WBCs count and blood indices.Diminished the level of serum malondialdehyde (MDA).Moreover, it ameliorates the activities of AST, ALT, cholesterol, TG, lipoproteins, uric acid, creatinine, glucose and sexual hormones includes FSH, LH, estrogen and testosterone.

Key words: pyrimidine-sulfanilamide complex; sodium nitrite; hematology toxicity; biochemical toxicity; Antioxidant

ISSN 2347-6893

INTRODUCTION

Sodium nitrite is an inorganic salt with wide spread applications in food industry as color fixative and preservative in meat and fish [1]. Sodium nitrite is also used as pharmacological agent in poisoning of cyanide. Recent studies suggest that the vasodilator effect of NaNO2 may be of therapeutic benefit in the treatment of pulmonary hypertension [2], post hemorrhagic cerebral vasospasm [3], and myocardial infarction [4]. The toxic effects of nitrites are well documented in mammalians including impairment of reproductive function, hepatotoxicity, dysregulation of inflammatory responses and tissue injury, growth retardation and endocrine disturbance [1]. In blood, nitrite undergoes a coupled oxidation reaction with oxyhemoglobin to produce methemoglobin (MetHb). Unlike hemoglobin, MetHb cannot exchange oxygen; hence, the presence of excess MetHb in the circulation proportionately reduces the ability of the blood to transfer oxygen [5] and can cause hemic hypoxia. The body reacts to hypoxia with adaptive responses, such as relaxation of smooth muscle, angiogenesis and vasodilatation, thus increasing blood supply to tissues, compensating for the lack of oxygen [6]. Moreover, some pyrimidine derivatives also possess hepato- protective effect which could be attributed to their anti oxidant activity [7], and many pyrimidine derivatives play vital role in many physiological actions. They are among those molecules that make life possible as being some of the building blocks of DNA and RNA [8]. Some pyrimidines of physiologically as well as pharmacologically importance [9].

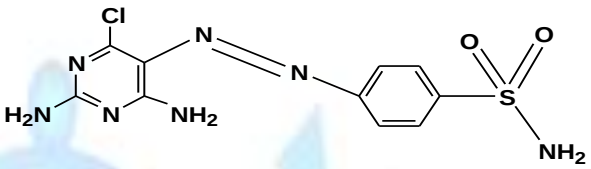


Fig.1: Chemical structure of 4-[(Z)-(2,4-diamino-6-chloropyrimidin-5-yl)diazenyl]benzene- sulfonamide (SPI)

MATERIALS AND METHODS

Nitrite-induce methemoglobuline in hemolysate:

Blood samples were obtained from healthy individuals by puncture of the vein, and kept in ethylene diamine tetra acetic acid (EDTA) containing tubes; then centrifuged at 2500 rpm for 10 minutes to remove plasma and the buffy coat of white cells. Washed the erythrocytes thrice with Phosphate Buffer Saline (PBS, pH 7.4) and lased by suspending in 20 volumes of 20mM Phosphate Buffer (PB, pH 7.4) to yield the required hemolysate concentration of 1:20. Finally, the lysate was centrifuged at 3000 rpm for 10 minutes to remove the stroma. The reaction was initiated by the addition of1.5 ml freshly prepared hemolysate, 1.0 ml of different concentrations of pyrimidine derivative-sulfanilamide complex (20µM, 10µM and 5µM) each time were added concomitantly with 0.1 ml sodium nitrite (final concentration 6.0 mM), and the formation of MetHb was monitored spectrophotometrically at 631 nm for 50 minutes using computerized UV-visible spectrophotometer [10].

Antioxidant in vivo

Animal and experimental design:

rats are divided into 5 groups (8 rats in each group) as following:

Control group: In this group, 10 male rats and 10 female rats were injected I.P with 0.9% normal saline (N.S) 0.5 ml daily for 21 days.

Treated 1 (T1) group: This group consisted of 10 male rats and 10 female rats which were injected intraperitoneally (I.P) with dimethyl sulfoxide (DMSO) 0.5 ml daily for 21 days.

Treated 2 (T2) group: This group consisted of 10 male rats and 10 female rats were injected I.P with 133 mg/kg sodium nitrite (NaNO2) daily for 21 days. (LD50 of NaNO2 is 180mg/kg B.W)

Treated 3 (T3) group: In this group, 10 male rats and 10 female rats were injected I.P with 1/10 of LD50 (742.15) mg/kg pyrimidine derivative-sulfanilamide complex daily after one hour of NaNO2 administration for 21 days.

Treated 4 (T4) group: In this group, 10 male rats and 10 female rats were injected I.P with 1/20 of LD50 (742.15) mg/kg pyrimidine derivative-sulfanilamide complex after one hour of NaNO2administration daily for 21 days.

Blood samples were collected from the heart by heart puncture by the use of the disposable syringes of 5cc capacity. After anesthesia of the rats, blood collected and analyzed according to Sood (1996) [11].

♦ 2 ml of blood was poured into a tube containing the ethylene diamin tetra acetic acid (EDTA) as an anticoagulant for RBC, Hb, PCV and WBC, differential WBC analysis.

♦3-4 ml of blood was poured into test tubes free from anticoagulant to isolate blood serum to estimate the biochemical parameters such as glucose, total cholesterol, HDL – cholesterol, LDL – cholesterol and triglycerol, VLDL, GOT, GPT, uric acid, creatinine, follicular stimulating hormone,leutinizing hormone, estrogen and testosterone. Haematological tests:

Erythrocytes count:

Hb, PCV, WBC, D. WBC and blood indices estimated according method of Schalm et al., (1975)[12].

Biochemical test:

Serum glucose, total cholesterol and Triglyceride (TG) estimated by the enzymatic method described by were measured according to the method described by (Wahlefeld, 1974). HDL –cholestrol obtained through the Burstine method [13]. Serum LDL was calculated according to Burstine method, formula and Serum VLDL was calculated according to Friedewald et al. [14] formula. MDA estimated according to Yagi method [15]. Serum uric acid and creatinine also measured via Wahlefeld method [16], while the liver enzymes ALT and AST estimated according to Reitman et al. [17]. In addition to the reproductive hormones FSH, LH, estrogen and testosterone.

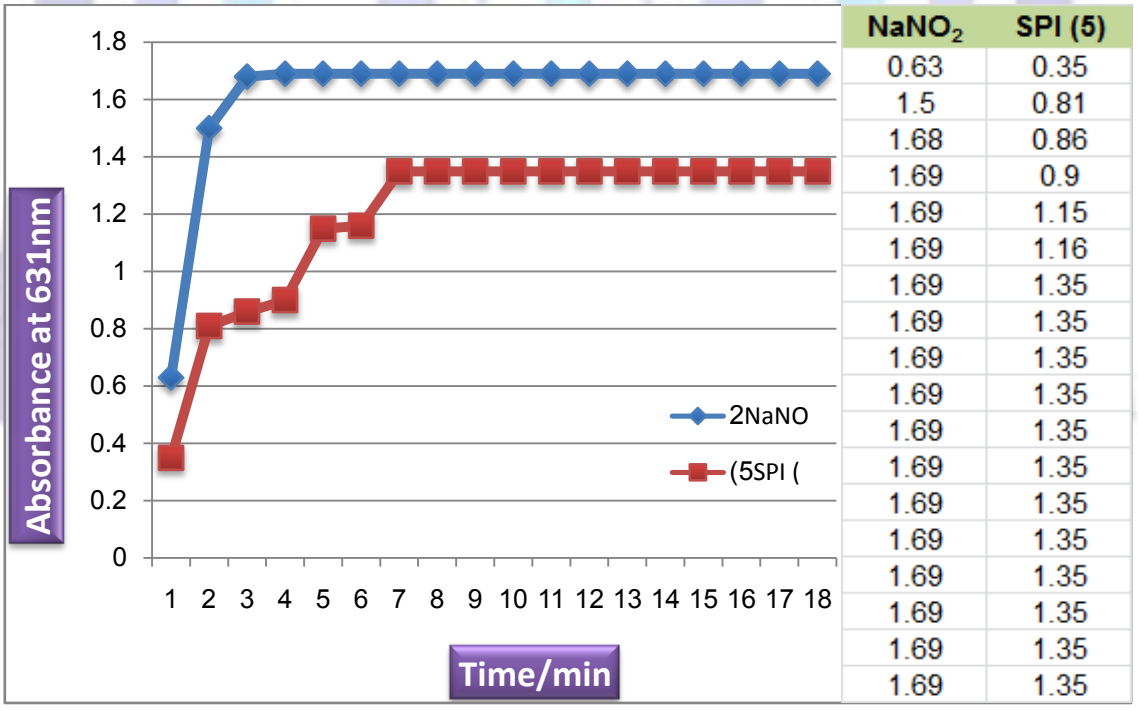
Statistical analysis:

Computerized SPSS (Statistical Package for Social Sciences) (V.13) program were used for analysis of results of the present study. The data were expressed as mean±standarddeviation (mean±SD).Least significant difference test (LSD) wasused to test the difference between means (groups); P≤ 0.05 was considered significant [18].

RESULTS AND DISCUSSION

In vitro antioxidant results

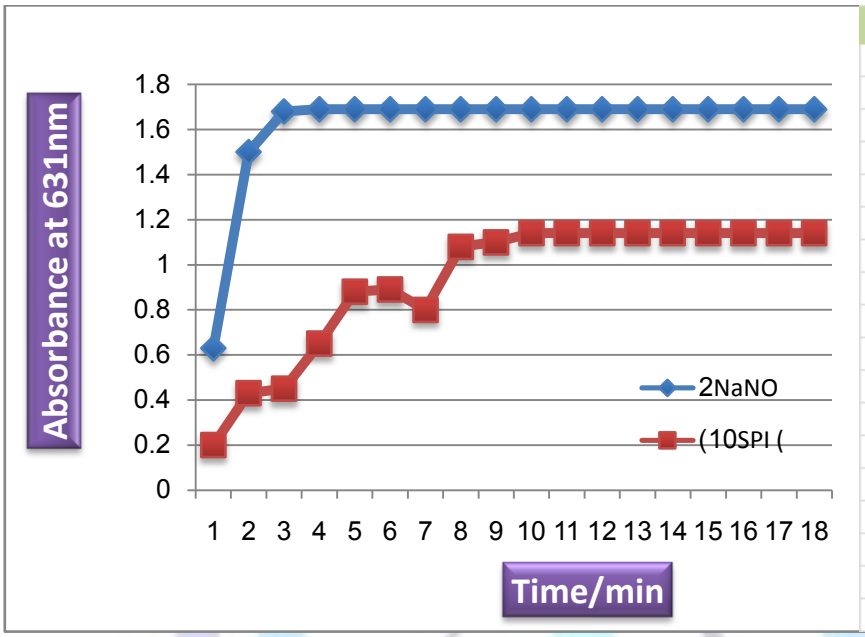
Sodium nitrite (NaNO2) caused rapid oxidation of hemoglobin (Hb) to methemoglobin (MetHb). Using SPI complex in a dose dependent manner, the oxidation process delayed (Fig.5).The results showed that the time required to convert the hemoglobin to methemoglobin was four minutes in the absence of the SPI complex, whereas using of 5µM SPI complex the time was increased to seven minutes (Fig.2). It seeded that not all Hb. convert to MetHb. That means, when the complex was added along with nitrite at 0 min, the formation of methemoglobin was been inhibited to wide extent. This observation also appeared in (Fig. 3) in which the time required to convert the hemoglobin to methemoglobin was 11 min. and that there was increased the inhibition of the conversion of Hb. to MetHb. The best result in our study is at concentration of 20 µM concentration of SPI complex in which it protected hemoglobin from oxidation by sodium nitrite to great extent, the conversion time of Hb to MetHb reached to 13 minutes compared with other SPI concentrations and NaNO2 oxidation samples(Fig. 4)



$NaNO_2$	SPI (5)
0.63	0.35
1.5	0.81
1.68	0.86
1.69	0.9
1.69	1.15
1.69	1.16
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35
1.69	1.35

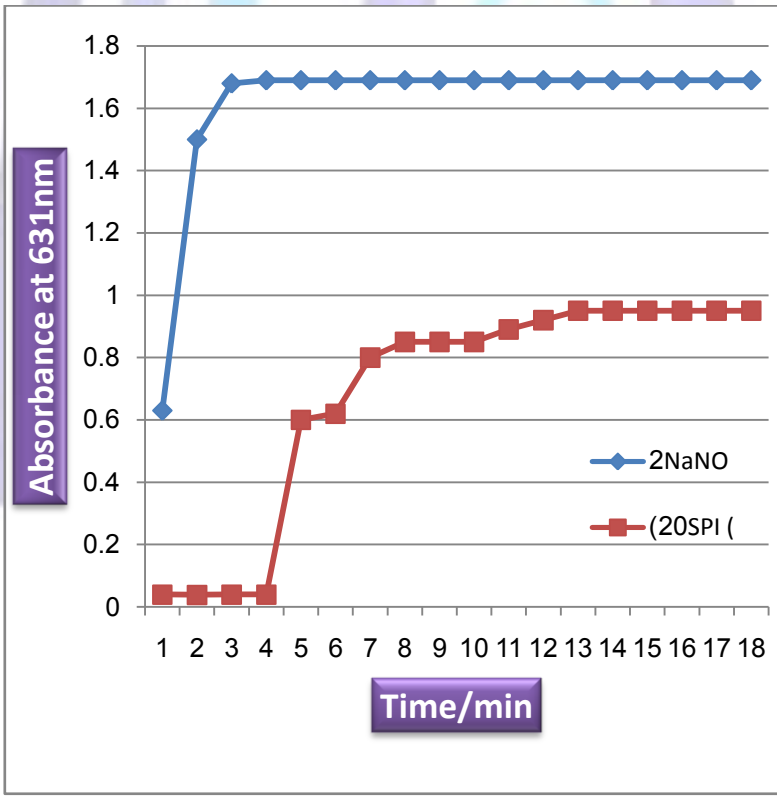
Figure 4.6: Shows antioxidant in vitro activity of SPI compound at (5µM).

ISSN 2347-6893



SPI (10)	$NaNO_2$
0.2	0.63
0.43	1.5
0.45	1.68
0.65	1.69
0.88	1.69
0.89	1.69
0.8	1.69
1.08	1.69
1.1	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69
1.14	1.69

Figure 4.7: Shows antioxidant in vitro activity of SPI compound at (10µM).



SPI (20)	$NaNO_2$
0.04	0.63
0.039	1.5
0.04	1.68
0.04	1.69
0.6	1.69
0.62	1.69
0.8	1.69
0.85	1.69
0.9	1.69
0.85	1.69
0.89	1.69
0.92	1.69
0.95	1.69
0.95	1.69
0.95	1.69
0.95	1.69
0.95	1.69
0.95	1.69

Figure 4.8: Shows antioxidant in vitro activity of SPI compound at (20µM).

ISSN 2347-6893

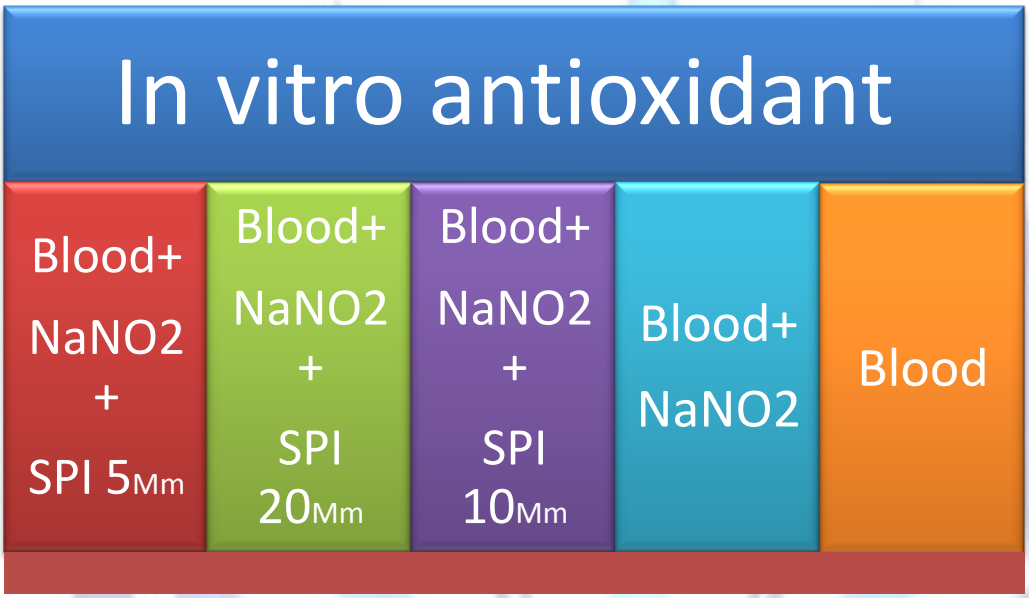


Figure 4.9: Show inhibition of nitrite-induced methemoglobin formation by SPI, at 5, 10 and 20 µm.

According to present findings, the free radicals liberation due to expose of sodium nitrite on erythrocyte lysate, leads to oxidative damage and production of MetHb [19] in which form of Fe3+ does not allow oxygen transport, owing to the strong binding of oxygen, therefore, methaemoglobin anemia can lead to cyanosis. Humans are considered to be more sensitive than rat in this respect. Hemoglobin regards a major aim for free radicals to be attack in RBCs due to it is a reactive molecule, capable of gaining and losing electrons relatively easily and interacts with variety of redox drugs and xenobiotics [20] in addition to its ability to give rise to reactive species to form of methemoglobin and consequent cell oxidative stress [21]. Oxidation of hemoglobin by nitrite was used as in vitro model to assess the concentration and time dependent protection of SPI compound nitrite-induced Hb oxidation. Initially the kinetic profile of Hb oxidation by nitrite was plotted under our experimental conditions, but there was not an initial slow phase, the reaction accelerates directly into a rapid phase of oxidation [22]. The in vitro results of SPI as antioxidant were agreed with earlier studies[23]. SPI compound can reduce ferric ions Fe3+ to the ferrous state Fe2+; it fails to reverse the oxidation of hemoglobin, suggesting that protection is not due to reduction

of methemoglobin to hemoglobin [24], but the SPI delay the oxidationof hemoglobin in concentration dependent pattern, attenuate sodium nitrite-induced Hb oxidation in vitro [19]. We believe that the SPI new compound acted as antioxidant activity was mainly related to the structure of SPI compound, which facilitate donation of electron and transfer of hydrogen atoms from hydroxyl moieties to free radicals, providing an efficient radical scavenging property to neutralize several reactive intermediates that promote continuous conversion of oxy-Hb to metHb.

ISSN 2347-6893

Invivo results:

Hematological Results: Statistical analysis of hematological indices is illustrated in Table (1-3).The results of this study did not showed any significant effect DMSO on the blood parameters including RBC, Hb, PCV, total and differential WBC, and blood indices; MCV, MCH and MCHC compared with the control group.DMSO is regard one of the most common solvents used experimentally because of its low toxicity for human and animal [25] in addition DMSO act as anti-inflammatory, anti-coagulant and reactive oxygen species scavenger activity [26] because of it has a high affinity for the hydroxyl radicals, and thus modulates the antioxidant enzyme system in the body, so it elevate the activities of blood antioxidant enzymes, i.e. glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PDH) to protect the cells against the hyper production of reactive oxygen species [27].

TABLE (1): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on the red blood cells count, Hb, PCV and total white blood cells count .

Parameters / Treatment	Red blood cell × 106 cell/mm3		Hb concentration g/dl		PCV %		WBC × 103 cell/mm3	
	Female	Male	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	aA 8.228 ± 0.224	aA 8.168 ± 0.234	ab A 11.440 ± 0.357	aA 11.320 ± 0.363	b A 34.500 ± 2.645	ab A 34.250 ± 2.217	c B 10.750 ± 1.318	c A 16.067 ± 1.318
Control 2 DMSO	b A 7.375 ± 1.089	b A 7.275 ± 0.820	aA 11.920 ± 0.228	ab B 11.040 ± 0.477	ab A 35.250 ± 3.594	ab A 35.750 ± 3.304	bc B 14.350 ± 1.318	b A 23.433 ± 1.318
NaNO2 133mg/kg	aA 8.935 ± 0.731	aA 8.585 ± 0.966	c A 9.480 ± 0.396	c A 9.800 ± 0.291	aA 40.000 ± 0.816	aA 38.500 ± 3.511	aA 24.400 ± 1.318	b A 23.200 ± 1.318
NaNO2 + SPI 1 (71.86 mg/kg)	b A 7.022 ± 0.470	b A 6.770 ± 0.554	b A 10.860 ± 0.581	bc A 10.360 ± 0.433	b A 33.250 ± 4.113	b A 32.000 ± 6.324	b A 17.300 ± 1.318	b A 20.633 ± 1.318
NaNO2 + SPI 2 (35.93 mg/kg)	aA 8.380 ± 0.882	aA 8.295 ± 0.808	b A 10.840 ± 0.477	b A 10.608 ± 0.662	b A 31.750 ± 3.774	ab A 34.500 ± 4.778	a B 24.917 ± 1.318	aA 31.017 ± 1.318
LSD	0. 873		0.700		4.875		4.167	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable.

ISSN 2347-6893

TABLE (2): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on differential white blood cells count.

parameters / Treatment	Neutrophil %		Basophil %		Acidophil %		Monocyte %		Lymphocyte %	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	aA 49.000 ± 9.899	a A54.500 ± 12.020	b A 0.500 ± 0.707	aA 0.500 ± 0.707	1.500 ± 0.707	1.000 ± 1.414	4.500 ± 0.707	4.000 ± 0.000	b A 38.500 ± 4.949	ab A 46.000 ± 8.485
Control 2 DMSO	aA 61.000 ± 8.485	a B 40.500 ± 21.920	b A 0.000 ± 0.000	aA 0.500 ± 0.707	1.500 ± 0.707	2.500 ± 0.707	5.000 ± 1.414	1.000 ± 1.414	b A 36.500 ± 0.707	ab A 50.500 ± 30.405
NaNO2 133mg/kg	b A 19.000 ± 5.656	b A 24.000 ± 2.828	aA 2.000 ± 1.414	aA 1.500 ± 0.707	2.500 ± 0.707	2.000 ± 1.414	3.500 ± 2.121	7.000 ± 1.414	aA 73.000 ± 9.899	aA 65.500 ± 2.121
NaNO2 + SPI 1 (71.86 mg/kg)	aA 48.000 ± 4.242	aA 55.500 ± 0.707	b A 0.500 ± 0.707	b A 0.000 ± 0.000	3.000 ± 1.414	2.500 ± 0.707	3.500 ± 0.707	6.500 ± 2.121	b A 45.000 ± 8.828	b A 38.500 ± 2.121
NaNO2 + SPI 2 (35.93 mg/kg)	24.000 b B ± 1.414	43.500 aA ± 0.707	0.000 b A ± 0.000	0.000 b A ± 0.000	1.000 ± 1.414	2.000 ± 1.414	3.500 ± 3.535	5.500 ± 2.121	aA 66.000 ± 8.485	ab A 49.000 ± 4.242
LSD	17.0		1.250		Not significant		Not significant		25.750	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable.

ISSN 2347-6893

TABLE (3): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on the blood indices

parameters / Treatment	MCVft		MCH(pg)		MCHC %	
	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	ab A 41.903 ± 2.386	b A 41.923 ± 2.201	bc A 13.975 ± 0.382	ab A 13.965 ± 0.710	aA 33.458 ± 2.457	aA 33.373 ± 2.206
Control 2 DMSO	aA 48.088 ± 3.651	aA 49.865 ± 9.475	aA 16.388 ± 2.354	aA 15.183 ± 1.742	aA 35.235 ± 3.497	ab A 30.765 ± 2.238
NaNO2 133mg/kg	aA 44.958 ± 3.225	ab A 47.908 ± 5.733	d A 10.755 ± 1.229	c A 11.580 ± 1.676	b A 23.898 ± 1.524	b A 25.688 ± 2.654
NaNO2 + SPI 1 (71.86 mg/kg)	aA 47.748 ± 8.950	ab A 47.708 ± 11.245	ab A 15.253 ± 0.459	aA 15.148 ± 1.472	aA 32.610 ± 4.794	ab A 32.803 ± 6.399
NaNO2 + SPI 2 (35.93 mg/kg)	b A 38.080 ± 4.920	b A 41.595 ± 3.106	c A 12.808 ± 0.952	bc A 12.710 ± 1.417	aA 33.923 ± 3. 553	ab A 30.665 ± 3. 836
LSD	6.595		1.591		7.501	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable

ISSN 2347-6893

TABLE (4): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on lipid profile .

parameters / Treatment	TC mg/dl		TGmg/dl		HDL-C mg/dl		LDL-C mg/dl		VLDL-C mg/dl	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	aA 88.612 ± 9.978	b A 78.917 ± 6.369	cd A 44.310 ± 7.827	b A 41.443 ± 11.311	aA 41.476 ± 3.150	aA 49.159 ± 7.738	b A 30.684 ± 3.073	c A 31.726 ± 3.855	d A 8.857 ± 1.565	b A 8.283 ± 2.261
Control 2 DMSO	c A 57.537 ± 15.922	c A 55.372 ± 5.467	b A 79.350 ± 15.454	b B 34.787 ± 11.608	aA 38.470 ± 1.080	aA 42.762 ± 1.639	bc A 29.210 ± 8.301	c A 29.210 ± 5.870	b A 15.935 ± 3.138	b B 6.955 ± 2.320
133mg/kg	aA 98.920 ± 13.529	bc B 67.865 ± 3.582	d A 38.862 ± 6.335	b A 40.517 ± 17.336	aA 34.768 ± 16.363	b B 11.110 ± 12.103	aA 56.042 ± 5.110	aA 56.795 ± 4.353	d A 7.842 ± 1.169	b A 9.067 ± 3.975
NaNO2 + SPI 1 (71.86 mg/kg)	ab B 90.358 ± 18.179	aA 111.635 ± 12.541	aA 100.542 ± 12.327	aA 106.298 ± 26.794	aA 51.770 ± 2.530	aA 39.140 ± 5.252	bc B 29.420 ± 4.820	b A 41.313 ± 3.307	aA 20.105 ± 2.464	aA 21.258 ± 5.356
NaNO2 + SPI 2 (35.93 mg/kg)	b A 78.618 ± 13.385	b A 81.762 ± 6.321	c A 58.127 ± 14.940	b B 43.088 ± 10.245	aA 37.567 ± 4.305	aA 43.387 ± 3.867	c A 21.030 ± 6.080	d A 19.302 ± 7.967	c A 11.770 ± 2.771	b B 8.610 ± 2.043
LSD	15.254		14.192		18.322		9.332		2.875	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable

ISSN 2347-6893

TABLE (4): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on lipid profile .

parameters / Treatment	Glucose mg/dl		ALT mg/dl		AST mg/dl		Uric acid mg/dl		Creatinine mg/dl	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	aA 132.60 ± 6.248	b B 112.26 ± 12.099	aA 35.33 ± 5.344	ab A 30.00 ± 9.899	aA 110.200 ± 20.584	c A 87.500 ± 48.098	aA 3.65 ± 0.421	aA 3.97 ± 0.392	aA 1.02 ± 0.264	aA 1.13 ± 0.255
Control 2 DMSO	ab B 129.05 ± 22.632	aA 169.83 ± 23.945	aA 33.16 ± 14.204	bc A 26.83 ± 7.250	aA 96.167 ± 23.224	bc A 110.00 ± 39.349	aA 3.70 ± 0.485	aA 3.87 ± 0.089	b A 0.78 ± 0.114	ab A 0.93 ± 0.098
NaNO2 133mg/kg	c A 27.76 ± 4.031	d A 14.38 ± 3.151	aA 39.25 ± 4.601	aA 38.08 ± 6.135	aA 122.16 ± 27.483	ab A 137.00 ± 5.567	b A 3.15 ± 0.455	b A 3.19 ± 0.491	ab A 0.90 ± 0.283	b A 0.70 ± 0.040
NaNO2 + SPI 1 (71.86 mg/kg)	b A 114.15 ± 13.921	c B 73.95 ± 5.634	b A 21.62 ± 5.707	c A 21.75 ± 5.909	a B 103.66 ± 26.515	aA 141.25 ± 12.120	d B 1.82 ± 0.433	ab A 3.59 ± 1.105	ab A 0.95 ± 0.295	ab A 0.93 ± 0.040
NaNO2 + SPI 2 (35.93 mg/kg)	b A 114.90 ± 24.629	b A 99.16 ± 11.352	b B 16.12 ± 3.065	b A 29.90 ± 7.003	aA 122.75 ± 11.324	c A 103.00 ± 24.931	c B 2.64 ± 0.386	b A 3.21 ± 0.377	aA 1.04 ± 0.103	aA 0.96 ± 0.177
LSD	16.9		8.544		29.293		0.456		0.231	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable

ISSN 2347-6893

TABLE (6): The effect of injection of DMSO, NaNO2 and SPI in both doses 1/10 and 1/20 on MDA, FSH, LH, estrogen and testosterone.

parameters / Treatment	MDA mg/dl		FSH mg/dl		LH mg/dl		Estrogen mg/dl		Testosteronemg/dl	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Control 1 0.9 % N.S	c A 3.310 ± 0.210	b A 3.212 ± 0.151	aA 1.702 ± 0.722	ab A 1.503 ± 0.225	aA 8.586 ± 0.252	c B 5.466 ± 0.420	b A 68.826 ± 8.874	d B 27.075 ± 10.618	a B 0.241 ± 0.051	aA 1.441 ± 0.133
Control 2 DMSO	c A 2.838 ± 0.240	c A 2.451 ± 0.172	b B 1.173 ± 0.163	ab A 1.496 ± 0.301	c B 7.512 ± 0.411	c B 4.712 ± 0.502	b A 62.392 ± 14.254	cd B 36.223 ± 12.795	a B 0.101 ± 0.04	aA 1.964 ± 1.07
133mg/kg	aA 7.503 ± 0.996	a B 6.189 ± 0.069	b B 1.353 ± 0.078	aA 1.712 ± 0.278	aA 8.584 ± 0.322	d B 0.592 ± 0.158	aA 102.107 ± 15.640	b B 48.077 ± 4.351	aA 0.279 ± 0.092	b A 0.213 ± 0.077
NaNO2 + SPI 1 (71.86 mg/kg)	b A 4.547 ± 0.270	b B 3.557 ± 0.405	aA 1.748 ± 0.290	b A 1.235 ± 0.190	c B 4.820 ± 0.201	b A 7.053 ± 0.335	c A 46.466 ± 18.134	b A 44.301 ± 7.942	aA 0.392 ± 0.299	b A 0.612 ± 0.010
NaNO2 + SPI 2 (35.93 mg/kg)	b A 4.087 ± 0.147	b B 3.417 ± 0.159	aA 1.520 ± 0.193	b A 1.395 ± 0.380	d B 1.052 ± 0.278	aA 8.640 ± 0.335	b A 72.918 ± 7.557	aA 67.138 ± 14.646	aA 0.170 ± 0.035	b A 0.719 ± 0.158
LSD	0.491		0.317		0.914		11.996		0.59	

The different letters refer to significant differences among groups at level of ($p \leq 0.05$). Small letters refer to vertical comparable and large letters refer to horizontal comparable

Erythrogram mean values of different experimental groups, in comparison to those of control group revealed the presence of macrocytic hypochromic anemia in sodium nitrite administered group which determined by significant decreases in hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC) and a significant increase in packed cell volume (PCV%) and mean corpuscular volume (MCV) values. It is known that nitrites convert the ferrous ion (Fe+2) of haemoglobin to ferric ion (Fe+3) both in vivo and in vitro [28]. This can explain the reduction of haemoglobin level. It seems that the administration of nitrite leads to haematopoietic tissue hypoxia resulting on the long term (21 days in the present study) to a decrease of red blood cell production and hence to reduction of blood haemoglobin level. The decrease of haemoglobin due to nitrite treatment has been reported using different animals [29]. Nitrite is known to cause free radical generation such as lipid peroxidation products which supposed to react with sulfhydryl groups of the lipid bilayer and protein components of erythrocyte membrane and change its structure, in addition nitrite-promoted Ca2+ influx

in blood cells activates phospholipases, which increase the proportion of phospholipids with a rigid structure in the membrane [30] and [31], and that lead to oxidative damage which might be a relevant cause of the initial decrease in RBC count which may be attributed to lysis or shrinkage of erythrocytes in the blood [32].

According to the present study the NaNO2 cause macrocytic hypochromic anemia in the treated rats, it could be possibly as a consequence of the toxic effect of NaNO2 on bone marrow, spleen and liver and this result in line with Abu Aita and Mohammed (2014). It's may be the cause of an increase PCV % results as of decrease of hemoglobin concentration and bone marrow tried to compensate the low level of Hb, therefor, the bone marrow product large number the immature form of RBC which is reticulocyte as mention National Toxicology Program [33], and it knowing the reticulocyte is larger than the volume of RBC and that lead to increase of PCV [34] and then MCV. Other explanations is as a result of effect of NaNO2 on the hemoglobin and cause its abnormality in shape and that lead abnormal shape of RBC, then the PCV value will be increase as described by Guyton and Hall [34]. It has been know that the PCV value depend on the shape of RBC. Results of total and differential leukocyte appear developing of inflammation with injection of sodium nitrite, that's clarified in total WBC count, and because of the inflammation was not in acute phase and not in chronic phase, the present study continued for 21 days and this duration of treatment regarding sub chronic toxicity of NaNO2 [35], this proved by the results of differential WBC in the present study in which there are decrease in neutrophil, increase in the lymphocyte and there was not significance in the monocyte. It has been known as true fact that the neutrophil regard as the first line defense against the inflammatory agents, therefore, neutrophil should be increase in the acute inflammation, in other hand, the monocyte increase in the chronic inflammation, and because of the period of current study is in the sub chronic phase of inflammation, the lymphocyte increased significantly with the control group. The effect of SPI appeared to improve the adverse effect of sodium nitrite on the hematopoietic system and blood indices relatively with dose graduated that's may be due to the effects of pyrimidine as an anti- inflammatory substance [36-37] and antioxidant activity [38-39]. SPI is reported in this study to inhibit lipid peroxidation as explained in in vitro study, lipid containing models such as liposomal membrane, liver microsomal system and human low density lipoprotein (LDL) exposed to oxidative damage [40], this protection was explained by the ability of SPI to scavenge free radicals and at the same time spare other antioxidants from oxidation suggesting the capacity of SPI to fight against the deleterious effect of nitrite overdose by protecting blood parameters and blood indices from free radical-induced oxidative damage.

Serum Biochemical results:

Statistical analysis of different serum biochemical values are illustrated in Table (6).Results of oxidative stress biomarkers revealed a remarkable increase in the values of serum malondialdehyde (MDA) of sodium nitrite administered group compared to control group. The present perturbations in oxidative markers of sodium nitrite rats could be attributed to the oxidative cytotoxicity and detrimental effect of nitrite [41]. (Abu Aita and Mohammed [1], which led to lipid peroxidation [41]. The results obtain from injection of SPI on the male and female rats was in line with De La Cruz et al. [42], and Al-Zamely and Al-Rikabi [43] in their studies, they have been proved that one of the pyrimidine compounds caused decrease of MDA level. The ameliorate of the MDA level after SPI injection was resulted from its ability to scavenge the free radicals [43]. The current results of MDA indicated the capability of SPI acts as antioxidant potential in vivo. Regarding hepato-renal markers, statistical analysis of our data, table (4-5), clarify the adverse effect of sodium nitrite on hepatic and renal functions. Sodium nitrite intoxicated rats exhibited an increase in the activities of serum AST and ALT enzymes but mostly not reach to the significantlyin comparison to control rat. The results after exposure to sodium nitrite were agreed with Ali et al. [44] and Helal [45] which give sodium nitrite for one month, while it's showed an increase in the ALT and AST levels in the study of Helal and Elsaid [46] and Abu Aita and Mohammed [1]. The cause of elevation of ALT and AST concentrations was due to sodium nitrite-induced oxidative stress. As it knowing the half-life of NaNO2 is only 11 minutes and both of the previous studies were significant increase and that's may be result from the period of administration, where they give the NaNO2 for three and two months respectively, so the toxic effect of NaNO2 persist for longer period. The route of administration appears has role in the effect of NaNO2, according to Hassan et al. [47] and Abu Aita and Mohammed [1] studies, the both AST and ALT enzymes increased in the serum due to NaNO2 orally treated rats, this could be attributed to the toxic effect of nitroso-compounds, formed in the acidic environment of the stomach to causing severe hepatic necrosis, in addition to that, when administered orally, the drug is reabsorbed by enterohepatic circuit. When injections kinetics completely different [48]. In case of uric acid and creatinine, NaNO2caused significant decrease in both sexed in the uric acid compare with control but the NaNO2 decrease the in creatinine level in the male treated rats only. This result was agreed with Dolomatov and Sataieva [48], that's due to increase creatinine clearance after treatment with sodium nitrite, the reason for this effect could be that the nitrites are resynthesis substrate for nitric oxide in the presence of hemoglobin. In turn, nitric oxide has angiotensin antagonist [48], and dis agreed with the findings obtained by Hassan, [47] which improved in his study that the sodium nitrite was increased creatinine level after giving the NaNO2 orally for one month due to kidney dysfunction.

The cause of low uric acid with sodium nitrite injection may be due to low blood glucose, resulting from sodium nitrite injection as occur in the present study, according to Čaušević et al. [49], it has been recognized that serum uric acid is positively associated with serum glucose levels, and this hypouricemia is associated with non adequate metabolic control, hyper filtration, and a late onset of overt nephropathy. The causes of decrease of creatinine after NaNO2 injection may be results from decreased of the protein synthesis results from the NaNO2 which reflected the biosynthesis of proteins that's due to the toxic effect of nitrite on the thyroid and adrenal glands that leads to block of protein synthesis [50]. Other cause of decreased creatinine level in the male rats injected with NaNO2 is may be due to the decrease level of testosterone in the male NaNO2, according to Guyton and Hall [51], the testosterone increase of protein formation and body musculature, and testosterone was decreased in the present study as appears in the result so that lead to decrease of creatinine level in the male while the female treated rats were not affected that may be due to the normal value of testosterone in the females in the present study. Injection of the new compound SPI was ameliorate the levels of liver enzymes, in addition,

the effects of SPI on the treated rats in the uric acid may results from the effect of the SPI compound which may act as other pyrimidine derivative, allopurinol, which is a clinically used as xanthine oxidase inhibitor [52]. It may be also due to increase of estradiol in the female and decreased of this hormone in the males treated rats, as it appeared from estradiol and testosterone results in current study after injected with SPI compound. The results have been confirmed by Leeling and Lata [53]. The results showed ameliorate the creatinine level in the SPI injection to female effects of NaNO2. As appears in the results of this study considering the effects of DMSO on the FSH and LH, table (6) the DMSO decreased the female treated rats but not affected on the male treated rats while in the estradiol and testosterone hormones the data appear no significant effect after DMSO injection for both sexes compared with control group. The results were agreed with Grachev et al. [54]. The data of the results after injection of NaNO2 were decreased FSH of the female treated rats and decreased LH in the male treated rats with increased estradiol value in both sexes' rats and decreased testosterone concentration in the male treated rats in comparison with control group.The decreased FSH after injection of sodium nitrite was agreed with Dubey et al. [55], which demonstrated in his study the cause of this decreasing might be due to that NaNO2 hinder follicular development via suppression of P450 aromatase activity in granulosa cells. The effect of sodium nitrite on LH was agreed with Campbell et al. [56] which clear that there was no evidence for adverse effects of sodium nitrite on female reproduction. While in case of the males the decreasing may occurs due to the negative feedback mechanism of increased estradiol values which cause decrease GnRH leading to decrease of FSH and LH [51]. The elevation in the estrogen level in the serum after treated with sodium nitrite could be due to the stimulation of the estrogen receptor alfa(ERα) by nitrite ions [57] or might be due to increase of the aromatase enzyme resulting from sodium nitrite, where aromatase enzyme regards the key step in the biosynthesis of estrogens, via converts and rostenedione to estrone and testosterone to estradiol, and then estrogens inhibits FSH via negative feedback. According to Fortepiani and Reckelhoff [58], the sodium nitrite is produced nitric oxide (NO) which inhibits steroidogenesis process [59] and [47], therefore, conversion of cholesterol to pregnenolone would be blocked, and this explained by directly inhibiting cholesterol side-chain cleavage enzyme (cytochrome P450) including different cytochromes P450 [60] and that leads to decrease of testosterone value. The increasing LH in the male treated rats after injection of SPI compound may be result from the same effect of one of pyrimidine derivatives, Org 43553 (hydrogen chloride salt of tert-butyl 5-amino-2-methylthio-4-(3-(2-(morpholin-4-yl)-acetamido)-phenyl)-thieno[2,3-d] pyrimidine-6-carboxamide). According to Lagemaat et al.[61] Org 43553 act as agonists to LH receptor and cause increase of LH in the treated rats, but its appear from the results of the present study the females have an opposite effect after injected with SPI compound. This amelioration in the SPI injection was clear also in the testosterone concentration compared with NaNO2 but it's not reach to the significant effect in comparison with control group for both sexes treated rats. According to the data of the results of FSH value after injection with SPI showed that a clear amelioration in the both sexes, the estradiol concentration in the female treated rats was ameliorated and mostly reach to the control group especially in the SPI 2, while in the male there was no significant difference in estrogen value compared with NaNO2, that's because the relation between estradiol and testosterone which showed in same result in this group, that's because of estrogen formed from testosterone by sertoli cells after stimulated by FSH [51].

REFERENCES

[1] Abu Aita, N. A. and Mohammed, F. F. Effect of Marjoram Oil on the Clinicopathological, Cytogenetic and Histopathological Alterations Induced by Sodium Nitrite Toxicity in Rats. Global Veterinaria 2014; 12 (5): 606-616.

[2] Hunter, C. J., Dejam A., Blood, A. B., Shields, H., Kim-Shapiro, D. B., Machado, R. F.,Tarekegn, S.,Mulla, N., Hopper, A. O., Schechter, A. N., Power, G. G. and Gladwin, M. T. Inhaled nebulized nitrite is a hypoxia-sensitive NO dependent selective pulmonary vasodilator. Nat. Med. 2004;10:1122-1127.

[3]Pluta, R. M., Dejam, A., Grimes, G., Gladwin, M. T. and Oldfield, E. H. Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage. JAMA: jou. Ameri.Med. Assoc. 2005; 293:1477-1484.

[4] Webb, A., Bond, R., McLean, P., Uppal, R., Benjamin, N. and Ahluwalia, A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. Proceedings of the National Academy of Sciences of the United States of America. 2004; Pp. 13683-13688

[5]Vorhees, CV, Butcher, RE, Brunner RL, Wootten V. Developmental toxicity and psychotoxicity of sodium nitrite in rats. Food Chem.Toxicol.198 ; 22(1):1-6.

[6]Farias, J. G., Bustos-Obregon, E., Orellana, R., Bucarey, J. L., Quiroz, E. and Reyes, J. G. Effects of chronic hypobaric hypoxia on testis histology and round spermatid oxidative metabolism. Andrologia.2005; 37: 47–52.

[7] Al –Okaily, B.N. ;Mohammed , R.S.; A. al-mzain, K. and Khalisa, K. Effect of Flavonoids Extract from blak Cumin (Nigella Sativa) and vitamin E in Ameliorating hepatic damage induced by sodium nitrite in adult male rats. Proceeding of the Eleventh veterinary scintfic conference, 2012; 172-181.

[8]Seada, M., Abdel-Megid, M. and El-Deen, I. M. Indian J. Heterocycl. Chem. 1993; 3: 81-86.

[9] Barnett, J. and Thomas, M. C. W. Chem. Abstr. 1999; 131:pp.28-30.

[10]Marouf, B. H., Aziz, T. A., Zalzal, M. H. and Hussain, S. A. Free Radical Scavenging Activity of Benfotiamine in Nitrite-induced Hemoglobin Oxidation and Membrane Fragility Models .J. Pharm. Biomed. Sci. 2010; 1(1): 13- 18.

[11]Sood, R. Hematology for students and practitions. 4th, ed., Jaypee brothers Medicial Publishers, (p) LTD.India, 1996; 318-325.

[12]Schalm, O.W.; Jain, N.C. and Carrol, E.J.Veterinary hematology. Lea and Febiger, 3ed. Philadelphia.1975;152-140.

[13]Burstine M., Scholnick H.R. and Martin, R. Rapid methods for the isolation of lipoproteins from human serum by precipitation with polyemions. J Lipid Res.1970; 11: 538-595.

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

[15]Yagi, K. Simple procedure for specific assay of lipid hydroperoxides in serum and plasma. Free Radical and Antioxidant Protocols.1998; 108: 101-106.

[16]Wahlefeld, A.W. Triglyceride determination after enzymatic hydrolysis. In: Bergmeyer HU, editor.Methods of Enzymatic Analysis. New York: Academic Press; 1974;Pp.:1831-1835.

[17] Reitman, S., Frankel, S., Amer, J. A. colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J ClinPathol. 1957;28(1):56-63.

[18] SPSS (Statistical Packages for the Social Sciences). (2001). Statistical software for windows version 13.0 Microsoft.SPSS®, Chicago, IL, USA.

[19] Aziz, T. A. Concentration-Dependant Antioxidant Activity of Pentoxifylline in Nitrite-induced Hemoglobin Oxidation Model. Iraqi J Pharm Sci, 2011; 20(1): 66-69

[20] French, J. K., Wininterbourn, C. C. and Carrel, R. W. Mechanism of oxy-hemoglobin breakdown on reaction with acetylphenylhydrazine. Biochem J.1978;173:19- 26.

[21] El-Agrody, A. M., Fouda, A. M. and Al-Dies, A. M. Studies on the synthesis, in vitro antitumor activity of 4H-benzo[h]chromene,7H-benzo[h]chromene[2,3-d]pyrimidine derivatives and structure–

activity relationships of the 2-,3- and 2,3-positions. Med. Chem. Res. 2014; 23(6): 3187-3199.

[22]Sulaiman, A. A. Concentration and Time - Antioxidant Effect Relationship of Anthocyanins in Sodium Nitrite and Azo Compounds-Induced Hemoglobin Oxidation and Erythrocytes Membrane Damage; In Vitro and In Vivo Studies. PhD thesis, 2012: P 114.

[23]Chaudhary, A., Sharma P. K., Verma, P. and Dudhe, R. Synthesis of novel pyrimidine derivative and its biological evaluation. Department of Physical Chemistry 4-12 Regina Elisabeta Blvd, District 3. 2011; 20(02):123 – 140.

[24]Oudah, S. K., Mohamed, H. T. and Al-Masoudi, W. A. Anti-oxidant activity of [6-chloro-2- (methylsulfanyl) pyrimidine-4-amine]cobalt(II)] complex. Scholars Research Library.DerPharmaChemica, 2015; 7(1):5-9.

[25]Kelava, T., Avarl, and Ulo, F. Biological actions of drug solvents. PERIODICUM BIOLOGORUM UDC. 2011; 113(3): 311–320.

[26] DI Giorgio, A. M., Hoi, Y., Zhao, X., Zhang, B., Lyeth, B. G., Russell, M. J. Dimethyl sulfoxide provides neuroprotection in a traumatic brain injury model. Restor.NeurolNeurosci. 2008; 26: 501-507.

[27]Fahim, F. A., Esmat, A. Y., Mady, E. A. and Ibrahim, E. K. Antitumor Activities of Iodoacetate and Dimethyl sulphoxide Against Solid Ehrlich Carcinoma Growth in Mice. Bio. Res. 2003;36(2): 253–262.

[28]Helal, E. G. E. and Elsaid, F. G. G. Management the Action of Sodium Nitrite on Albino Rats by Aqueous Garlic Extract. Res. J..Med. and Medical Sci. 2006; 1(3): 85-89.

[29] Kohn, M. C., Melnick, R. L., YeF, .and Portier, C. J. Pharmacokinetics of sodium nitrite-induced methemoglobinemia in the rat. Drug MetabDispos. 2002; 30(6):676-83

[30]Zavodnikal, B., Lapshinaa, E. A., Rekawieckab, K., Zavodnika, L.B., Bartoszb, G. and Bryszewskab M. Membrane effects of nitrite-induced oxidation of human red blood cells. BiochimicaetBiophysicaActa (BBA) - Biomembranes. 1999;1421(2): 306–316.

[31]Gluhcheva, Y., Ivanov, I., Petrova, E., Pavlova, E. and Vladoval. Sodium nitrite-induced hematological and hemorheological changes in Rats.Series on Biomechanics, 2012; 27, No.3-4, 53- 58.

[32]Rahman, M. M. I., Kim, G. B., Hong, C. U., Lee, Y. U., Kim, S. Z., Kim, J. S., and Kang, H. S. Nitrite induced methemoglobinaemia affects blood ionized and total magnesium level by hydrolysis of plasma adenosine triphosphate in rat. Basic Clin.Pharmacol.Toxicol. 2009; 105(5): 294-300.

[33] National Toxicology Program.Toxicology and carcinogenesis studies of sodium nitrite (CAS

NO. 7632-00-0) in F344/N rats and B6C3F1 mice (drinking water studies).NatlToxicolProgramTech Rep Ser. 2001; 495:P. 7.

[34] Guyton, M.D. and Hall, J.E. Text book of medical physiology 12th ed. 1074 pp. W.B. Saunders Company.2011.

[35]Velayudam, D. R., Ilavarasan, D. R. and Amuthan, D. R. A. ACUTE AND 28-DAY SUBCHRONIC ORAL TOXICITY STUDY OFKADUKKAI MAATHIRAI, AN IRON BASED SIDDHA HERBAL FORMULATION IN WISTAR ALBINO RATS. Inter. J. Pharm. and Pharmac. Sci. 2013; 5 (4): Pp.15-18.

[36]Bano, T., Nitin Kumar, N. and Dudhe, R. Free radical scavenging properties of pyrimidine derivatives. Org Med ChemLett.2012; 2:34-37.

[37] Mohammed, A., Hussein, Refaat, H., Omar; and Hatem, S. Farghaly. Design, Synthesis And Biochemical Evaluation of Some Novel Anti-inflammatory Pyrazolo [3, 4-d] Pyrimidine-4-ones. International Journal of Academic Research. 2011; 3(1): p 454

[38]Bhalgat, C. M., Ali, M. I., Ramesh, B. and Ramu, G. Novel pyrimidine and its triazole fused

derivatives: Synthesis and investigation of antioxidant and anti-inflammatory activity. Arab.J. Chem.2014; 7 (6): 986–993.

[39]Kachroo, M., Panda, R. and Yadav, Y. Synthesis and biological activities of some new pyrimidine derivatives from chalcones. Der PharmaChemica, 2014; 6(2):352-359.

[40] Garcia-AlonsoM., RimbachG., Rivas-GonzaloJ, C. and De Pascual-Teresa, S. Antioxidant and cellular activities of anthocyanins and their corresponding vitisins A-studies in platelets, monocytes, and human endothelial cells. J Agric Food Chem. 2004; 52:3378-3384.

[41]Çiftçi, S.Y., Yalovaç, A., Hekimoğlu, R., Gümüşel, B., Korkusuz, P. and Uçar, G. Effects of nitrite on hepatic ischemia-reperfusion injury.Turk. J. Biochemistry–Turk J Biochem.2012; 37 (4): 392–404.

[42] De la Cruz, J.P., Moreno, F. A., Mérida, J., García-Campos F., Sánchez de la Cuesta. The pyrimido- pyrimidine derivatives, dipyridamole and RA-642, reduce opacification of crystalline lens in diabetic rats. Pharm. Tox. 1994; 75(5):250-254.

[43] Al- Zamely, O. and Al-Rikabi, I. Verification of [4(1,2-Di Hydro -4,6-Dihydroxy Pyrimidine -2- YhThio)-3-Hydroxy-5-(2,2-Dimethyl-1,3-Dioxolan-4-Yl) Furan- 2(5H)One] Properties as Antioxidants in ExperimentalAnimals Induced with Diabetes. Chem. Proc. Engin. Res. 2013; 13.Pp. 25-26

[44] Ali, S. A., Aly, H. F., Faddah, L. M. and Zaidi, Z. F. Dietary supplementation of some antioxidants

against hypoxia. World J Gastroenterol.2012;18(44): 6379-6386.

[45] Helal, E. G., Progressive effects of the interaction of Sodium nitrite and sunset yellow on different physiological parameters in albino rats. Egyp.J. of Hosp. Med. 2001; 2: 23 – 46.

[46] Helal, E. G. and Elsaid, F. G. Management the Action of Sodium Nitrite on Albino Rats by Aqueous Garlic Extract. Res. J..Med. and Medical Sci. 2006; 1(3): 85-89.

[47] Hassan, H.A. THE POSSIBLE PROTECTIVE ROLE OF BEES HONEY AGAINST HAZARD EFFECTS OF SOME SYNTHETIC FOOD ADDITIVES ON THE KIDNEY FUNCTIONS OF MALE RATS. J. Egypt. Soc. Toxicol.2007; 36: 13-21.

[48] Dolomatov, S. I., and Sataieva, T. P. Effect of ras-blockers and no-cycle metabolites on the renal functions of rats exposed to thyroxine injections. J. Edu. Health and Sport. 2015;5(1):41-55.

[49] Čaušević, A., Semiz, S., Macić-Džanković, A., Cico, B., Dujić, T., Malenica, M. and Bego, T. RELEVANCE OF URIC ACID IN PROGRESSION OF TYPE 2 DIABETES MELLITUS. BOSNIAN J. BASIC MED. SCI. 2010; 10 (1): 54-59.

[50] El-Wakf, A. M., Elhabiby, E. M.; El-kholy, W. M. andAbd El-Ghany, E. Use of Tumeric and Curcumin to Alleviate Adverse Reproductive Outcomes of Water Nitrate Pollution in Male Rats.*Nat. and Sci*. 2011; 9(7): 229–239.

[51] Guyton, M.D. and Hall, J.E. Text book of medical physiology 12th ed. 1074 pp. W.B. Saunders Company. 2011.

[52] Yoon, K. N., Alam, N., Lee, K. R., Shin, P. G., Cheong, J. C.,Yoo, Y. B. and Lee, T. S. Antioxidant andAntityrosinase Activities of Various Extracts from the Fruiting Bodies of Lentinuslepideus. Molecules, 2011; 16: 2334-2347.

[53] Leeling, J.L. and Lata, G.F. Sex Difference in Rat Liver Uric Acid Metabolism. Published Online.US National Library of Medicine, 2013; 77(6):1075-84.

[54] Grachev, P., Li, X. F., Hu, M. H., Li, S. Y., Millar, R. P., Lightman, S.L. and O'Byrne, K. T. Neurokinin B Signaling in the Female Rat: a Novel Link Between Stress and Reproduction. Endocrinology, 2014; 155(7):2589 –2601.

[55] Dubey, P. K., Tripathi, V., Singh, R. P. and Sharma, G. T. Influence of nitric oxide on *invitro*growth, survival, steroidogenesis, and apoptosis of follicle stimulating hormone stimulated buffalo (*Bubalusbubalis*) preantral follicles. J Vet Sci., 2011; 12(3): 257–265.

[56] Campbell, M., Dunn, A., Donald, J., Morgan, J., Golub, M., Zeise, L. and Alexeeff, G. EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF SODIUM NITRITE.Reproductive and Cancer Assessment Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.2000.Pp. 70

[57] Veselik, D. J., Divekar, S., Dakshanamurthy, S., Storchan, G. B., Turner, Jasmine, M.A., Graham

K. L., Huang, Li., Stoica, A. and Martin, M. B. Activation of Estrogen Receptor-α by the Anion Nitrite. J. cancer Res.2008; 68(10): 3950-3985.

[58] Fortepiani, L.A. and Reckelhoff, J. F.Treatment with tetrahydrobiopterin reduces blood pressure in male SHR by reducing testosterone synthesis. American J. Physiology - Regulatory, 2004; 288 (3):P. 6

[59] Kostić, T., Andrić, S., Kovačević, R. and Marić, D. The involvement of nitric oxide in stress-

impaired testicular steroidogenesis. Europ.J. .Pharma.1998;346, Issues 2–3: 267–273.

[60] Del Punta, K., Charreau, E. and Pignataro, O. Nitric oxide inhibits Leydig cell steroidogenesis Endocrinology, 1996; 137*:* 5337–5343*.*

[61] Lagemaat, R.V., Timmers, C.M., Kelder, J., Van Koppen, C., Mosselman, S. and Hanssen, R.G.J.M. Induction of ovulation by a potent, orally active, low molecular weight agonist (Org 43553) of the luteinizing hormone receptor. Human Reproduction,2009; 24(3): 640–648.