Protective Effects of Magnesium and Pyridoxine on Sodium Nitrite-Induced Oxidative Stress in Rats

Diaa B. Al-Azhary, Nagy S. Tawfik, Abdalla A. Abdalla and Mohamed S. Farghaly

Department of Zoology, Faculty of Science, Minia University, El-Minia, Egypt

ABSTRACT

The present study was carried out to examine the protective effects of magnesium and pyridoxine on sodium nitrite-induced oxidative stress and hepatotoxicity in albino rats. Forty male rats were divided into five groups: (1) normal control group, (2) sodium nitrite treated group (50mg/Kg b.wt. daily), (3) magnesium treated group (150 mg/Kg b.wt.), (4) sodium nitrite and pyridoxine (300 mg/Kg b.wt.) treated group and (5) sodium nitrite, magnesium and pyridoxine treated group. At the end of 4 weeks - experimental period, animals were sacrificed to obtain blood and liver samples for hematological, biochemical and histopathological investigations. The results revealed that sodium nitrite induced a significant increase in malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) concentrations and induced a significant decrease in glutathione peroxidase (GPX) and reduced glutathione (GSH) as compared to control. Elevated hemoglobin concentration, erythrocyte number and liver injury were also observed. On the other hand, magnesium and pyridoxine treatments caused decreased levels of MDA, AST, ALT, GGT and increased GPX and GSH concentrations. They also lowered liver tissue damage and cellular alterations enhanced by exposure to sodium nitrite, a process which was more pronounced in rats exposed to both magnesium and pyridoxine. It could be concluded that co-treatment of magnesium and pyridoxine reduces oxidative stress parameters suggesting their potential antioxidant activity.

Key words: Oxidative stress, antioxidants, sodium nitrite, Magnesium, Pyridoxine.

Introduction

Oxidative stress (OS) is an imbalance between free radical production and antioxidant defense. Evidence from experimental and clinical studies suggests a major role of OS increasing levels in initiation and progression of many diseases by triggering a number of potentially damaging biochemical reactions. Oxidative reactions contribute too many consequences of aging cardiovascular disease, pulmonary diseases, diabetes, neurodegenerative disorders, and cancer (Ciriolo *et al.,* 1997) .Oxidative stress is caused by overload of oxidants, in particular reactive oxygen species (ROS) which tend to disrupt cell structures and functions maintained and mediated by critical redox balance (Salama *et al.,* 2016) .

There are several sources by which the ROS are generated. Reactive oxygen species come either from endogenous sources as by-products of normal and essential metabolic reactions and detoxification reactions in liver or from exogenous sources including environmental pollutants and toxicants such as exposure to radiation, infection, chemical solvents, food containing chemicals and food additives (Al-Gubory et al., 2010) . Nitrates and nitrites have been reported to induce free radical generation in vivo (Kashko *et al.,* 1993). Reactive nitrogen species are produced by exposure to nitrite is considered one of the most important causes of carcinogenesis through its reaction with body tissues and triggering lipid peroxidation, DNA lesions, enzyme inactivation and damage of different organs (Ismaeil *et al.,* 2007).

Sodium nitrite has been used as color fixative and preservative of meat and fish products (Aboulgasem *et al.,* 2013). It is also used as vasodilator, bronchial dilator, intestinal relaxant and antidote for cyanide poisoning (Gluhcheva *et al.,* 2012). Industrialization and unchecked use of nitrate/nitrite salts for various purposes has increased human exposure to high levels of sodium nitrite

Corresponding Author: Diaa B. Al-Azhary, Department of Zoology, Faculty of Science, Minia University, El Minia, Egypt. E-mail: diaa_elazhary@yahoo.com

which can act as a pro-oxidant and pro-carcinogen (Ansari *et al.,* 2015). Sodium nitrite toxic effect are documented in mammalians, including impairment of reproductive function, hepatotoxicity, methemoglobinemia, deregulation of inflammatory responses, tissue injury, growth retardation, and endocrine dysfunction. The harmful effect of sodium nitrite derives from the reaction of nitrites with amines to produce nitrosamines and with amides to produce nitrosamides. It exerts its effect by generation of free radicals that impair oxidant / antioxidant balance (Hassan *et al.,* 2009). It has been reported that while acute poisoning usually occurs following intake of large doses of nitrates and nitrites, chronic poisoning occurs also as a result of intake of small toxic doses over long periods Ansari *et al.,* 2015).

Magnesium plays an important role in the activities of various enzymes, possesses antioxidant properties, scavenging free radicals by affecting the rate of spontaneous dismutation of the superoxide ion and reduction of lipid peroxides (Hans *et al.,* 2003). Magnesium is required at a number of steps during the synthesis of nucleic acids and also required by enzymes participating in carbohydrates and lipids synthesis. Glutathione, an important antioxidant requires magnesium for its synthesis (Bhutto *et al.*, 2005). Also, Pyridoxine (vitamin B6) is an enzymatic co-factor required for many biochemical reactions. These are related to amino acid biosynthesis and degradation, sugar, fatty acid and nucleic acids metabolism. In addition, pyridoxine may also have a crucial role in antioxidant mechanisms (Hsu *et al.,* 2015). Therefore, the purpose of the present study was to evaluate the beneficial effects of magnesium and pyridoxine on sodium nitrite –induced toxicity and oxidative stress in rats.

Materials and Methods

Experimental Animals:
Forty male albino rats (weighing 170 - 200 gm) were housed in isolated polypropylene cages, kept under good ventilation, room temperature, received a balanced diet (commercial rodent pellets) and water under good hygienic laboratory conditions. They were maintained for two weeks as an acclimation period before the beginning of the experiment.

Chemicals and reagents:
Sodium nitrite ($NaNO_2$), magnesium sulphate ($Mg\ SO_4$) and pyridoxine (pyridoxine hydrochloride) were purchased from Oxford Company, Cairo, Egypt. Freshly prepared solutions were applied to be used for treatments at dose concentrations of 50 mg $NaNO_2$/kg body wt. (Gluhcheva *et al.,* 2012), 150 mg $MgSO_4$/kg body wt. (Abayomi *et al.,* 2011) and 300 mg pyridoxine /kg body wt. (Kuypers and Hoane, 2010).

Experimental design:
Forty male albino rats were divided into five groups. The first group served as a control group. Rats of the second group received only intraperitoneal injection of $NaNO_2$. The third group animals were injected with both $NaNO_2$ and $MgSO_4$.The fourth group received $NaNO_2$ and pyridoxine. The fifth group received $NaNO_2$, $MgSO_4$ and pyridoxine. While $NaNO_2$ were daily injected, both $MgSO_4$ and pyridoxine were injected each alternative day. At the end of experimental period (4 weeks), rats were sacrificed under mild diethyl ether anesthesia 24 hr after cessation of treatment. Blood samples were obtained in tubes containing EDTA as anti-coagulant to be used for hematological investigations while other samples were centrifuged at 4000 r.p.m for 15 minutes to get plasma for different biochemical assays. Moreover, samples of liver were taken for preparation of tissue homogenates and others were fixed in 10% formalin and subsequently processed for histopathological examinations.

Preparation of tissue homogenate:
Liver tissue was perfused with a phosphate buffered saline solution (pH7.4), homogenized (one gram of the tissue in 5 ml cold buffer) and centrifuged at 4000 r.p.m for 15 minutes. The supernatant was removed and stored on at -80°C until used for biochemical assay.

Hematological measurements:

Hematological measurements were performed in blood samples using hematological analyzer (ERMA PCE-210 N). Hematological evaluation included red blood cells count (RBC), hemoglobin concentration, hematocrit value or packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), total and differential white blood cells counts and platelet count (PLT).

Biochemical assays:

Plasma and liver tissue ALT and AST were determined according to Henry *et.al*. (1960). Alkaline phosphatase was determined according to Tietz *et al.* 1993). Gamma-glutamyltransferase (γ-GT) was determined according to Persijn and Szasz (1974) using reagent kits purchased from Egyptian Company for Biotechnology. Determination of lipid peroxidation level was based on the method of Satoh (1978) and Ohkawa *et al.* (1979). Analysis of glutathione peroxidase level was based on the method of Paglia and Valentine (1967). Determination of glutathione reduced level was were performed according to the method of Beutier *et al*. (1963) using Bio-diagnostic kits.

Histological investigations.

Formalin fixed liver tissues were dehydrated in different grades of alcohol, embedded in paraffin, sectioned with microtome at 5μ thickness and finally stained with hematoxylin and eosin (H&E) according to Bancroft and Gamble, (2008).

Statistical analysis:

The data was analyzed using the statistical package for social science program (S.P.S.S.). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by post hoc. The results were expressed as means ±SD and $p<0.05$ was considered to be statistically significant.

Results

As shown in table (1) and figure (1), sodium nitrite treatment caused significant increases in hemoglobin concentration, hematocrit value and insignificant increase in RBCs count as compared to the control groups. Non-significant decrease in MCV and MCH and insignificant increase in MCHC were also observed. Significant reduction in total white blood cells count and percentage of neutrophils and significant rise in lymphocytes as compared to control were recorded. Moreover, insignificant decrease in platelet number of $NaNO_2$ treated group compared to control was noticed.

Table 1: Effect of sodium nitrite, magnesium sulfate and pyridoxine hydrochloride on hematological parameters.

Groups / Parameters	Control	NaNO$_2$	NaNO$_2$ & MgSO$_4$	NaNO$_2$ & Pyridoxine	NaNO$_2$ & MgSO$_4$ & Pyridoxine
Hemoglobin (mg/dl)	11.75±1.60	14.45±1.55 #	15.36±1.22 #	15.84±1.26 #	15.58±1.05 #
RBCs (106/cum)	7.29±1.08	8.28±0.79	8.23±1.50	8.88±0.73 #	8.89±0.87 #
Hematocrit (%)	39.24±7.06	48±4.90 #	46.64±8.23 #	50.36±4.40 #	48.19±6.54 #
MCV (fl)	58±11.53	55.88±2.47	56.63±1.19	56.88±3.27	53.75±1.39 #
MCH (Pg.)	18.81±5.04	17.1±0.76	19.1±3.28	17.74±0.76	17.16±0.26
MCHC (%)	29.75±3	30.64±0.80	33.73±5.48 #*	31.48±0.94	32.31±0.80 #
Platelets (103/cu.mm)	468.25±123.88	387.75±185.58	426.63±173.84	513±214.07*	614.25±154.57 *
WBCs (103/cu.mm)	8.48±1.89	5.14±1.23 #	6.60±2.10#	7.16±1.82*	8.18±1.36 *
Lymphocyte %	60.63±16.21	72.5±9.26 #	84.25±6.09 #*	77.63±6.21 #	75±5.55 #
Monocyte %	4.25±1.16	4.75±1.39	5±1.85	5.63±3.70	5±1.60
Eosinophil %	1.5±0.76	2.63±1.30	2.25±1.4	1.75±1.04	2.88±1.46 #
Basophil %	0.25±0.46	0.13±0.35	0±0	0.13±0.35	0.63±0.52 *
Neutrophil%	34.38±19.12	20±10.70 #	7.75±3.77 #*	14.75±6.92 #	15.25±6.30 #

*Values are expressed as mean ±SD. # denotes significant value vs. control group and * denotes value vs. NaNO$_2$ -treated group. Values are considered significant at P<0.05.*
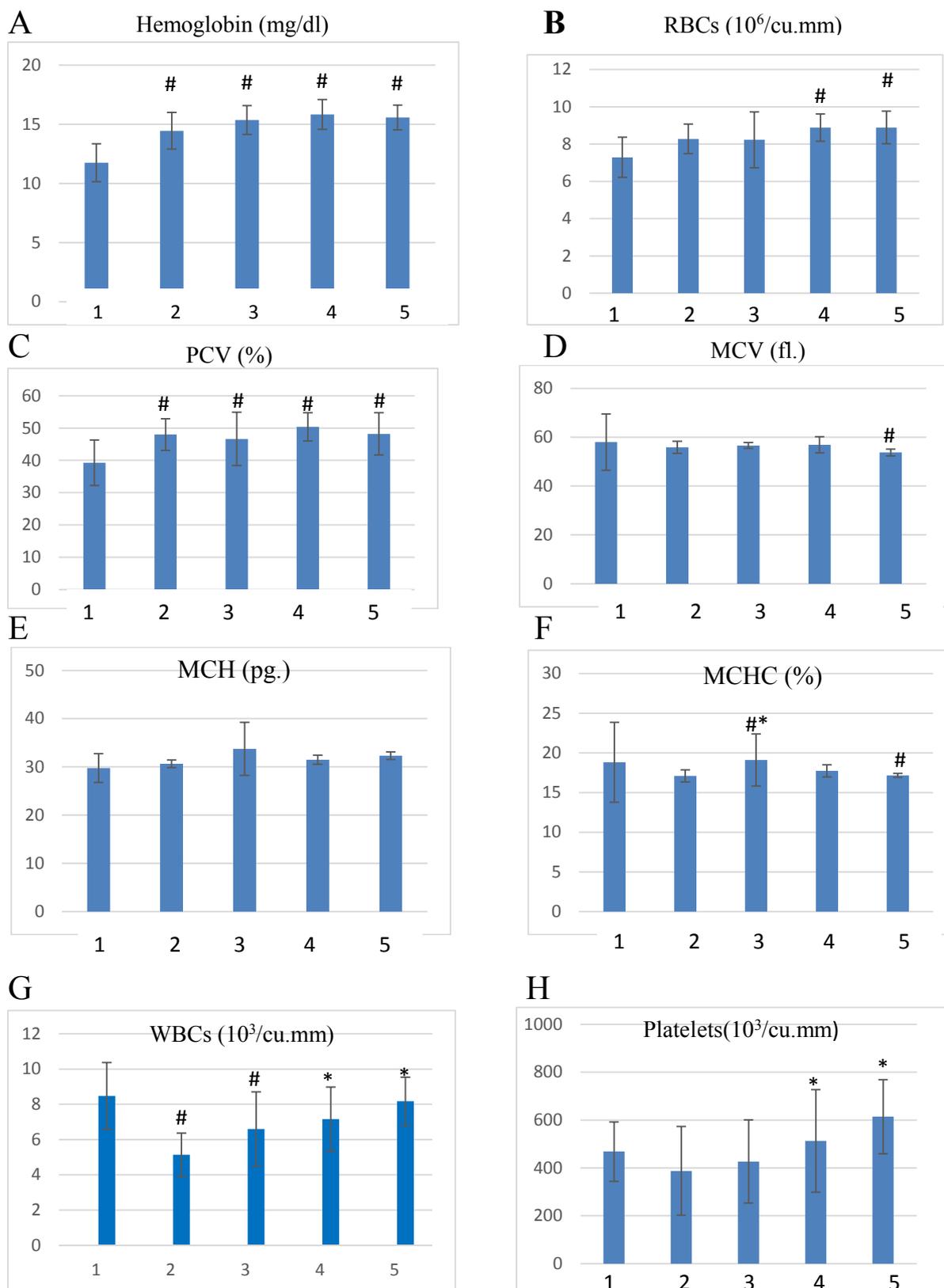
Fig. 1: (A) Hemoglobin concentration (mg/dl), (B) RBCs (106/cu.mm), (C) PCV (%), (D) MCV (fl.),(E) MCH (pg.), (F) MCHC (%),(H) platelets (103/cu.mm) and (G) WBCs (103/cu.mm) in (1) control group, (2) NaNO2, (3) NaNO2& MgSO4, (4) NaNO2& pyridoxine (5) NaNO2 & MgSO4& pyridoxine treated groups.. # denotes significant value vs. control group and * indicates denotes value vs. NaNO$_2$ treated group.Values were considered significant at P<0.05.

On the other hand, rats treated with NaNo$_2$ in addition to magnesium or /and pyridoxine showed adverse changes of blood indices as compared to control or NaNO$_2$ group. A significant increase of hemoglobin concentration and hematocrit value was observed in all treated groups. This was also reflected on the significant increase of RBCs count in different groups except magnesium treated group which showed insignificant increase in RBCs count as compared to control group. When compared to NaNO$_2$ group, the changes of the above parameters were not statistically significant. No significant change was found in MCH in all groups, but a significant decrease in MCV was observed in the animal group receiving all treatments. MCHC raised significantly in groups receiving magnesium either alone or combined with pyridoxine treatment. The drop of WBCs and platelet count noticed in NaNO$_2$ group was recovered particularly in animal groups receiving pyridoxine (Table.1).

Data of table (2) and figure (2) showed highly significant increase (P<0.001) in plasma MAD level in NaNO$_2$ group as compared to the control group, and high significant decrease in its level in magnesium and/or pyridoxine treated groups as compared to NaNO$_2$ group. Moreover, the results demonstrated a highly significant increase in GGT, ALP, AST and ALT levels in sodium nitrite treated group compared to control group. On the other hand, plasma GGT, ALP AST activities decreased significantly in magnesium and/or pyridoxine treated groups as compared to NaNO$_2$ group. Insignificant decrease of ALT level was observed in magnesium + NaNO$_2$ group and pyridoxine + NaNO$_2$ group but highly significant decrease in magnesium + pyridoxine + NaNO$_2$ compared to sodium nitrite treated group and became within the normal range of control group (Table 2 & Figure 2).

Table 2: Effect of sodium nitrite, magnesium sulfate and pyridoxine hydrochloride on the plasma levels of some biochemical parameters.

| Group Parameters	Control	NaNO$_2$	NaNO$_2$ & MgSO$_4$	NaNO$_2$& pyridoxine	NaNO$_2$& MgSO$_4$& pyridoxine
MDA (nmol/ml)	7.15±1.44	23.29±5.06 #	19.34±5.56 #*	8.95±2.29 *	9.06±1.91 *
GGT (U/L)	5.69±1.28	11.75±2.91 #	8.05±2.11*	8.68±3.32#*	7.43±1.54 *
ALP (U/L)	8.3±3.74	19.55±5.05 #	11.01±5.37 *	8.88±3.16 *	8.49±3.49 *
AST (U/L)	22.63±5.88	41.75±7.61 #	26.13±8.83*	27.5±20.75*	30.12±15.56
ALT (U/L)	15.25±3.24	23 ±3.89 #	18.5±3.74	18.5±5.24	16.4±5.62*

*Values are expressed as mean ±SD. # denotes significant value vs. control group and * denotes value vs. NaNO$_2$ -treated group. Values are considered significant at P<0.05*

Examination of liver tissue oxidative stress markers like MAD, GPX and GSH revealed that sodium nitrite caused significant increase in MAD level and significant decrease in GPX and GSH as compared to control group. On the other hand, treatment with magnesium and/or pyridoxine caused significant reduction in MAD level significant increase in GPX and insignificant increase in GSH as compared to NaNO$_2$ group, but still lower than control group (Table 3 & Figure 2).

Table 3: Effect of sodium nitrite, magnesium sulfate and pyridoxine hydrochloride on oxidative markers in liver tissue.

Group Parameter	Control group	NaNO2	NaNO$_2$ & MgSO$_4$	NaNO$_2$& pyridoxine	NaNO$_2$& MgSO$_4$& pyridoxine
MDA (nmol/g)	0.76±0.14	2.63±0.48 #	1.27±0.66 *	1.81±0.83 #*	1.59±0.41#*
GPX (U/gt)	61.60±6	36.48±20.19 #	53.50±12.85*	56.74±11.88 *	56.74±9.65*
GSH (mg/g)	14.11±0.84	10.11±2.10 #	11.36±3.40 #	10.52±2.85 #	11.60±3.01

*Values are expressed as mean ±SD. # denotes significant value vs. control group and * denotes value vs. NaNO$_2$ -treated group. Values are considered significant at P<0.05.*
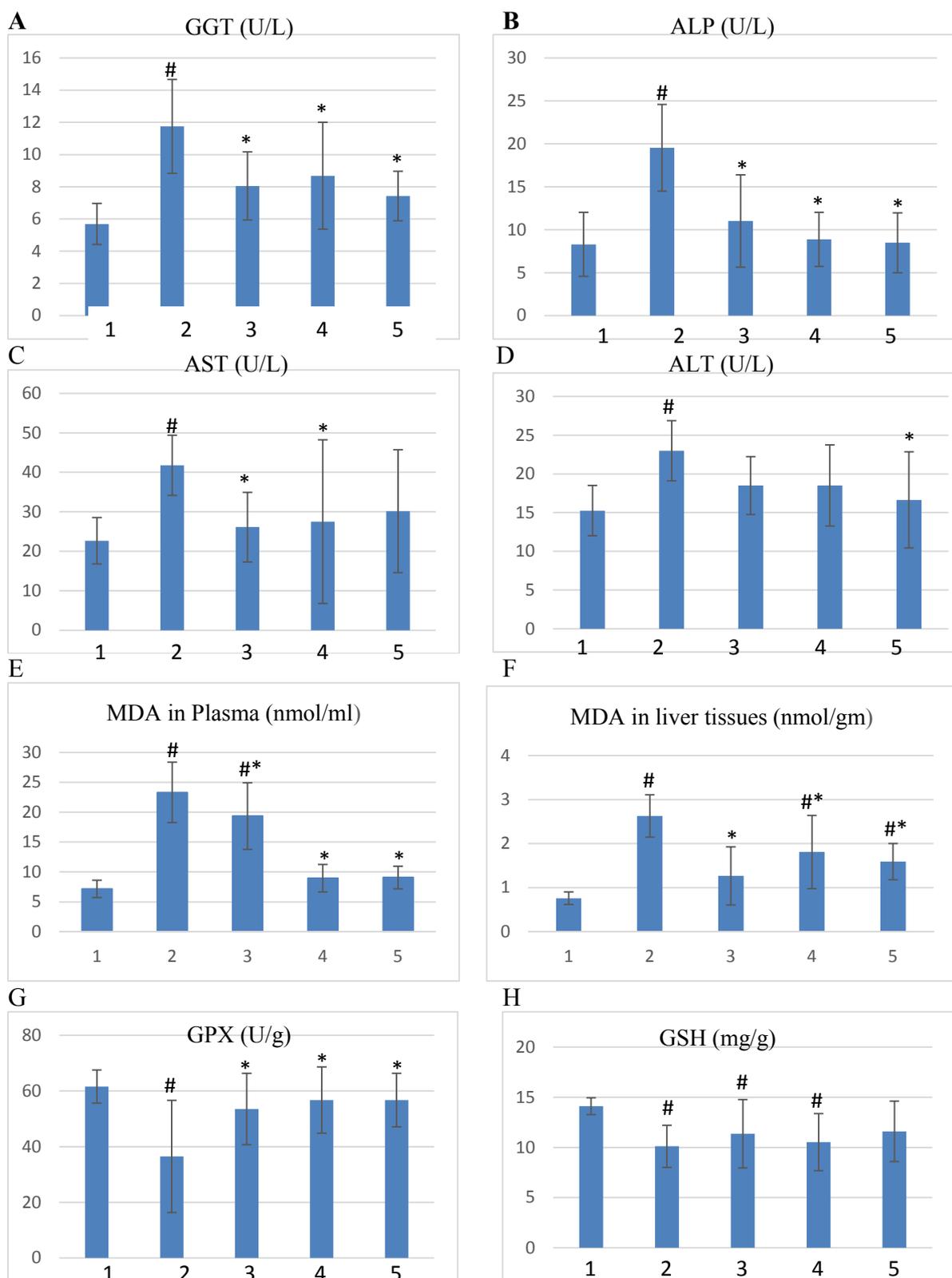
Fig. 2: (A) Levels of plasma gamma-glutamyletransferase (U/L), (B) plasma alkaline phosphatase (U/L), (C) aspartate amino transferase (U/L), (D) alanine amino transferase activity (U/L), (E) plasma malondialdehyde (nmol/ml), (F) malondialdehyde in liver tissue (nmol/gm) (G) liver glutathione peroxidase (U/g) and (H) liver reduced glutathione (mg/g) in (1) control group, (2) $NaNO_2$, (3) $NaNO_2$& $MgSO_4$, (4) $NaNO_2$& pyridoxine (5) $NaNO_2$ & $MgSO_4$& pyridoxine treated groups. # denotes significant value vs. control group and * indicates denotes significant value vs. $NaNO_2$ treated group. Values were considered significant at P<0.05.

Histopathological investigation showed that liver of sodium nitrite –treated group caused different histological and cellular alterations characterized by marked change in its characteristic architecture, sinusoidal dilatation, disrupted endothelial lining of hepatic central vein and hepatocellular damages. This was also associated with the appearance of vacuolar and granular cytoplasm and nuclear fragmentation or pyknosis in hepatocytes. Moreover, some disintegrated or completely damaged hepatocytes appeared in some areas indicating liver tissue necrosis (Fig 3 B&C). The influence of treatment by magnesium and pyridoxine, either alone or in combination, in amelioration of cellular alterations induced by sodium nitrite is shown in figures (Fig. 3 D, E&F).
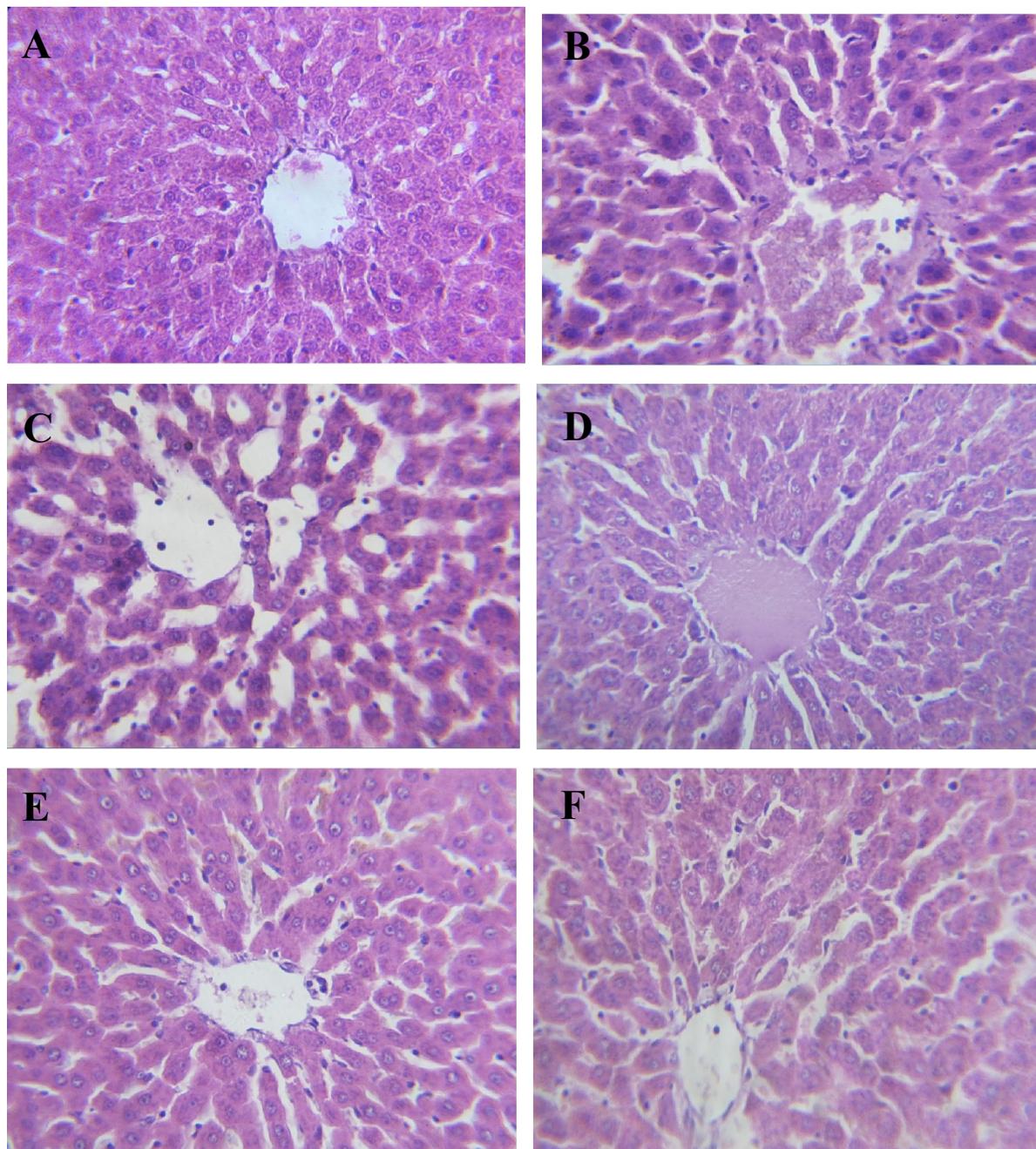
Fig. 3: liver sections of (A) control group showing normal architecture of hepatocyte strands surrounding central vein, (B) $NaNO_2$ treated group showing sinusoidal dilatation, disrupted endothelial lining of hepatic central vein, dark stained pyknotic and disintegrated hepatocytic nuclei, (C) $NaNO_2$ group showing more sinusoidal dilatation, and vacuolated, injured or completely damaged hepatocytes , (D) $NaNO_2$ & $MgSO_4$ showing less degradation and rearrangement of liver architecture, (E) $NaNO_2$ & pyridoxine showing approximate normal hepatocytic features and less sinusoidal dialitation and (F) $NaNO_2$ & $MgSO_4$ & pyridoxine group with less cell injuries and necrotic areas (stained with H&E, X 40).

This effect was represented by less degradation in hepatocellular compartments and preserving liver architecture in NaNO$_2$ & MgSO$_4$ group (Fig. 3 D), approximate normal hepatocyte features and less sinusoidal dialitation and disruption in NaNO$_2$ & pyridoxine group (Fig. 3 E) and less cell injuries and necrotic areas in NaNO$_2$ & MgSO$_4$ & pyridoxine treated group (Fig. 3 F) as compared to sodium nitrite treated group.

Discussion

The results of the present study revealed that NaNO$_2$ induced some adverse effects on the hematological parameters. It caused an increase in erythrocyte number, hemoglobin concentration and hematocrit value as compared to control. Although this is in contrary to data obtained from other studies, it may be in agreement with that of Glucheva *et al.* (2012) who suggested that the increase may be through the erythropoiesis and via the release of RBC from the hematopoietic tissues by β-adrenergic action.

Nitrites may cause alteration of thyroid gland function and elevation the rate of catabolic transformations in tissues of animals which is reflected in oxygen demand and increasing methemoglobin formation leading to polycythemia as a physiological response to tissue hypoxia and increasing production of erythropoietin (Bailer *et al.,* 2000). RBCs and MCHC increase was also observed as a result of tissue anoxia or increase of body demand for oxygen (Hoffbrand *et al.,* 2004).

On the other hand, the significant decrease of WBCs in NaNO$_2$ treated animals in the present study may be due to the failure of hematopoietic tissue to produce new WBCs as suggested by Glucheva et *al.*(2012). Also, decreased platelets number might be a result of a selective megakaryocyte depression as previously suggested by Hoffbrand *et al.* (2004) . However, other studies conducted in rats have also provided evidence for adverse effect of NaNO$_2$ on the hematological parameters. The effect seems to be dependent on dose concentration, duration, tolerance and adaptation to NaNO$_2$ toxicity as reported by Bensoltane *et al.* (2006).

Potential effects of NaNO$_2$ toxicity on liver and plasma biochemical parameters were clearly observed in the present study. The significant increase in the marker enzymes such as ALT, AST GGT and ALP in sodium nitrite treated rats in the present study indicates hepatic disorders and liver tissue damage as also revealed by microscopic investigation. Previous investigations suggested that increasing levels of such enzymes in nitrite treated rats could be attributed to the toxic effect of formed nitroso-compounds causing hepatic necrosis or it may be due to anemia and methaemoglobinemia inducing hypoxic injury to hepatocytes that consequently cause enzyme leakage (Aboulgasem *et al.,* 2013; Hassan *et al.,* 2009).

GGT is an enzyme responsible for the metabolism of extracellular GSH, promoting GSH hydrolysis and facilitating its reuse for intracellular synthesis so increase in GGT causes decrease in GSH. GSH is a substrate for GPx and glutathione S-transferases (GST), playing a key role in protecting against oxidative stress and detoxification/metabolism of endogenous and exogenous compounds (Avelar *et al.,* 2015).

Data of the present study showed also that malondialdehyde (MDA), the major product of lipid peroxidation and high oxidative stress indicator, increased significantly in plasma and liver tissue in NaNO$_2$ treated rats. This may be in concordance with the results of previous studies (Ismaeil *et al.,* 2007; Krishnamoorthy and Sangeetha, 2008). This indicates increased activity of lipid peroxidation, which was suggested to be increased by nitrosamines and free radical formation as a result of reacting sodium nitrite and other food additives (Hassan *et al.,* 2009; Salama *et al.,* 2013).

Data obtained from this study showed raised levels of some hematological parameters in groups of animals treated with magnesium and/or pyridoxine as compared to control group. Although mechanisms of interaction are not completely understood, some studies indicated that magnesium deficient diet leads to significant decreases in the concentration of red blood cells (RBC) and hemoglobin (Sanchez-Morito *et al.,* 2000) and that pyridoxine improves MCV and HGB level of rats feed protein deficiency diet (Lewicka *et al.,* 2012).

Our results indicated also that magnesium and pyridoxine caused clear improvement in liver functions represented by significant reduction of liver enzymes and lipid peroxidation markers as compared to sodium nitrite treated group. Also they lowered oxidative stress via increasing reduced glutathione GSH and antioxidant enzyme GPX. Magnesium possesses antioxidant properties,

scavenging oxygen radicals by affecting the rate of spontaneous dismutation of the superoxide ion (Guerrero-Romero and Rodrı́guez-Mora, 2005). Glutathione, requires magnesium for its synthesis (Bhutto *et al.,* 2005). So, antioxidant effects of magnesium in this study may coincide with that of Scibior *et al.* (2013) and Ghaffarian-Bahram, *et al.* (2014) who stated its beneficial role in limiting lipid peroxidation and antiradical activity. This protective effect of magnesium was further confirmed by its amelioration of liver tissue as shown by microscopic investigation in the present study.

Pyridoxine has been reported to inhibit oxidative stress because of its ability to quench reactive oxygen species (Hashmi *et al.,* 2011). Moreover, Anand (2005) found that pyridoxine increased GPX activity by enhancing the incorporation of selenium in GPX in liver and GSH by acting as a cofactor in cysteine synthesis. It has been proposed that the presence of -OH, -NH2 and pyridine in pyridoxine can react with the peroxy radicals and thereby lowering oxidative stress (Hsu *et al.,* 2015 ;Anand (2005).

Histopathological examination of liver tissue of different treated groups reflected the alterations of biochemical parameters and confirmed the potential protective effect of magnesium and pyridoxine against NaNO$_2$ – induced liver injury. Liver of sodium nitrite - treated group showed different histological and cellular alterations characterized by marked change in its characteristic architecture, sinusoidal dilatation, congestion, disrupted endothelial lining of hepatic central vein and hepatocellular damages. This was also associated with the appearance of vacuolar and granular cytoplasm and nuclear fragmentation or pyknosis in hepatocytes. Moreover, some swollen disintegrated or completely damaged hepatocytes appeared in some areas indicating liver tissue necrosis (Fig.3B, C). These observations were consistent with that of previous study Hassan *et al.* (2009) and Salama *et al.* (2013). The influence of treatment by magnesium and pyridoxine, either alone or in combination, in attenuating or preventing cellular alterations induced by sodium nitrite are shown in figures (Fig.3D, E, F). Generally, amelioration observed on the level of histopathology of liver tissue and that of biochemical data was more obvious in animals exposed to both treatments.

Conclusion:

The present study provides evidence that combination of magnesium and pyridoxine administration ameliorates oxidative stress as well as hepatotoxicity in rats exposed to repeated doses of sodium nitrite.

References

Abayomi, A.L., E.O.Adewoye, S.B. Olaleye, and A.T. Salami, 2011. Effect of Magnesium pre-treatment on Alloxan induced hyperglycemia in rats. African health science, 11(1), 79 – 84.

Aboulgasem, G., A.Azab, and M.Almaky, 2013. Sodium nitrite induced biochemical alterations in the blood serum and its amelioration by aqueous extract of libyan propolis in guinea pigs. Int. J. of Science and Research, 4(8), 1040-1048.

Al-Gubory, K.H., P.Fowler, and C.Garrel, 2010. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int. J. Biochem. Cell Biol., 42(10), 1634-1650.

Anand, S.S., 2005. Protective effect of vitamin B6 in chromium-induced oxidative stress in liver. J. Appl. Toxicol. , 25, 440-443.

Ansari, F.A., S.N.Ali and R.Mahmood, 2015. Sodium nitrite-induced oxidative stress causes membrane damage, protein oxidation, lipid peroxidation and alters major metabolic pathways in human erythrocytes. Toxicology in Vitro, 29, 1878-1886.

Avelar, T. M. T., A. S.Storch, L. A. Castro, G. V. M. M. Azevedo, L.Ferraz and P. F. Lopes, 2015. Oxidative stress in the pathophysiology of metabolicsyndrome: which mechanisms are involved? J. Bras. Patol. Med. Lab., 51(4), 231-239.

Bailer, A.J., J.S. Bus, L.A. Chatman, J.M. Cullen, H.Davis, N.R.Drinkwater, S.M. Fischer, S.S. Hecht, M. Medinsky and J.Russo, 2000. National toxicology program board of scientific counselors technical reports review subcommittee. Online (https:// ntp.niehs.nih. gov/ntp/htdocs/lt_ rpts/tr495.pdf).

Bancroft, J. and M.Gamble, 2008. Theory and practice of histological techniques (6th ed), Churchill

Livingstone.

Bensoltane, D., M.Kammerer, M. Larhantec, N. Pilet and C. Thorin, 2006. Nitrate and nitrite concentrations in rabbit saliva Comparison with rat saliva. Environ. Toxicology. Pharmacol. , 23(1), 132-134.

Beutier, E., O. Duron and B. M. Kefly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882-888.

Bhutto, A., A.Mastoi, A. M. Sikander and G. A. Qureshi, 2005. Magnesium and its essential role in health. J. of Liaquat University of Medical and Health Sciences, 33-35.

Ciriolo, M. R., A. T.Palamara, S.Incerpi, E.Lafavia, M. C.Bue`, P. D. Vito, E. Garaci and G. Rotili, 1997. Loss of GSH, oxidative Stress and decrease of intracellular pH as sequential steps in viral infection. The J. of biological chemistry, 272 (5), 2700–2708.

Ghaffarian-Bahraman, A., I.Shahroozian, and A.Jafari, 2014. Protective effect of Magnesium and Selenium on Cadmium toxicity in the isolated perfused rat liver system. Acta. Medica. Iranica, 52(12), 872-878.

Glucheva, Y., I.Ivanovo, E.Petrova, E.Pavlov and I.Vladov, 2012. Sodium nitrite –induced hematological and hemorheological changes in rats. Series on biomechanics, 27 (3-4), 53-58.

Guerrero-Romero, F. and M. Rodrı´guez-Mora´n, 2005. Complementary therapies for diabetes: The Case for chromium, magnesium, and antioxidants. Archives of Medical Research, 36, 250–257.

Hans, C. P., D. P. Chaudhary, and D. D. Bansal, 2003. Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. Magnesium Research, 16 (1), 9-13.

Hashmi, A., L.Wang, K. Juneja, Y.Ye, Y.Zhao, and L.J. Ming, 2011. Vitamin B6 inhibits oxidative stress caused by Alzheimer's disease-related Cu (II)-ß-amyloid complexes-cooperative action of phosphor-moiety. Bioorg. Med. Chem. Lett., 21, 6430-6432.

Hassan, H. A., S. M.El-Agmy, G. L. Guar, A. Fernando, M.H .G. Raj and A.Quhtit, 2009. In vivo evidence of hepato-and reno-protective effect of garlic oil against sodium nitrite –induced oxidative stress. Int. J. of Biological Sciences, 5(3), 249-255.

Henry, R. J., N.Chiamori, O. J.Golub and S.Berkman, 1960. Revised spectrophotometric methods for the determination of glutamic oxalacetic transaminase, glutamic pyruvic transaminase and lactic acid dehydrogenase. Amer. J. clin. Path., 34, 381-398.

Hoffbrand, A.V., J.E. Petit, and P.A.H. Moss, 2004. Essential Haematology. Blackwell, Oxford, 252-253.

Hsu, C., C.Cheng, C. Hsu, W.Lee, S. Huang and Y.Huang, 2015. Role of vitamin B6 status on antioxidant defenses, glutathione, and related enzyme activities in mice with homocysteine-induced oxidative stress. Food and Nutrition Research, 59, 1-7.

Ismaeil, N., A.A. Osman, E.Ali, L.Rashed and M.Saleh, 2007. The potential role of mesenchymal stem cells in a hypoxia model induced by sodium nitrite in testes of male albino rats. Biomedical Research and Therapy, 4(2), 1128-1146.

Kashko, M., A.Khokha, S.Antsulevich, N. Doroshkevich, and P.Voronov, 1993. Influence of ethanol and ethanol-induced lipid peroxidation on the steroidogenic activity of testicles. Ukr- Biochim-Zh, 65, 89-94.

Krishnamoorthy, P. and M.Sangeetha, 2008. Hepatoprotective effect of vitamin C on sodium nitrite – induced lipid peroxidation in albino rats. Indian Journal of Biochemitry and Biophysics, 45, 206-208.

Kuypers, N. and M.Hoane, 2010. Pyridoxine administration improves behavioral and anatomical outcome after unilateral contusion injury in the rat. Journal of Neurotrauma, 27, 1275-1282.

Lewicka, A., I. S.Lewick, R.Zdanowski, P.Rutkowski, M.Turkowska, A. Klos, and J. Bertrandt, 2012. The effect of vitamin B6 supplementation of protein deficiency diet on hematological parameters in the blood of rats subjected/non subjected to physical exertion. Centr. Eur. J. Immunol., 37 (3), 187-192.

Ohkawa, H., N. Ohishi and K.Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem, 95, 351-358.

Paglia, D. E. and W. N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Cm. Med., 70,158-169.

Persijn, J.P. and G.Szasz, 1974. A Kinetic colorimetric method for determination of gamma-glutamyl transpeptidase in serum. Zeitschirft fur kliniische chemie und kliniische bio chemie, 12, 228-

232.

Sadowska, J. and M.Kuchlewska, 2011. Effect of diet composition and mixture of selected food additives on the erythrocytic system and iron metabolism in peripheral blood of male rats. Acta. Sci. Pol., Technol. Aliment. 10(4), 497-506.

Salama, A. K., A. O.Khaled, A.S. El-Bakary and M. S. Salama, 2016. Oxidativ tress biomarkers in rats exposed to Bromoxynil. Int. J. Cur. Res. Rev., 8 (9), 1-6.

Salama, M., A.Abbas, M. Darweish, A.El-Hawwary and M. Al- Gayyar, 2013. Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. Pharmaceutical Biol., 51(11), 1435-1443.

Sanchez-Morito, N., E.Planells, P.Aranda and J.Llopis, 2000. Influence of magnesium deficiency on the bioavailability and tissue distribution of iron in the rat. J. Nutr. Biochem, 11(2), 103-108.

Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorder determined by a new colorimetric method. Clin. Chim. Acts. , 90, 37-43.

Scibior, A., D.GoBwbiowska, and I.Niedfwiecka, 2013. Magnesium can protect against Vanadium-induced lipid peroxidation in the hepatic tissue. Hindawi Publishing Corporation, Oxidative Medicine and Cellular Longevity, 2013, 1-11.

Tietz, N.W., C.A. Burtis, and P.Duncan, 1983. A reference method for measurement of alkaline phosphatase activity in human serum. Clin. Chem., 29(5), 751–756.