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AMELIORATIVE ROLE OF SCHIFF-BASE DERIVED FROM PHENYL ETHYL AMINE AGAINST SODIUM NITRATE TOXICITY IN LABORATORY MALE RATS

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ABSTRACT

This study was designed to Evaluate the biological activity of Schiff base derived from phenyl ethyl amine produced novel compound used in this experiment. Thirty adult male rats age (10-14) weeks (150-200) gm weights were included and separate randomly into 5 groups (6 rats / group) and treated for 21days intraperitoneally (I.P) as follows:- Group 1 (control): 0.5ml/day of DMSO daily ,Group 2: 18mg/kg of $NaNO_2$, daily ,Group 3 94 mg/kg of phenyl ethyl amine daily ,Group 4 18 mg/kg of $NaNO_2$, then after one hour 0.5ml/day from 66.8mg/kg of synthesis compound daily , Group 5: 0.5ml/day from 66.8mg/kg of synthesized compound. According to finding Results there was significant ameliorative effects of Schiff base against sodium nitrate toxicity in several physiological parameters as (R.B.C. count , Hb. concentration , PCV. ,W.B.C. count MID%, TSH,T3 andT4 levels) which the result showed there were significant decreased in this parameters in group 5 where administrate sodium nitrate and after one hour novel compound in compared with group 2 sodium nitrate alone .

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INTRODUCTION

Concentrate products of primary amines with carbonyl compounds are called Schiff bases were first reported as Schiff in 1864 by (1) . according to important characteristic features in catalysis. and biological activity, such as, antibacterial, antifungal activities Schiff bases have been studied (2). Schiff bases metal complexes have been widely studied for the reason that, they have anticancer and herbicidal effects . Schiff bases show clinical properties (3, 4). At

higher doses Sodium nitrite ($NaNO_2$) have toxic effect because of it exerts on red blood cells. MtHb. levels below 2%. in mammals blood are normally, While levels exceed 70% usually become fatal, by creating a lethal deficit of oxygen in cardiac muscle and the brain, and that lead to rapid lack of oxygen to the brain and other vital organs and according respiratory failure this goes to quickly death (5).

Severe metha-hemoglobinaemia are common sign in mammals that include shortness of breath, cyanosis, lethargy, loss of consciousness and blue coloring of the skin mainly in high blood supply areas like lips, gums, hands/paws and nose . There is various acute NaNO2 toxicity data, however very limited are data available on risk of secondary poisoning (5, 6). It was concluded that the biological and ameliorative action of this novel compound that derived from phenyl ethyl amine and 2-hydroxy naphthaldehyde. against sodium nitrate toxic effect.

MATERIAL AND METHODS

The LD 50 of synthesized new compound was studied by the "up-and-down" procedure on the mice that described by (7)

Experimental design

Thirty adult male rats age (10-14) weeks (150-200) gm weights were included and separate into 5 groups (6 rats /group) randomly and treated for 21 days were injected intraperitoneally (I.P) with:-Group 1 (control): 0.5ml/day of DMSO daily, Group 2: 18mg/kg of $NaNO_2$, daily, Group 3: 94 mg/kg of phenyl ethyl amine daily , Group 4: 18 mg/kg of $NaNO_2$, then after one hour injected with 0.5ml/day from 66.8 mg/kg of synthesis compound daily ,Group 5: 0.5ml/day from 66.8mg/kg of synthesized compound.

Finally, blood was obtained from the heart by cardiac puncture with a 10 ml disposable 22 G needle syringe (8). Divided into two sections, 1ml was transferred to the tube contain anti-coagulant for hematological analysis, other part of blood was added in plain tube and centrifuge for 15 minutes at 3000 rpm to extract the serum, which was collect to Eppendorf tube and store at-20^{o}C until used for biochemical and hormonal analysis.

Statistical Analysis

Experiments data expressed as (Mean ±SD), and analyzed by One-way ANOVA using computerized program Special Program for Statistical System (SPSS) version 21.0. least

significant difference test (LSD) to determine the significant differences between groups in ANOVA-test (9).

RESULT

It seems from Table (1) that the RBC count, hemoglobin concentration (Hb), PCV, and MCH decreased significantly (P≤0.05) in sodium nitrite compared as the control group. However, RBC, Hb, PCV, MCHC, and MCH increased significantly (p≤0.05) in phenyl ethylamine group, injection of synthesized compounds after one hour of NaNO2 increased RBC a count (Hb), PCV, and MCH significantly (p≤0.05). In contrast, there was a decrease of MCHC as compared with the NaNO2 group and control group. Injection of synthesized compounds indicated that a substantially (p≤0.05) increases RBC count, hemoglobin conc., PCV% , MCHC, and MCH level compared with the $NaNO_2$ group.No significant difference was seen in treated rats with (MCV)in all treated groups.

Table (1). Effects of different treatment on hematological parameters in adult male rat (mean±SD).

Group	RBCs $\times10^6/\mu L$	Hb (g/dL)	PCV $\times10^3/\mu L$	MCH (pg/cell)	MCHC (g/dL)	MCV (fL)
Control	6.40 ± 0.35 a	12.66 ±0.37 a	37.10 ± 2.43 a	19.03 ± 0.68 a	32.84 ± 0.89 a	58.65 ±0.85 a
$NaNO_2$	5.67 ± 0.66 b	9.39 ±0.69 b	24.92 ±1.04 b	16.82 ± 1.03 b	30.82 ± 0.82 b	54.58 ±2.41a
Phenylethylamine	6.11 ± 0.56 a	11.53 ±0.89 a	36.15 ±2.40 a	18.85 ± 0.47 a	31.83 ± 0.60 ab	59.33 ±2.09a
$NaNO_2$synthesized compound	6.24 ±0.75 a	11.53 ±1.07 a	36.81 ±3.52 a	18.46 ± 0.81 a	31.30 ±1.00 b	59.23 ±2.99a
synthesized compound	6.53 ± 0.84 a	12.63 ±1.50 a	39.13 ±5.73 a	19.31 ± 0.71 a	32.3 ±0.87 a	59.53 ±2.32a

*different letters denote significant P<0.05

Total WBC count increased significantly ($p \leq 0.05$) in NaNO2 (group2) and phenyl ethylamine (group3) when make comparison with the control group. injection of synthesized compounds (group 5) alone or after an hour of injection NaNO2 (group4) was elevated but not significant of WBC value than control groups. The administration novel compound (group5) WBC value decreased significantly ($p \leq 0.05$) as compared with the (group2) group. Simultaneously, there was a decrease in MID % in treated (groups 2 and 3) than control groups. un- substantially changed was seen in granulocyte and lymphocytes value in male treated rats in all treated groups. (Table 2),

Table (2). Effects of different treatment on WBC count in adult male rat. (mean ± SD) .

Group	WBC ×103/µL	MID%	GRAN%	LYM%
Control G1	10.15 ± 1.04 b	15.38 ± 3.57 a	36.71 ±2.87 a	47.90 ±4.14 a
NaNO2 G2	15.90 ± 1.93 a	8.26 ±1.85 b	40.00 ±5.86 a	51.73 ±6.80 a
Phenylethylamine G3	15.51 ± 1.77 a	6.03 ±3.17 b	44.21 ±7.65 a	49.75 ±10.48 a
NaNO2+ synthesized compound G4	11.67 ± 0.83 b	6.08 ±4.11 bc	41.30 ±14.62 a	52.61 ±18.44 a
synthesized compound G5	10.23 ± 1.14 b	5.78 ±2.36 bc	44.03 ±5.49 a	50.18 ±7.19 a

*different letters denote significant P<0.05

Thyrotropin (TSH) level increase significantly ($p \leq 0.05$) in (group2) compared with all treated groups and showed decrease but not substantial in (group3 4 and 5) while significant reduction ($p \leq 0.05$) in groups (3. 4 and 5) is compared with NaNO2. Simultaneously, there were non-significant changes between groups 3 and 4 also between groups 4 and 5. Table (3)

Also, Table (3) showed that Triiodothyronine (T3) value increased in all groups except (group4) there was an increased but not significant in comparison with the control group. No significant difference was seen in Thyroxine level (T4) in all treated groups. Table (3) showed that testosterone value increased significantly($p \leq 0.05$) in (groups 3, 4, 5) compared with the control group while there was an increase but not significant in (group2) than control group.

Table (3). Effects of different treatment on thyroid hormones in adult male rat. (mean ± SD).

Group	TSH µlU/ml	T3 ng/dl	T4 µg/dl	Testosterone pg/ml
Control(DMSO) G1	1.8 ± 0.24 b	0.91 ± 0.10 b	7.80 ±0.60 a	1.12 ± 0.19 a
NaNO2 G2	3.03 ±0.52 a	1.22 ±0.13 a	8.25 ±1.60 a	1.36 ±0.08 ab
Phenylethylamine G3	1.86 ±0.15 b	1.15 ± 0.12 a	7.65 ±1.33 a	1.59 ± 0.10 a
NaNO2+Synthesis compound G4	1.69 ±0.09 b	1.06 ± 0.17 ab	7.99 ±0.55 a	1.61 ±0.34 a
Synthesized compound G5	1.79 ±0.16 b	1.19 ± 0.21 a	6.76 ±0.49 a	1.54 ± 0.28 a

*different letters denote significant P<0.05

DISCUSSION

The results findings in a table (1) were consistent with previous research (10, 11) on the decrease in RBCs, while the other hematological outcomes, including the percentage of PCV, MCH, and MCHC, were not consistent with the work (12)

Nitrite created free radicals, such as lipid peroxidation products, which react with lipid bilayer sulfhydryl groups erythrocyte membrane protein components change its structure. Besides, nitrite-promoted Ca2 + influx stimulates phospholipases in blood cells, which raises the proportion of phospholipids in the cell membrane to a rigid frame (13, 14).

Nitrites believed to transform hemoglobin's ferrous ion (Fe+2) to ferric ion (Fe+3) both *in vivo* and *in vitro* (12). This conversion can explain the lowering of hemoglobin levels. Nitrite

administration tends to lead to hypoxia of the hematopoietic tissue, resulting in a long-term (21 days in the current study) decrease in blood hemoglobin levels. Different animals have reported reducing hemoglobin due to nitrate treatment (14).

According to this experiment, in treated rats, NaNO2 causes macrocytic hypochromic anemia. It may result from NaNO2's harmful effect on the bone marrow, liver, and spleen, and it is compatible with this finding (11). It may be the reason for a percentage rise in PCV (15). The PCV value is known to depend on the RBC structure.

Sodium nitrite causes oxidative damage to type meta globulin, which induces modifications and oxidation of ferrous ions in oxyhemoglobin (15, 16). Another mechanism for decreasing hematological values was expressed as reductions in erythropoietin hormone secreted from kidney tissues due to their damage by reducing RBC production (17).

Erythrocytes are highly susceptible to oxidative damage than other cells due to the high cellular concentration of oxygen; also, hemoglobin is considered potentially as a powerful promoter for the oxidative processes (18).

The results show that the injection of phenyl ethylamine group3 causes a significant increase in the RBC count, MCHC, and increase in MCH, whereas no significant difference observed in packed cell volume and MCV compared with the control group.

It seems from the table (1) that the RBC count, Hb, MCV, MCH MCHC, and PCV did not change by the injection of NaNO2 and then injected with synthesized compound after one hour to rats. Hence, it gives evidence that the synthesized compound, when injected after one hour NaNO2, has ameliorative effects that reduce the harmful effects of $NaNO_2$ when compared with the control group.

Because of its low toxicity to humans and animals, the synthesized compound (group5) raises the RBC can be known to be one of the most common solvents used experimentally (19). Because it has a high affinity with hydroxyl radicals and modulates the antioxidant enzyme system in the body, the synthesized compound can also serve as an anti-inflammatory, anti-coagulant reactive oxygen species scavenger activity. Increases the activity of blood antioxidant enzymes, i.e., (glucose-6-phosphate dehydrogenase (G6PDH) and glutathione peroxidase (GPx), to shield the cells from hyper production of reactive oxygen species (20). The hydroxyl radical is

also neutralized and reduces cellular toxicity as one of the most toxic side products of superoxide production (21).

The findings indicate a sustainable increase in total leukocyte count after animal injection with sodium nitrate. The increases in the overall and differential count of leukocytes may be due to the inflammatory reaction to sodium nitrite; this causes the development of a high number of bone marrow cells, accompanied by the release of significant amounts of possible oxidant agents that may trigger damage to the tissues and cells around them (22).

The current findings do not agree with (23), which indicates a substantial decrease in the overall leukocytes' count of the group treated with sodium nitrite than the control group.

The phenyl ethylamine effect on WBC was a little increased in the treated rats than the control. The trace amine receptors that β-phenyl ethylamine is known to influence (TAAR1 and 2) appear to be expressed on leukocytes, and trace amino acids appear to control leukocyte migration concentration, which is relevant even without nutritional supplementation (24). Including both T and B cells and the activation by β-phenyl ethylamine of both receptors and the cause chemotaxis of immune cell, This is a dose that is already lower at rest than human plasma, therefore below physiological thresholds and thus tends to be important physiologically. Similar actions have also been found with T1AM (3-Monoiodothyronamine) and tyramine trace amines (25).

While there was an increase in WBC but not significantly in (groups 4 and 5)The effect of the synthesized compound on the hematopoietic system and blood indices tended to enhance the destructive impact of sodium nitrite compared to the graduated dosage, which may be attributed to the effects of the novel compound as an anti-inflammatory agent and antioxidant activity, Also, there was a significant decrease in MID (Basophile, Eosinophile, and monocytes) when treated by $NaNO_2$ and phenyl ethylamine, group4 (NaNO2+synthesized compound) and group 5(synthesized compound) than control and other groups. As explained in the study, synthesized compounds reported inhibiting lipid peroxidation in this study. This defense defined the ability of the synthesized compound to scavenge free radicals and, at the same time, spare other antioxidants from oxidation, suggesting the power of a synthesized compound to counteract the deleterious effect of nitrite overdose by shielding blood parameters and blood indices from oxidative damage caused by free radicals (26, 27).

A significant increase in Thyrotropin (TSH) T3and T4 after injected rats with $NaNO_2$ than in all other treated groups; Such data indicate $NaNO_2$, which causing blocked protein synthesis, fast break down, increased rate of Free amino acids, and lowering protein turnover, and this will require stimulation of thyroid and adrenal glands (28, 29),

Thyrotropin (TSH) and Triiodothyronine and Thyroxine (T4)value decrease in group ($NaNO_2$ + synthesized compound) and synthesized compound group than the $NaNO_2$ group by the effect on the thyroid gland which may cause enlargement of the thyroid gland and cause hypothyroidism,through inhibiting iodine absorption through tyrosine thyroglobulin residue and thereby ending the thyroid hormone, (T3) and (T4), biosynthesis (30, 31).

Increased serum testosterone in (Group 2) $NaNO_2$ resulted from elevated hypoxia as a result of the influence of $NaNO_2$ hydrogen peroxide-induced oxidative stress in rats, resulting in testicular degeneration (32, 33, 43).

The table showed that testosterone value increases significantly in group3 (phenyl ethylamine) compared with the control group. This increase in phenyl ethylamine injection was also apparent in the concentration of testosterone relative to $NaNO_2$.

Synthesized compound expected to decrease these harmful effects of ($NaNO_2$) when injected after one hour from $NaNO_2$ injection, and its effects were apparent where seminiferous tubules and epididymis tubules have somewhat retained to their typical histological architecture, increased spermatogenic cells, and increased the number of mature spermatozoa in epididymis tubules (23).

Ethics

All animal experiments are subjected to established stander ethics in the Veterinary Medicine College of Basrah University.

REFERENCES

1-Cimerman, Z.; Miljanic, S.; and Galic, N.; Croatica, and Chemica Acta.(2000). Schiff bases derived from aminopyridines as spectrofluorimetric analytical reagents. Croatica Chemica Acta 73 (1), 81- 95.

2-Verma, M.; Pandeya, S.N.; Singh, K N.; Stabler, J P.; and Acta Pharm. (2004). Microwave-Assisted synthesis of some nitro-benzimidazoles and their salicyl and is at in Schiff bases. United States Patent, 54, 49.

3-Singh H L, Sharma M & Varshney A K, (1999). Synthesis,characterization and antimicrobial studies of tin(II) complexeswith Schiff base of sulpha drugs, A C G C Chem Res Commun,8 (1998) 35-40; Chem Abstr, 131 110367.

4- Hatim, I. H., Al-Masoudi, W. A., & Ghadhban, R. F. (2019). Synthesis, characterization and acute toxicity of new Schiff base derived from phenylethyl amine and 2-hydroxy naphthaldehyde. European Journal of Chemistry, 10(1), 26–29.

5- Eason, CT.; Murphy, E.; Hix, S.; and Mac Morran , D. (2010). The effectiveness of an encapsulated formulation of a new pig toxin in pen trials. Connovation Ltd Report for Regional Councils. 7 p.

6- Shapiro, L.; Eason, CT.; Bunt, C.; Hix, S.; Aylett, P.; and MacMorran, D. (2016). Encapsulated sodium nitrite as a new tool for possum control in New Zealand. New Zealand Journal of Ecology 40: 381–385.

7- Dixon, W. J. (1980). Ann. Rev.Toxicol., 20: 441 – 462.

8- Parasuraman, S.; Raveendran, R.; and Kesavan, R. (2010). Blood sample collection in small laboratory animals. J Pharmacol Pharmacother.1 (2):87-93.

9- Abo-Allam, R.M.; (2003). Data statistical analysis using SPSS Program. 1st ed. Publ. for the U. Cairo.

10- Sulaiman, A.A. (2012). 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.

11- Abu Aita, N. A. and Mohammed, F. F. (2014). Effect of Marjoram Oil on the Clinicopathological, Cytogenetic and Histopathological Alterations Induced by Sodium

Nitrite Toxicity in Rats. Global Veterinaria., 12 (5): 606-616

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

13-Zavodnika, I.B.; Lapshinaa, E.A.; Rekawieckab, K.; Zavodnika, L.B.; Bartoszb, G.; and Bryszewskab, M. (1999). Membrane effects of nitrite-induced oxidation of human red blood cells. Biochimica et Biophysica Acta (BBA) - Biomembranes., 1421(2): 306–316.

14-Guyton, M. D. and Hall, J.E. (2011). Text book of medical physiology 12th ed. J. Egypt. Soc. Toxicol, 1074.

15- Gladwin, M.T.; J.H. Crawford and R.P.; Patel, (2004). The biochemistry of nitric oxide, nitrite and hemoglobin : Role in blood flow regulation. Free Radic. Biol. Med., 36: 707-717.

16- Baky, N.A.; Z.F.; Zaidi, A.J; Fatani, M.M.; Sayed- Ahmed, and H.; Yaqub. (2010). Nitric oxide pros and cons: The role of l-arginine, a nitric oxide precursor and idebenone, a coenzyme-Q analogue in ameliorating cerebral hypoxia in rat. Brain Res. Bull., 83: 49-56.

17- Koury, S.T., Bondurant, M.J.; and Semenza, G.L. (1999). Localization of cell producible erythropoietin in Marine liver by in six hybridization blood. Drug Metabolism and Disposition . (77) 2497-2503.

18- Adam, A.; Magdalena, S.; Arkadiusz, G.; and Halina, M. Z. (2012). The Effects of Gamma - irradiation and Quercetin on Human Red Blood Cells. Electronic International Interdisciplinary Conference. Boll. Chem. Farm Pp: 563-567.

19- Kelava, T.; Avar, I.; and Ulo, F. (2011). Biological actions of drug solvents. Priodicum Biologorum ., 113(3): 311–320.

20- Fahim, F.A.; Esmat, A.Y.; Mady, E.A.; and Ibrahim, E. K.; (2003). Antitumor Activities of Iodoacetate and Dimethylsulphoxide Against Solid Ehrlich Carcinoma Growth in Mice. Bio. Res., 36(2): 253-262.

21- Kucuk, C.; Ok, E.; Yilmaz, Z.; Sozuer, E.; Muhtaroglu, S.; and Arar, M.(2003). The Effects of Dimethylsulfoxide in Experimental Obstructive Jaundice. Acta chir belg., 103(4): 392-395.

22- Rebaur, A.H., Mcwilliams, P.S.; Feldman, B.F.; Metazger F.L.; Pollock, R.V.; H.; and Roche, J.(2005). Neutrophils: Overveiw quality and morphology. Int.Vet. inform. Serv.

(IVS). Thuca.13305-0305.

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

24- Nelson DA.. (2007). Expression of neuronal trace amine-associated receptor (Taar) mRNAs in leukocytes. J Neuroimmunol. nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicology. Egyptian Journal of Hospital Medicine 49: 857-878 .

25- Babusyte, A.; Kotth off, m.;Fiedler, J.; Kreutwurst, D.; (2013). Amines activate blood leukocytes via trace amine-associated receptors TAAR1 and TAAR2. J Leukoc Biol. 93: 387. 394.

26-Bhalgat, C.M.; Ali, M. I.; Ramesh, B.; and Ramu, G. (2014). Novel pyrimidine and its triazole fused derivatives: Synthesis and investigation of antioxidant and anti-inflammatory activity. Arab. J. Chem., 7(6): 986–993.

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

28- Yousif, Y.Y.; Al – Rufaie, I.A.;and Al – Mansori, H.A. (2004). Effect of Dietary Zinc Chloride on Glycogen, Protein and Cholesterol Contents in Liver, Kidney and Muscle of Mice. Department of Biology, College of Science, University of Basrah, Basrah, Iraq. Basrah J. Science. B, Vol. 20 No. 1: 87-96.

29- Abdeen, AM.; El-Shayeb, AF.; Othman, AI.; and El-Agamy, SH. (2008). Histopathological and histochemical studies of the influence of garlic oil against sodium nitrite induced toxicity in the liver and lungs of albino rat J. Egypt. Ger. Soc. Zool. 55C, 261–287.

30- Biassoni, B.; Ravera, G.; and Bertocchi, J.(1998). Influence of dietary harpit on thyroid status of nomadic people, the Bororo shepherds, roaming a central African region affected a sever iodine deficiency. Eur J Endocrinol; 138:681-5.

31- Mariela, J.C.; Yamila, V. C.; B.; Liliana, O.; and Maria, S. G. (2012). Hypothyroidism on Lipid Metabolism. Instituto Multidisciplinario de Investigaciones Biológicas-CONICET,

San Luis. Universidad Nacional de San Luis. Argentina.,3-34,

32-Rahim, S. M.; Taha, E. M.; Mubark, Z. M.; Aziz, S. S.; Simon, K. D.; and Mazlan, A. G. (2013). Protective effect of Cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. Syst Biol Reprod Med., 59(6):329-336.

33-National Toxicology Program. (2001). Toxicology and carcinogenesis studies of sodium nitrite (CAS NO. 7632-00-0) in F344/N rats and B6C3F1 mice (drinking water studies). Natl Toxicol Program Tech Rep Ser., 495:273-277.

34-Okumura, A.; Fuse, H.; Kawauchi, Y.; Mizuno, I. and Akashi, T. (2003). Changes in male reproductive function after high altitude mountaineering. High Alt Med Biol.,4 (3):349-353.