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Original Communication

Protective effect of green tea against the hematological, biochemical, histopathological and ultrastructural changes in rat liver induced by subchronic exposure to melamine

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ABSTRACT

Melamine (MA) is considered as a natural nitrogen heterocyclic substance that is added to soil to improve the soil fertility and used to manufacture plastics, dyes and cloths. In recent years many milk processing companies have started adding melamine (MA) to milk to increase its protein content and this led to toxic effects to many organs such as liver, brain, kidney etc. and caused bladder cancer in experimental animals. Recent studies have proved that green tea extract (GTE) has a hepatoprotective role and has health benefits against a variety of toxins associated with oxidative stress. The aim of the present study is to evaluate the protective effect of green tea against the hematological, biochemical, histopathological and ultrastructural changes in rat liver induced by subchronic exposure to melamine. Eighty adult albino rats (200-250 gm) were equally divided into four groups; group I received normal distilled water orally, group II received 1.5% w/v GTE, group III received MA (50 mg/kg b.wt/day), and group IV received MA (50 mg/kg b.wt/day) plus 1.5% w/v GTE. All rats were given GTE by gastric gavage for 2 months. The animals were sacrificed under general anesthesia, and the blood and liver were sampled and prepared for haematological, biochemical, histopathological and ultrastructural examinations. The results revealed that MA treatment (group III) caused significant alterations in haematological and biochemical parameters in comparison with groups I and II. Also, histopathological and ultrastructural examination of the liver revealed severe degenerative changes. Co-administration of GTE with MA ameliorated most of the toxic effects of melamine (group IV). Our results demonstrated that GTE exerts protective effect by alleviating the toxic pathological changes caused by MA in the liver of albino rats.

KEYWORDS: green tea extract, melamine, liver, histopathology, rat.

1. INTRODUCTION

Melamine (MA) is an organic substance that contains 66% nitrogen and is commercially synthesized from urea; cyanic acid is obtained as a by-product during the synthesis of melamine. In industry, melamine is combined with formaldehyde to produce melamine resin (a durable thermosetting plastic), dry erase boards, cloths, flame retardants, adhesive substances and housewares [1]. It is one of the contents of yellow pigment which is used as a colorant in inks and plastics. Melamine derivatives of arsenic drugs are used for the treatment of African trypanosomiasis. MA is being used in fertilizers since 1958 and is sometimes fed to cattle as a non-protein nitrogen source [2].

In recent years, a large number of accidental deaths of domestic animals following ingestion of

MA-tainted food have been reported [3, 4]. Moreover, there have been cases of MA poisoning due to consumption of MA-contaminated infant formula in various countries [5]. Several adverse effects due to MA consumption such as bladder cancer [6], kidney inflammation [7], nerve destruction [8], and toxicity to reproductive systems [9] have also been found. Children ingesting MA-contaminated powdered infant formula have shown symptoms of immunocompromised disorders [10, 11].

It has been found that MA-cyanurate caused erythrocyte deformation and aggregation [12]. Also Yin *et al*. [13] reported that MA caused cytotoxic effects on lymphocytes of spleen when administered at a dose of 50 mg/kg/day for 14 consecutive days.

Recently, herbal plant extracts have been used as natural protective agents against various toxins [14]. Green tea extracts (GTEs) have health benefits against a variety of toxins associated with oxidative stress [15]. Recent experiments revealed that GTE could be used as a hepatoprotective agent [16, 17] because it had high amounts of catechins such as epicatechin, epicatechin gallate (ECG), epigallocatechin, and epigallocatechin gallate (EGC). These catechins are responsible for GTE's antioxidant activity [18, 19]. Also GTE contains additional amounts of vitamins E and C [20] as well as minerals which function as co-factors of antioxidant enzymes.

2. MATERIALS AND METHODS

2.1. Materials

MA (99.5% purity) was purchased from Alpha Chemica (Mumbai, India). It was diluted in distilled water to working stock concentrations to be used in this experiment [21].

Fifteen grams of instant green tea powder was dissolved in 100 ml of boiled distilled water for 5 min. The solution was filtered to make 1.5% GTE. The dose is 1.5% w/v [22].

2.2. Animals

80 healthy albino rats of both sexes weighing 200-250 gm were obtained from the Animal House of University of Taif, Saudi Arabia and housed, six in a cage, in a room with a controlled temperature of 22-24 °C, relative humidity of about 60%, and artificial lights on from 7:00 a.m. to 16:00 p.m. All animal procedures were carried out in accordance with the international laws and policies under the Ethics of Committee of Animal Experiments. The rats were kept in the room for fifteen days to adapt to the above-mentioned conditions and supplied with standard diet and water.

2.3. Experimental design

At the end of the adaptation period, the rats were divided into four groups namely, I, II, III, and IV.

- Group I, consisting of 20 rats, received normal distilled water orally (served as control).
- Group II, consisting of 20 rats, received 1.5% w/v GTE [22].
- Group III, consisting of 20 rats, received MA (50 mg/kg b.wt/day) [23].
- Group IV, consisting of 20 rats, received MA (50 mg/kg b.wt/day) [23] plus 1.5% w/v GTE [22].

The rats were given GTE by gastric gavage for 2 months.

2.4. Sample collection

2.4.1. Blood samples

Twenty-four hours after the end of the experimental period, the animals were slightly anesthetized with ether, and rapidly decapitated. Blood was collected and divided into two samples. The first sample collected in an ethylenediaminetetraacetic acid (EDTA) tube (heparinized tube) was used for the determination of hematological parameters and the second sample was left to clot at 37 °C and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was collected and stored at -20 °C for evaluating the activities of transaminase alanine amino transferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase, total protein, albumin and total bilirubin.

2.4.2. Tissue samples

Small slices of rat liver were excised, cleaned, washed with normal saline and grouped into three: the first group was kept at -80 °C until use for the assessment of oxidative stress and antioxidant

enzyme activity; the second group was homogenized for histological examination and the third group was processed for ultrastructure examination.

2.5. Hematological studies

The first blood sample, i.e. the heparinized blood sample collected in EDTA tubes, was analyzed for the number of red blood cells (RBCs), white blood cells (WBCs), and hemoglobin concentration (Hb%), and the differential count of polymorphs and lymphocytes according to standard methods using an Animal Blood Counter-ABC vet (Horiba ABX, France).

2.6. Biochemical assays

2.6.1. Liver biomarker assessment

The second blood sample was used for determining the levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) and these were estimated according to the method described in [24] using reagent kits purchased from "Human Diagnostics" (Germany). Serum albumin was determined by using a commercial kit and total serum bilirubin (TSB) was assayed according to the method described in [25].

2.6.2. Liver samples

2.6.2.1. Assessment of oxidative stress and antioxidant enzyme activity

The first group of liver samples was used to determine the activities of catalase enzyme (CAT) and glutathione (GSH), the content of malondialdehyde (MDA) and superoxide dismutase (SOD) [26-29].

2.7. Histological studies

2.7.1. Light microscopy

The second group of liver samples was fixed in 10% buffered formalin solution for 24 h for histological studies and then they were dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax. 5 mm thick sections were stained with haematoxylin and eosin for histopathological studies, with Masson's trichrome stain for connective tissue staining, and with Periodic acid Schiff (PAS) stain to demonstrate mucopolysaccharides as PAS-positive materials [30].

2.7.2. Electron microscopy

The third group of liver samples was cut into small pieces of 1 mm thickness and fixed in phosphate buffer solution (pH 7.2) for 3 h at 4 °C for electron microscopy, after which the tissues were removed and post-fixed in buffered 2% OsO4 for one hour at 4 °C. Post-fixed tissues were rinsed in the buffer and dehydrated at 4 °C in a graded series of ethanol, then embedded in epon-araldite mixture in labeled beam capsules. Ultrathin sections (50 nm thick) were cut, collected on naked copper-mesh grids and stained with uranyl acetate for 1/2 hour and lead citrate for 20-30 min [31].

2.8. Statistical analyses

Results were expressed as mean ± SEM. Comparison of means was done by the Student's t-test (One way analysis of variance (ANOVA)) and the Mann-Whitney U test. Values of $P < 0.05$ were considered statistically significant. Statistical evaluation was conducted using SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Hematological findings

Data in Table 1 reveal that the rats in group III showed significant decrease in RBC count, hemoglobin level, total WBC count and lymphocyte but neutrophils and monocytes were significantly elevated in comparison with groups I and II. While rats in group IV showed significant increase in RBC count, hemoglobin level, total WBC count and lymphocyte, neutrophils and monocytes were significantly decreased in comparison with group III.

3.2. Liver biomarkers of rats

Data in Table 2 show significant increase in serum AST, ALT and TSB levels and significant reduction in serum albumin in rats of group III when compared to groups I & II, while rats in group IV showed significant reduction in serum AST, ALT and TSB levels and significant increase in serum albumin when compared to group III.

3.3. Antioxidants and lipid peroxidation

Data in Table 3 show that rats in group III showed significant reduction in SOD, CAT and GSH level

Table 1. Effect of MA used alone or with GTE on mean ± SD of blood counts of rat.

Parameters	Group I (control)	Group II (GTE)	Group III (MA)	Group IV (GTE + MA)
RBCS (x 10^6/µL)	5.80 ± 0.71	5.44 ± 0.72	3.97 ± 0.82*	5.09 ± 0.21**
Hb (g/dl)	11.98 ± 0.02	11.12 ± 0.03	8.81 ± 0.72*	11.01 ± 0.04**
HCT (%)	50.91 ± 0.23	49.98 ± 0.26	42.31 ± 0.33*	51.02 ± 0.39**
WBCS (x 10^3/ul)	5.06 ± 0.46	5.18 ± 0.23	3.77 ± 0.37*	5.01 ± 0.20**
Lymphocytes (%)	31.16 ± 0.51	35.20 ± 0.51	26.44 ± 0.69*	31.71 ± 0.54**
Eosinophils (%)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Neutrophils (%)	52.25 ± 0.51	52.95 ± 0.51	62.41 ± 0.79*	52.87 ± 0.43**
Monocytes (%)	3.21 ± 0.21	3.13 ± 0.21	8.42 ± 0.21*	0.30 ± 0.32**

Number per group = 20; SD = standard deviation.
Group I (control) received equal volume of distilled water/day.
Group II received 1.5 % w/v GTE [22].
Group III received MA (50 mg/kg b.wt/day) [23].
Group IV received MA (50 mg/kg b.wt/day) plus 1.5 % w/v GTE for 2 months [22].
* = $p < 0.05$ (significant difference in comparison with group I & II).
** = $p < 0.05$ (significant difference in comparison with group III).

Table 2. Effect of melamine used alone or with green tea extract on mean ± SD of liver functions of rat.

Parameters	Group I (control)	Group II (GTE)	Group III (MA)	Group IV (GTE + MA)
AST (U/L)	51.34 ± 0.66	52.64 ± 0.66	70.23 ± 0.45*	52.34 ± 0.18**
ALT (U/L)	83.14 ± 0.76	84.04 ± 0.76	132.27 ± 0.82*	85.23 ± 0.04**
TSB (mg/dl)	1.01 ± 0.01	1.21 ± 0.01	2.86 ± 0.02*	1.17 ± 0.04**
Albumin (mg/dl)	4.01 ± 0.06	4.39 ± 0.06	2.94 ± 0.07*	4.47 ± 0.05**

Number per group = 20; SD = standard deviation.
Group I (control) received equal volume of distilled water/day.
Group II received 1.5 % w/v GTE [22].
Group III received MA (50 mg/kg b.wt/day) [23].
Group IV received MA (50 mg/kg b.wt/day) plus 1.5 % w/v GTE for 2 months [22].
* = $p < 0.05$ (significant difference in comparison with groups I & II).
** = $p < 0.05$ (significant difference in comparison with group III).
TSB: total serum bilirubin.
AST: Aspartate amino transferase enzyme.
ALT: Alanine amino transferase enzyme.

Table 3. Effect of melamine used alone or with green tea extract on mean ± SD of hepatic antioxidant enzymes in rats.

Parameters	Group I (control)	Group II (control)	Group III (MA)	Group IV (GTE + MA)
SOD	5.65 ± 0.23	5.78 ± 0.23	2.02 ± 0.76*	5.92 ± 0.03**
CAT	9.38 ± 0.16	9.33 ± 0.16	5.01 ± 0.82*	9.69 ± 0.08**
GSH	55.14 ± 0.44	55.24 ± 0.44	29.97 ± 0.06*	55.34 ± 0.23**
MDA	34.13 ± 0.12	34.52 ± 0.12	49.78 ± 0.44*	34.45 ± 0.12**

Number per group = 20; SD = standard deviation.

Group I (control) received equal volume of distilled water/day.

Group II received 1.5 % w/v GTE [22].

Group III received MA (50 mg/kg b.wt/day) [23].

Group IV received MA (50 mg/kg b.wt/day) plus 1.5 % w/v GTE for 2 months [22].

* = $p < 0.05$ (significant difference in comparison with groups I & II).

** = $p < 0.05$ (significant difference in comparison with group III).

GSH: glutathione.

SOD: superoxide dismutase.

CAT: catalase.

MDA: malondialdehyde.

but significant increase in MDA level as compared to groups I & II. But rats in group IV showed significant increase in SOD, CAT and GSH levels and significant reduction in MDA level in relation to group III.

3.4. Light microscopic observations

The liver of rats in groups I & II showed a normal structure of liver cells containing large spherical nucleus with marked nucleolus, and hepatocytes were arranged into hepatic cords running radiantly from the central vein and the spaces between the cell cords called blood sinusoids were lined by Kupffer cells (Figures 1 and 2).

The administration of MA in group III showed obvious signs of hepatic alterations; these include congested blood vessels, extensive vacuolar degeneration of hepatocytes, and dilation of the walls of blood sinusoids with numerous Kupffer cells. In a few liver areas, necrotic changes in most of the hepatocytes were observed in the form of a small and pycknotic cellular nucleus with condensed chromatin, and lack of nucleolus; in addition to this, portal fibrosis was also observed (Figures 3 and 4).

Liver of rats treated with GTE in combination with MA (group IV) showed marked recovery and restoration to almost normal hepatic configuration (Figures 5 and 6).

3.5. Histochemical observations

The liver tissues of rats of groups I & II showed strong positive PAS reaction in the liver cells and cytoplasm due to the presence of large amount of glycogen (Figure 7).

The liver tissues of the rats exposed to MA alone (group III) showed marked reduction in PAS reaction in both liver cells and cytoplasm (Figure 8).

The liver tissues of the rats exposed to MA in combination with GTE (group IV) appear to have a strong positive PAS reaction in the liver cells and cytoplasm due to the presence of large amount of glycogen when compared to group III (Figure 9).

3.6. Transmission electromicroscopic observations

The liver cell of rats of groups I & II showed normal ultrastructure in the form of a normal

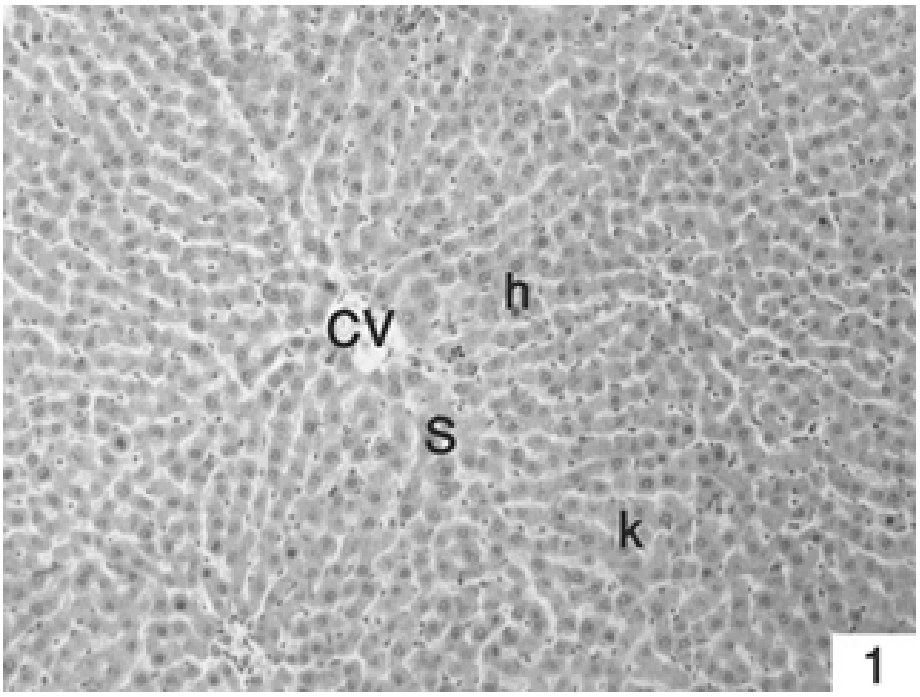

Figure 1. Light photomicrography of liver of control rats showing that the hepatocytes (h) are hexagonal in shape, arranged into hepatic cords running radiantly from the central vein (CV) and are separated by adjacent blood sinusoids (S) containing Kupffercells (k) (H&E x 400).

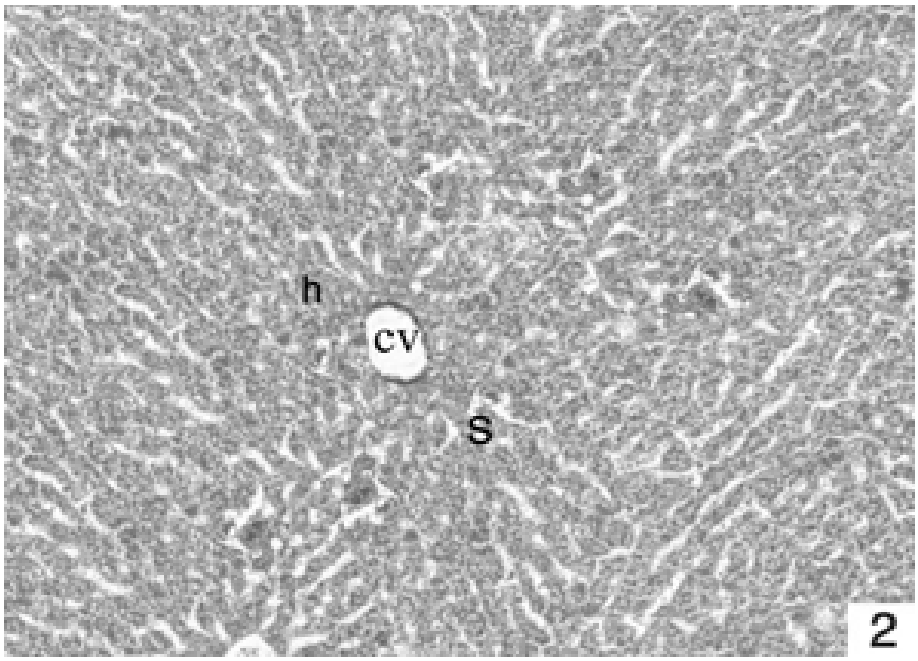

Figure 2. Light photomicrography of liver of control rats showing normal hepatocytes (h) arranged into hepatic cords running radiantly from the central vein (CV) and are separated by adjacent blood sinusoids (S) (Mallory's x 400).

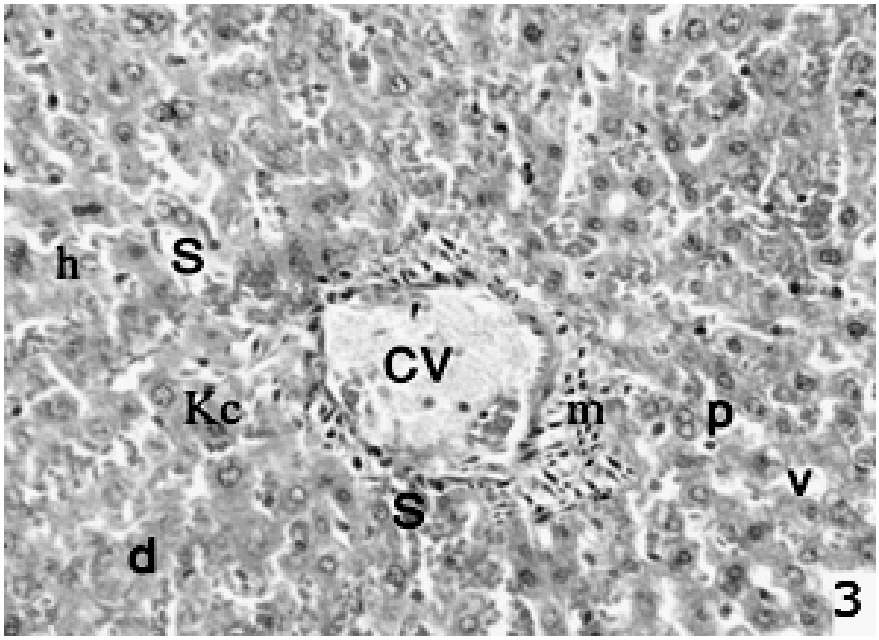

Figure 3. Light photomicrography of liver of rats after eight weeks of exposure to MA showing shrunken hepatocyte (h) with vacuolated cytoplasm (v), widening of blood sinusoids with accumulation of mononuclear cells (m) in the nearest areas of sinusoids (S) and the central vein (CV). The sinusoid walls have numerous Kupffer cells (Kc). Notice that there are degenerated areas (d) containing pyknotic nuclei (p) (H&E x 400).

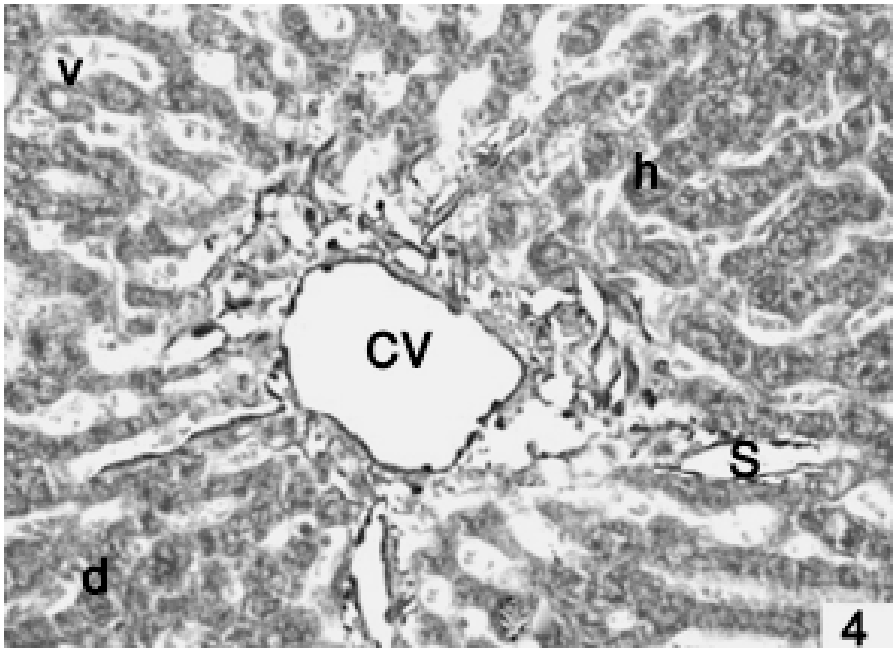

Figure 4. Light photomicrography of liver of a rat after eight weeks of exposure to melamine showing widening of central vein (CV) and sinusoidal spaces (S) with portal fibrosis, degenerated area (d), vaculated cytoplasm (v), and degenerated hepatic cells (h) (Mallory's x 400).

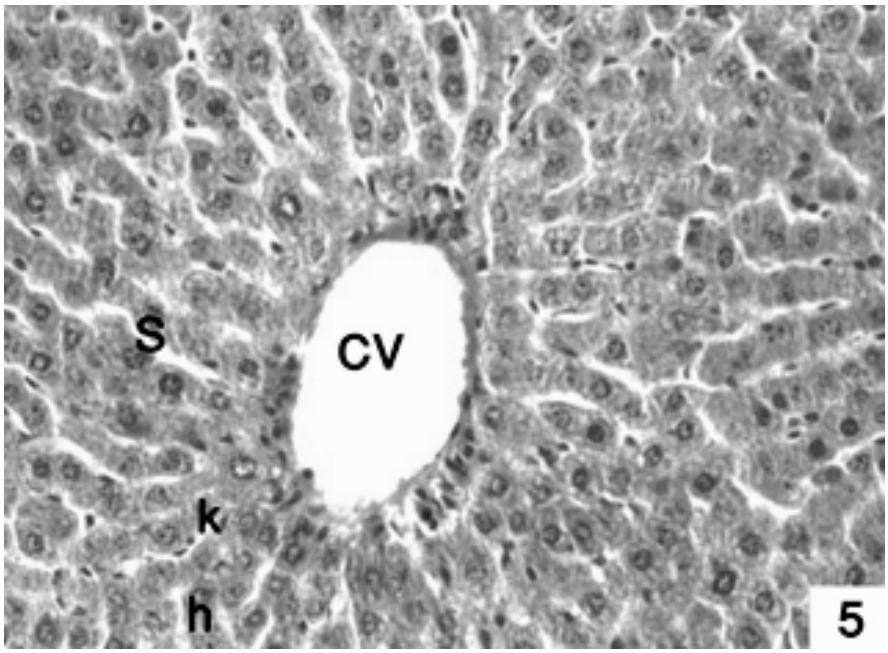

Figure 5. Light photomicrography of liver of rats after eight weeks of exposure to green tea extract in combination with melamine. The liver organization appears normal with nearly normal hepatocytes (h) around the central vein (CV), and decreased widening of blood sinusoid (S) containing Kupffer cells (k) (H&E x 400).

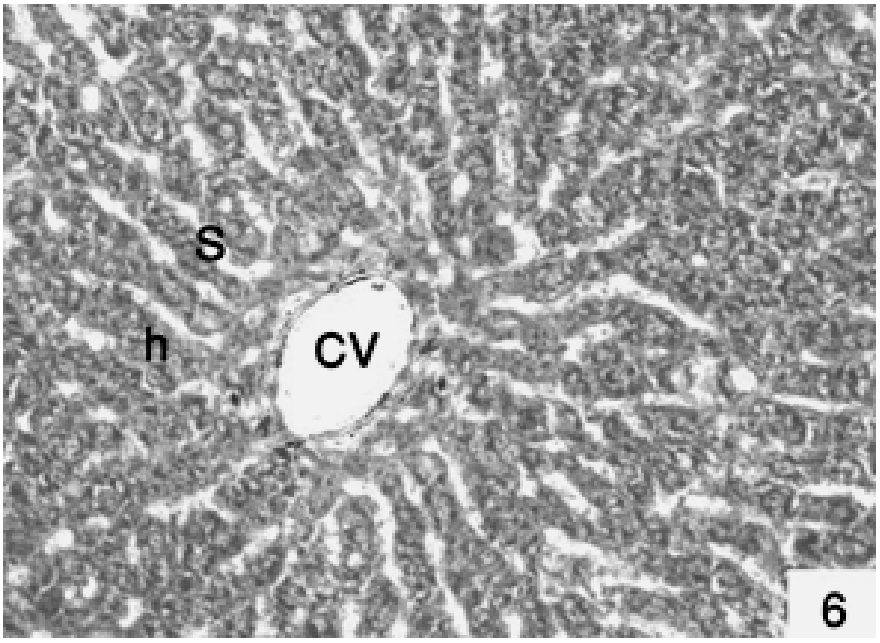

Figure 6. Light photomicrography of liver of rats after eight weeks of exposure to green tea extract in combination with melamine, showing marked decreased in degenerated hepatic cells (h), normal-sized central vein (CV) and sinusoidal spaces (S) with marked absence of portal fibrosis around the central vein (Mallory's x 400).

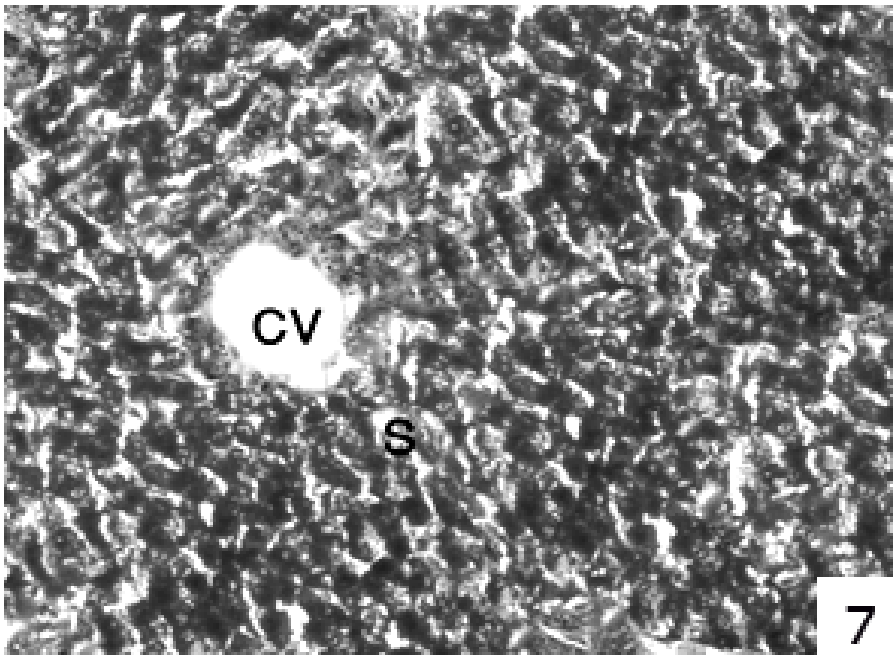

Figure 7. Light photomicrography of liver of a control rat, showing strong positive PAS reaction in all of its components particularly the hepatocytes, central vein (CV) and blood sinusoid (S) (Periodic acid-Schiff's x 400).

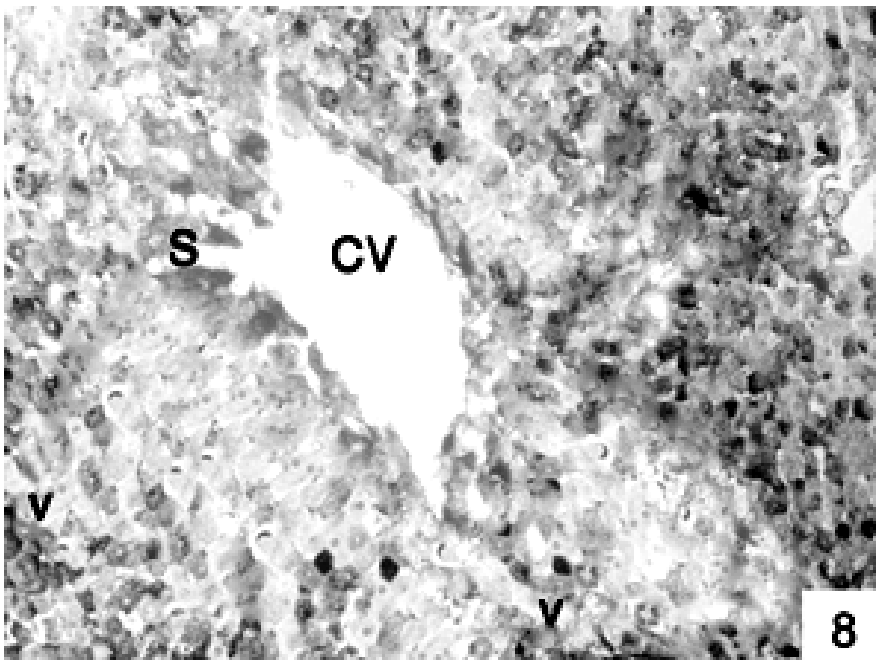

Figure 8. Light photomicrography of liver of rats after eight weeks of exposure to melamine showing marked reduction in PAS reaction in all areas of the liver tissues inside the hepatocytes, around central vein (CV) and blood sinusoid (S) due to depletion of glycogen in hepatocytes with vacuolated cytoplasm (V) (Periodic acid-Schiff's x 400).

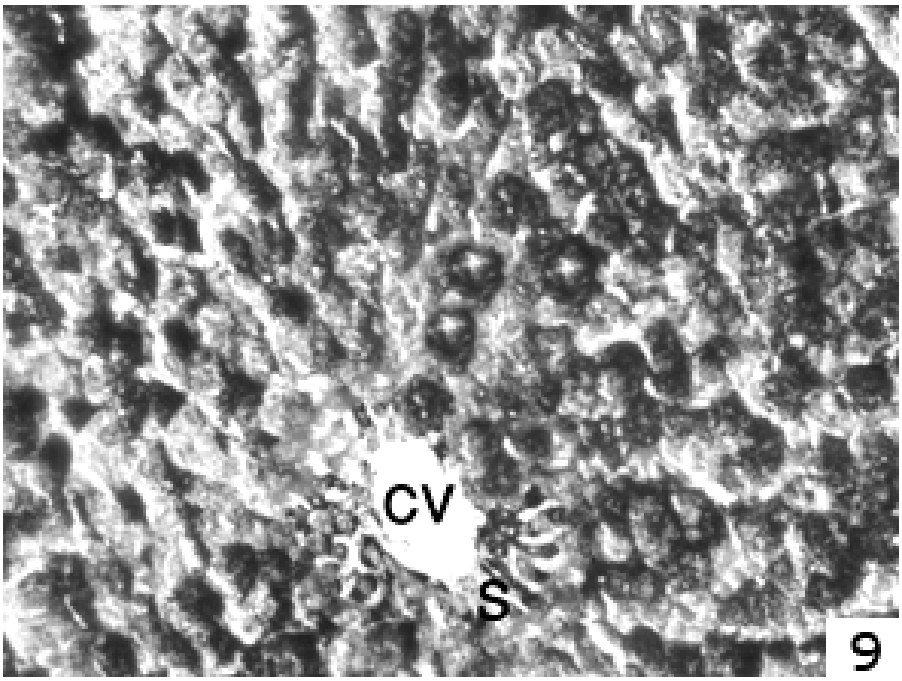

Figure 9. Light photomicrography of liver of rats after eight weeks of exposure to green tea extract in combination with melamine, showing marked increased in PAS reactions in all its components, particularly the hepatocytes, central vein (CV) and blood sinusoid (S) (Periodic acid-Schiff's x 400).

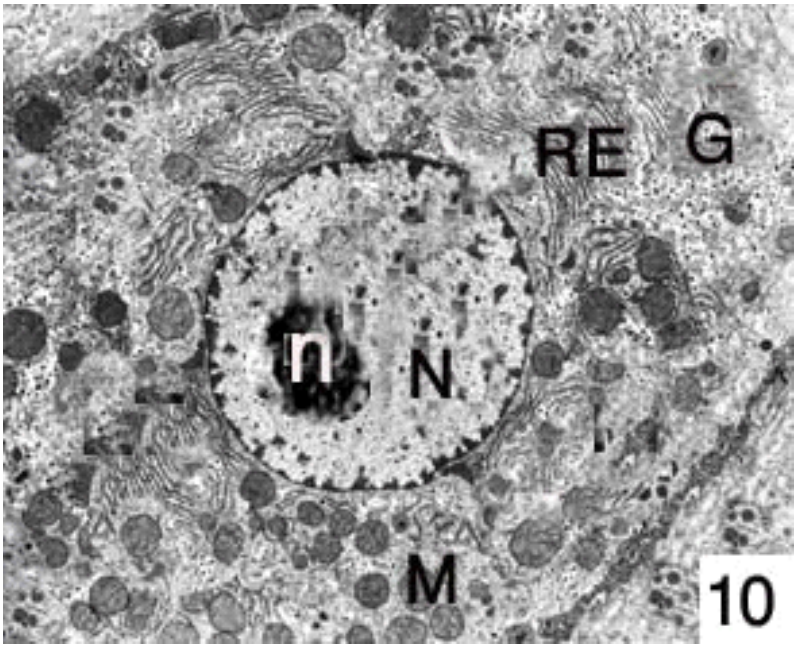

Figure 10. Transmission electron microscopic picture of a section of liver of control rats showing normal euchromatic nucleus (N), nucleolus (n) with Golgi apparatus (G), rough endoplasmic reticulum (RE) and many mitochondria (M) (TEM mag. = 8000X).

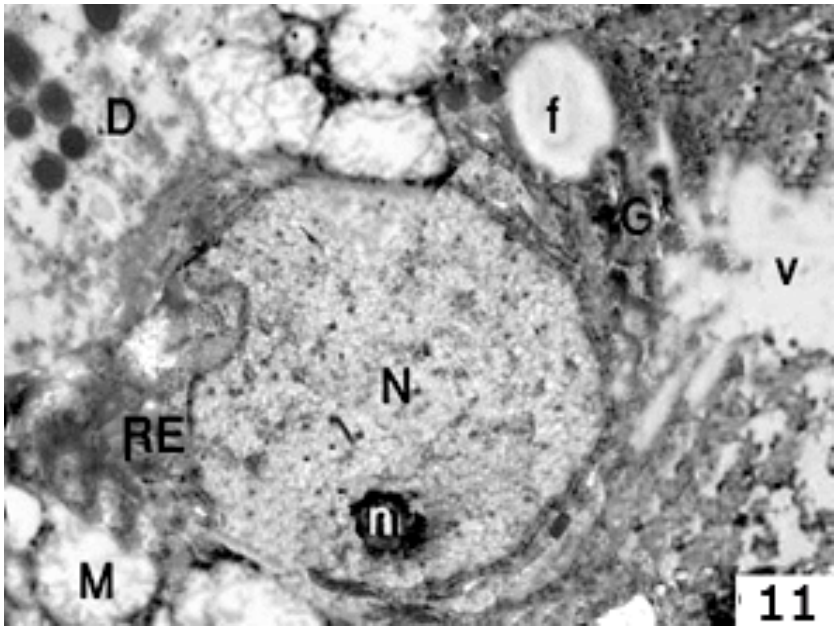

Figure 11. Electron micrographs of hepatocytes of rats treated with melamine for 8 weeks showing destruction of cell membrane of liver cell with decreased size of its nucleus (N) and less prominent nucleolus (n), swollen and vacuolated mitochondria (M), large fat droplets (f), many cytoplasmic vacuoles (V), swollen in rough endoplasmic reticulum (RE) and degenerated Golgi apparatus (G) and some degenerated hepatocytes (D) (TEM mag. = 10000X).

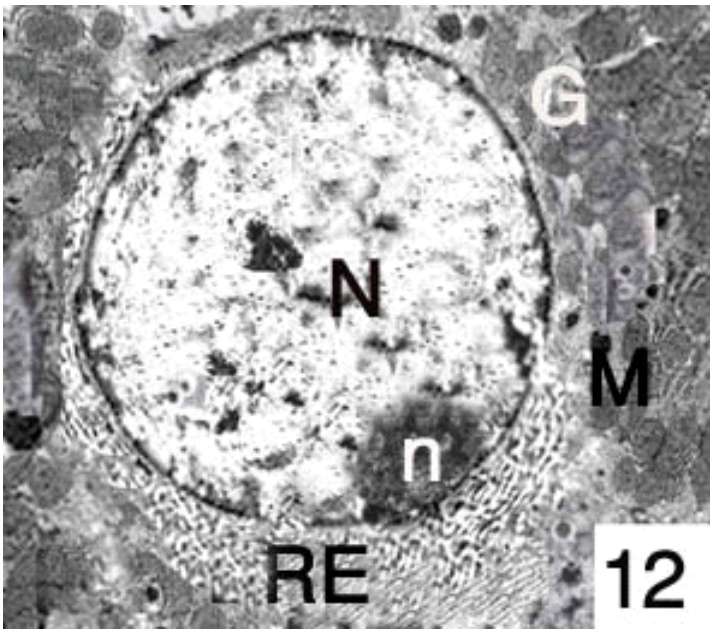

Figure 12. Transmission electron microscopic picture of a section of liver of rats exposed to green tea extract in combination with melamine for 8 weeks, showing euchromatic nucleus (N) and prominent nucleolus (n), Golgi apparatus (G), rough endoplasmic reticulum (RE) and numerous mitochondria (M) (TEM mag. = 12000X).

euchromatic nucleus and nucleolus with Golgi apparatus, numerous rough endoplasmic reticulum and many mitochondria (Figure 10). In the liver of rats in group III which were exposed to MA alone, the transmission electromicroscopic observation of the liver cells showed destruction of cell and nuclear membranes, less prominent nucleolus, destruction of mitochondria and rough endoplasmic reticulum, with increase in cytoplasmic fat droplets, and vacuoles (Figure 11). Rats of experimental group IV which were exposed to MA in combination with GTE showed marked recovery and restoration (Figure 12).

4. DISCUSSION

Hematopoietic system is considered as a sensitive system to evaluate the hazardous effects of poisons in humans and animals [32]. In the present study, the data in Table 1 show that the rats treated with MA had significant decrease in RBC count, hemoglobin level, total WBC count and lymphocyte but neutrophils and monocytes were significantly elevated in comparison with groups I and II. These findings are in agreement with those reported by Abd-Elhakim *et al*. [21] who reported that mean values for RBCs, Hb, and MCHC were significantly lower in MA-treated mice as compared to those in the control mice. MA caused reduction in the number of peripheral blood lymphocytes in mice which may be due to the toxic effect of MA on lymphoid organs. Our results are also parallel with the findings of Haddad *et al*. [33] who reported that MA caused decrease in RBC, Hb, and MCHC counts and also caused decrease in leucocytes and increase in neutrophils and lymphocytes.

GTE also induced an increase in erythrocyte count which might be due to an increase in erythropoiesis [34]. Our findings revealed that co-administration of GTE with MA ameliorated the toxic effects of MA on rat hematology and blood biochemistry; our results are consistent with the findings of Albokhadaim [35] who reported that GTE improved the hematological and biochemical alterations induced by carbon tetrachloride toxicity. Also, our results are parallel with the results of Abou-Zeid [36] who reported that co-administration of GTE with ethephon ameliorated the toxic effects of ethephon on mice hematology and blood biochemistry.

Regarding the liver function, the AST and ALT are considered as biomarker enzymes used in the evaluation of the function and integrity of liver cells. Both enzymes are present mainly in the cytoplasm of hepatocytes [37]. In the present study, as shown in Table 2, the rats treated with MA showed elevation in serum levels of ALT, AST, TSB and reduction in the serum level of albumin as compared with groups I & II. The elevation in the serum liver enzymes indicates a necrotic lesion in the liver cells while the decrease in serum albumin level indicated that there was an impairment in both synthetic and execratory activities of liver cells. Our results are parallel with the biochemical, histopathological findings of Tolbaa *et al*. [38]. Also our results are in agreement with the results of Lijuv *et al*. [32] who reported that MA toxicity increased the levels of AST, ALT, and ALP, compared to the negative control group. The present study indicated that there were reduction in serum albumin, and these results are in agreement with the results of [33, 38], who reported that there was decrease in mean values of serum total protein and albumin of male rats after MA supplementation in diet for 28 days; these results are concordant with the results of Chen *et al*. [39] who reported that there was significant decline in albumin levels in the pet which was given food contaminated with MA and cyanuric acid.

Oral administration of GTE prior to and after MA caused significant reduction in its toxic effect on serum levels of AST & ALT enzymes compared to untreated rats; these results agree with the results of [36].

Regarding antioxidants and lipid peroxidation, as shown in Table 3, MA in the liver tissue of rats of group III induced significant increase in MDA level, while it caused significant decrease in SOD, CAT activities and GSH level as compared with groups I & II. These results are in agreement with the results of Adaramoye *et al*. [37] who reported that treatment with high dose of melamine formaldehyde for 14 days caused a highly significant elevation in the content of MDA while decreasing GPx activity by 77.4%, as compared to those of the control animals. Oral administration of GTE prior to and after MA caused significant reduction in MDA level and elevation in SOD,

CAT activities and GSH level in the liver tissues of rats compared to groups I & II, and these results are in agreement with the results of [36].

With regard to histopathological changes, in the present study, liver cells of rats treated with MA (group III) showed hepatic tissues degeneration, necrosis, massive fatty and lymphocyte infiltration. There were destruction of cell membrane of liver cells with shrinking of the nucleus, swollen, vacuolated mitochondria and many cytoplasmic vacuoles. These results are in agreement with the results of [33] who reported that MA caused degenerated hepatic tissues, necrotic changes, massive fatty changes and broad infiltration of the lymphocytes. In addition to that Tolbaa *et al*. [38] confirmed that histological examination of liver of rats treated with MA showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes, fatty infiltrations, leucocytic infiltrations, congestion of blood vessels, fibrosis and liver necrosis. Our results are also in agreement with the results of Abdulbasit *et al*. [40] who reported that the liver of rats supplied with 5,000 and 10,000 ppm melamine showed massive fatty changes, necrosis, and broad infiltration of the lymphocytes; also rats supplemented with moderate doses of melamine (15,000 and 20,000 ppm) showed more or less severe degree of necrosis and slight lymphocyte infiltration of liver.

With regard to histochemical changes a strong PAS-positive reaction was observed in the hepatocytes of the groups I & II, indicating the presence of large amount of glycogen. On the other hand, marked reduction in PAS reaction was observed in group III indicating the reduction of amount of glycogen due to MA administration. However, administration of GTE after treatment of MA in group IV revealed an obvious increase in glycogen which resembled that of the liver of groups I & II. These results are in parallel with El-Beshbishy *et al*. and Tarek *et al*. [41, 42] who reported that addition of GTE could be useful in alleviating tamoxifen and insecticide-induced liver injury in rats.

Our results revealed that administration of GTE with MA ameliorates the toxic side effects of MA through its antioxidant, radical-scavenging and antiperoxidative activities; these results are in agreement with [41] who reported that addition of GTE could be useful in alleviating tamoxifen-induced liver injury in rats. Also [42] concluded that the use of GTE could improve the damage caused by insecticide exposure.

5. CONCLUSION

The present study revealed that administration of MA caused hepatotoxic effect in the form of disturbance and changes in hematological parameters, liver biomarkers, lipid peroxidation biomarker (MDA), histological parameters, and in the levels of antioxidant enzymes including CAT, SOD, and GSH. On the contrary the pre-treatment with GTE had a beneficial role in these MA-induced prior changes through its antioxidant and free radical-scavenging activities. Thus, we conclude that GTE could be considered as a useful dietary supplement to patients exposed to MA. This provides a cheap protective strategy in the management of acute hepatotoxicity induced by MA.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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