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PROTECTIVE EFFECT OF AQUAEOUS GREEN TEA EXTRACT ON TOXICITY CAUSED BY SODIUM NITRITE

(With 2 Tables and 12 Figures)

By

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التأثير الوقائى لمستخلص الشاى الأخضر على السميه التى تسببها نتريت الصوديوم

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الاضافات الغذائية سواء الصناعية منها أو الطبيعية تضاف إلى الغذاء على مستوى العالم ولذلك فان سلامة التعرض المتكرر للاضافات الصناعية المسموح بها قيد الاختبارات. فى هذا البحث تم استخدام الصوديوم نتريت بجرعة ٨٠ مج/كج من وزن الجسم / يوم للفئران البيضاء عن طريق الفم إما وحدها أو فى اقتران مع مستخلص الشاى الأخضر المائى (١٠%) لمدة شهرين. بعد هذه المدة تم تجميع عينات الدم والأعضاء للفحص البيوكيميائى والهستوباثولوجى. وقد وجد أن تجريع الصوديوم نتريت ادى إلى انخفاض ملحوظ فى مستوى كل من الجلوتاثيون، السعة الاجمالية لمضادات الأكسدة ، اليوريا والكرياتينين ، الكتيك ديهيدروجيناز ، كرياتين فسفوكيناز فى الدم. وقد عضد الفحص الهستوباثولوجى النتائج حيث ظهرت التغيرات فى شكل موت للخلايا ونخر محيط للوريد البابى للكبد ، مخترقة بالخلايا وحيدة النواة فى بعض الاحيان مما يعطى ما يشبه الورم الحبيبى. بالاضافة إلى تنكس فجوى حاد. اما فى الكليه ، ظهرت مناطق من النخر الانبوبى والتنكس الفجوى الحاد. نخر عضلة القلب أيضاً ظهر نتيجة تجريع الصوديوم نتريت للفئران. ولكن لحسن الحظ، قد تحسنت جميع التغيرات السابقة سواء البيوكيميائية منها أو الهستوباثولوجية بالاستخدام المتزامن للشاى الأخضر كمصدر وحيد للشرب للفئران وهذه النتائج تشير إلى أهمية استخدامه كمستخلص وقائى ضد الاثار السمية الناتجة عن استخدام الصوديوم نتريت وذلك قد يكون لما يحتويه الشاى الاخضر من مضادات الاكسدة.

SUMMARY

Food additives are substances internationally added to food, this may be natural or synthetic. The safety of repeated exposure to permitted synthetic food additives (colorants or preservatives) has been questioned. Sodium nitrite (NaNO2, a food preservative agent) was used at 80 mg/kg/day and orally administered to the rats either alone or in conjugation with aqueous green tea extract (GTE, 10%) for 2 months. Blood and organs samples were collected for biochemical and histopathological examinations. Ingestion of NaNO2, induced a significant decline in blood glutathione reduced (GSH), plasma total antioxidant capacity (TAC) and glutathione -S- transferase (GST). Furthermore, significant increase was recorded in the level of serum lipid peroxide (MDA) and plasma nitric oxide (NO). Moreover, serum alanine transaminase (ALT), aspartate aminotransferase (AST) as well as bilirubin levels were increased significantly, while albumin level was decreased. Also, serum urea and creatinine levels were significantly increased. Plasma lactate dehydrogenase (LDH) and serum Creatine phosphokinase (CPK) activities were increased. Histopathological examination in sodium nitrite group rats revealed marked alterations in liver as apoptosis, periportal necrosis Sometimes, there were areas of focal necrosis which infiltrated with mononuclear cells giving the picture of granuloma like lesions, in addition to severe vacuolar degeneration. Also, kidney showed severe vacuolar degeneration and massive areas of tubular necrosis. Furthermore, heart showed necrosis of myocardial muscle cells. Fortunately, administration of green tea extract in conjugation with sodium nitrite showed significant amelioration of the investigated parameters and pathological changes. The results indicated that green tea extract has a potential to be developed as a preventive extract against sodium nitrite induced toxicity and the mechanism involved in the protection could be due to its antioxidant activity.

Key words: *Sodium nitrite, Green tea extract, Toxicity, Antioxidant activity*

INTRODUCTION

In relation to the toxicological limit, the FAO/WHO, Joint Expert Committee on Food Additive (JECFA) established acceptable daily intakes (ADIs) of 0-0.07 mg kg^{-1} body weight for sodium nitrite (expressed as nitrite ion) (WHO, 2003). It is widely used in food and drug industries as a preservative (Hill, 1991), and in medicine as antidote for cyanide poisoning (Filvo *et al.*, 1993). Approximately 40% of absorbed nitrite is excreted unchanged in the urine and the fate of the rest 60% is not accurately know (Hill, 1991). The major metabolites of NaNO2 are nitric oxide and nitrosamine (Reisser *et al.*, 1998). The later is highly carcinogenic and associated with a high risk of stomach, liver and esophagus carcinomas (Kim *et al.*, 2002). Nitric oxide (free radicals) can cause DNA damage by inhibiting DNA synthesis and cell cycle arrest (Wink *et al.*, 1991). The hazardous effect of NaNO2 derives from the reaction of nitrites with amines to produce nitrosamines, and with amides to produce nitrosamides. The toxic effects of nitrates and nitrites are well documented in mammalians, including impairment of reproductive function (Sleight *et al.*, 1972), hepatotoxicity (Swann, 1975), dysregulation of inflammatory responses and tissue injury (Blanquat *et al.*, 1983), growth retardation (Prasad, 1983), and endocrine disturbance (Jahries *et al.*, 1986). A moderate and significant acceleration of leukemia development was observed in sodium nitrite treated mice (Ilntisky and Kolpakova, 1997). Also, NaNO2 inhibits a number of anti-tumor cytotoxic effector cell types as natural killer cells against pathogens and tumor cells (Abuharfiel *et al.*, 2001). Children who ate more than 12 nitrite-cured hot dogs per month showed an increased risk of developing childhood leukemia (Peters *et al.*, 1994). Anyway, nitrite when present at high concentration in blood, it can react with iron (III) of the hemoglobin, forming methaemoglobin which has no oxygen-carrying ability. This fatal disease is called methaemogobenemia (Sanchez-Echaniz *et al.*, 2001).

Sodium nitrite was used at 10 mg/kg/day and orally administered to the rats for 30 days by Eman and Fahmy (2006) who reported that ingestion of NaNO2 significantly decreased RBCs%, WBCs%, hematocrit values%, Hb%, serum albumin, globulin and total protein contents in the treated rats while, serum alanine transaminase (ALT),

aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) were highly increased. Also, the authors observed elevation in triglycerides, total cholesterol and total lipids in serum and in the investigated tissues as well as hyperglycemia in food additive treated rats. Furthermore, sodium nitrite was found to significantly increase the lipid peroxidation and decrease the activities of antioxidant enzymes in liver such as superoxide dismutase, catalase and reduced glutathione level in rats received sodium nitrite orally for a month at dose rate of 300 mg/kg b.w. (kirshnamoorthy and Sangeetha, 2008). Concerning kidney function, it was reported that sodium nitrite produces significant elevation in serum urea and creatinine and significant decrease in renal urea and creatinine levels in rats dosed orally with 80 mg/kg b.w. for 3 months (Hanaa *et al.*, 2009).

Sodium nitrite intake studied by National Toxicology Program (2001) revealed that mean body weight at the highest dose was lower than that of control groups and showed the incidence of squamous cell papilloma and carcinoma of the forestomach in female mice. Szemes and szamado (1991) found that rats received sodium nitrite showed histopathological changes in striated muscles, liver and kidney. Also, Alam *et al.* (2005) observed that mice exposed to nitrosodibutylamine showed cell destruction and extensive necrosis.

Green tea, from the steamed dried leaves of camellia sinesis, is widely consumed in eastern Asia. It comprises many types of catchins especially epigallocatchin-3-gallate (EGCG) which is the major polyphenol component and it is primarily responsible for the beneficial effects of green tea. Substantial evidence suggests that EGCG elicit anti-oxidant properties by attenuating the lipid peroxidation (LPO) caused by various forms of free radicals (Guo *et al.*, 1999). It has been demonstrated that the chemically induced LPO in liver and kidney could be inhibited by the intake of tea catechins (Sano *et al.*, 1995). In a recent study, supplementation of green tea extract (GTE) attenuated cyclosporine A-induced oxidative stress in rats (Mohamadin *et al.*, 2005). Moreover, it can reduce the risk of colorectal and pancreatic cancers (Brown, 1999), inflammatory diseases and muscle necrosis (Benelli *et al.*, 2002). In addition, green tea polyphenol might be a useful cancer chemopreventive agent in the human population (Lung *et al.*, 2002).

The aim of this work was to study the biochemical effects, antioxidant profile and histopathological features exerted by green tea extract (GTE) supplementation to sodium nitrite-induced toxicity in rats.

MATERIALS and METHODS

1. Chemicals

NaNO2 (Sigma Aldrich, St Louis, MO) was applied as a freshly prepared solution. GTE was prepared from a hot-water extract of green tea (Twining and CO.LTD. LONDON) as described by Byung *et al.* (2009). Ten g of green tea leaves were soaked in 100 mL of boiling distilled water for 5 min. The solution was filtered to make 10 % green tea extract (GTE). This solution was provided to rats as their sole source of drinking water.

2. Kits

Glutathione reduced (GSH), Total antioxidant capacity (TAC), glutathione -S- transferase (GST) lactate dehydrogenase (LDH), nitric oxide (NO) and lipid peroxide (MDA) kits were obtained from *Biodiagnostic*® Company. Alanine aminotransfrase (ALT), aspartate aminotransfrease (AST) kits were purchased from *Biosystem*® Company. Albumin, bilirubin, urea and creatinine were obtained from *Human*® Company. Creatine phosphokinase (CPK) kit was purchased from bioMerieux® Company.

3. Animals

Forty mature healthy Sprague-Dawley rats of both sexes weighing 120-150 g (purchased from Animal House Colony, Giza, Egypt) were housed in stainless-steel cages with hard wood shavings as bedding. Animals were accommodated to the laboratory conditions for one-week before experiment. They were maintained on balanced ration and given water ad-libitum throughout the experimental period (2 months).

4. Experimental design

The animals were divided into four groups (10 rats each) as follows:

(1) **Group 1:** Rats fed on the basal diet and normal water, left in normal conditions for 2 months which served as control group.

(2) **Group 2:** Rats orally administered green tea extract (GTE) (10%) as their sole source of drinking water for 2 months and served as GTE group.

(3) **Group 3:** Rats were treated with sodium nitrite by gavages at a dose of 80 mg/kg body weight daily as previously described by (Hanaa *et al.*, 2009) for 2 months and served as Sodium nitrite - intoxicated group.

(4) **Group 4:** Rats were orally administered both GTE as their sole source of drinking water, sodium nitrite by gavages at a dose of 80 mg/kg body weight daily for 2 months and served as Sodium nitrite–GTE group.

4.1. Biochemical analysis

Two blood samples were collected from each animal via the retro-orbital venous plexus after fasting for 12 h. The first blood sample was collected on EDTA for estimation of GSH in whole blood. TAC, GST, LDH and NO were estimated in plasma. The second blood sample was left to clot then centrifuged at 5000 rpm for 10 min to separate serum for measuring liver ALT, AST, albumin and bilirubin, kidney urea and creatinine functions in addition to CPK and MDA activities. GSH content was estimated according to Beutler *et al.* (1963). TAC was determined according to Koracevic *et al.* (2001). GST was estimated according to Habig *et al.* (1974), in addition to oxidative biomarkers (NO) which was determined according to Montgomery and Dymock (1961) and MDA was determined according to method described by Ohkawa *et al.* (1979). LDH activity was determined as previously described by Raabo (1963). ALT and AST activities were determined according to the method described by Reitman and Frankel (1957). Albumin was measured as previously reported by Tietz (1990), whereas bilirubin, urea and creatinine were estimated as by the method of Henry (1974). CPK was performed according to the method of Oliver (1955).

4.2. Histopathological examination

Specimens from liver, kidney and heart were preserved in 10% buffer neutral formalin and processed through paraffin embedding technique. Sections of about 5-7 microns thickness were cut and stained with hematoxylin and eosin (H&E) for histopathologic examination according to Culling (1983).

5. Statistical analysis

Statistical analysis of data was conducted using statistical analysis system SAS (2001).

RESULTS

Table 1: The effect of sodium nitrite (80 mg/kg b.w. daily) and aqueous green tea extract (10%) on some antioxidant and oxidative biomarkers of rats.

Rat group / Parameters	Control	Green Tea	Sodium nitrite	Sodium nitrite plus Green tea
GSH, mg/dl	30.33 ± 0.33 b	33.26 ± 0.84 a	23.34 ± 0.47 d	27.47 ± 0.65 c
TAC, mMol/L	0.31 ± 0.01 b	0.37 ± 0.01 a	0.22 ± 0.01 c	0.28 ± 0.01 b
GST, U/L	283.75 ± 9.44 a	297.00 ± 13.40 a	215.00 ± 5.40 c	252.75 ± 3.04 b
MDA, nmol/ml	60.78 ± 0.96 c	54.28 ± 0.50 d	97.72 ± 1.78 a	79.58 ± 1.20 b
NO, µmol/L	3.50 ± 0.14 c	3.03 ± 0.06 d	4.62 ± 0.14 a	3.94 ± 0.07 b

- Values are mean ± standard error.
- Means in a row without a common letter differ significantly (P<0.05).

Table 2: The effect of sodium nitrite (80 mg/kg b.w. daily) and aqueous green tea extract (10%) on liver, kidney and heart functions parameters of rats.

Rat group / Parameters	Control	Green Tea	Sodium nitrite	Sodium nitrite plus Green tea
ALT, U/L	41.50 ± 0.96 c	38.25 ± 1.38 c	56.50 ± 0.65 a	47.25 ± 1.93 b
AST, U/L	136.75 ± 1.65 c	131.50 ± 0.96 d	170.50 ± 1.55 a	148.50 ± 1.55 b
Albumin, g/dl	5.15 ± 0.07 a	5.28 ± 0.03 a	3.98 ± 0.10 c	4.86 ± 0.04 b
Bilirubin, mg/dl	0.46 ± 0.01 c	0.45 ± 0.01 c	0.67 ± 0.01 a	0.51 ± 0.01 b
Urea, mg/dl	36.25 ± 0.48 b	35.25 ± 0.48 b	47.00 ± 0.71 a	37.25 ± 0.85 b
Creatinine, mg/dl	0.86 ± 0.02 c	0.84 ± 0.02 c	1.37 ± 0.01 a	1.01 ± 0.05 b
CPK, U/L	391.00 ± 4.81 c	377.25 ± 6.97 c	598.75 ± 4.53 a	508.25 ± 2.87 b
LDH, U/L	742.75 ± 3.04 b	739.75 ± 5.81 b	802.50 ± 12.33 a	758.50 ± 2.53 b

- Values are mean ± standard error.
- Means in a row without a common letter differ significantly (P<0.05)

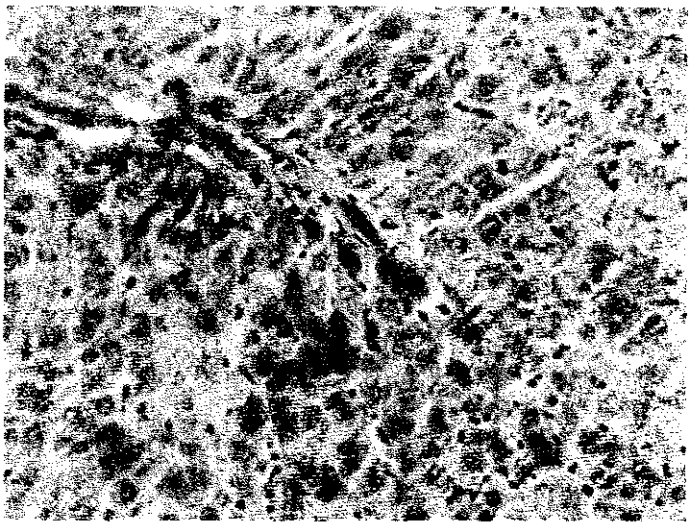

Fig. 1: Liver (group2), showing apoptotic figures which were collected either in groups or in individual scattered in hepatic parenchyma. (H&E) (10x20).

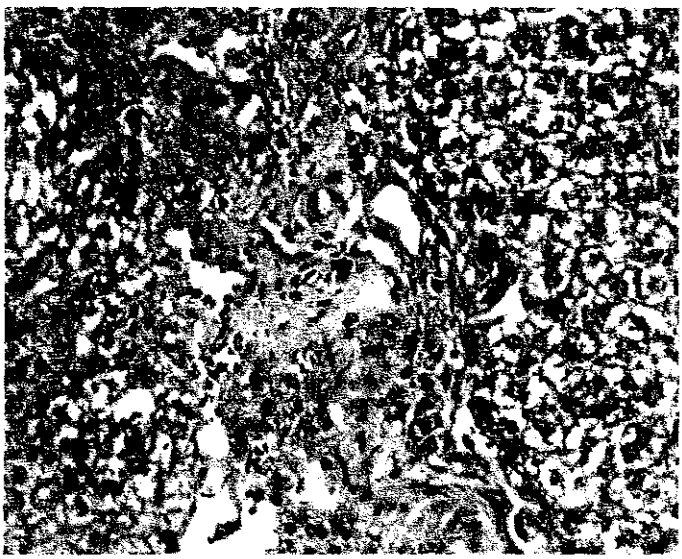

Fig. 2: Liver (group2), showing periportal necrosis of hepatic parenchyma. (H&E) (10x20).

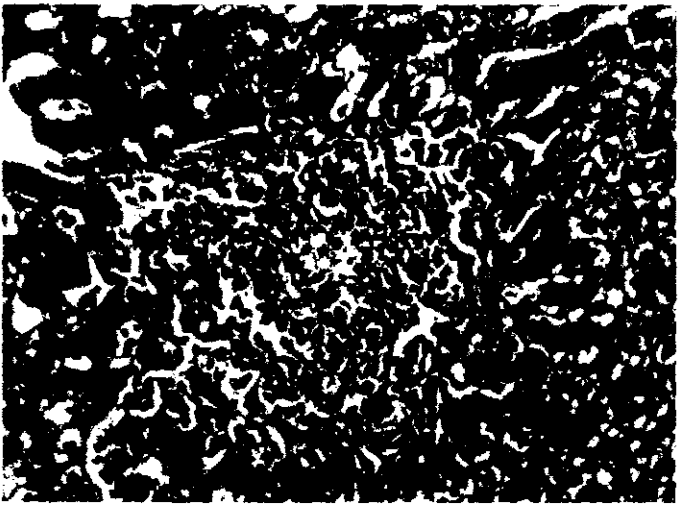

Fig. 3: Liver (group2), showing focal area of necrosis heavily infiltrated with mononuclear cells giving the picture granuloma like lesion. (H&E) (10x20).

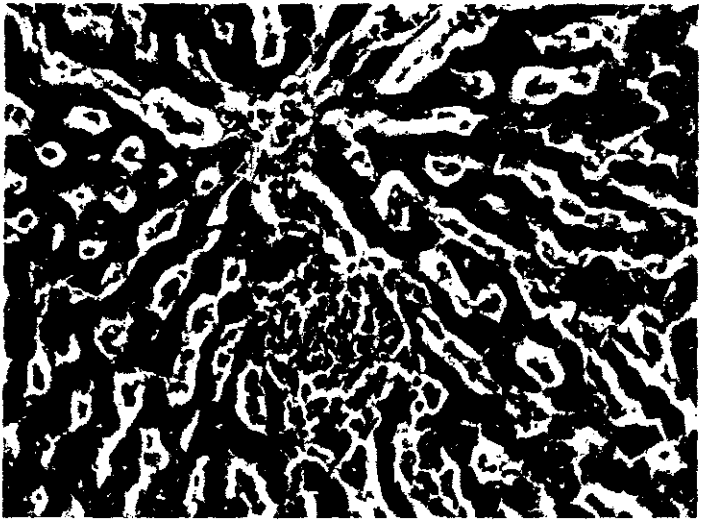

Fig. 4: Liver (group2), showing midzonal mononuclear cell infiltration which accompanied with peripheral single cell necrosis. (H&E) (10x20).

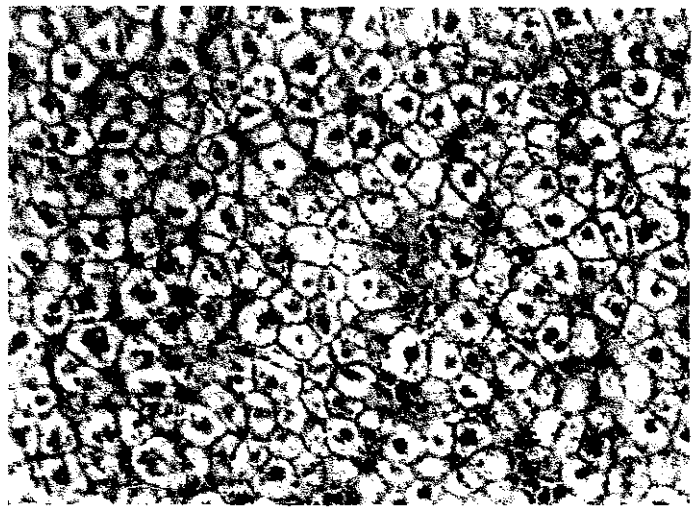

Fig. 5: Liver (group2), showing severe vacuolar degeneration in the form of ill-defined vacuoles leading to rarefaction of cytoplasm with pycknotic nuclei. (H&E) (10x20).

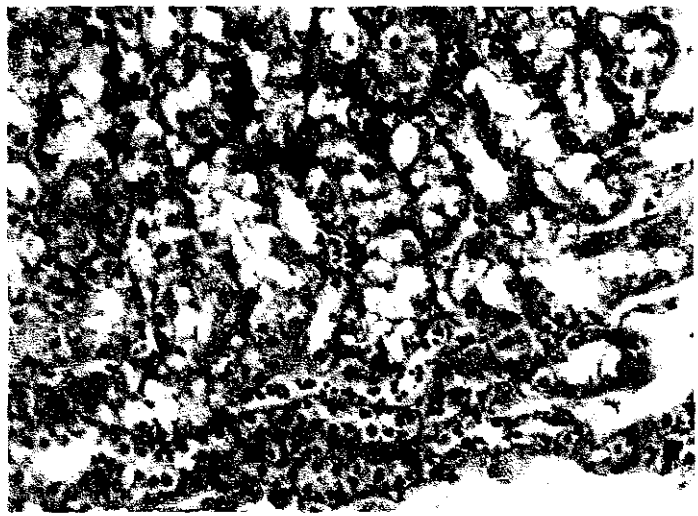

Fig. 6: Kidney (group2), showing severe vacuolar degeneration in the form of ill-defined vacuoles with pycknosis of nuclei of some renal tubular cells. (H&E) (10x20).

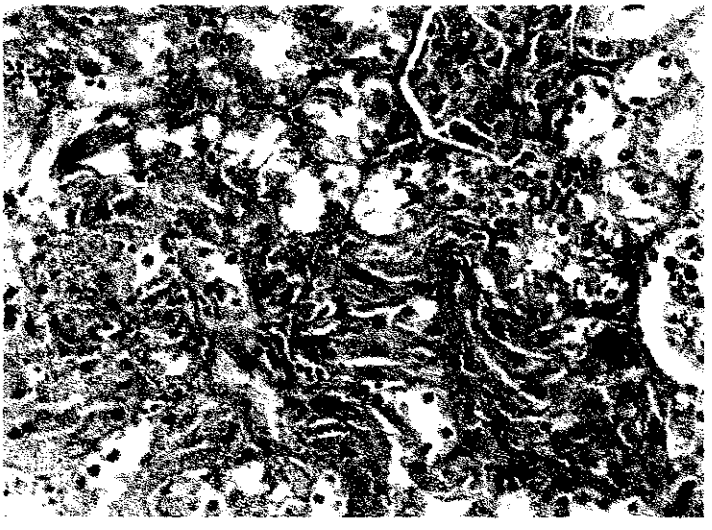

Fig. 7: Kidney (group2), showing massive areas of tubular necrosis (H&E) (10x20).

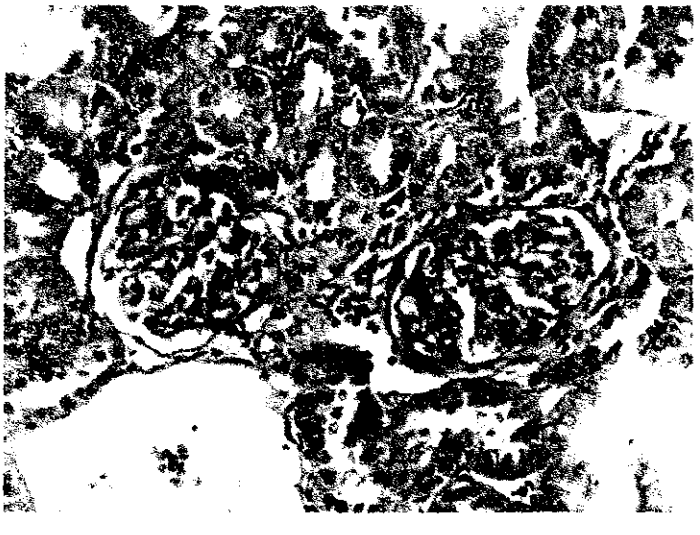

Fig. 8: Kidney (group2), showing slight thickening of basement membrane of glomerulus and pycknosis of nuclei of mesangial cells. (H&E) (10x20).

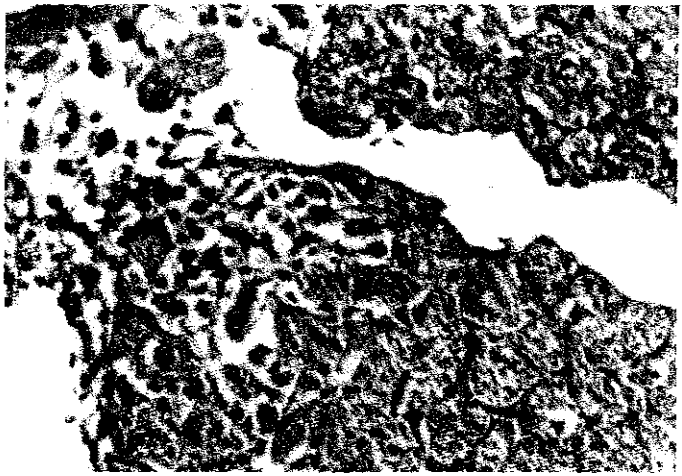

Fig. 9: Heart (group2), showing necrosis of myocardial muscle cells. The necrotic area was infiltrated with mononuclear cells including macrophages engulfing necrotic cells (myophagia). (H&E) (10x20).

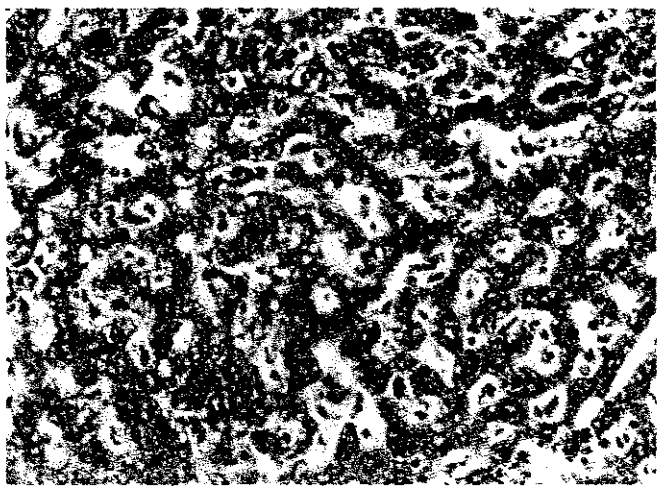

Fig. 10: Liver (group3), showing sinusoidal dilatation and multiple microvacuoles in cells. (H&E) (10x20).

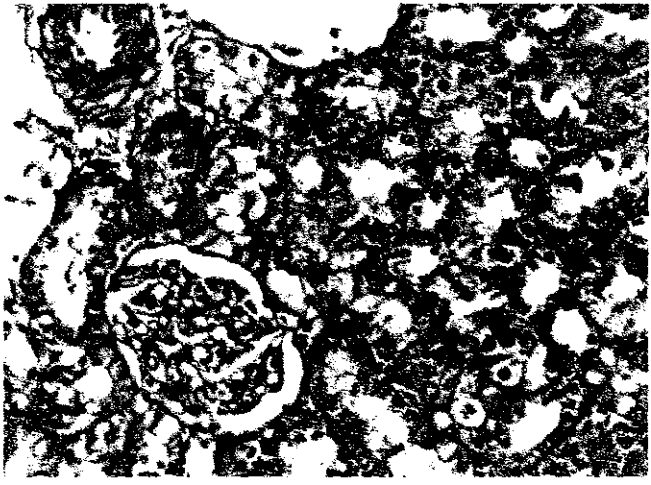

Fig. 11: Kidney (group3), showing slight vacuolar change in renal epithelial. (H&E) (10x20).

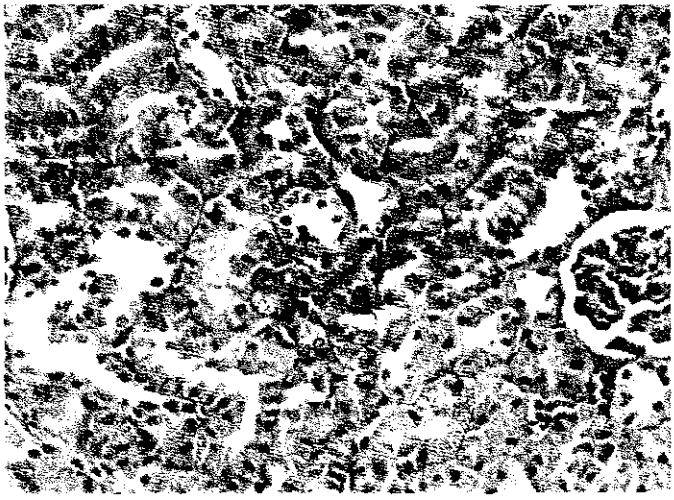

Fig. 12: Kidney (group3), showing pycknotic nuclei of some cells of renal tubules. (H&E) (10x20).

DISCUSSION

Oxidative stress has been implicated in the development of many toxic conditions. Sodium nitrite and other additives may react with amines of the foods in the stomach and produce nitrosamines and free radicals. Such products may increase lipid peroxidation, which can be harmful to different organs including liver, kidney and heart (Choi *et al.*, 2002). On the other hand, these free radicals, known to cause oxidative stress, can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products (Aruoma, 1998).

In present study, a number of antioxidant biomarkers were examined and the results are summarized in Table 1. The administration of sodium nitrite orally to rats at dose of 80 mg/kg body weight daily for 2 months, resulted in a dramatic decline ($P<0.05$) in blood GSH, plasma TAC and GST; these results are supported by previous data obtained by (kirshnamoorthy and Sangeetha, 2008). These results may be attributed to the observed induction of lipid peroxidation (Shahjahan *et al.*, 2005). Glutathione plays a crucial role in the cellular antioxidant defense system by scavenging free radicals and other reactive oxygen species, removing hydrogen and lipid peroxides and preventing oxidation of biomolecules (Wu *et al.*, 2004). The oxidative stress caused by sodium nitrite can lead to significant depletion of GSH when compared with control animals. The downfall of blood GSH content in this study might be due to its enhanced utilization for scavenging free radicals. Moreover, estimation of total antioxidant capacity (TAC) may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids (Koracevic *et al.*, 2001).

In the current study, administration of sodium nitrite elicited a significant increase ($P<0.05$) in the level of oxidative biomarkers including plasma NO and serum MDA compared to normal control group. MDA is an end product of lipid peroxidation, and it is considered a late biomarker of oxidative stress and cellular damage (Carampin *et al.*, 2003). Furthermore, there is a significant increase in plasma nitric oxide free radical ($NO\cdot$) due to its generation from the nitrite by nonenzymatic method and $O\cdot$ formation which found in acidic environment such as

stomach and oral cavity (Mcknight *et al.*, 1997). Both NO· and oxygen radicals could react further to produce other oxidant and nitro compounds such as peroxynitrite which is a powerful oxidant and causes tissue damage (Teppema *et al.*, 2002).

Histopathological findings confirmed the toxic effects of sodium nitrite and are consistent with biochemical determination. In sodium nitrite group, there were marked alterations in liver, kidney and heart. Sodium nitrite treated animals showed increased apoptotic figures. The apoptotic cells were collected either in groups or in individuals scattered in hepatic parenchyma. The necrotic cells showed more acidophilic cytoplasm and pyknotic nuclei (Fig.1). The increase in apoptotic figures may be due to induction of oxidative stress and mitochondrial alteration (Fladmark *et al.*, 1999).

Hepatic necrosis has been the most frequently reported lesion associated with sodium nitrite induced liver damage. Furthermore, liver of the same animals showed periportal necrosis of hepatic parenchyma mostly observed in portal areas in which the cytoplasm became more acidophilic with pyknosis or completely disappearance of nuclei (Fig.2). In other cases, there were focal areas of necrosis heavily infiltrated with mononuclear cells giving the picture of granuloma like lesions (Fig3). These findings are in agreement with that mentioned by Hassan *et al.* (2009) who recorded liver necrosis induced by sodium nitrite and attributed the liver damage to the toxic effect of nitros compounds formed in acidic environment of stomach causing hepatic necrosis. These obtained results are confirmed by the biochemical data which showed significant increase in AST, ALT and bilirubin in serum of sodium nitrite treated rats indicating hepatic disorder and damage.

Our results also indicated an inhibitory effect of sodium nitrite on albumin synthesis, this may be due to stimulation of thyroid and adrenal glands which can lead to a blockade in protein synthesis, fat break down, increased rate of free amino acids and decreased protein turnover (Eremin and Yocharina, 1981). In addition, the release of NO· can inhibit protein synthesis through inhibiting oxidative phosphorylation process and the availability of the energy source for protein synthesis (Anthony *et al.*, 1994).

Another histopathological finding in sodium nitrite group of rats was mononuclear cell infiltration in hepatic parenchyma either perivascular, periportal or midzonal which sometimes accompanied with peripheral single cell necrosis (Fig.4). This finding is in accordance with the view that the NO· originally known to be important in the modulation of tissue inflammation (Rees *et al.*, 1990). Moreover, Sarsour and Hassuneh (2001) reported that sodium nitrite causes inflammatory reaction and infiltration of inflammatory cells that subsequently release large quantities of potential oxidants as H_2O_2 that might induce damage to surrounding tissue and cells. Alternatively, the hepatic cells in some animals of sodium nitrite group of rats showed severe vacuolar degeneration in the form of ill-defined vacuoles leading to rarefaction of cytoplasm with pyknotic nuclei (Fig5). This finding indicates increased production of free radicals especially reactive oxygen species that attack the cell membranes and other molecules inside the cells.

In present study the results of assessment of kidney function (urea and creatinine levels) as recorded in Table 2, indicated that serum urea and creatinine levels increased significantly in sodium nitrite group, suggesting an impairment of kidney function. These results were in agreement with those obtained by Eman and Fahmy (2006) and correlated with the results of the histopathological examination of kidney of sodium nitrite group of rats.

The kidney showed severe vacuolar degenerative changes in the form of ill-defined vacuoles mostly observed towards the luminal border of tubules together with pyknosis of nuclei of some renal tubular cells (Fig.6). Some rats showed massive areas of tubular necrosis (Fig.7) and very few revealed slight thickening of the basement membrane of glomeruli and pyknosis of nuclei of mesangial cells (Fig.8). Sodium nitrite induced renal toxicity was in accordance with that obtained by (Hanaa *et al.*, 2009), this toxicity may be attributed to increased generation of NO· which is the major metabolite of sodium nitrite (Reisser *et al.*, 1998) that leads to increased lipid peroxidation (Goligosky *et al.*, 2002) or may be due to direct effect of sodium nitrite which alters transport of sodium and chloride in the distal nephron and may also adversely affect the renal function (Zaki *et al.*, 2005) or

through changes in the threshold of tubular re-absorption, renal blood flow and glomerular infiltration rate (Zurovsky and Haber, 1995).

In current study, the heart function markers (CPK and LDH) increased significantly indicating cellular damage of the heart. This may be due to the increase in the production of free radicals. The histopathological findings of the heart in sodium nitrite group of rats confirmed these biochemical results. There was focal necrosis of myocardial muscle cells in which the necrotic cells had more acidophilic cytoplasm with pyknotic nuclei and loss of muscle fiber striations. The necrotic area was infiltrated with mononuclear cells including macrophages engulfing necrotic cells (myophagia) (Fig.9).

The main components of green tea are catechins, which have a polyphenol structure, including [(−)-epigallocatechin-3-gallate (EGCG)], [(−)-epigallocatechin (EGC)], [(−)-epicatechin-3- gallate (ECG)] and [(−)-epicatechin (EC)]. All these catechins have strong antioxidant activity (Higdon & Frei, 2003) and are considered as potent scavengers of reactive oxygen species (ROS) and nitrogen species such as superoxide, lipid peroxide, peroxynitrite, hydroxyl radicals and nitric oxide produced by various chemicals (Schroeder *et al.*, 2003). Green tea prevents the loss of lipophilic antioxidant a-tocopherol, by repairing tocopheryl radicals and protection of the hydrophilic antioxidant ascorbate (Skrzydlewska *et al.*, 2002). Therefore, it may decrease the concentration of lipid free radicals and terminate initiation and propagation of lipid peroxidation (Guo *et al.*, 1999). Catechins have beneficial effects in prevention of cardiovascular diseases including LDL oxidative susceptibility, serum lipids and lipoprotein concentrations (Wan *et al.*, 2001).

The administration of green tea extract (10%) in conjugation with sodium nitrite ameliorated the nitrite adverse effects as evidenced by restored TAC as well as increased GSH level and GST activity. In addition to significant reduction in NO and MDA levels when compared with normal control and sodium nitrite group. Also, supplementation of sodium nitrite intoxicated rats with green tea extract declined the AST, ALT and bilirubin levels when compared to normal control and sodium nitrite rats. Moreover, albumin level was significantly increased in Sodium nitrite-GTE rats.

The results of histopathological examination were in accordance with the liver function biochemical results. There was marked improvement in liver upon treatment of sodium nitrite intoxicated rats with green tea. The most hepatic changes induced by sodium nitrite disappeared except some sinusoidal dilatation and multiple microvacuoles in hepatic cells (Fig.10). These findings simulate those of Ostrowska *et al.* (2004) who detected that green tea protected liver and caused disappearance of necrotic areas induced by ethanol intoxication. Also green tea protected hepatic cells against tamoxifen intoxication (Elbeshbishy, 2005) and against microcystin –LR induced hepatotoxicity (Xu *et al.*, 2007). More over, Relja *et al.* (2010) proved that green tea reduced liver injury and necrosis induced by Hemorrhage resuscitation (H-R) in rats. The apoptosis of hepatic cells was decreased when compared with sodium nitrite group. This finding was in agreement with Hockenberg *et al.* (1990) who mentioned that B-CL-2 protein residue in the mitochondrial outer membrane, has been implicated in the regulation of mitochondria permeability transition (MPT) and release of apoptogenic proteins from mitochondria into cystol, B-CL-2 protein is an important gene product to control apoptosis, and it can inhibit apoptosis through blocking apoptotic signal transmitted system. The present study, suggests that the modulation of B-CL-2 protein expression by green tea might be an important factor in signal transduction mediating the protective effect against sodium nitrite induced apoptosis of hepatic cells.

In current study, supplementation of sodium nitrite intoxicated rats with green tea extract restored the normal urea level as well as declined creatinine level when compared to normal control and sodium nitrite rats. These obtained data were confirmed by histopathological examination of kidney of rats treated with both sodium nitrite and green tea extract. There was marked improvement in kidney picture and absence of most pathological alterations which were detected in sodium nitrite rat group except there was slight vacuolar change in renal epithelium (Fig.11) and pyknotic nuclei of some cells of renal tubules (Fig.12). Similar results were detected by Ozer *et al.* (2008) who reported that EGCG supplementation improved the histopathological pictures in kidney of rats exposed to sevoflurane where green tea decreased renal degeneration and caused disappearance of cortical

necrosis in kidney tissues. This protection may be attributed to phosphorylation and activation of endothelial nitric oxide synthase in endothelial cells through modulation of protein kinase C, A signaling pathways by green tea resulting in endothelial dependant vasorelaxtion (Lorenz *et al.*, 2004)

In the present study, heart function markers (serum CPK and LDH) showed marked decrease in the group of rats received both sodium nitrite and green tea, these biochemical results are in accordance with histopathological picture of the heart. There was absence of necrosis and the heart showed more or less normal appearance except congestion.

In the present study, GTE rats did not show any significant changes in the majority of the estimated parameters and histopathological examinations except AST level showed significant decrease when compared to normal control rats. Further more, GTE rats showed significant increase in GSH and TAC activities as well as significant decrease in NO and MDA levels, while there is no significant change in GST level when compared with normal control rats.

In conclusion, from the results achieved, it can be concluded that the administration of green tea extract has an extremely beneficial role in overcoming the adverse effects of ingestion of sodium nitrite, which is probably through its excellent antioxidant properties and enhancing the antioxidant potency of studied organs cells and organelles.

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