Green Tea Extract Ameliorates Liver and Pituitary Gland Toxicity Induced by Diethylnitrosamine in Male Rats

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Abstract: Diethylnitrosamine, one of the most important environmental carcinogen, has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury. The aim of this work was to evaluate the effect of the green tea extract (GTE) as an antioxidant on the toxicity of DEN on liver and pituitary gland. Consequently, the study was carried out in four groups as follows: G1, control animals; G 2, administrated green tea only; G 3, rats injected only with a single dose of 200 mg/kg DEN and G 4, rats received a single dose of 200 mg/kg DEN and green tea extract (100 mg/kg b.wt) for the same period (30 days). It was found that DEN-related changes occur in the histology, histochemistry and ultrastructure of the liver and pituitary gland. The histopathological changes of the liver included clear signs of severe hepatic injury. Ultrastructural changes showed destructed cell membrane, Pyknotic nuclei, vacuolated cytoplasm, dilated endoplasmic reticulum reduction or complete loss of cristae in the mitochondria. Ultrastructural study of the pituitary gland showed cellular degeneration, which seemed to involve many cell types containing secretory granules. As regard in the pituitary gland and liver marked increase in collagen fibers and marked decrease in protein, carbohydrates and DNA contents were observed. Treatment with green tea as antioxidant and free radical scavenger caused improvement of these changes in the liver as well as the pituitary gland.


Key words: diethylnitrosamine, liver, pituitary gland, histopathology, histochemistry and ultrastructure.

1. Introduction

Diethylnitrosamine (DEN) is a potent hepatocarcinogenic nitrosamine present in tobacco smoke, ground water having high level of nitrates, cheddar cheese, cured and fried meals, alcoholic beverages, occupational settings, cosmetics, agricultural chemicals and pharmaceutical agents (Sivaramk Krishnan, et al., 2008 and Gupta et al., 2010). The International Agency for Research on Cancer (IARC) (1972) and Verna et al., (1996) has classified DEN as a probable human carcinogen, despite the lack of epidemiologic data. Administration of DEN to experimental animals has been shown to cause cancer in liver and at lower incidences, in other organs as well (Schuller, 1992; Poirier & Beland, 1994; Bansal et al., 2005 and Ghosh et al., 2012). It is also reported to be a hepatotoxic agent causing hepatocellular necrosis in experimental animals (Liao et al., 2001). Diethylnitrosamine is reported to undergo metabolic activation by cytochrome P450 enzyme to form reactive electrophiles which cause oxidative stress leading to cytotoxicity, mutagenicity and carcinogenicity (Archer, 1989).

DEN treatment at a necrogenic dose can cause acute toxicity to various cell types in the adenohypophysis of the pituitary gland and alter serum levels of several hormones in rats (Liao et al., 2001). A single necrogenic dose of DEN to rats can also cause liver necrosis and a decrease in the level of hepatic growth hormone (GH) receptor (Roitman et al., 1986), the expression of which is partly regulated by GH (Kelly et al., 1991). Furthermore, degenerating and dying somatotropes have been reported in the pituitary from rats bearing malignant hepatomas induced by long-term, low-dose treatment of DEN (Ingleton & Hancock, 1976). Considering the above factors, it is likely that human exposure to DEN is inevitable. Hence, the development of an effective agent against DEN induced toxicity has become the need of the day.

Attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially when free radical generations are involved. Green tea has attracted significant attention recently, both in the scientific and in consumer communities for its health benefits for a variety of disorders, ranging from cancer to weight loss (Chandra et al., 2011). This publicity has led to the increased consumption of green tea by the general and patient population, and to the inclusion of green tea extract as a featured ingredient in several nutritional supplements, including multivitamin supplements. Historically, green tea has been consumed by the Japanese and Chinese populations for centuries, and is probably the most consumed beverage besides water. Tea has many advantages over chemicals since it contains several antioxidants including polyphenols of catechin and theamine (Mohamadin et al., 2005). Green tea has received a lot of attention owing to its role as an antioxidant and free radicals scavenger (Jung et al., 2001 and Tsubono et al., 2001). It is well known that green tea has anti-cancer, anti-diabetics and...
allowed drinking water conditions, fed with standard laboratory diet and Egypt. They were kept under normal day/light Research Institute of Ophthalmology, El-Giza, norvegicus 30 of all experimental groups were sacrificed on the

by gastric intubation for 30 day. Green tea was dissolved in distilled water and administrated daily (Ojo dose of green tea at a dosage of 100 mg/kg b. wt. injected intraperitoneally with a single dose of diethylnitrosamine (200 mg/kg) on day zero; group 2 (G2): 12 rats served as control green tea group and received a daily dose of green tea extract exhibited normal hepatic cords (Figs. 1a&b). Liver from rat treated with green tea (Fig. 1c). Rats treated with DEN alone architecture, indicating the non-toxic effect of with green tea extract exhibited normal

An experimental model: Forty-eight adult male albino rats (Rattus norvegicus) weighing 130-150g were used in this study. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under normal day/light conditions, fed with standard laboratory diet and allowed drinking water ad libitum. The animals were divided into four groups. Group 1 (G1): 12 rats served as controls and were treated with saline (i.p.) on day zero; group 2 (G2): 12 rats served as control green tea group and received a daily dose of green tea at a dosage of 100 mg/kg b. wt. dissolved in distilled water; group 3 (G3): 12 rats were injected intraperitoneally with a single dose of diethylnitrosamine (200 mg/kg) on zero day of the experiment according to Goldsworthy and Hanigan (1986). Diethylnitrosamine was purchased from Sigma Chemical Company, USA. It was prepared immediately before use by dissolving it in saline. Group 4 (G4): 12 rats were injected with diethylnitrosamine as G3 and then received a daily dose of green tea at a dosage of 100 mg/kg b. wt. (Ojo et al., 2006) for 30 day. Green tea was dissolved in distilled water and administered daily by gastric intubation for 30 day. Green tea was purchased from Arab Co. for Pharmaceuticals & Medicinal Plants Mepaco-Pharma, Egypt. The rats of all experimental groups were sacrificed on the 30th day.

Chemicals and drugs: Histological preparations:

For the histological preparations, animals were anaesthetized under light diethyl ether and dissected to remove the liver and pituitary gland after 30 day of treatment. Liver and pituitary gland tissues were cut into small pieces and then fixed in 10% neutral buffered formalin for 24 hours. The tissue was routinely processed and sectioned at 4 to 5 μm thickness with a microtome and stained with haematoxylin and eosin (Banchroft et al., 1996) for histopathological studies. Collagen fibers were demonstrated by Masson trichrome stain (Drury et al., 1976).

Histochemical studies:

After 30 day, liver and pituitary gland sections of all groups were stained with mercuric bromophenol blue (Hg-Bb) method (Mazia et al., 1953) for demonstration of total proteins, periodic acid Schiff (PAS) procedure (Hotchkiss, 1948) for demonstration of general polysaccharides and Feulgen method (Feulgen and Rossenbeck, 1924) for demonstration of DNA material.

Electron Microscopic Preparations:

Rats were sacrificed by decapitation after 30 day of the experiment. Liver and anterior pituitary tissues of control, diehtylnitrosamine and treated rats were immediately fixed in 3% glutaraldehyde buffer at PH 7.4. Liver and anterior pituitary tissues were removed and further fixed in 1.3 % osmium tetroxide in phosphste buffer (PH 7.4) for 1 hour. The samples were then processed and embedded in araldite CY212 (Glauert, 1965). Semi-thin sections (1 μm) of liver and anterior pituitary tissues were cut with an LKB ultramicrotome, stained with toluidine blue and examined on light microscope to determine the area of interest prior to ultra-thin sectioning. Ultrathin sections (70 - 90 nm) were prepared and stained with uranyl acetate and lead citrate according to the method of Reynolds (1963). Stained grids were then examined on a Joel CX 100 TEM operated at 60 kV.

3. Results
A-Liver:- Histopathology:

The control liver showed normal lobular architecture with central veins and radiating hepatic cords (Figs. 1a&b). Liver from rat treated with green tea extract exhibited normal architecture, indicating the non-toxic effect of green tea (Fig. 1c). Rats treated with DEN alone showed clear signs of severe hepatic injury manifested as area with periportal and perivascular inflammatory infiltrates (Fig.1d). The cytoplasm of most hepatocytes appeared vacuolated (Fig.1e&f) with pyknotic, karyolytic or enlarged nuclei (Figs.1e.f&g). Congested blood vessels (Figs.1d&f) and focal necrotic area infiltrated with mononuclear leukocytes (Fig.1e) was also observed. Some hepatocytes appeared with

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vacuolated or ruptured nuclei (Figs. 1f&g). On treatment with green tea the architecture of the liver was restored showing regeneration of normal hepatocytes (Figs. 1h& i).

After Masson's trichrome staining there was marked increase of the collagen fibers deposition around the blood vessels after DEN injection as compared with the control one (Figs. 2a&b). After treatment with green tea the distribution of the collagen fibers appeared near to the control (Fig. 2c).

Histochemistry:

In the hepatocytes of normal rats, total proteins appeared as intensely dark blue colored inclusions in the cytoplasm (Fig. 3a). In hepatocytes of rats exposed to DEN, protein content was found to exhibit an obvious reduction and their remnants were mainly located at the peripheries of the hepatic cells which showed sever cytoplasmic vacuolation (Fig. 3b). After treatment with green tea a marked increase in protein content was observed (Fig. 3c).

The PAS preparations of the normal liver of rats, revealed that polysaccharides were observed in the cytoplasm of the hepatocytes as indicated by large number of magenta red fine granules (Fig. 4a). After exposure of rats to DEN, polysaccharides content of the liver cells obviously decreased. In these specimens polysaccharides content displayed faint stainability and became hardly detectable (Fig. 4b). PAS reaction showed marked increase in PAS positive materials in the hepatocytes of rats treated with DEN and green tea (Fig. 4c) as compared to that of DEN treated rats.

The DNA content showed a noticeable decrease in the nuclei of hepatocytes of rats treated with DEN (Fig. 5b) when compared with that of control (Fig. 5a).

Many cells appeared anucleated and others with vacuolated nuclei (Fig. 5b). However, the hepatic DNA amount exhibited an increase with the simultaneous administration of green tea which retained a nearly normal distribution of DNA (Fig. 5c).

No obvious variations were determined in protein, polysaccharides and DNA contents in the control group treated with green tea only.

Ultrastructure:

The ultrastructural picture of the hepatic tissue in control rat liver revealed, normal hepatic cell with euchromatic nucleus, numerous intact mitochondria, granular endoplasmic reticulum profiles and glycogen deposits (Fig. 6a). In DEN treated rats some cells showed destructed cell membrane and the nuclei frequently appeared with irregular shapes and chromatin condensation (Fig. 6b). Other damaged cells appeared with pyknotic nuclei, vacuolated cytoplasm, dilated endoplasmic reticulum and little or no glycogen granules (Fig. 6c). The mitochondria showed a reduction or complete loss of cristae and an amorphous or granular matrix (Fig. 6c). Marked increase in the deposition of collagen fibers was observed as compared to the control (Figs. 6b&6c).

Examination of liver from rats treated with DEN and green tea revealed hepatocytes having nuclei with normal chromatin pattern, mitochondria with closely parallel cristae and the endoplasmic reticulum showed normal appearance (Fig. 6d).

B-Pituitary gland:

Histopathology:

There was no histopathological alterations observed in the pars nervosa, pars intermedia and pars distalis of control rats and rats administrated green tea and the histological cellular details of different regions were recorded in (Figs. 7a&b).

In rats treated with DEN focal hemorrhage with haemolysed blood was detected inbetween the pars intermedia and pars distalis (Fig. 7c). Appearance of signet ring cells (vacuolated cytoplasm and eccentric nucleus) (Fig. 7d), congested and dilated blood vessels were observed in both pars nervosa (Fig. 7d), and pars distalis (Fig. e). In rats administrated DEN and green tea the pituitary showed marked improvement in both pars nervosa and pars intermedia except the presence of hyperemia in pars distalis (Fig. 7f). After Masson's trichrome staining there was marked increase of the collagen fibers deposition inbetween the pituitary cells and around the blood vessels after DEN as compared with the control group (Figs. 8a&b). After treatment with green tea the distribution of the collagen fibers appeared near to the control (Fig. 8c).

Histochemistry:

DEN induced great depletion of proteins, polysaccharides and DNA contents in the pituitary cells (Figs. 9b, 10b and 11b respectively) compared with control group (Figs. 9a, 10a and 11a respectively). However, the simultaneous administration of green tea plus DEN resulted in moderate increase of protein content (Fig. 9c), retaining near normal distribution of polysaccharides (Fig. 10c) and DNA (Fig. 11c) content in pituitary cells compared to DEN-treated rats. No obvious variations were determined in protein, polysaccharides and DNA contents as a result of treatment with green tea only.

Ultrastructure:

Examination of pituitary gland of control rats revealed that most of the granular cells were of the storage type, characterized by many secretory granules which have characteristic size, shape and electron density by which the different cell types can be recognized. The cells had obviously rounded or oval nuclei with smooth nuclear envelopes and homogenously distributed nuclear
chromatin (Fig.12a). Moreover, normal blood sinusoid was observed (Fig.12b). The specific granules in the secretory cells of pars distalis were stored but were apart from the cells membrane by small distance till being exocytosed.

After DEN treatment moderate congestion and hemorrhage was observed in some areas and few secretory cells were desquamated inside the blood sinusoids (Fig.12c). Cellular degeneration, which seemed to involve many cell types containing secretory granules, was evident (Figs.12c, d&e). The degenerative alterations were characterized by degraded organelles, karyolytic or pyknotic nuclei, destructed cell membranes (Figs.12c&d) and dilated ER (Fig.12d). The secretory granules in non-degenerated cells were often located along the cell membrane (Fig.12e).

Marked increase in the deposition of collagen fibers was observed (Fig.12f). The simultaneous administration of DEN and green tea revealed nearly normal secretory cells with their rounded or oval nuclei. The cytoplasm contained multiple electron dense secretory granules with normal appearance of rough ER and mitochondria (Figs.12g&h).

Figs. (1a&b): Photomicrographs of section in the liver of control rat showing: (a): Central vein (CV) and trabeculae of hepatocytes separated by blood sinusoids (H&E; original magnification: X 200). (b): Higher magnification of the previous photo showing central vein (CV) and sinusoids (S) (H&E; original magnification: X 400).

Fig. (1c): Photomicrograph of section in the liver of green tea treated rat showing: normal lobular architecture. (H&E; original magnification: X 200).

Figs. (1d,e,f&g): Photomicrographs of section in the liver of rat treated with DEN showing: (d): Periportal inflammatory infiltrates (arrow) and congested portal vein (PV) (H&E; original magnification: X 200). (e): Vacuolated cytoplasm and mononuclear cell infiltrates (arrow) (H&E; original magnification: X 200). (f): Congested sinusoids (S), area of dissolution in cytoplasm and karyolytic (thin arrows) and ruptured nuclei (thick arrow) (H&E; original magnification: X400). (g): Karyomegalic (K) and vacuolated nuclei (V) (H&E; original magnification: X 1000).

Figs. (1h&i): Photomicrographs of section in the liver from rat treated with DEN and green tea showing regeneration of normal hepatocytes.  (h): (H&E; original magnification: X 200) and (i): (H&E; original magnification: X 400).
Figs. (2a,b&c): Photomicrographs of section in the liver of rat showing the distribution of collagen fibers. (a): Normal liver (Masson’s Trichrom; original magnification: X 200). (b): Liver of DEN treated rat showing marked increase in the amount of collagen fibers around the portal tract (Masson’s Trichrom; original magnification: X 200). (c): Liver of DEN and green tea treated rat showing marked decrease in the amount of collagen fibers (Masson’s Trichrom; original magnification: X 200).

Figs. (3a,b&c): Photomicrographs of section in the liver of rat showing the variability in the hepatic protein content. (a): Normal liver (bromophenol blue; original magnification: X 200). (b): Liver of DEN treated rat showing marked decrease in protein content of most hepatocytes (bromophenol blue; original magnification: X 200). (c): Liver of DEN and green tea treated rat showing marked increase in protein content of most hepatocytes (bromophenol blue; original magnification: X 200).

Figs. (4a,b&c): Photomicrographs of section in the liver of rat showing variation in the hepatic polysaccharides (glycogen) content. (a): Normal liver (PAS; original magnification: X 200). (b): Liver of DEN treated rat showing decreased staining reaction (PAS; original magnification: X 200). (c): Liver of DEN and green tea treated rat showing polysaccharides content similar to control (PAS; original magnification: X 200).

Figs. (5a,b&c): Photomicrographs of section in the liver of rat showing the variability in the hepatic DNA content. (a): Normal liver (Feulgen; original magnification: X 200). (b): Liver of DEN treated rat showing decreased DNA content (arrows) (Feulgen; original magnification: X 200). (c): Liver of DEN and green tea treated rat showing nearly normal DNA content (Feulgen; original magnification: X 200).
Fig. (6a): Electron micrograph of a hepatocyte of control rat showing mitochondria (M), nucleus (N), rough endoplasmic reticulum (rER) and glycogen rosettes (arrows). Scale bar = 1 µm.

Figs. (6b&c): Electron micrographs of a hepatocyte of rat treated with DEN showing (b): Shrunken nucleus (N) with irregular shape and chromatin condensation. Note also vacuolated cytoplasm (V), collagen fibers (C), destructed cell membrane (arrow) and rough endoplasmic reticulum (rER). Scale bar = 1 µm. (c): Vacuolated mitochondria (M), dilated endoplasmic reticulum (ER) and pyknotic nucleus (P). Notice the absence of glycogen. Scale bar = 2 µm.

Fig. (6d): Electron micrograph of a hepatocyte of rat treated with DEN and green tea showing nucleus (N) with normal chromatin pattern and large nucleolus. Note the normal rough endoplasmic reticulum (rER) and mitochondria (M) with closely parallel cristae compared to DEN treated rats. Scale bar = 2µm.
Figs. (7a & b): Photomicrographs of sections in the pituitary gland of (a): Control rat showing normal histological structure of the three pars, pars nervosa (N), pars intermedia (I) and pars distalis (D) in contact with each other (H&E; original magnification: X40). (b): Green tea treated rats showing normal histological structure of pars nervosa (N), pars intermedia (I) and pars distalis (D). (H&E; original magnification X 40).

Figs. (7c, d & e): Photomicrographs of sections in the pituitary gland of DEN treated rat showing (c): Hemorrhage (H) in between the pars intermedia and pars distalis (H&E; original magnification: X 160). (d): congested and dilated blood vessels (V) and vacuolated cells (thin arrow) in the pars nervosa (H&E; original magnification: X 40). Notice the presence of signet ring cells (thick arrows) (e): Dilated and congested blood vessels (v) of pars distalis (H&E; original magnification: X 160).

Fig. (7f): Photomicrograph of cross section in the pituitary gland of rat treated with DEN and green tea showing only hyperemia in blood vessels (V) of pars distalis (H&E; original magnification: X 64).
Figs. (8a, b & c): Photomicrographs of cross sections in the pituitary gland of (a): Control rat showing some collagen fibers (Masson’s Trichrom; original magnification: X 200). (b): DEN treated rat showing marked increase in the amount of collagen fibers in between the cells and around the blood vessels (Masson’s Trichrom; original magnification: X 200). (c): DEN and green tea treated rat showing marked decrease in the amount of collagen fibers (Masson’s Trichrom; original magnification: X 200).

Figs. (9a, b & c): Photomicrographs of cross sections in the pituitary gland of rat showing variation in the hepatic protein content. (a): Normal pituitary, (b): Pituitary of DEN treated rat showing decreased protein content, (c): Pituitary of DEN and green tea treated rat showing protein content similar to control. (Bromophenol blue; original magnification: X 200).

Figs. (10a, b & c): Photomicrographs of cross sections in the pituitary gland of rat showing variation in the hepatic polysaccharides (glycogen) content. (a): Normal pituitary, (b): Pituitary of DEN treated rat showing decreased staining reaction, (c): Pituitary of DEN and green tea treated rat showing polysaccharides content similar to control. (PAS; original magnification: X 200).

Figs. (11a, b & c): Photomicrographs of cross sections in the pituitary gland of rat showing the variability in the DNA content. (a): Normal pituitary, (b): Pituitary of DEN treated rat showing decreased DNA content, (c): Pituitary of DEN and green tea treated rat showing nearly normal DNA content. (Feulgen; original magnification: X 200).
Figs. (12a&b): Electron micrographs of an ultra-thin section of control rat adenohypophysis (pars distalis) showing (a): Corticotrophs (C) with rounded nuclei, sparse secretory granules, located at the extreme periphery (arrows) and mitochondria (M). Scale bar = 1µm. (b): Normal sinusoid (S) and somatotroph cell (Sm) with normal rough endoplasmic reticulum (rER) and numerous spherical dense and large secretory granules. Scale bar = 2µm. Inset photo showing gonadotroph cell (G) with circular secreting granules which variable in their size and density within the same cell. Scale bar = 2µm.

Figs. (12c-f): Electron micrographs of an ultra-thin section of adenohypophysis of rat treated with DEN showing (c): Moderate congestion and hemorrhage, few secretory cells desquamated inside the blood sinusoids, karyolytic nucleus (K) and destructed cell membranes (arrows). Scale bar = 2µm. (d): Markedly dilated cisterns of endoplasmic reticulum (ER), cytoplasmic vacuoles (V) and nucleus (N) with hypercondensed chromatin. Scale bar = 2µm. (e): Secretory granules located along the cell membrane (arrows) and vacuolated mitochondria (M). Scale bar = 1µm. (f): Collagen fibers (C) and destructed cell membrane (arrow). Scale bar = 200 nm.

Figs. (12g&h): Electron micrographs of an ultra-thin section of adenohypophysis of rat treated with DEN and green tea showing (g): Nearly normal somatotroph (Sm) with electron dense secretory granules (arrows), normal nucleus, rER and mitochondria (M). Scale bar = 2µm. (h): Corticotrophs (C) with nearly normal mitochondria (M) and nuclei. Scale bar = 1µm.

4. Discussion
Diethylnitrosamine, one of the most important environmental carcinogen, has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury (Bartsch et al., 1989 and Pradeep et al., 2010). As liver is the main site of diethylnitrosamine metabolism, the production of ROS in liver may be responsible for its carcinogenic effects (Bansal et al., 2005). The involvement of oxidative stress in diethylnitrosamine induced hepatotoxicity and carcinogenicity underscores the need for development of novel compounds with potent
antioxidant activity. The present study aimed at the investigation of the protective action of GTE in alleviating the adverse toxic effect of DEN. The present results showed that DEN administration to rats created liver toxicity which was manifested by marked architectural disturbances of hepatic lobules as well as hepatic necrosis, nuclear degeneration manifested by pyknosis and karyolysis, rising of the leucocytic infiltrations with congested blood vessels and increased collagen fibers. The increase of inflammatory cells of leucocytic infiltration might be powerful allies in body's defense against DEN-induced tissue destruction and hepatic necrosis at which scavenger macrophages engulf dead cells as previously reported by Cotran et al. (1999).

Hepatocellular necrosis occurred probably due to the direct attack of the cell membranes by hepatotoxin or by interacting with some specific components of the metabolic pathways leading to the alteration of their structure and function (klatskin and Conn, 1993). In agreement with the present study Shaarawy et al. (2009) demonstrated that the injection of DEN to rats lead to a marked elevation in the levels of serum AST, ALT and ALP which is indicative of hepatocellular damage. Accumulation of connective tissue proteins, especially collagen, have been reported in DMN-induced liver injury (Ala-Kokko et al., 1987 and George & Chandrakasan, 1996). The accumulation of collagen in the hepatocytes may be due to the decreased synthesis of collagenolytic enzymes by the impaired hepatocytes as reported by George et al. (2001).

Concerning the histochemil changes described in the present investigation as a result of DEN administration, it was found that polysaccharides, nucleic acids (DNA) and proteins were markedly decreased in both liver and pituitary gland cells. The present data agreed with work by Barbisan et al. (2003) who found severe hepatic necrosis and DNA damage in liver following administration of the identical dose of DEN to rats, in addition to liver damage observed by Koul et al. (2006) in mice. DEN can alkylate DNA molecule with itself being converted to highly reactive molecule by P-450 dependent oxygenases (Li et al., 2005). DEN injection caused depletion of polysaccharides granules in the hepatocytes. It possibly due to disruption in calcium metabolism as reported by Koizumi et al. (1995) in liver of DEN treated rats. Further more, calcium has been demonstrated to be associated with gluconeogenesis (Nagata and Rasmussen, 1970).

Protein disruption after DEN treatment might be due to detachment of ribosomes from rough endoplasmic reticulum and hence reduction of protein syntheses corresponded to stress of the DEN which caused enzymatic damage and free radical (Yamada et al., 2006). Inhibition of protein synthesis is known to stop DNA synthesis almost immediately (Verbin et al., 1969). Inhibition of DNA synthesis is also brought about by many carcinogens and this is considered to have some significance in the carcinogenic process (Farber, 1976).

Histological and histochemical disturbance occurred in liver tissue were confirmed by electron microscopic changes. In the present study DEN showed to be a destructive compound for the most intracellular organelles. The toxic effects of DEN on the liver were represented by cytoplasmic vacuolization, mitochondrial alterations, dilated endoplasmic reticulum and decrease of glycogen granules. The endoplasmic reticulum is particularly liable to the free radical attack, not only because it is considered as a site of radical production but also due to the enrichment of its membrane with polyunsaturated fatty acids which are susceptible to free radical attack (Slater, 1984). It has been observed as an early event in the process of toxicity as a result of exposure to certain chemicals (Cortan et al., 1994).

Therefore, the dramatic ultrastructural changes observed in the present study may be attributed to increased oxidative stress as reported by (Pradeep et al., 2010). Our results were confirmed by previous study (Braunbeck et al., 1992; Chandra et al., 2011 and Gosh et al., 2012) that showed a significant morphological and ultrastructural changes following treatment of DEN.

The pituitary gland is refractory to the toxicity of most substances other than hormone analogues or antagonists (Saeger, 1992). The present study showed that rats treated with DEN showed focal haemorrhage in between the pars intermedia and pars distalis. Vacuolation and sever hyperemia was observed in both pars nervosa, and pars distalis. Appearance of signet ring cells and increase in collagen fibers deposition were also observed. In accordance to the ultrastructural study of pituitary DEN treatment showed moderate congestion and hemorrhage, desquamation of some secretory cells inside the blood sinusoids and cellular degeneration. The degenerative alterations were characterized by degraded organelles, karyolytic or pyknotic nuclei, and marked increase in the deposition of collagen fibers. These results are in accordance with Liao et al. (2001) who reported that DEN treatment at a necrogenic dose can cause acute toxicity to various cell types in the adenohypophysis and alter serum levels of several hormones. Roitman et al. (1986) observed liver necrosis and a decrease in the level of hepatic growth hormone (GH) receptor, the expression of which is partly regulated by GH (Kelly et al., 1991), after a single necrogenic dose of DEN to rats. Furthermore, degenerating and dying somatotropes have been reported in the pituitary from rats bearing malignant hepatomas.
induced by long-term, low-dose treatment of DEN (Ingleton & Hancock, 1976).

Accumulation of ROS exerts a potent damaging effect on the cells especially phospholipids of biological membrane, proteins and DNA. Mitochondria are the main site of ROS generation in cell as well as the main target for the free radical attack. In turn this results in damage of mitochondrial respiratory chain with subsequent increase in ROS generation and a vicious cycle is formed (Szewczyk & Wojtczak, 2002). In this work it was found that the most affected organelle in the pars distalis cells was the mitochondria, which might explain this ROS generation mechanism.

In addition to cellular damage, dilated ER and peripheral localization of secretory granules in the non degenerated cells were observed in DEN-treated rats. Similar alterations have been reported in the pituitary from male rats one to several days after partial hepatectomy (Shiino & Rennels, 1975 and Hirano & Shiino, 1991) and from the male rats receiving chronic feeding of liver carcinogen 2-AAF (Ingleton & Hancock, 1976) or 3′-methyl-4-dimethylaminoazobenzene (Varey & Ingleton, 1984). In these other reports, these alterations were considered signs of increased cellular activities and exocytosis in the pituitary cells. Therefore, it is possible that the similar changes observed in the present study may reflect increased cellular activities of non degenerated pituitary cells and may be caused by the loss of liver tissue or the need for liver regeneration, both of which occur after partial hepatectomy or treatment with 2-AAF, or 3′-methyl-4-dimethylaminoazobenzene.

The simultaneous administration of DEN and green tea ameliorates the unfavorable effects produced in the liver and pituitary gland by DEN activity. GTE enhances glycogen, protein and DNA content. The increase of protein and glycogen content may be due to the presence of polyphenols that stimulate the transcription of phase II detoxifying enzymes mediated by an antioxidant response element (Ranjbar et al., 2005). Green tea can suppress the DNA adduction, and hence act as inhibitors of cancer and it is a rich source of polyphenols, which are antioxidants in natural and their amelioration effect on genital organs were recorded (Ogura et al., 2008 and GAWISH et al., 2010). Consuming high levels of GTE over a long period reduced the DNA damage caused by nicotine which is a nitrosamine precursor (GAWISH et al., 2010). In the other hand, it was previously reported that GTE activities prevent damage to DNA structure (HIDER et al., 2001) and liver cirrhosis (NOORI et al., 2009). WALTNER-LAW et al. (2002) explain the lowered blood glucose to the decrease in expression of genes control gluconeogenesis in liver cells while, KHAN et al. (2007) elucidated that GTE causes selective adaptive alterations in the activities of certain mitochondrial enzymes involved in glycolysis, gluconeogenesis and glycogenesis in the liver cells. GTE showed an improvement of the cell membranes destructed by DEN and so cytoplasmic organelles, intact cell membranes of the hepatocytes and pituitary cells, in this regard OSTROWSKA et al., (2004) found that enhancement in lipid peroxidation was associated with disruption of cell membranes. So Green tea protects phospholipids from better peroxidation and prevents morphologic changes. The results of this work support the suggestion that green tea protects membranes from peroxidation of lipids associated with DEN consumption in rat liver and pituitary by decreasing oxidative stress (Augustyniak, et al., 2005). Chemopreventive intervention by different phytochemicals, particularly tea polyphenols found in green tea showed 20 times more powerful antioxidant activity than vitamin C (Craig, 1999). It enhances the expression of intracellular endogenous antioxidants such as glutathione, glutathione peroxidase, glutathione peroxidase catalase by reducing the generation of reactive radicals (KHAN et al., 1992 and VALERIO et al., 2001).

GTE probably arrest the harmful mechanism of liver and pituitary injury through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals and stimulate the regeneration of damaged tissues and cells (FENG et al., 2001 and Jimenez-Lopez and Cederbaum, 2004). The result confirmed by many authors (ALSCHLER, 1998; LIAO, 2001; FADHEL & AMRAN, 2002; KHAN & MUKHTAR, 2007 and HININGER-FAVIER et al., 2009), they stated that consumption of GTE has many beneficial effects on human health, particularly polyphenols, chiefly catechins and their derivatives that retard various forms of cancers due to its antimutagenic, anticarcinogenic and antioxidant properties, cardioprotective, neuroprotective, antidiabetic and antibacterial.

Conclusion:
The results of the current study demonstrated that DEN may induce production of free radical that caused oxidative stress to the liver and pituitary cells and their organelles particularly mitochondria and cell membranes. Green tea reduced this oxidative damage by its antioxidant properties and ameliorated DEN-induced liver and pituitary toxicity. Further studies are necessary to establish the structures and functions correlations.

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