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Adverse effects of melamine formaldehyde on the liver, kidney and brain in rats

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ABSTRACT

Melamine became an adulterant and associated with the pets deaths in some country. The aim of the present study is to evaluate the adverse effects of administration of melamine formaldehyde on the liver, kidney and brain of Wister rats. Rats were randomly designed in to 14 groups (6 rats each) as follows: Groups 1 & 2 were given distilled water (10 ml/kg) for 7 &14 days and served as normal controls, Groups 3-5 were treated orally with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for seven days. Groups 6-8 were fed on food mixed with the same three doses of melamine formaldehyde for 7 days. Groups 9-11 treated orally with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 14 days. Groups 12-14 were fed on food mixed with the same three doses of melamine formaldehyde for 14 days. Liver enzymes and kidney functions were affected and malondialdehyde (MDA) content, glutathione peroxidase (GPx) and acetyl cholinesterase activities in brain was altered by the melamine. The histological examination of liver of melamine showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes, fatty infiltrations, leucocytic infiltrations, congestion of blood vessels and fibrosis and kidney necrosis, atrophy some glomerular and tubular epithelium. Chromosomal aberration showed fragmentation, polyploidy, breaking gaps, deletion, and multiple aberrations. In conclusions, melamine should be absent from diet at milligram concentrations to safe our health.

Key words: melamine, glutathione peroxidase , acetylcholine, chromosomal aberration, rats

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INTRODUCTION

Modern industries introduce many hazardous and carcinogenic chemicals to our environment. The toxic behavior of these chemicals may produce adverse effects on living cells such as mutagenicity and carcinogenicity, the problem being faced by toxicologists and biotechnologists is the early identification of the potentials effect of chemicals.

Melamine formaldehyde is a member of the amino resin family. Originally developed in the 1930s and prized for its toughness, chemical resistance, and relative ease of manufacture, MF is incorporated into a wide variety of products that are still in use today. Familiar products include Formica and melamine dinnerware. Commercial applications have included fabric impregnation, adhesives, paints, electrical mouldings, glass-reinforced substrates and engineered wood products. These condensed amino-plastic products are generally stable with low emitted formaldehyde ratio [1].

Melamine belongs to the triazine family. Other members of this family have been used extensively as nitrogen fertilizers [2] and their accumulation and persistence in the environment is well known [3]. Triazine residues have been detected in many soils and sediments [4]. MF is introduced to the environment from many industrial effluents. Both melamine and formaldehyde are known human health threats and MF releases monomers of both [5]. Thus the biodegradation of MF in industrial effluents is important.

Melamine is a category III carcinogen. Its illegal addition to milk and other protein products has led to the pet melamine poisoning in USA and infant renal calculus cases in China [6]. Melamine (MEL) became an adulterant of much discussion after it was associated with the deaths of pets in America and six children in China in 2007 and 2008, respectively [7]. The adverse health effects of formaldehyde exposure, including carcinogenicity, for professionals exposed to formalin-based fixatives such as pathologists, anatomy students, nurses and embalmers, and for workers exposed to formaldehyde in manufacturing [8].

A well established protocol for chemical safety evaluation as well as genetic toxicology estimation of this chemical may be of great importance in giving a clear environmental and genetic picture when dealing with it both industrially and domestically. Therefore, we examine the hepatic, renal and brain toxicities of melamine formaldehyde.

MATERIALS AND METHODS

Animals:
Albino Wistar male rats, weighing 150-160g were used throughout the experiments. They were purchased from Animal House Lab., National Research Centre, and Giza, Egypt. Animals received human care in compliance with the guidelines of the animal care and use committee of National Research Centre, and Giza, Egypt, experiments were performed according to the National Regulation of Animal Welfare and Institutional Animal Ethical Committee.

The animals were kept in a quiet place and were allowed free access water and standard food pellets throughout the period of investigation.

Chemicals:
Melamine formaldehyde was purchased from Sigma-Aldrich, Germany.

Experimental design:
Rats were randomly designed in to 14 groups (6 rats each) as follows: Groups 1 & 2 were given distilled water (10 ml/kg) for 7 and 14 days and served as normal controls, Groups 3-5 were treated orally with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 7 days. Groups 6-8 were feed on food mixed with the same three doses of melamine formaldehyde for 7days. Groups 9-11 treated orally with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 14 days. Groups 12-14 were feed on food mixed with the same three doses of melamine formaldehyde for 14 days.

Methods:
Preparation of blood sample and tissue homogenate:
Blood samples were withdrawn from the retro-orbital vein of each animal, under light anesthesia by diethyl ether. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15min. All groups were injected intraperitoneally two hour before scarified with 0.5 ml (0.002%)of colchicine / 20gm body w of rats (3mg/kg body weight of rat) for chromosomal preparation, then animals were sacrificed by cervical dislocation and the brain tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. A weighed brain was homogenized, using a homogenizer (Medical instruments, MPW-120, Poland), with ice-cooled saline to prepare 20% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. at 4°C in a cooling centrifuge to remove cell debris (Laborzentrifugen, 2k15, Sigma, Germany).

Biochemical analysis
The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in serum according to Reitman, and Frankel [9]. Alkaline phosphatase and gamma glutamyl transpeptidase (GGT) were done according to Belfield and Goldberg [10] and Szasz [11] respectively. Levels of creatinine, total protein and albumin in serum were done according to Bartles et al. [12], Gornal et al. [13] and Doumas et al. [14] respectively. Malondialdehyde (MDA) and glutathione peroxidase (GPx) contents were determined according to Ohkawa et al. and Beutler [15-16], respectively, using Biodiagnostic kits, Egypt. Brain acetyl cholinestrase content was measured by ELISA using Glory Science Co., Ltd.

Histopathological studies:
Sections measuring approximately 0.2 cm x 0.2 cm were taken from the kidney and liver of each rat. They were dehydrated through graded solutions of alcohol ending in two changes of absolute alcohol for 2 hours each. They were cleared in 2 changes of xylene, infiltrated in 2 changes of paraffin wax for 2 hours each using the automatic

tissue processor obtained from Sakura fine tek, Netherlands and embedded in molten paraffin wax. Sections were cut at 4µ with the rotary microtome

The organs (liver and kidney) were sectioned in the plane that best demonstrate the lesions. Sections of adjacent grossly uninvolved tissue were also collected. The collected samples were rapidly fixed in 10% neutral buffered formalin solution, and then passed through the paraffin embedding technique. Paraffin sections of 3-5 microns thickness were prepared and stained with hematoxylin and eosin (X20) according to Bancroft and Gamble [17].

Preparation of bone marrow metaphase chromosome

Each rat/group was injected IP with 1.0 ml of colchicine (3 mg\kg body weight) 2 hr prior to sacrifice at the end of the exposure period. The rats were sacrificed by cervical dislocation and their femoral bones surgically removed. The femurs were immediately put in normal saline, the epiphyses were cut off and the bone marrow was aspirated using 2.2 % (w/v) solution of sodium citrate. The suspension was then centrifuged for 10 minutes at 2000 rpm. The supernatant was decanted and replaced with 0.075 M potassium chloride solution and allowed to stand for 30 minutes. The mixture was centrifuged again at the same speed and time. The supernatant was decanted and replaced with freshly-prepared cold fixative (methanol:glacial acetic acid, 3:1 v/v). This was allowed to stand for 10 minutes after which it was centrifuged for 10 minutes at 2000 rpm. The supernatant was decanted and replaced with fresh fixative. The process of fixing and centrifuging was done thrice. Slides were prepared by dropping the fixed cells from a height of 30-40 cm onto clean, dry, grease-free slides. Finally, the slides were air-dried and stained with 5 % Giemsa (v/v, stock Giemsa stain/distilled water) for 10 minutes. All slides were analyzed blind to treatment at x1000 for chromosomal aberrations.

Statistical analysis

Data are expressed as mean ± S.E. Data analysis was done using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test for multiple comparisons. Difference was considered significant when *p* is less than 0.05. SPSS (version 11) program was used to carry out these statistical tests.

RESULTS

Effects of oral administration and feeding of melamine formaldehyde on serum liver function tests:

Animals treated with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 7 days by oral gavage caused a highly significant elevation (P<0.05) in the activities of ALT by 131.73%, 239.48% and 281.55%, AST by 576.49, 703.12% and 927.20%, ALP 5.65%, 9.68% and 57.26%, GGT by 546.55%, 721.62% and 893.77%, respectively, also treatment with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 7 days by feeding caused a highly significant elevation (P<0.05) in the activities of ALT by 114.02%, 209.96% and 214.76%, AST by 511.90, 524.93% and 552.41% and GGT by 477.98%, 692.44% and 725.60%, respectively, as compared to normal control animals (Table 1).

Table 1: Effects of oral administration and feeding of melamine formaldehyde for 7 days on serum liver enzymes and kidney function tests

	Normal control	Oral melanine formaldehyde (0.8 mg/kg)	Oral melanine Formaldehyde (1.6 mg/kg)	Oral melanine Formaldehyde (2.3mg/kg)	Feeding melanine Formaldehyde (0.8 mg/kg)	Oral melanine formaldehyde (1.6 mg/kg)	Oral melanine Formaldehyde (3.2mg/kg)
ALT(U/L)	54.20±0.20	125.60±0.40*	184.00±0.63*	206.80±0.49*	116.00±0.63*	168.00±0.32*	170.60±0.40*
AST(U/L)	70.60±0.40	477.60±0.40*	567.00±0.63*	725.20±0.20*	432.00±0.32*	441.20±0.20*	460.60±0.40*
ALP(U/L)	24.80±0.20	26.20±0.20*	27.20±0.20*	39.00±0.32*	24.80±0.20	25.20±0.20	25.40±0.24
GGT(U/L)	30.16±0.12	195.00±0.06*	247.80±0.06*	299.72±0.08*	174.32±0.08*	239.00±0.06*	249.00±0.06*
Creatinine (mg/dl)	0.10±0.00	0.61±0.00*	0.64±0.01*	4.38±0.06*	0.18±0.00*	0.63±0.01*	0.85±0.01*
Total protein (g/dl)	6.22±0.14	6.24±0.16	5.50±0.02*	5.30±0.03*	6.30±0.13	6.14±0.19	6.12±0.35
Albumin (g/dl)	3.98±0.00	3.96±0.01	2.08±0.01*	2.06±0.03*	3.97±0.01	2.68±0.01*	2.17±0.01*

Statistical analysis was carried out by one-way ANOVA followed by LSD test.
* *Significantly different from normal control (Saline) at P<0.05.*

Animals treated with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 14 days by oral gavage caused a highly significant elevation (P<0.05) in the activities of ALT by 214.29%, 236.63% and 369.23%, AST by 567.60%, 698.04% and 926.54%, ALP by 232%, 356% and 400% and GGT by 551.46%, 1302.12% and 1387.17%, respectively, also treatment with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 14 days by feeding caused a highly significant elevation (P<0.05) in the activities of ALT by 146.15%, 206.96% and 221.98%, AST by 519.27%, 537.71% and 545.25%, ALP by 4.80%, 68% and 208% and GGT by 527.65%, 704.63% and 764.02%, respectively, as compared to normal control animals (Table 2).

Table 2: Effects of oral administration and feeding of melamine formaldehyde for 14 days on serum liver enzymes and kidney function tests

	Normal control	Oral melanine formaldehyde (0.8 mg/kg)	Oral melanine Formaldehyde (1.6 mg/kg)	Oral melanine formaldehyde (2.3mg/kg)	Feeding melanine formaldehyde (0.8 mg/kg)	Oral melanine Formaldehyde (1.6 mg/kg)	Oral melanine formaldehyde (3.2mg/kg)
ALT(U/L)	54.60±0.40	171.60±1.03*	183.80±0.58*	256.20±0.49*	134.40±0.60*	167.60±0.68*	175.80±0.80*
AST(U/L)	71.60±0.93	478.00±0.32*	571.40±1.17*	735.00±1.84*	443.40±0.98*	456.60±1.66*	462.00±0.00*
ALP(U/L)	25.00±0.32	83.00±0.00*	114.00±0.00*	125.00±0.00*	26.20±0.20*	42.00±0.00*	77.00±0.00*
GGT(U/L)	30.24±0.12	197.00±0.06*	424.00±0.13*	449.72±0.23*	189.80±0.13*	243.32±0.21*	261.28±0.45*
Creatinine (mg/dl)	0.11±0.00	0.62±0.01*	0.97±0.00*	4.50±0.10*	0.21±0.01*	0.73±0.00*	0.95±0.00*
Total protein (g/dl)	6.08±0.05	5.28±0.20*	5.22±0.01*	5.32±0.01*	6.16±0.07	6.08±0.05	6.04±0.04
Albumin (g/dl)	3.98±0.00	2.11±0.06*	1.76±0.07*	1.58±0.01*	2.86±0.01*	2.52±0.01*	1.95±0.04*

Statistical analysis was carried out by one-way ANOVA followed by LSD test.
** Significantly different from normal control (Saline) at P<0.05.*

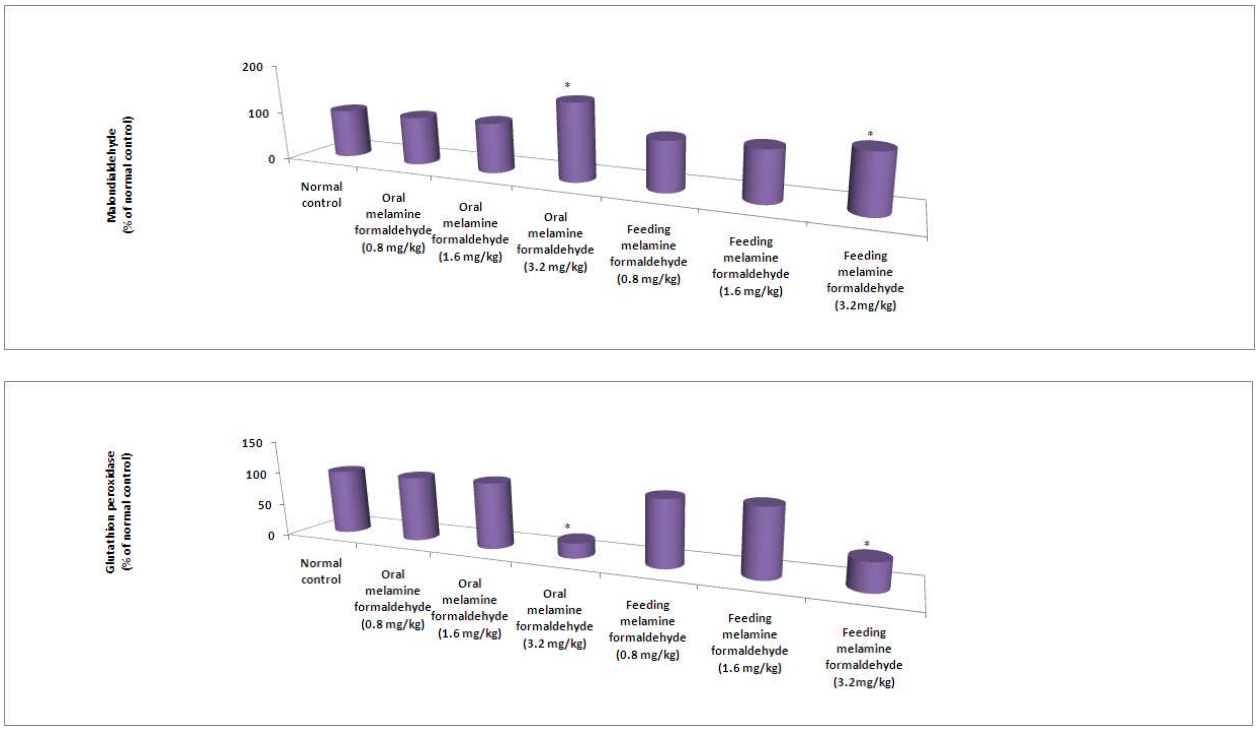


Fig 1: Effects of oral administration and feeding of melamine formaldehyde for 14 days on brain malondialdehyde and glutathione Peroxidase

Statistical analysis was carried out by one-way ANOVA followed by LSD test.
** Significantly different from normal control (Saline) at P<0.05.*

Effects of oral administration and feeding of melamine formaldehyde on serum kidney function tests:

Animals treated with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 7 days by oral gavage caused a highly significant elevation (P<0.05) in the levels of serum creatinine by 498.04%, 525.49% and 4194.12%, while melamine formaldehyde (1.16, 3.2 mg/kg) decreased levels of serum total protein by 11.51% and 14.75% and albumin by 47.74% and 48.39% respectively, in addition, treatment with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 7 days by feeding caused a highly significant elevation (P<0.05) in the concentration of creatinine by 72.55%, 521.57% and 733.33%, did not change levels of serum total protein and melamine formaldehyde (1.16, 3.2 mg/kg), decreased levels of serum albumin by 32.68% and 45.53%, respectively, as compared to normal control animals (Table 1).

Animals treated with melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) for 14 days by oral gavage caused a highly significant elevation (P<0.05) in the serum levels of creatinine by 486.79%, 815.09% and 4145.28% and resulted in a decrease in total protein by 13.23%, 14.18% and 12.44% and albumin by 47.00%, 55.82% and 60.34% respectively, in addition, treatment with melamine formaldehyde for 14 days by feeding caused a highly significant elevation (P<0.05) in the serum levels of creatinine by 96.23%, 588.68% and 798.11%, did not change levels of serum total protein and decreased levels of serum albumin by 28.31%, 36.75%and 51%, respectively, as compared to normal control animals (Table 2).

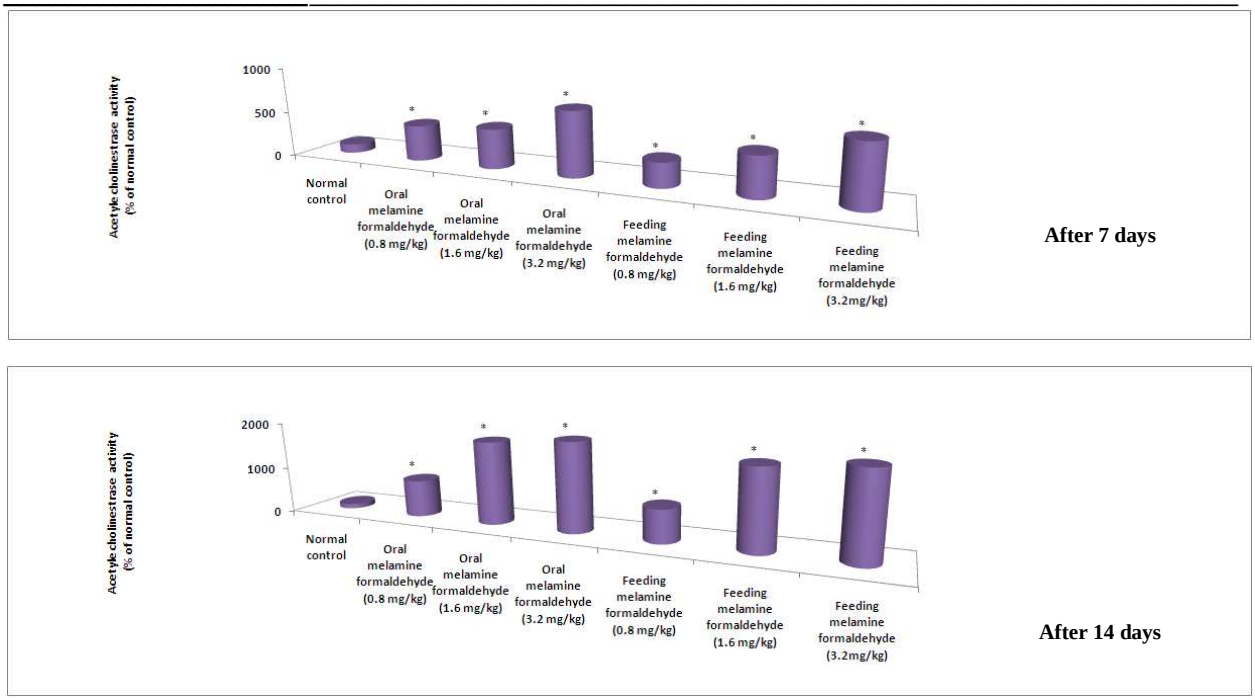


Fig 2: Effects of oral administration and feeding of melamine formaldehyde on brain cholinestrase
Statistical analysis was carried out by one-way ANOVA followed by LSD test.
** Significantly different from normal control (Saline) at P<0.05.*

Effects of oral administration and feeding of melamine formaldehyde on brain Malondialdehyde (MDA) and glutathione Peroxidase (GPx):

Animals treated with melamine formaldehyde for 7 days by oral gavage or feeding did not change the content of MDA and GPx activity. Treatment with high dose of melamine formaldehyde for 14 days by oral gavage caused a highly significant elevation in the content of MDA by 57.5 % while decreasing GPx activity by 77.4 % , also treatment with high dose of melamine formaldehyde for 14 days by feeding caused a highly significant elevation in the content of MDA by 15 % while decreasing GPx activity by 58.6% , as compared to those of the control animals (Figure 1).

Effects of oral administration and feeding of melamine formaldehyde on brain cholinestrase:

Animals treated for 7 days with oral melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) caused a highly significant elevation in the activity of cholinestrase by 294%, 335% and 609% respectively, also treatment for 7 days with feeding melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) caused a highly significant elevation in the activity of cholinestrase by 168%, 334% and 555%, respectively, moreover animals treated for 14 days with oral melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) caused a highly significant elevation in the activity of cholinestrase by 697%, 1676% and 1808% respectively, also treatment for 14 days with feeding melamine formaldehyde (0.88, 1.16, 3.2 mg/kg) caused a highly significant elevation in the activity of cholinestrase by 609%, 1599% and 1722%, respectively as compared to those of the control animals (Figure 2).

Results of Histopathological Examination

The liver of normal control rat appeared with normal tissue structure **(Fig 3A)**. Liver of rats treated with melamine Formaldehyde (0.8mg/kg) for 7 days by oral gavage showed congestion of central vein (A) and single cell coagulative necrosis (D) with congestion of hepatic sinusoids (B) and proliferation of kupffer cells(C) **(Fig 3B)**. Liver of rats treated with melamine Formaldehyde (1.16 mg/kg) for 7 days by oral gavage showed coagulative necrosis of hepatocytes (B), sever fibrosis in portal area with congestion of portal blood vessels (A)and lymphocytic infiltration (D) **(Fig 3C)**. Liver of rats treated with melamine Formaldehyde (3.2 mg/kg) for 7 days by oral gavage showed sever coagulative necrosis of hepatocytes (A), vaculation of the cytoplasm, congestion of central vein (B) and hepatic sinusoids (C) **(Fig 3D)**. Liver of rats feeding with melamine Formaldehyde (0.8 mg/kg) for 7 days showed impairment of the normal structural organization of the hepatic lobules and sinusoidal spaces were enlarged. Intrahepatic veins, central and portal were dilated and congested (A) and single cell coagulative necrosis (B) was observed **(Fig 3E)**. Liver of rats feeding with melamine Formaldehyde (1.16 mg/kg) for 7 days showed Leucocytic infiltrations and congestion of central vein (B) and single cell coagulative necrosis (C) with mild congestion of hepatic sinusoids (A) were observed **(Fig 3F)**. Liver of rats feeding with melamine Formaldehyde (2.3 mg/kg) for 7

days showed congestion of central vein (A) and single cell coagulative necrosis with congestion of hepatic sinusoids (B) were observed **(Fig 3G)**.

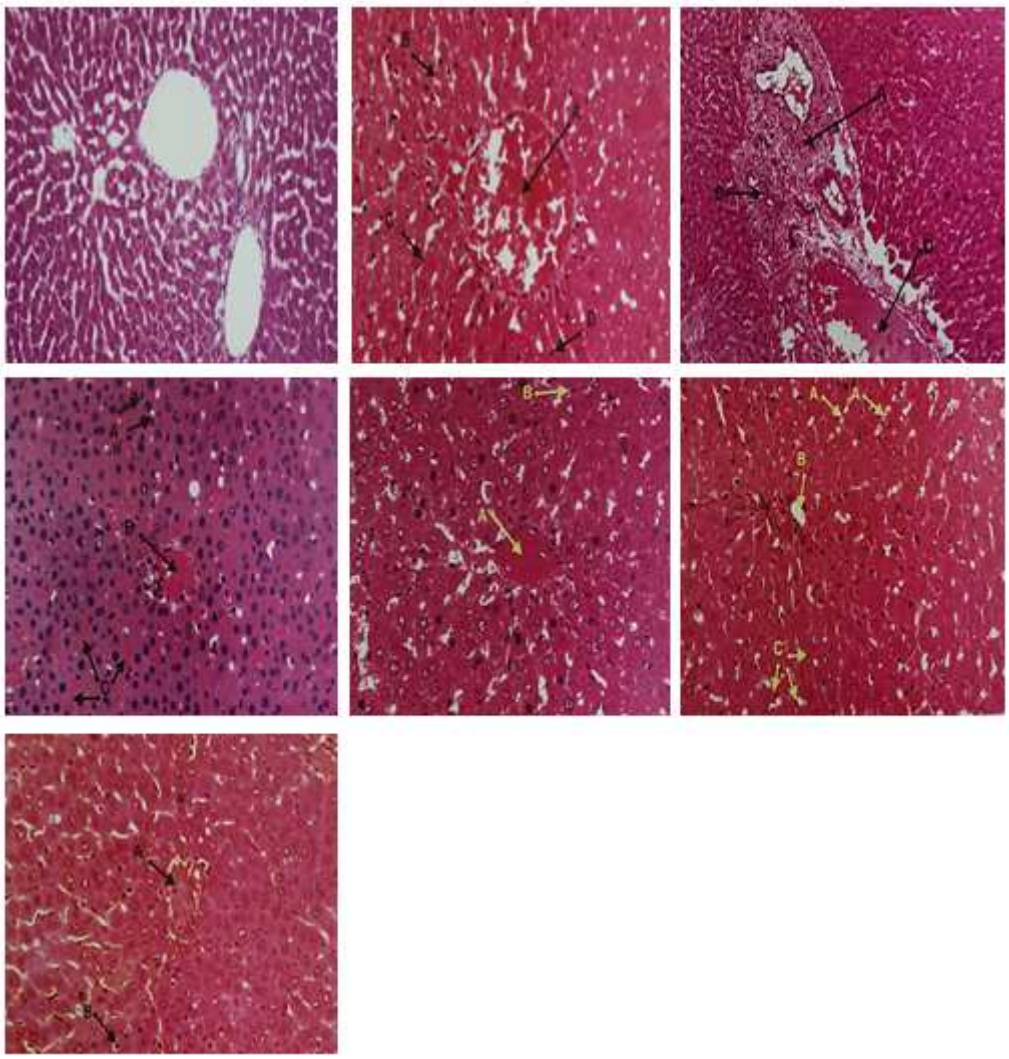


Fig 3: The liver of normal control rat (A). Liver of rats treated with oral melamine Formaldehyde (0.8mg/kg) for 7 days (B). Liver of rats treated with oral melamine Formaldehyde (1.16 mg/kg) for 7 days (C). Liver of rats treated with oral melamine Formaldehyde (3.2 mg/kg) for 7 days (D). Liver of rats feeding with melamine Formaldehyde (0.8 mg/kg) for 7 days (E). Liver of rats feeding with melamine Formaldehyde (1.16 mg/kg) for 7 days (F). Liver of rats feeding with melamine Formaldehyde (3.2 mg/kg) for 7 days (G)

The liver of normal control rat appeared with normal tissue structure **(Fig 4A)**. Liver of rats treated with melamine Formaldehyde (0.8 mg/kg) for 14 days by oral gavage showed the liver cells were degenerated and suffered saculer dilation and congestion of central vein (A), congestion of hepatic sinusoids (C), coagulative necrosis of hepatocytes (B)(**(Fig 4B)**. Liver of rats treated with melamine Formaldehyde (1.16 mg/kg) for 14 days by oral gavage showed sever engorgement and dilation of central vein (B), coagulative necrosis of hepatocytes (C), lymphocytic infiltration (A) **(Fig 4C)**. Liver of rats treated with melamine Formaldehyde (3.2 mg/kg) for 14 days by oral gavage showed sever coagulative necrosis of hepatocytes (C), vaculation of the cytoplasm (B), congestion of central vein and hepatic sinusoids (A) **(Fig 4D)**. Liver of rats feeding with melamine Formaldehyde (0.8 mg/kg) for 14 days revealed that a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells - frequently forming a narrow peripheral rim was left focal proliferation of lymphocytes and single cell coagulative necrosis with dilation of hepatic sinusoids **(Fig E**). Liver of rats feeding with melamine Formaldehyde (1.16 mg/kg) for 14 days showed sever engorgement and dilation of central vein (B), destruction and desquamation of endothelium lining the central vein (A) were observed **(Fig 4F)**. Liver of rats feeding with melamine Formaldehyde (3.2 mg/kg) for 14 days showed congestion of central vein (A), destruction and desquamation of endothelium lining the central vein (B), coagulative necrosis of hepatocytes, dilation of hepatic sinusoids and proliferation of kupffer cells were observed (C) **(Fig 4G)**.

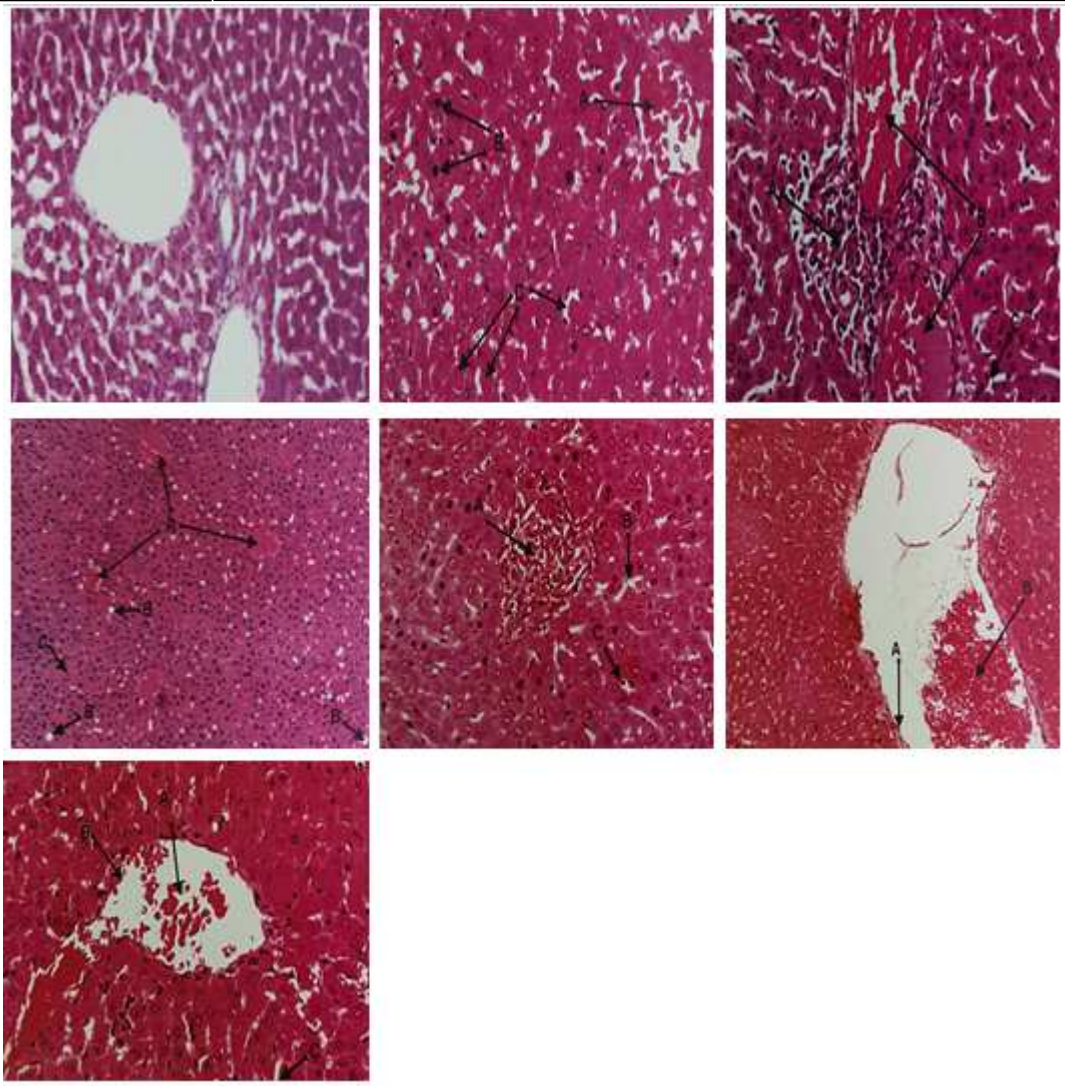


Fig 4: The liver of normal control rat (A). Liver of rats treated with oral melamine Formaldehyde (0.8mg/kg) for 14 days (B). Liver of rats treated with oral melamine Formaldehyde (1.16 mg/kg) for 14 days (C). Liver of rats treated with oral melamine Formaldehyde (3.2 mg/kg) for 14 days (D). Liver of rats feeding with melamine Formaldehyde (0.8 mg/kg) for 14 days (E). Liver of rats feeding with melamine Formaldehyde (1.16 mg/kg) for 14 days (F). Liver of rats feeding with melamine Formaldehyde (3.2 mg/kg) for 14 days (G)

Examination of Kidney sections obtained from control rats showed normal kidney structure **(Fig 5A).** Oral gavage Administration of melamine Formaldehyde (0.8mg/kg) for 7 days resulted in cloudy swelling (A) and necrosis of the tubular epithelium with tubular cast (B), congestion of glomerular capillaries (C), deformity of the tubular lumen taken star or slit shape, slight hemorrhages (D) **(Fig 5B).** Oral gavage Administration of melamine Formaldehyde (1.16 mg/kg) for 7 days resulted in congestion of glomerular capillaries and intertubular blood vessels (A), necrosis of the tubular and glomerular epithelium (B) with tubular cast, lymphocytic infiltration (C) **(Fig 5C).** Oral gavage Administration of melamine Formaldehyde (3.2 mg/kg) for 7 days resulted in necrosis of the tubular and glomerular epithelium with tubular cast, congestion of glomerular capillaries and intertubular blood vessels, sever hemorrhages were observed **(Fig 5D).** Rats treated with melamine Formaldehyde (0.8 mg/kg) by feeding for 7 days revealed cloudy swelling and necrosis of the glomerular and tubular epithelium (A), Narrowing of the tubular lumen (TL) **(Fig 5E).** Rats treated with melamine Formaldehyde (1.16mg/kg) by feeding for 7 days revealed atrophy of some glomeruli (A), decrease of Bowman's space (B), Cloudy swelling (C) and necrosis of the glomerular and tubular epithelium(D), Narrowing of the tubular lumen was observed **(Fig 5F).** Rats treated with melamine Formaldehyde (3.2 mg/kg) by feeding for 7 days revealed sever cloudy swelling (A) and necrosis of the glomerular and tubular epithelium (B), decrease of Bowman's space (C) **(Fig 5G).**

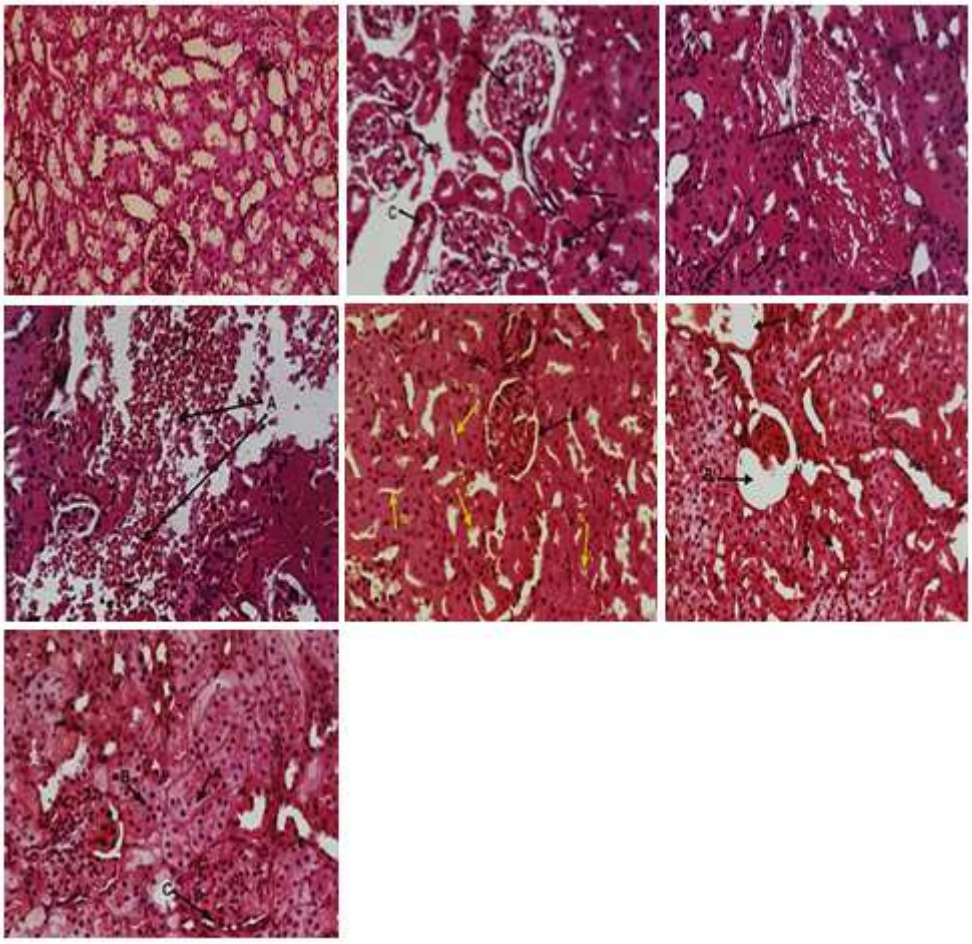


Fig 5: Kidney of control rats (A). Oral gavage Administration of melamine Formaldehyde (0.8mg/kg) for 7 days (B). Oral gavage Administration of melamine Formaldehyde (1.16mg/kg) for 7 days (C). Oral gavage Administration of melamine Formaldehyde (3.2 mg/kg) for 7 days (D). Rats treated with melamine Formaldehyde (0.8mg/kg) by feeding for 7 days (E). Rats treated with melamine Formaldehyde (1.16 mg/kg) by feeding for 7 days (F). Rats treated with melamine Formaldehyde (3.2 mg/kg) by feeding for 7 days (G)

Control rats showed normal kidney structure after 14 days **(Fig 6A).** Animals treated with melamine Formaldehyde (0.8mg/kg) by oral gavage for 14 days showed necrosis of the tubular and glomerular epithelium (A), congestion of glomerular capillaries (G), congestion of the blood vessels with thickening of its wall (C), fibrosis and edema around the blood vessels (D) (**Fig 6B).** Animals treated with melamine Formaldehyde (1.16 mg/kg) by oral gavage for 14 days showed sever necrosis of the tubular (A) and epithelium with destruction of renal tubules, congestion of the blood vessels (B) with thickening of its wall (C), fibrosis and edema around the blood vessels (D) (**Fig 6C).** Animals treated with melamine Formaldehyde (2.3 mg/kg) by oral gavage for 14 days showed sever necrosis of the tubular and epithelium with destruction of renal tubules, congestion of the blood vessels with thickening of its wall, fibrosis and edema around the blood vessels, hemorrhages were observed (**Fig 6D).** Animals treated with melamine Formaldehyde (0.8mg/kg) by feeding for 14 days the histological structure of the kidney appeared with cloudy swelling and necrosis of the glomerular and tubular epithelium, deformity of the tubular lumen taken star (C) or slit shape (A), tubular cast (B) (**Fig 6E).** Kidney of rats treated via feeding of (1.16 mg/kg) melamine formaldehyde for 14 days. The kidney tissue showed (A) cloudy swelling and necrosis of the glomerular and tubular epithelium and congestion of glomerular capillaries, (B) narrowing of the tubular lumen(**Fig 6F).** Rats treated via feeding of (3.2 mg/kg) melamine formaldehyde for 14 days. The kidney tissue showed (D) cloudy swelling and necrosis of the tubular epithelium with tubular cast and narrowing of the tubular lumen, (A) congestion of the blood vessels with(B) thickening of its wall, (C)fibrosis and edema around the blood vessels (**Fig 6G).**

Chromosomal aberration

The histological examination of liver sections of melamine formaldehyde irradiated rats resulted in many pathological criteria such as (A) fragments, (B) breaks, polyploidy and(C) gaps **(Fig 7)**.

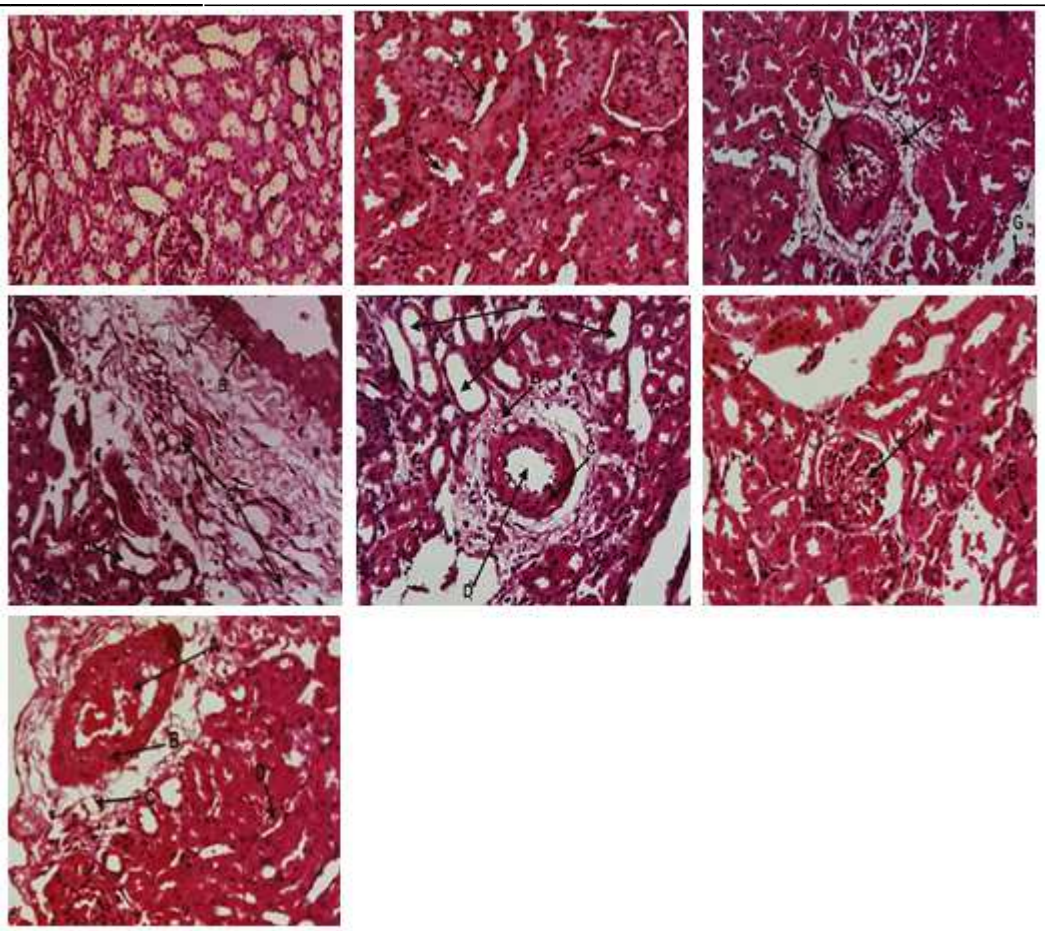
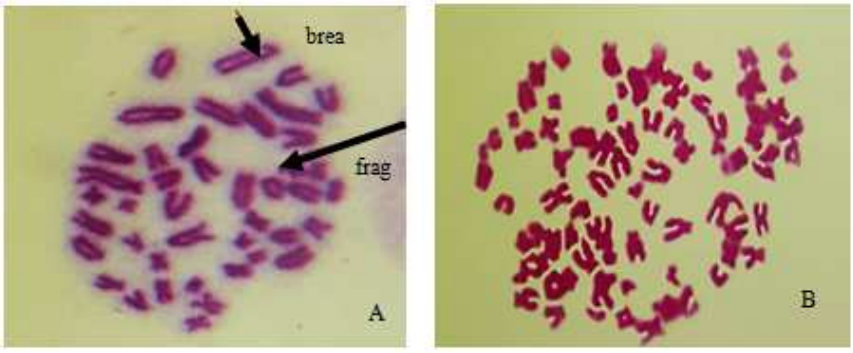
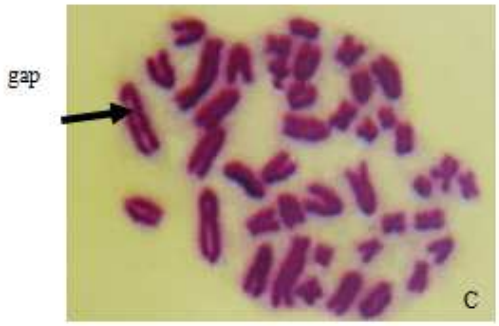


Fig 6: Kidney of control rats (A). Oral gavage Administration of melamine Formaldehyde (0.8mg/kg) for 14 days (B). Oral gavage Administration of melamine Formaldehyde (1.16 mg/kg) for 14 days (C). Oral gavage Administration of melamine Formaldehyde (3.2 mg/kg) for 14 days (D). Rats treated with melamine Formaldehyde (0.8mg/kg) by feeding for 14 days (E). Rats treated with melamine Formaldehyde (1.16 mg/kg) by feeding for 14 days (F). Rats treated with melamine Formaldehyde (3.2 mg/kg) by feeding for 14 days (G)





many pathological criteria Fig 7: The histological examination of liver sections of melamine formaldehyde irradiated rats resulted in such as (A) fragments, (B) breaks, polyploidy and(C) gaps

DISCUSSION

The present investigation has been focused on testing the adverse effects of oral administration or feeding with melamine on liver, kidney and brain, the mean values of serum ALT, AST and ALP in all melamine groups were significantly higher than normal control. These results agree with data of serum chemistry after one month of melamine contaminated artificial food that indicated severe renal and mild hepatic failure [18] and our results are in contrast with Al- Sieni et al. [19] who reported the effects of melamine supplementation (5000, 10000, 15000 and 20000 ppm), for 28 days on serum liver enzymes (ALT, AST, ALP, GGT) in rats that were not higher than the negative control.

The alteration in biochemical parameters was supported by the histopathological study of liver of melamine formaldehyde-treated animals that showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes, fatty infiltrations, leucocytic infiltrations, congestion of blood vessels, and fibrosis. These results may indicate an increase in the release of the liver enzyme in the blood stream. Similar results were obtained by Jaramillo-Juarez et al. [20] who stated that liver sections of rats supplemented with 20000 ppm melamine showed degenerated hepatic tissues, necrosis, and changes in massive fatty and broad infiltration of the lymphocytes.

Studies concerning the melamine toxicity taken orally in humans are nonexistent. Toxicity data mainly come from studies in sheep, cat, dog, mice, and rat. Toxicity can be classified as acute or chronic. The most common toxicity is renal toxicity, which is also the area of most concern to nephrologists [21]. In this study, the mean values of serum creatinine were significantly higher while total protein and albumin were significantly lower than normal control. While El Rabey et al. [22] reported that creatinine, total protein and albumin were non significantly higher in melamine supplementation at a dose of 20000 ppm in the diet for 28 days than those of the negative control.

Lower concentration of total protein observed in this work may be due to liver and/or kidney disorder in which protein is not digested or absorbed properly and is hence, excreted in urine also, decreasing albumin concentration in instances of liver disease and nephritic syndrome since, albumin is made in the liver, these results are in line with Shehu et al. [23].

Studies reported that melamine affect potassium channels and sodium channels in hippocampal neurons, induce cognitive deficits associated with impairments of synaptic plasticity in the hippocampus and was able to pass through the blood-brain barrier (BBB) and take up in the hippocampus [24]. Therefore, studies are urgently needed to identify neurotoxic adverse effect after exposure to melamine on CNS.

Our data may indicate that there is underlying neurotoxicity of melamine. Melamine not only elevated the levels of malonaldehyde (MDA) but also reduced the activity of glutathione peroxidase (GSH-Px), moreover we investigated the effect of melamine on the AChE activity of rats. Our results showed that AChE activity were sensitive to melamine exposure. These results are in accordance with An et al. [25] using melamine at a dose of 300 mg/kg for 28 days, that induced spatial cognitive deficits associated with impairments of hippocampal long-term depression and cholinergic system as well as oxidative damage in Wister rats.

Results obtained in the present work indicated that melamine formaldehyde induced chromosomal aberrations in liver and kidney of Wister rats. Formaldehyde alone induced chromosomal aberrations and apoptosis in peripheral blood lymphocytes of personnel working in pathology departments [26]. Inaddition, melamine induces sperm DNA damage and abnormality [6].

CONCLUSION

This work revealed adverse effects of melamine in liver and kidney in rats; and the toxicity may not be limited to these but there is brain toxicity also. This was concluded from the alteration in all biochemical tests, histopathological signs and Chromosomal aberration compared with the normal control rats.

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