Original Research Article

EFFECT OF FOOD ADDITIVES (MONOSODIUM GLUTAMATE AND SODIUM NITRITE) ON SOME BIOCHEMICAL PARAMETERS IN ALBINO RATS

Eman Salah Abdel-Reheim[1], Hanan Abdel-Hameid Abdel-Hafeez[2], Basant Mohamed Mahmoud[2]* and Eman Nazeem Abd-Allah[2]
[1]Department of Zoology, Faculty of science, Beni-Suef University, Beni-Suef, Egypt
[2]Department of Chemistry, Faculty of science, Beni-Suef University, Beni-Suef, Egypt

Abstract: Food additives are substances used in food industry in order to improve the food's taste and appearance by preserving its flavor and preventing it from souring. We study the adverse effect of some food additives on the biochemical parameters in adult male albino rats by daily oral exposure of (15mg/kg body weight),(5mg/kg body weight) of Monosodium glutamate and Sodium nitrite respectively, for 4 weeks and show the alteration of the results of these biochemical parameters in serum of adult male albino rats associated with liver and kidney functions, lipid profile, cardiac enzymes and the effect of these additives on the biomarkers of the oxidative stress and antioxidant enzyme activities in tissue homogenates of liver and kidney. In comparison with the normal rats we found that body weight increased in rats treated with MSG while decreased in rats consumed $NaNO_2$; In the liver functions parameters there is an increase in GPT, GOT, ALP, total protein, Albumin, Globulin and billirubin of MSG while, in $NaNO_2$ there is an increase in GPT, GOT, ALP and billirubin activity while, there is a decrease in total protein, albumin and globulin. In our view of lipid profile there is an increased level in total cholesterol and TG concentration in MSG and decreased level of total cholesterol and TG in $NaNO_2$ while, there is a decrease in HDL concentration in both MSG and $NaNO_2$. In cardiac enzymes there is an increase in LDH enzyme in both additives and there is a decrease in CK enzyme activity in MSG while, increased in serum of $NaNO_2$. Our investigations showed an increase in kidney functions in both types of additives. In the oxidative stress observation there is an increase in LPO while, there is a decrease in CAT, GSH, and SOD activity in MSG and $NaNO_2$.

Key Words: Food Additives, Monosodium Glutamate and Sodium Nitrites

INTRODUCTION

Food additives are used widely for various purposes; including preservation, coloring and sweetening. Some food additives however, have been prohibited from use because of their toxicity (IARC, 1983). Food additives are divided into five broad categories according to their function: 1) *Taste Enhancers:* taste is comprised of the rudimentary sensations of sweet, sour, bitter, and salty, 2) *Antioxidants:* that protect oily and fatty foods from spoilage by inhibiting lipid peroxidation and preventing the disintegration of lipid-soluble vitamins, 3) *Preservatives:* enhance food safety and extend shelf-life by limiting viral, bacterial, and fungal growth. They are added to a wide variety of foods, especially those with high carbohydrate content, 4) *Stabilizers and Emulsifier:* these include lecithin, gelatins, corn starch, waxes, gums, propylene glycol, and cation scavengers such as ethylenediamine tetra acetic acid (EDTA) (Wilde *et al.,* 2004), 5) *Coloring Agents:* they are derived from multiple animal, vegetable and mineral sources (Green, 1988).

MSG improves the palatability of meals and thus influences the appetite center positively with its resultant increase in body weight (Gobatto *et al.,* 2002). L-Glutamic acid (Glu) is a widespread amino acid present in foodstuffs as the free and protein-bound form. Foods containing large amounts of free Glu (tomatoes, mushrooms and cheese) are traditionally used to obtain savoury dishes (Yamaguchi and Ninomiya, 2000).

Sodium nitrite, with the chemical formula $NaNO_2$, is a white to slightly yellowish crystalline powder. It is used as an antimicrobial agent, a preservative and a color fixative in meats and fish. In the European Union (EU) and, when labelled 'for food use', sodium nitrite may only be sold in a mixture with salt (NaCl) or a salt substitute. Sodium nitrite is known as E250. As a food additive it stabilizes the color of preserved fish and meats and also it inhibits the growth of *Clostridium botulinum,* the bacterium, which produces the botulinum toxin. Experimental studies have shown that a number of N-nitrosamines are carcinogenic in animal species, with a wide range of potencies, although the evidence on whether dietary N-nitrosamines cause cancer in humans has long been considered equivocal. In 2006, for example, IARC concluded that an industrial food is associated with an increase in the incidence of stomach cancer (Grosse *et al.,* 2006).

*Corresponding Author:
Basant Mahmoud Mohamed Morsy
Department of Zoology,
Faculty of science,
Beni-Suef University, Beni-Suef, Egypt.

Reactive nitrogen species produced by exposure to nitrate 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 (El-Wakf *et al.,* 2009). The chemistry of nitrite in cured meat is an extremely complex mixture of interactive chemical reactions involving several different reactants. Nitrite is a highly reactive compound that can function as an oxidizing, reducing or a nitrosylating agent, and can be converted to a variety of related compounds in meat including nitrous acid, nitricoxide and nitrate (Honikel, 2004).

MATERIALS AND METHOD

Experimental Animals

White male albino rats (*Rattus norvegicus*) weighting about 100 – 120 g were used as experimental animals in the present investigation. They were obtained from the animal house of National Research Institute, El-Giza, Egypt.
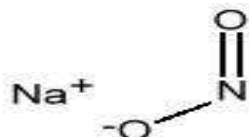
Food additives used:

Two types of food additives were used in the experiment (Monosodium glutamate and Sodium nitrite).

- **Monosodium glutamate:** white colored substance used as flavor enhancer.
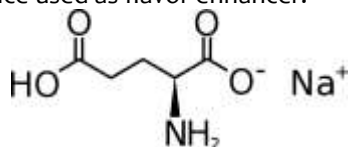
(Chemical structure of Monosodium glutamate)

- **Sodium nitrite:** white colored substance used as preservative.

(Chemical structure of Sodium nitrite)

Animal grouping and doses:

The considered rats were divided into three groups each group consists of 6 rats:

1. **The first group**: was regarded as control and orally given distilled water.

2. **The second group**: was orally injected with monosodium glutamate (*MSG*) at a dose level (15 mg/kg body weight) (Egbuonu *et al.,* 2011) that dissolves in 5ml distilled water by gastric intubation daily for 30 days.

3. **The third group**: was orally injected with sodium nitrite (*NaNO$_2$*) at a dose level (5 mg/kg body weight) (EFSA, 2009) that dissolved in 5ml distilled water by gastric intubation daily for 30 days.

All the injections were performed orally and daily between 8.00 am and 10.00a.m. By the end of the experimental period, normal and induced blood were taken and centrifuged under diethyl anesthesia. Blood samples were taken and centrifuged at 3000r.p.m. for 30 minutes. The clear, non hemolysed supernatant were quickly removed, divided into three portions for each individual animal, and kept in deep freezer at -30°C till used.

Biochemical Examination:

Lipid profile tests determination: Serum total lipids concentration was determined according to the method of Frings *et al.,* (135), Serum triglycerides concentration was determined according to the method of Fossati and Prencipe (1982), Serum cholesterol concentration was estimated according to the method of Deeg and Ziegenohrm (1983) and Serum HDL-cholesterol concentration was measured according to the method of Burstein *et al.,* (1970) and all determined using reagent kit purchased from Reactions Spin react Company (Spain).

Serum LDL-cholesterol concentration was determined according to Friendewald *et al.,* (1972) formula:
LDL-Cholesterol = total cholesterol – triglyceride/5 – HDL – cholesterol

Serum vLD-cholesterol was calculated according to Norbert (1995) formula:
vLDL-cholesterol = triglycerides / 5

Cardiac enzymes determination: The cardiac risk factors expressed by R1, The ratio of serum Total cholesterol / HDL-cholesterol ratio was calculated according to (Medic *et al.,* 2006 and R2).The ratio of serum LDL-cholesterol / HDL-cholesterol ratio was calculated according to (Hanafi*et al.,* 2008).

Creatine Kinase CK-NAC (UV-Rate) activity in serum was determined according to the method of Young, (1975) and Kachmar and Moss, (1976).Using reagent kits purchased from Stanbio Laboratory

Lactate dehydrogenase (E.C.; 1.1.1.27) activity in serum was determined according to the method of Witt and Trendelenburg (1982) and Schumann *et al.,* (2002). Using reagent kits purchased from Human Diagnostics Chemical Company (Germany).

Hepatic enzymes determination: ALT activity in liver was determined according to the kinetic method of Schumann and Klauke (2003), AST activity in liver was determined according to the kinetic method of Schumann and Klauke (2003), Serum alkaline phosphatase activity was determined according to the method of Belfield and Goldberg (1971).

Kidney function tests determination: Serum uric acid concentration was measured according to the method of Barham and Trinder (1972), Serum urea concentration was determined according to the method of Patton and Crouch (1977), Serum creatinine concentration was determined according to the method of Henry (1974) and all these parameters were determined using reagent kits obtained from Human Diagnostics Chemical Company (Germany).

The ratio of serum urea / creatinine ratio was determined as serum urea / creatinine level.

Serum total protein concentration was determined according to the method of Henry and Row (1964), Serum albumin concentration was determined according to the method of Doumas *et al.,* (1971) using kits obtained from Human Diagnostics Chemical Company (Germany).

Serum globulin concentration was determined according to the formula:

Globulin (mg/dl) = total protein (mg/dl) –albumin (mg/dl)

The ratio of serum albumin/ globulin was determined as albumin / globulin level. Total and direct Bilirubin in serum was measured by colorimetric method using kits obtained from Human Diagnostics Chemical Company (Germany).

Serum indirect billirubin concentration was calculated according to the formula:

In direct billirubin (mg/dl) = total billirubin (mg/dl) – direct billirubin (mg/dl).

Oxidative Stress and Antioxidant Enzyme determination: Glutathione level in liver and kidney homogenates were determined according to the method of Beutler *et al.,* (1963).

The SOD activity was measured in liver and kidney homogenates according to the method described by Kakkar *et al.,* (1984) based on the inhibition of the auto-oxidation of pyrogallol.

Lipid peroxidation was determined according to the method of Preuss *et al.,* (1998).

Catalase activity determined according to Cohen *et al.,* (1970) which follows the first-order kinetics as given by the equation:

$$K = \log (S_0 / S_3) \times 2.3 / t.$$

Statistical analysis

The Statistical Package for the Social Sciences (SPSS for WINDOWS, version 20.0; SPSS Inc., Chicago) was used for the statistical analysis. Results were articulated as mean ± SE and all statistical comparisons were made by means of one way ANOVA test followed by Duncan's multiple range test post hoc analysis.

Values of $P > 0.05$ were considered statistically non-significant, while values of $P < 0.05$ were considered statistically significant, values of $P < 0.01$ were considered statistically highly significant and $P < 0.001$ were considered statistically very highly significant.

RESULTS

The effect of monosodium glutamate and sodium nitrite on body weight change, biochemical parameters (ALT, AST, ALP, bill, TP, alb, glob, urea, creat, uric acid, lipid profile, LDH, CK) and oxidative stress (CAT, GSH, SOD and MDA) of liver and kidney homogenates of normal and induced rats is presented in the following figures after one month of experimental work.
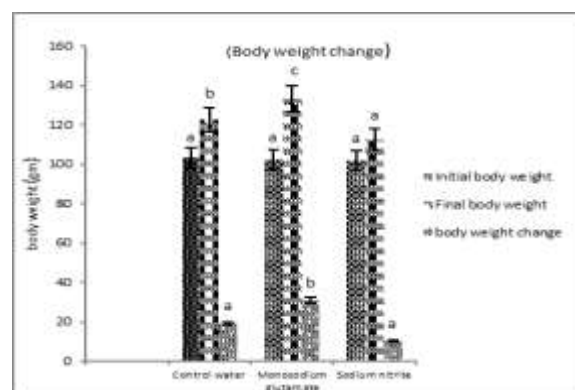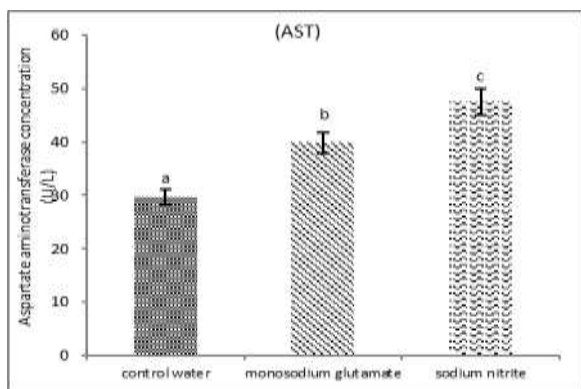

Fig. 1: Effect of food additives (MSG and NaNO₂) on body weight change of normal and treated rats.Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
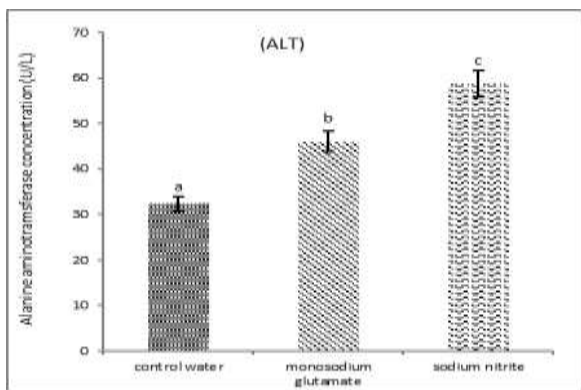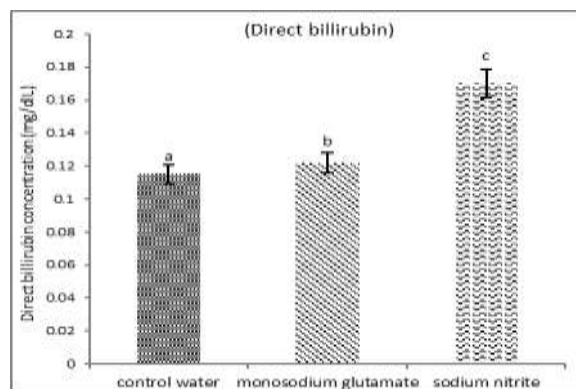
Fig. 2: Effect of food additives (MSG and NaNO$_2$) on serum alanine aminotransferase of normal and treated rats.Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
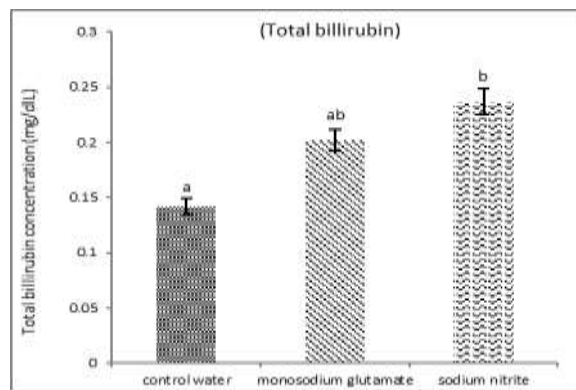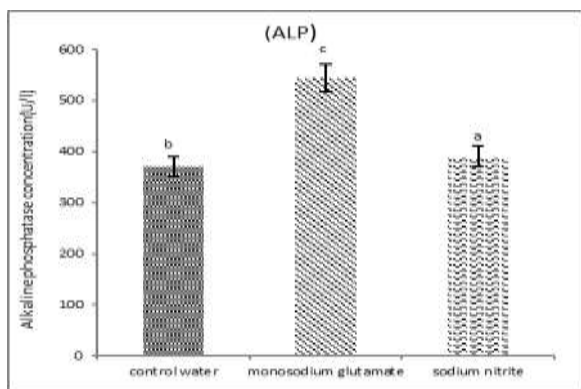

Fig. 5: Effects of food additives (MSG and NaNO$_2$) on serum total billirubin concentration of normal and treated rats.Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
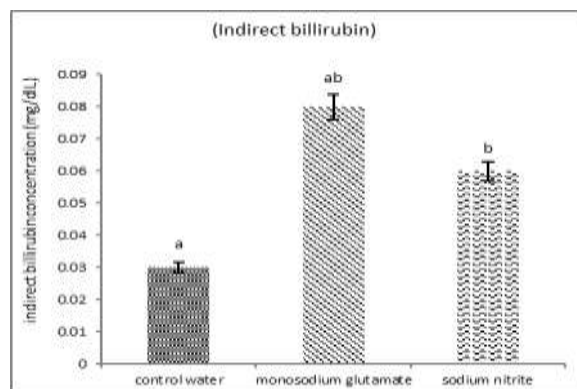

Fig. 3: Effect of food additives (MSG and NaNO$_2$) on serum aspartate aminotransferase of normal and treated rats.Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 6: Effect of food additives (MSG and NaNO$_2$) on serum direct billirubin concentration of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
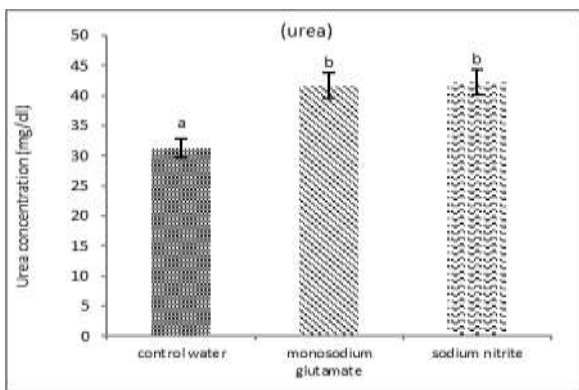

Fig. 4: Effect of food additives (MSG and NaNO$_2$) on serum alkaline phosphatase of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 7: Effect of food additives (MSG and NaNO$_2$) on serum indirect billirubin concentration of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 8: Effect of food additives (MSG and $NaNO_2$) on serum urea concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

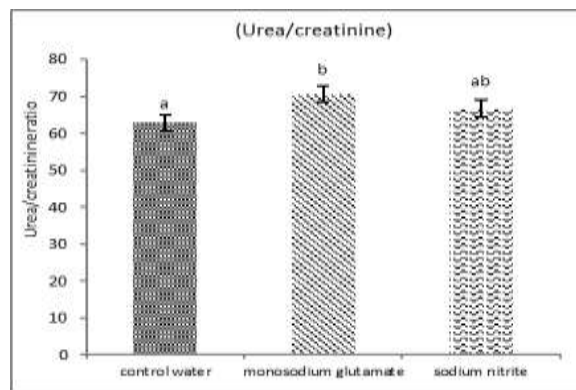

Fig. 11: Effect of food additives (MSG and $NaNO_2$) on serum urea/creatinine ratio of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
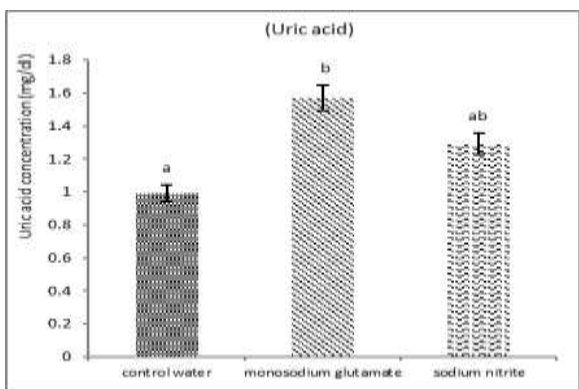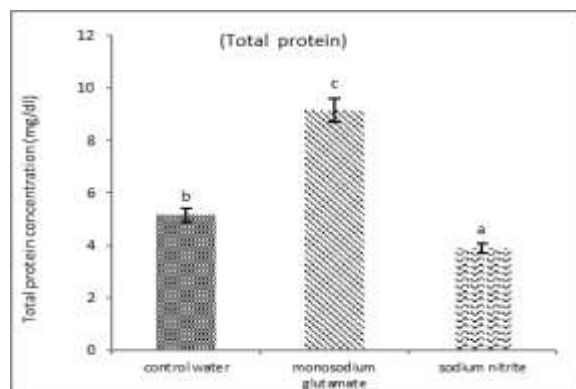

Fig. 9: Effect of food additives (MSG and $NaNO_2$) on serum uric acid of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 12: Effect of food additives (MSG and $NaNO_2$) on serum total protein concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
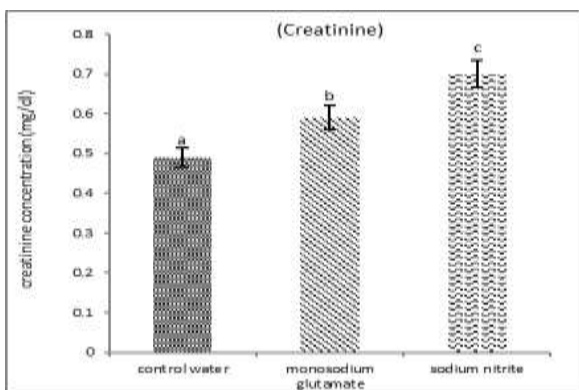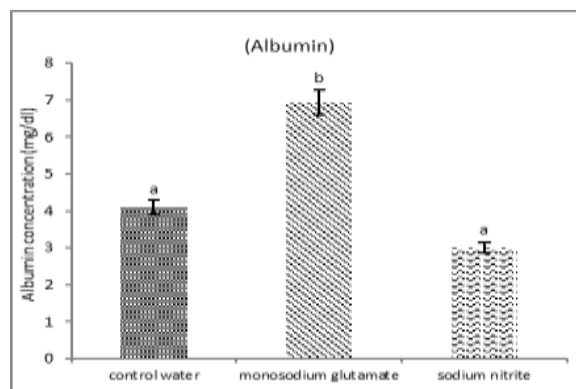

Fig. 10: Effect of food additives (MSG and $NaNO_2$) on serum creatinine concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 13: Effect of food additives (MSG and $NaNO_2$) on serum albumin concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 14: Effect of food additives (MSG and $NaNO_2$) on serum globulin concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
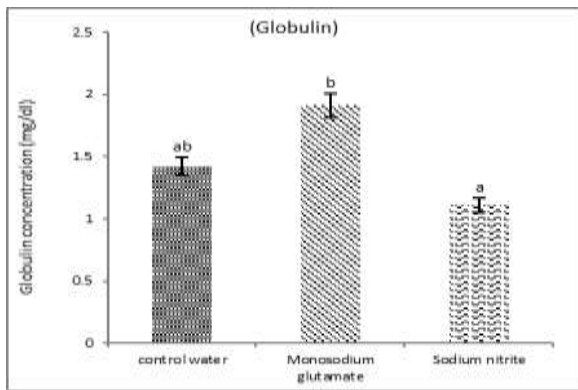

Fig. 17: Effect of food additives (MSG and $NaNO_2$) on serum triglycerides concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
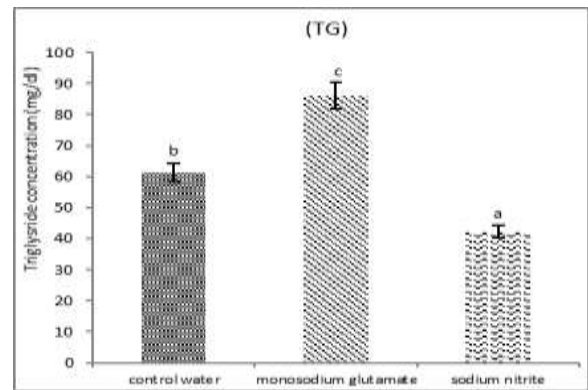

Fig. 15: Effect of food additives (MSG and $NaNO_2$) on serum albumin/globulin ratio of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 18: Effect of food additives (MSG and $NaNO_2$) on serum HDL-cholesterol concentration of control and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
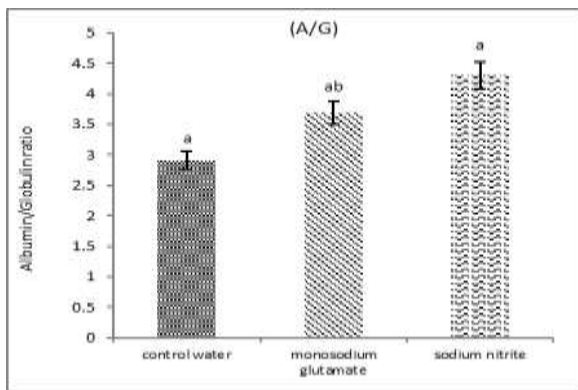

Fig. 16: Effect of food additives (MSG and $NaNO_2$) on serum total cholesterol concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
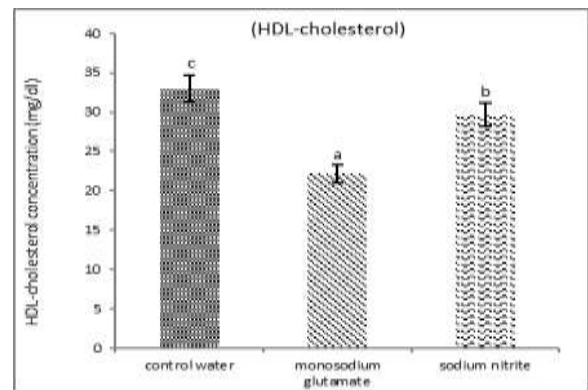

Fig. 19: Effect of food additives (MSG and $NaNO_2$) on serum LDL-cholesterol concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
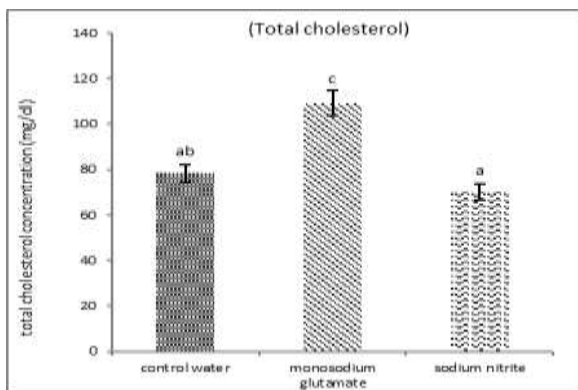
Fig. 20: Effect of food additives (MSG and NaNO₂) on serum vLDL-cholesterol concentration of control and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 23: Effect of food additives (MSG and NaNO₂) on serum creatine kinase concentration of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
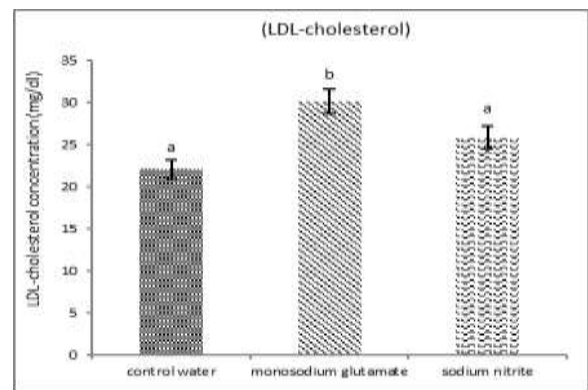

Fig. 21: Effect of food additives (MSG and NaNO₂) on serum total cholesterol/HDL-cholesterol ratio of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
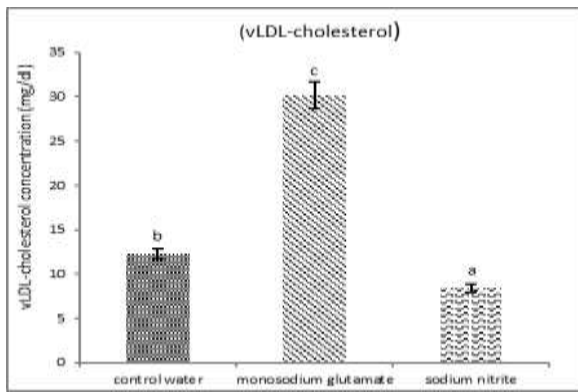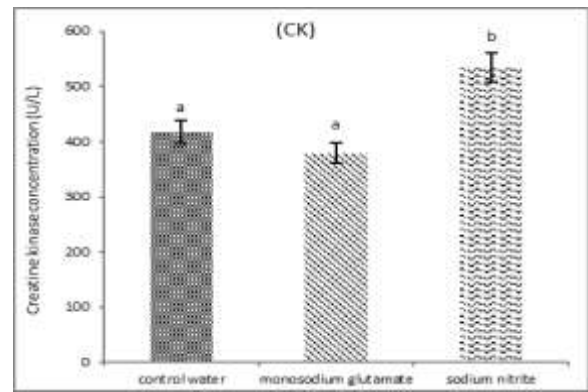

Fig. 24: Effect of food additives (MSG and NaNO₂) on serum lactate dehydrogenase concentration of control and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 22: Effect of food additives (MSG and NaNO₂) on serum LDL-cholesterol/HDL-cholesterol ratio of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig.25: Effect of food additives (MSG andNaNO₂) on liver catalase of normal and tested rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
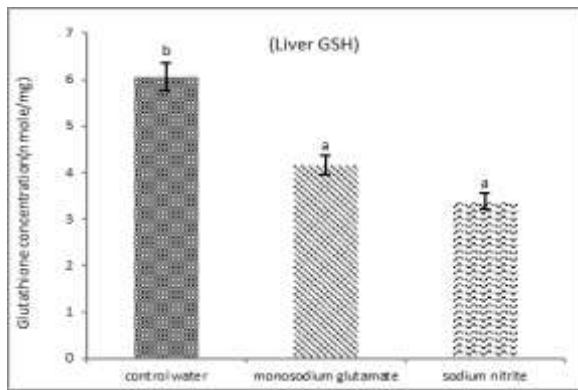
Fig. 25: Effect of food additives (MSG and NaNO$_2$) on liver glutathione of normal and tested rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
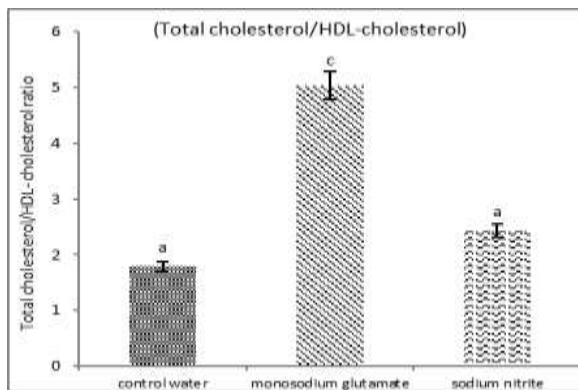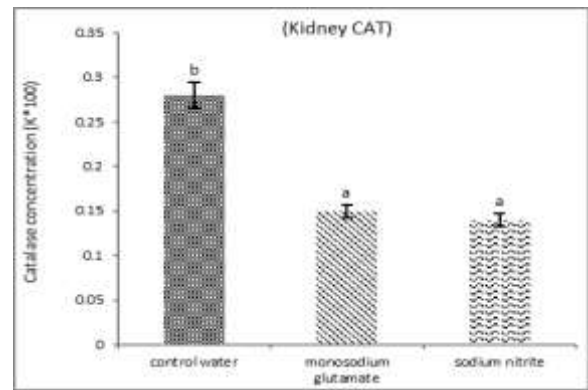

Fig. 29: Effect of food additives (MSG and NaNO$_2$) on kidney catalase of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
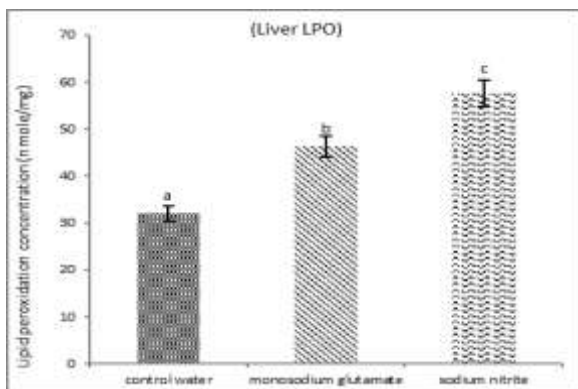
Fig. 27: Effect of food additives (MSG and NaNO$_2$) on liver lipid peroxidation of control and tested rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
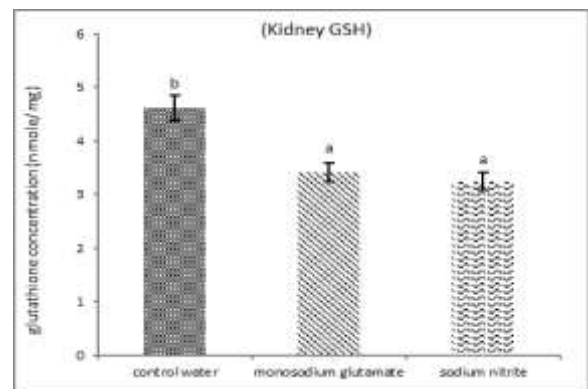
Fig. 30: Effect of food additives (MSG and NaNO$_2$) on kidney glutathione of control and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
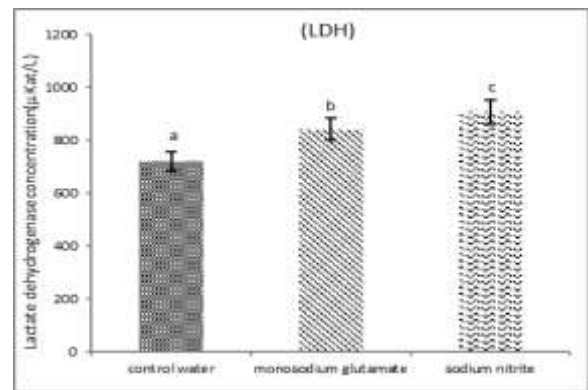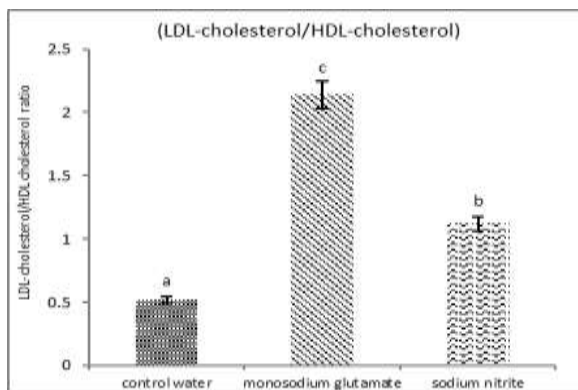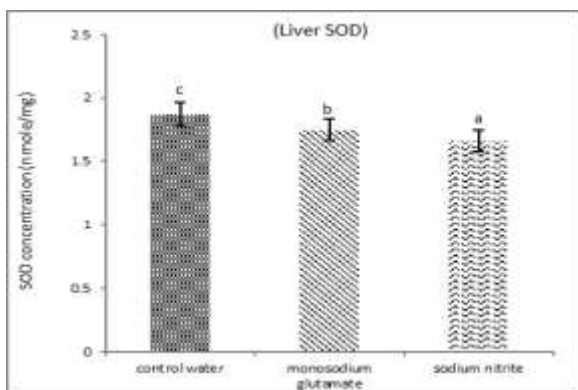

Fig. 28: Effect of food additives (MSG and NaNO$_2$) on liver superoxide dismutase of normal and treated rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.

Fig. 31: Effect of food additives (MSG and NaNO$_2$) on kidney lipid peroxidation of normal and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
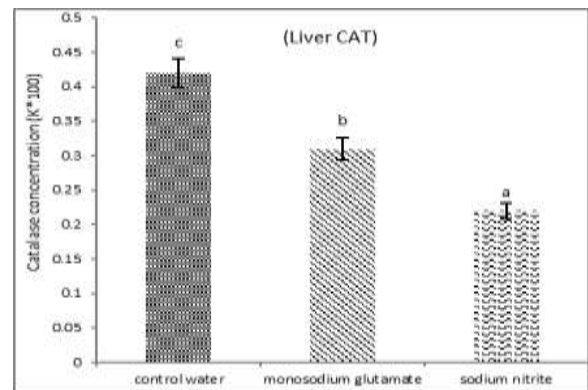
Fig. 32: Effect of food additives (MSG and NaNO₂) on kidney superoxide dismutase of control and induced rats. Data are expressed as mean ± SE, means which share the same superscript symbol (s) are not significantly different.
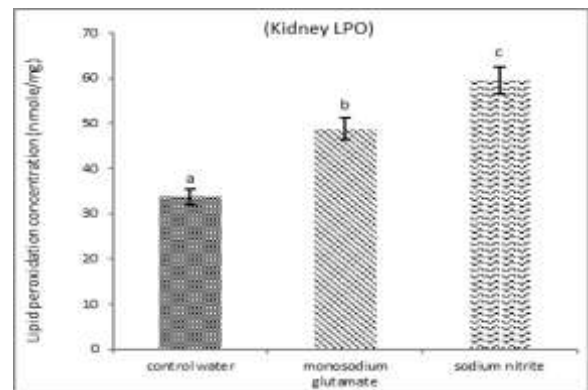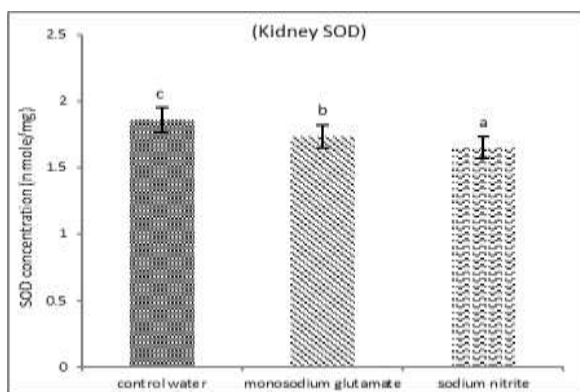
DISCUSSION

The objective of this study was to assess the side effects including biochemical changes in some constituents in serum, oxidative stress and antioxidant changes in induced rats treated with two types of food additives (MSG and NaNO₂), the white albino rats were given doses (15 mg/kg body weight and 5 mg/kg body weight) for MSG and NaNO₂ respectively, and it is injected orally for 30 days. The present investigation revealed that body weight increased in rats treated with MSG and on the other side decreased in rats consumed NaNO₂ as compared with the normal ones. These results are in agreement with (Makoto *et al.,* 2002, Hermanussen *et al.,* 2006). As shown later the elevated serum cholesterol concentration indicate hyper lipidemia and obesity so body weight increased in rats treated with MSG (Schummer *et al.,* 2008), in the same concern, body weight loss is considered by some authors to be a good reliable sensitive toxicity indicator as shown in NaNO₂ rats (Ezeuko *et al.,* 2007). The daily intake of NaNO₂ and MSG exhibited an increase in ALT, AST and ALP when compared to control rats. These results are in accordance with (Ibrahim *et al.,* 2010; Egbuonu *et al.,* 2011). Liver enzymes activities were used as important biomarkers for detection of hepatotoxic nature of different drugs. Serum hepatic marker enzymes (ALT, AST and ALP) were evaluated for hepatotoxicity. The liver is the most sensitive organ to pre-oxidative damage because it is rich in oxidizable substances. The more severe the liver damages the higher the release of the liver enzymes (El-Khayat *et al.,* 2009). Increase in serum level of ALT, AST and ALP as observed in groups induced with these additives may reflect damage of liver cells and cellular degeneration or destruction in this organ and the increase in the activities of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis. When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are

released into the blood stream. Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or for gluconeogenesis and is used to determine liver damage (Etim *et al.,* 2006). Also, the elevation in ALP level in case of MSG suggests an increase in lysosomal mobilization and cell necrosis due to toxicity (Kalender *et al.,* 2005). The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and necrosis of hepatocytes (Pari and Arumugam, 2008).

The current investigation demonstrates that the daily intake of MSG and NaNO₂ exhibited an increase in serum total, direct and indirect billirubin when compared to control rats. The present findings are in agreement with (Ibrahim *et al.,* 2010). El-Sharaky *et al.,* (2007) reported that the elevated levels of bilirubin (hyper-bilirubenimia) are observed in diseases of the liver due to increased production, possible erythrocyte haemolysis, decreased uptake by the liver and decreased conjugation. Oxidation products could cause destruction of the membrane structure with consequent erythrocyte haemolysis (Senthilkumar *et al.,* 2006).

Our results demonstrated that the daily intake of MSG and NaNO₂ exhibited an increase in serum creatinine, urea, uric acid and urea/creatinine ratio concentrations when compared with the control group, these results are in agreement with (Piacenza *et al.,* 2009, Vinodini *et al.,* 2010). El-Demerdash *et al.,* (2005) observed that food preservatives caused changes in kidney convoluted tubules cell lining as well as in Bowman's corpuscles. Khadiga *et al.,* (2009) observed that there is an elevation in kidney functions parameters after administration of MSG and lead to alterations in kidney functions, these impairments could also be attributed to the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate (GFR) (El-sheikh and khalil, 2011). Our study demonstrated that rats treated with MSG and NaNO₂ showed that there is an elevation in serum total protein, albumin, globulin concentrations in MSG's rats and a reduction in total protein, albumin and globulin concentrations in rats treated with NaNO₂ when compared to the control ones, These results are in accordance with (Abdeen *et al.,* 2008, Hassan *et al.,* 2010 and El-sheikh and Khalil, 2011). These data suggest a stimulation of thyroid and adrenal glands by NaNO₂ which lead to a blocked protein synthesis, fast break down, increased rate of free amino acids and decreased protein turnover (Abdeen *et al.,* 2008). Yousef *et al.,* (2004) indicated an inhibitory effect of some food additives on the biosynthesis of protein and albumin which in turn reflects that the liver is unable to perform its functions.

The present observation elucidated that total cholesterol and TG levels increased in MSG's rats and reduced in NaNO₂ group while HDL concentration in all induced rats showed a reduction in its level. LDL concentration in rats consumed both types of additives is elevated when compared to control rats, these results run parallel with (Khadiga *et al.*, 2009, Hassan *et al.,* 2010). The cholesterol pool in the intestine comes from dietary cholesterol and the majority from biliary excretion. Approximately 50% of the intestinal cholesterol pool is reabsorbed by the intestine and recirculated through the body (via the entero-hepatic circulation), with the remainder excreted in feces (Grigore *et al.*, 2007). The deviation from normal values of cholesterol, in the blood serum is considered as symptoms of liver diseases (Singh *et al.,* 1988).

The recent studies recorded that the higher plasma TG could be linked to the increased number of medium vLDL particles. It has been shown that medium vLDL particles are associated with visceral fat area in obese individuals (Okasi *et al.*, 2005). It has also been shown that large and medium vLDL particles are precursors of small dense LDL (Krauss, 2004). Thus there was a significant correlation between medium vLDL particles and small LDL. It has been hypothesized that low HDL-C due to high plasma TG is linked to vLDL metabolism. When vLDL is excreted by the liver, it is normally rich in TG. In plasma, vLDL can exchange TG for CE with HDL, a process mediated by cholesterol ester transfer protein (CETP) (Guerin *et al.*, 2001).The exchange of lipids between these two lipoproteins leads to the production of TG-rich HDL particles (Huesca *et al.,* 2004).

In our work, the concentration of serum CK exhibited a decrease in rats induced with MSG and there is an increase in NaNO₂groupwhile LDH activity showed an elevation of its level in all treated rats when compared to the normal ones, these results are in agreement with (Jevtovic *et al.*, 2003). This elevation could be attributed to a generalized increase in membrane activity due to the increase of one of isoenzymes of LDH (Perry *et al.*, 1997) and significant increase in serum LDH activity indicating the significant cellular damages. The extracellular appearance of LDH is used for detecting cell damage or cell death (Das *et al.,* 2004).

Lactate dehydrogenase is a marker enzyme of lactate production by anaerobic glycolysis (Broglio *et al.,* 1999). Several lines of evidence indicate that glycolysis may be especially important for the maintenance of cellular ion homeostasis and by implication, for diastolic relaxation. Lactate is produced even under aerobic conditions and solely from exogenous glucose (Chowdhury *et al.,* 2005)

supporting the idea that energy from glycolysis is preferentially used to myocardial ion transport (Okamoto *et al.,* 2001). Thus having an important role in cell membrane integrity (Seiva *et al.,* 2008). Cytosolic enzymes of lactate dehydrogenase and creatine kinase, which serve as the diagnostic markers of myocardial tissue damage, leak out from the damaged tissues to the blood stream when the cell membrane becomes permeable or rupture (Gürgün *et al.,* 2008). The amount of these cellular enzymes presented in plasma reflects the alterations in plasma membrane integrity and/or permeability. This could be due to their action on maintaining membrane integrity thereby restricting the leakage of these enzymes (Zhou *et al.,* 2008).

An antioxidant is any substance that when present at low concentration significantly delays or prevents oxidation of cell content like proteins, lipid, carbohydrates and DNA (Irshad *et al.,* 2002).The present investigation revealed that oral administration of MSG and NaNO₂suppressed the activity of catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) in liver and kidney homogenates While, there is an elevation in the activity of lipid peroxidation (LPO) in liver and kidney tissues in both types of additives these results agreed with those reported by (Voja*et al.,* 2007, Hassan and Yousef, 2010). Lipid peroxidation could also be increased due to the increases in the blood glutamate and glutamine which are reported to favour lipogenesis (Malik and Ahluwalia, 1994) .In liver, glutamine degradation yields glutamate which then undergoes oxidative deamination to produce ammonium ions, α-ketoglutarate and NADH. Hence, the increased level of glutamine could also initiate lipid peroxidation by changing the redox potential of the cell (Vinodini*et al.,* 2010).

The high MDA level in serum may reflect the oxidative stress exerted different tissues as it has been reported that oxidant/antioxidant status may reflect the extracellular response to the external agents or the tissue status. These findings are consistent with elevated lipid peroxide, malondialdehyde (Kalaivanam *et al.,* 2006).

NaNO₂-inhibited glutathione content and catalase enzyme activity in the plasma may be attributed to the observed induction of LPO and may be explained according to their function as a free radical scavenger, which suppress the formation of the reactive oxygen species (ROS) and/or oppose their action (Shahjahan *et al.,* 2005). The decreased GSH content in the present study may be attributed to the increased LPO rather than reduced synthesis (Kalaivanam *et al.,* 2006), this also in accordance with (El sheikh and Khalil, 2011). DNA damage was induced by radicals formed in the reaction mixtures of phenol

and nitrite. Some researchers have reported that combined treatment of antioxidants and NaNO$_2$ generates reactive oxygen species (ROS) in vitro (Kuroiwa *et al.,* 2007).

Glutathione (GSH) is a major non-enzymatic antioxidant molecule that is involved in the second line of defense against free radical damage in the body. GSH donates an electron in the reduction of peroxides catalyzed by (GSHpx.) as a component of the enzyme system containing GSH oxidase and reductase (Hong and Lee, 2009). Amin *et al.*(2010) observed a decreased level of GSH , this is due to the most hepatic reduced glutathione (GSH) is converted to its oxidized form (GSSG) by the enzyme glutathione reductase to protect the cells from damage by the toxic materials and free radicals and this explain why GSH is depleted as a result of their toxicity.

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxido-reduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage (Valko *et al.,* 2005). The major portion of the long term effects is inflicted damage on DNA (Evans and cooke, 2004). Most of these oxygen derived species are produced at low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. Increased generation of ROS or free radicals is able to cause auto-oxidation of the hepatic cells, resulting in marked hepatic lesions (Suzuki *et al.,* 1998). SOD converts superoxide radicals into hydrogen peroxide, whilst CAT breaks down hydrogen peroxide into water and oxygen (Limon and Gonsebatt, 2009). Thereby, toxicoxygen species are converted into less harmful or harmless products (Quinlan *et al.,* 1994). Some biomarkers are used to demonstrate the oxidative damage caused by free radicals in biomolecules. The most commonly used biomarker for the determination of lipid peroxidation is the generation of malonaldehyde (Migliore and Copped, 2009).

The reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and H$_2$O$_2$ could be produced in the metabolism of nitrosamines and increase oxidative stress (Bansal, 2005). The extent of oxidative stress-induced damage depends not only on the nature and amount of ROS involved but also on the duration of ROS exposure and ROS scavengers (El-Tohamy, 2012). Excessive ROS production that exceeds critical levels can overwhelm all antioxidant defense strategies, causing oxidative stress (Sikka, 2001). As a result of the ROS formation, the antioxidant defense mechanism of the cells including CAT, SOD, MDA and GSH began to be consumed to prevent the cell death by these toxic radicals (Amin *et al.,* 2010). Lipid peroxidation has been implicated in the toxic effect of many chemicals and in many tissue injuries and disease processes. It has been suggested that ROS could be involved in neuronal damage by inducing lipid peroxidation (Marcio *et al.,* 2008).

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