Original Article

Evaluation of The Role of Buffalo Milk on Acute Toluene Induced Hepatotoxicity and Nephrotoxicity in Adult Male Albino Rats

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ABSTRACT

Background: Toluene is a volatile organic compound that is commonly used in a variety of applications around the world, including paints, inks, solvents, varnishes, plastics, thinners, fabrics, and dyes. This enhances the probability of exposure in occupational and environmental settings. **Aim of the work:** This research was carried out in adult male albino rats to look into the hepatic and renal histopathological changes caused by acute toluene exposure, the possible underlying mechanisms, and the role of buffalo milk in reducing the toxic effects of toluene. **Methodology:** The study was conducted for 7 days on seventy-two adult male albino rats. They were divided into five groups as follow: Group I (negative control): 12 rats were fed with regular diet and water to test basic parameters .Group II (positive control) 24 rats subdivided into 2 equal groups: Subgroup IIa (corn oil group)12 rats received 1 ml of corn oil (vehicle of toluene) by oral gavage . Subgroup IIb (distilled water group) 12 rats received 1ml of distilled water by oral gavage. Group III (pasturalized buffalo milk group - PBM) 12 rats received 1ml of buffalo milk by oral gavage. Group IV (toluene group) 12 rats received toluene 900mg/kg dissolved in corn oil by oral gavage. Group V (toluene and pasturalized buffalo milk group) 12 rats received toluene 900mg/kg followed by 1ml of buffalo milk by oral gavage for 7 days. At the end of the study, blood samples were collected for estimating (serum ALP, ALT, serum albumin, serum creatinine, serum MDA, TAC). Then livers and kidneys were extracted for histopathological (H&E) and immunohistochemistry (caspase-3) examination. **Results:** The result of the study revealed that administration of toluene for 7 days induced significant increase in ALP, ALT, creatinine, serum MDA and significant reduction in serum albumin and TAC. It also induced histopathological alterations in liver and kidney with increased caspase-3 immunoreactivity. Administration of PBM with toluene for 7 days improved toluene induced histopathological alterations in liver and kidney with decreased caspase-3 immunoreactivity. Also, PBM caused significantly beneficial effect on liver and kidney parameters; In addition, it decreased serum MDA level and increased serum TAC. **Conclusion:** Buffalo milk has partial protective effects against toluene induced hepatotoxicity, nephrotoxicity, oxidative stress and apoptosis.

Keywords: Toluene, Buffalo Milk, Hepatotoxicity, Nephrotoxicity.

I. INTRODUCTION

Toluene is one of aromatic hydrocarbons with ($C_6H_5CH_3$) formula, that is a clear, colourless and also known as methyl benzene, phenyl methane, and toluol (Tormoehlen et al., 2014).

Toluene is a volatile organic compound that is commonly used in a variety of industrial applications such as paints, coatings, adhesives, varnishes, plastics, thinners, leather, and dyes (Meydan et al., 2012).Toluene is considered as an abused organic solvent and is extremely potential for various types of abuse(Laio et al., 2019).

Toluene is absorbed by inhalation, ingestion, and dermal absorption to a lesser degree. Toluene is primarily metabolised in the liver, with the kidney serving as the primary organ for toluene elimination. (Tas et al., 2011).

Exposure to toluene can damage variety of organs including liver, kidney, lung, heart and nervous system (Meydan et al., 2016). The affinity of toluene with lipid-rich nervous tissue structures results in toxic effects of CNS within minutes (Yoon et al., 2016).

Acute toxicity occurs due to exposure to high amounts of toluene that may lead to headache, exhaustion, drowsiness, nausea, and unconsciousness. Toluene causes death from respiratory failure or arrhythmias if exposure continued for a long time. (Yasar et al., 2016).

Toluene can cause oropharyngeal irritation, abdominal pain, nausea, vomiting, and hematemesis when inhaled or ingested. Ascites, jaundice, hepatomegaly also are manifestation of toluene induced hepatotoxicity . (Malaguarnera et al., 2012).

Toxic effects of acute toluene toxicity on the kidney include renal tubular acidosis, hypokalemia, hypophosphatemia, azotemia, hematuria, proteinuria, and pyuria. (Neghab et al., 2015).

It has been reported that acute toluene toxicity associated with severe metabolism disturbances, including distal renal tubular acidosis (RTA-1), hypokalemic paralysis, metabolic acidosis, rhabdomyolysis, and proteinuria (Camara-Lemarroy et al., 2012).

The inability of the distal tubules to excrete hydrogen ions as ammonium, caused by reduced proton conductance via the active conduction pathway, and overproduction of hippuric acid by toluene metabolism are the key proposed mechanisms in toluene-induced distal RTA-1. (Camara-Lemarroy et al., 2015).

Toluene could damage the structure of both glomerular and tubular systems. Toluene poisoning is associated with Fanconi syndrome, hematuria and acute renal oliguria (Meydan et al., 2016).

Proteins, fat, lactose, minerals, vitamins, and antioxidants are all abundant in buffalo milk. hydroxyl radicals, superoxide radicals, and peroxide radicals can all be inhibited by milk's antioxidant systems. (Usta et al., 2013).

II. MATERIALS AND METHODS

II.1. Materials:

II.1.1. Chemicals:

Toluene was purchased as anhydrous colourless liquid substance with pungent aromatic odour ,molecular weight 92.13 g/mol CAS No. is 108-88-3 from El-Gomhouria Company for pharmaceutical, Egypt ,buffalo milk used as commercial pasturalized milk and corn oil was obtained from sekem,cairo.

II.1.2. Animals:

Seventy two adult healthy male albino rats were used in this study, each weighing 150-200 gm, with an average age of 6-8 weeks, obtained from the Animal House of Faculty of Medicine, Zagazig University. All animals were acclimatized for 2 weeks prior to the beginning of the experiment with free access to solid food and water in their home cages and proper ventilation. The room was maintained with 12h light/dark cycle. The Institutional Review Board (IRB) committee for scientific research of Faculty of Medicine, Zagazig University approved the design of the experiment according to the established guidelines for the care and use of laboratory animals.

II.1.3. Experimental design:

The rats were divided into 5 groups as the following:

- Group1 (negative control group):12 rats received regular diet and water for 7days.
- Group II (positive control): contained 24 rats subdivided into 2 equal groups Subgroup IIa (corn oil group): 12 rats received 1 ml of corn oil (vehicle of toluene) by oral gavage (stomach tube) to test the effect of the vehicle for 7days .Subgroup IIb (distilled water group): 12 rats received 1ml of distilled water by oral gavage for 7days .
- Group III (pasturalized buffalo milk group): 12 rats received 1ml of buffalo milk daily by oral gavage for 7days .
- Group IV (toluene group): 12 rats received toluene 900mg/kg(1/10 of oral LD50)(ASTDR 2000). dissolved in corn oil by oral gavage for 7days.
- Group V (toluene and pasturalized buffalo milk group): 12 rats received toluene 900mg/kg followed by 1ml of buffalo milk by oral gavage for 7 days.

II.1.4. Sampling

At the end of the study (24 hours from the last dose of treatments) rats were anaesthetized with ether then blood samples were collected from the retro-orbital plexuses. The blood samples were used for estimating alkaline phosphatase(ALP),alanine amino transferase (ALT), serum albumin. serum creatinine, serum malondialdehyde (MDA) and total antioxidant capacity (TAC) then the animals were sacrificed, the liver and kidneys samples were dissected . Liver and kidney samples were preserved in 10% neutral buffer formalin then prepared for histopathological examination by light microscope and immunehistochemical examination.

II.2. Methods:

II.2.1. Blood samples collection:

Animals' venous blood samples were taken from the retroorbital

plexus using micro–capillary glass tubes in according to Johnson (2007).

Blood samples (approximately 3ml) were collected in clean test tubes without anticoagulant and allowed to clot at 25°C for 30 minutes. after which serum was separated by centrifugation of blood 3000 rpm for 15 min. The supernatant sera were pipette off using fine tipped automatic pipettes and stored at –20°C until used for estimating

1. Liver function tests (alkaline phosphatase(ALP), alanine amino transferase (ALT), serum albumin)assayed according to spectrophotometric technique of Rosalki et al., 1993, Reitman and Frankel, 1957 and Doumas et al ., 1971 respectively.
2. Kidney function tests (serum creatinine) assayed according to Schirmeister et al., 1964 technique.
3. Serum malondialdehyde (MDA) and total antioxidant capacity (TAC) aasyed according to Ohkawa et al., 1979 and Koracevic et al., 2001 technique respectively.

II.2.2. Histopathological examination :

Liver and kidney were fixed in 10% formalin saline solution. After fixation, liver and kidney were embedded in paraffin blocks and processed for the preparation of 5 µ thickness sections. These sections were subjected for hematoxylin and eosin stains (Kiernan, 2001)

II.2.3. Immunohistochemical studies:

Caspase-3 immunohistochemistry was performed on parts of liver and kidney from adult male albino rats. It is a cytosolic protein that is present in cells as an inactive proenzyme that is only activated when cells undergo apoptosis by proteolytic cleavage into two active subunits. (Krajewska et al., 2005).

II.2.4. Statistical Analysis:

SPSS Software program was used. Mean values ± Standard Deviation (SD) were calculated, ANOVA (F) test followed by least significant difference test (LSD test) & chi square test were performed. P value of less than 0.05 was considered to be significant.

III. RESULTS

III.1. Biochemical results

III.1.a. Biochemical parameters of control groups

Serum ALP, ALT, albumin, creatinine, MDA and TAC in control and vehicle groups (distilled water and corn oil)showed no significant changes among these groups($P > 0.05$) (table 1). So, we used negative control group as a control group to be compared with other treated groups.

III.1.b. Biochemical parameters of treated groups.

The results of the study showed a highly significant increase in mean values of serum levels of ALP,ALT,creatinine ,MDA and TAC and highly significant decrease in mean values of serum levels of albumin in toluene group when compared to negative control group and PBM group($P < 0.001$).Administration of PBM with toluene caused highly significant reduction in mean values of serum levels of ALP, ALT,creatinine ,MDA and TAC and highly significant increase in serum albumin in toluene+PBM group when compared with toluene group ($P < 0.001$) (table 2)

(Table 1): Statistical comparison between negative and positive control groups after7 days as regard mean values of (ALP, ALT, serum albumin, serum creatinine, serum MDA and serum TAC) using ANOVA test.

Variables	Control groups			F	P
	Negative mean ± SD	Dis water mean ± SD	Corn oil mean ± SD		
Alkaline phosphatase enzyme(ALP): (IU/L)	157.4 ± 1.2	157.6± 1.05	158.4± 0.86	1.04	0.4
Alanine transeferase enzyme(ALT): (IU/L)	47.5 ± 1.04	46.9 ± 0.63	47.3±1.2	0.17	0.8
Serum albumin: (g/dl)	3.77 ± 0.076	3.72± 0.147	3.68±0.156	1.61	0.2
Serum creatinine: (mg/dl)	0.43±0.02	0.44 ± 0.02	0.43 ± 0.02	0.37	0.7
Serum malondialdhyde (MDA)(nmol/ml):	0.14±0.01	0.13 ± 0.04	0.14 ± 0.01	0.1	0.9
Total anti-oxidant capacity(TAC): (ng/ml)	0.22±0.01	0.23 ± 0.01	0.21 ± 0.01	0.13	0.8

All values are expressed as mean±SD. (SD: standard deviation).
Number of rats in each group=12 rats.

(Table 2): Comparison of serum levels of ALP, ALT, serum albumin, serum creatinine, serum MDA and serum TAC of control and experimental groups of rats (ANOVA test).

	Control group Mean ± SD	PBM group	Toluene group Mean ± SD	Toluene + PBM group (V) Mean ± SD	P	LSD
ALP (IU/L)	157.4 ± 1.2	157.3 ± 3.5	217.5 ± 4.6	163.3 ± 5.4	< 0.05	<0.001
ALT (IU/L)	47.3± 1.4	48.0 ± 0.8	80.9 ± 10.6	54.3 ± 3.6	< 0.05	<0.001
Albumin (IU/L)	3.8 ± 0.1	3.7 ± 0.2	2.3 ± 0.4	3.4 ± 0.5	< 0.05	<0.001
Creatinine (U/L)	0.44 ± 0.02	0.43 ± 0.02	0.74 ± 0.12	0.50 ± 0.03	< 0.05	<0.001
MDA (U/L)	0.14 ± 0.01	0.13 ± 0.01	0.34 ± 0.1	0.18 ± 0.01	< 0.05	<0.001
TAC	0.22 ± 0.01	0.23 ± 0.01	0.14 ± 0.02	0.20 ± 0.01	< 0.05	<0.001

All values are expressed as mean±SD. (SD: standard deviation).Number of rats in each group=12 rats.LSD=least significant difference. P<0.001 highly significant difference. P >0.05= non significant

III. Histpathological results:

III.2.1 Liver:

Examination of liver sections of Control and PBM groups by H&E stains showed the hepatic lobular architecture; the standard hexagonal or pentagonal lobules with central veins and peripheral hepatic triads (portal areas) contained branches of the portal vein, hepatic artery, and bile duct within connective tissue stroma. Hepatocytes are arranged radially from the central vein in cords. They had an acidophilic cytoplasm with stippled appearance and massive pale vesicular nuclei. Narrow sinusoidal spaces separated these cords. (figure 1a).

Histopathological examination of liver of toluene group showed some hepatocytes with pyknotic nuclei and ballooning degeneration, interstitial haemorrage , congestion of central vein , loss of cytoplasmic density , homogenous structurless hepatic tissue denoting necrosis **(**figure1b,c,d).

The examination of H&E stained liver parts of toluene + PBM group under a light microscope revealed interstitial hemorrage and no central vein congestion. However, few partial improvement in histopathological changes in form of disappearance of necrotic areas,

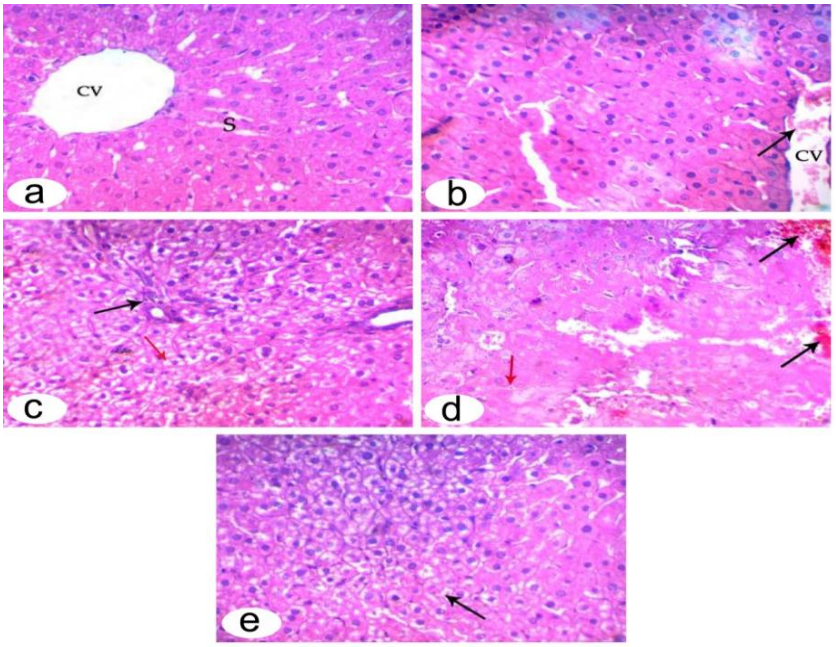
hepatocytes with hydropic degeneration were shown **(**figure 1e).

III.2.2. Kidney

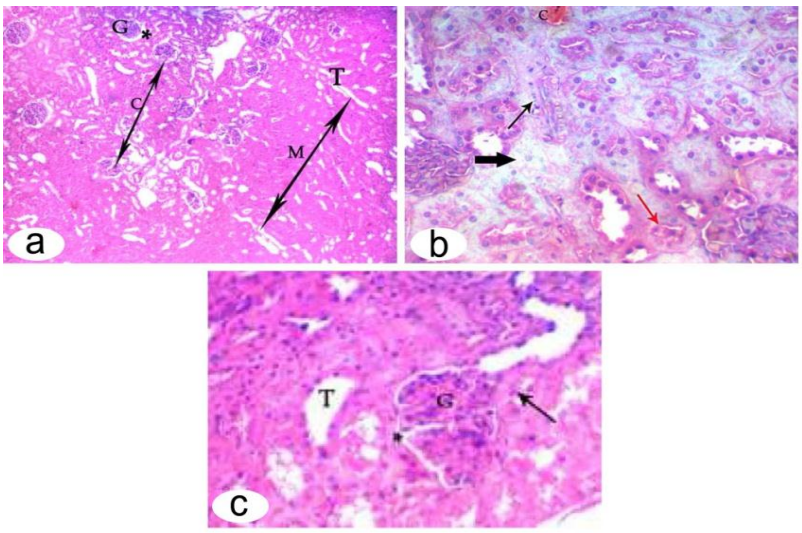
The normal architecture of renal tissue of control and PBMgroups which composed of renal cortex and medulla was found after examination of H&E stained sections of kidney. The renal cortex comprised renal corpuscles and closely packed renal tubules. Renal corpuscles and tightly packed renal tubules made up the renal cortex. Bowman's capsules enclosed the renal corpuscles, which were made up of glomerular tufts of capillaries with an outer parietal layer and an inner visceral layer separated by Bowman's space. The convoluted tubules lined by cuboidal cells. The lumen in the proximal ones was narrow and irregular. Furthermore, the distal tubules had a transparent lumen. Tubules of various types were found in the renal medulla. (figure 2a).

Exposure of rats to toluene resulted in several histological alterations in kidney structure. Renal tubules showed cloudy swelling, some renal tubules were distorted and lined by cells with dark pyknotic nuclei with faint cytoplasm. The interstitium showed congestion and loss of density capacity (figure 2b).

Examination of H&E stained sections of renal medulla of toluene + PBM group under alight microscope revealed partial improvement in histopathological changes in form of normal density capacity with no pyknotic nuclei in tubular epithelial lining. However, some renal tubules showed cloudy swelling (figure 2c).



(Figure 1):H&E staining micrograph of liver tissues showing :a) section from control group showing normal lobular architecture. Central vein (CV) is surrounded by radiating cords of hepatocytes separated by blood sinusoids(s).Hepatocytes are polyhedral with acidophilic cytoplasm and round vesicular nuclei. Sections from toluene group showing b) congestion of central vein (arrow) (H&Ex400). c) hepatocyte with pyknotic nuclei (black arrow) and ballooning degeneration of hepatocytes (red arrow)(H&Ex400). d) interstitial haemorrage (black arrow) and homogenous structurless pattern of hepatocyte denoting hepatic necrosis (red arrow)(H&Ex400). e) section from toluene +PBM group showing ballooning degeneration of hepatic cells (arrow)(H&Ex400)



(Figure 2): H&E staining micrograph of renal tissues showing :a) section of negative control group showing the renal cortex (C) containing renal corpuscles (arrow) surrounded by Bowman`s capsule(star) and renal medulla (M) containing different types of tubules (T) (H&E×400). b) section in kidney of an adult male albino rat of toluene group after 7 days , showing cloudy swelling of renal tubules(red arrow)and some renal tubules are lined by cells with dark pyknotic nuclei (black arrow). Loss of density capacity (thick arrow) and congestion (C)are also seen (H&E x400). **C)** section in kidney of an adult male albino rat of toluene+PBM group after 7 days , showing normal renal glomeruli (G) surrounded by Bowman`s capsule(star) and cloudy swelling of some renal tubules (arrow) (H&E x400).

III.3. Immunohistochemical results:

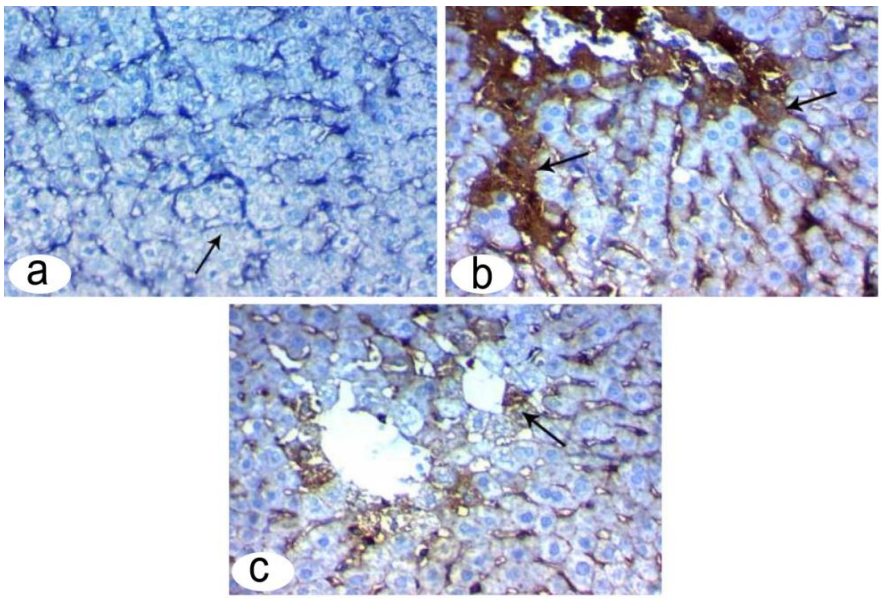
III.3.1. Liver:

Immunohistochemical examination of the liver sections of control and PBM group showed negative caspase-3 immunoreactivity in the cytoplasm of hepatocytes (figure 3a). In comparison to the negative control group, light microscopic analysis of immunohistochemically stained parts of the liver of the toluene group revealed high positive caspase-3 immunoreactivity in the cytoplasm of hepatocytes after 7 days. (figure 3b).

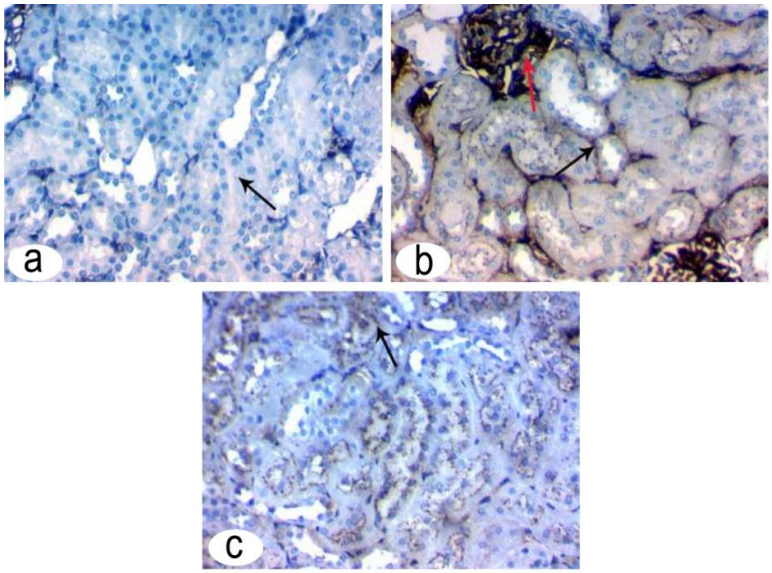
After 7 days, immunohistochemically stained portions of the liver from the toluene + PBM group showed poor positive caspase-3 immunoreactivity in the cytoplasm of hepatocytes, relative to the toluene group. (figure 3c).

III.3.2. Kidney:

Throughout the analysis, immunohistochemical review of kidney parts from control groups revealed negative caspase-3 immunoreactivity in the cytoplasm of renal corpuscular cells and tubular lining cells in the cortex and medulla. (figure 4a). After 7 days, immunohistochemically stained parts of kidney from the toluene group showed high positive caspase-3 immunoreactivity in the cytoplasm of renal corpuscular cells and tubular lining cells in the cortex and medulla, compared to the negative control group (figure 4b). After 7 days, immunohistochemically stained parts of the kidney from the toluene +PBM group showed faint positive caspase-3 immunoreactivity in the cytoplasm of renal tubules, relative to the toluene group (figure 4c).



(Figure 3): Immunohistochemical staining micrograph of liver tissues showing:a) section from negative control group, showing negative caspase-3 immunoreactivity in cytoplasm of hepatocytes (arrow) (Immunohistochemical x400).b) section of hepatic lobule from toluene group, showing strong positive caspase-3 immunoreactivity in cytoplasm of hepatocytes (arrow) (Immunohistochemical x400).c) section of hepatic lobule of toluene+ BPM group showing weak positive caspase-3 immunoreactivity in cytoplasm of hepatocytes (arrow) (Immunohistochemical x400).



(Figure 4): immunohistochemical staining micrograph of renal tissues showing: a) section of renal medulla from negative control group, showing negative caspase-3 immunoreactivity in cytoplasm of renal tubular lining cells (black arrow) (Immunohistochemical x400).b) section of renal cortex obtained from an adult male albino rat of toluene group, showing strong positive caspase-3 immunoreactivity cytoplasm of the renal corpuscular cells (red arrow) and in cytoplasm of renal tubular lining cells (black arrow) (Immunohistochemical x400).c) section of renal medulla obtained from toluene+PBM group, showing weak positive caspase-3 immunoreactivity cytoplasm of the renal tubular lining cells (black arrow) (Immunohistochemical x400)

IV. DISCUSSION

Toluene is one of aromatic hydrocarbons that used as gasoline mixer and found in many commercial and household products such as paint thinner, adhesives, lacquers, varnishes and glues (Lim et al., 2014). Toxic exposure to toluene may result from inadvertent or intentional inhalation of fumes, ingestion, or transdermal absorption. Since it is readily available and inexpensive, toluene abuse or "glue sniffing" is common, particularly among children and adolescents. (Alsharif et al., 2018).

Toluene metabolism occurs mainly in the liver and about 80% of ingested or inhaled toluene excreted by the kidney so their role in metabolism and excretion of toluene predispose them to toluene toxicity (Tas et al., 2011).

When compared to the negative control group, the results of this study showed a highly significant increase in the mean values of serum ALP, ALT, creatinine, MDA, and TAC, as well as a highly significant decrease in serum albumin after 7 days.

The findings of this study matched those of Tas et al. (2011), who found a significant increase in ALT and ALP, as well as a significant reduction in serum albumin, in rats exposed to 3000 ppm toluene daily for 30 days. In contrary to the results of present study, Kim et al. (2013) reported a non -significant differences of serum levels of ALP and ALT in rats received 436 mg/kg toluene intraperitoneally (i.p.)

once daily for 3 days. While the serum levels of ALT and ALP were significantly increased in rats received both dimethylformamide (DMF) and toluene.
Cámara-Lemarroy et al. (2012) reported an elevation of serum levels of ALP and ALT in all patient with acute toluene inhalation admitted to the emergency department of Monterrey's University Hospital "José Eleuterio Gonzalez" in case series study. Also, Cámara-Lemarroy et al. (2015) assessed 20 patients with acute toluene intoxication and reported normal levels of serum ALT but serum ALP was elevated

Toluene induced hepatotoxicity can be explained by highly lipophilic activity of toluene. It is rapidly spread to heavily perfused tissues such as the brain and liver, with accumulation in lipid-rich tissues. Liver is one of the primary organs for toluene accumulation, irrespective of the route of exposure. Toluene can generate excessive oxidative stress and cell apoptosis (Tas et al., 2011 and Ayan et al., 2012). The results of this study matched those of Ahmadizadeh et al. (2014), who found that rats given 600mg/kg and 900mg/kg toluene for 7 days had significantly higher creatinine levels compared to rats in control group but the highest levels were with rats received 900mg/kg. These results are supported by Meydan et al. (2016) who reported highly significant increase in serum creatinine in rats received 500mg/kg toluene intraperitonially for 14 days.

Other clinical reports recorded an elevation of serum levels of creatinine in all patients with acute toluene inhalation (Cámara-Lemarroy et al., 2012; Cámara-Lemarroy et al., 2015).

MDA is generally recognised as a responsive biomarker of lipid peroxidation, according to Oboh et al. (2012), and is considered a valuable indicator of oxidative stress status. Kamel et al. (2008) reported significant increase in serum MDA among rats received 650mg /kg toluene daily for 15, 30 and 45 days.

MDA levels in liver tissues were significantly higher in toluene-exposed rats than in the control group, according to Tas et al. (2011), but superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) values were higher in toluene-exposed rats than in the control group. They were not, however, statistically important. It was reported that MDA levels in the kidneys of rats given different doses of toluene were found to be significantly higher. In rats given 600 mg/kg and 900 mg/kg toluene, the reduction in SOD and CAT enzyme activities was statistically important. (Afravy et al., 2017).

When rats given toluene were compared to rats in the control group, the reduction in CAT and SOD enzyme activities was found to be highly statistically important. (Meydan et al., 2016). The administration of pasturalized buffalo milk with toluene resulted in highly significant reduction in mean values of serum ALT and ALP, creatinine, MDA and TAC and highly significant increase in mean values of serum albumin. As a result, the importance of natural antioxidants as a strategy for preventing oxidative damage as a factor in the pathophysiology of various health disorders has recently piqued interest. Buffalo milk is rich in antioxidants such as vitamin C, E, selenium, zinc,

tyrosine, and cysteine, which are all essential for antioxidant activity. (Zulueta et al., 2009).

The findings of this study were similar to those of Ahmadizadeh et al. (2017), who found a substantial reduction in serum levels of ALT and ALP in rats given buffalo milk by oral gavage ten minutes before receiving intraperitonial xylene for seven days. The findings of present study were in line with that of Afravy et al. (2017) reported significant reduction in serum creatinine in rats received toluene +buffalo milk group when compared to toluene groups values.

In agreement with present study, In xylene-treated rats, Ahmadizadeh et al. (2017) discovered that the levels of enzymatic (superoxide dismutase, SOD, and catalase, CAT) and non-enzymatic glutathione (GSH) antioxidant system components were reduced in a dose-dependent manner. Pre-treating animals with Buffalo milk, on the other hand, significantly increased the amount of antioxidant components in xylene-treated rats. Similar histopathological results of present study were also reported by Meydan et al. (2019) who found that toluene induced a pathological damage in the liver manifested by sinusoid dilation, hemorrhage, vacuolization and necrosis. Furthermore, Ayan et al. (2012) found that after a single oral dose of 5200mg/kg toluene, toluene caused hepatocyte degeneration as well as a small, focal infiltration of mononuclear inflammatory cells in the parenchyma and portal areas in toluene-treated rats. During the acute time, high doses of toluene induce apoptosis in rat liver via mitochondrial pathways, according to the conclusion of the researchers.

In the present study, administration of pasturalized buffalo milk with toluene revealed partial improvement in histopathological changes of liver in form of disappearance of necrotic areas, interstitial hemorrage and no central vein congestion. However, few hepatocytes showed hydropic degeneration.

These results are supported with Ahmadizadeh et al. (2017) who reported that buffalo milk restored kidney and liver structures and prevent xylene induced cytotoxicity in rats pretreated with buffalo milk.

Toluene exposure caused many histological changes in the kidney structure in rats. Renal tubules showed cloudy swelling, some renal tubules were distorted and lined by cells with dark pyknotic nuclei with faint cytoplasm. The interstitium showed congestion and loss of density capacity. Ahmadizadeh et al. (2014) confirmed the findings of this study, reporting pathological changes in toluene-treated rats, including swelling of renal tubular cells, loss of staining capability, nuclei that appeared to be dilated, and the presence of blood clot.

The current study's histopathological changes matched those recorded by Afravy et al. (2017), who found swelling of renal tubular cells, loss of staining ability, nuclei dilatation, and the presence of a blood clot in toluene-treated rats..

In the present study, administration of pasturalized buffalo milk with toluene revealed partial improvement in histopathological changes of kidney in form of normal density capacity with no pyknotic nuclei in tubular epithelial lining. However, few renal

tubules showed cloudy swelling. According to Afravy et al. (2017), when buffalo milk pretreated rats were compared to toluene-only treated rats, the degree of toluene-induced nephrotoxicity was reduced. After 7 days, light microscopic analysis of immunohistochemically stained portions of the liver of the toluene group revealed strong positive caspase-3 immunoreactivity in the cytoplasm of hepatocytes, and weak positive caspase-3 immunoreactivity in the cytoplasm of hepatocytes of the toluene + pasturalized buffalo milk group revealed weak positive caspase-3 immunoreactivity in the cytoplasm of hepatocytes, compared to the negative control. In a research conducted by Kamel et al. (2008), oral administration of 650 mg/kg toluene resulted in an increase in Caspase 3 activity in rat liver tissues.

Caspase-3 staining in liver tissues was also significantly higher in rats given toluene compared to rats in the negative control group. (Ayan et al., 2012).

V. CONCLUSION:

From these observations, it can be concluded that acute toluene exposure for 7 days induced toxic effects on liver and kidney of adult male albino rats with histopathological changes and apoptosis. Administration of pasturalized buffalo milk with toluene for 7 days produced partial improvement in the hepato- and nephrotoxic effects of toluene.

VI. RECOMMENDATIONS:

On the light of the results of the study, Strict precautions during use and disposal of toluene are recommended to prevent unwanted environmental impacts. Development of necessary safety rules for people working with materials containing toluene and for those who might be exposed to toluene during commercial or industrial applications.Continuous monitoring of serum MDA, liver and kidney function tests should be done on a routine basis in toluene exposed workers. If there is abnormality in these functions, workers should be excluded from work place until return to normal level. It is recommended to use pasturalized buffalo milk during treatment of acute toluene toxicity.

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تقييم دور الحليب الجاموسي علي التسمم الكبدي والكلوي الحاد الناتج عن التولوين في ذكور الجرذان البيضاء البالغه

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يعتبر التولوين أحد الهيدروكربونات العطريه ويتميز بانه عديم اللون ويعرف ايضا باسم ميثيل بنزين وفينيل ميثان وتولول. و.يستخدم التولوين على نطاق واسع في جميع أنحاء العالم كمركب عضوي متطاير ويتم تصنيعه بكميات كبيرة للاستخدام في التطبيقات الصناعية والتجارية المختلفة بما في ذلك الدهانات والأحبار والمواد اللاصقة والورنيش والبلاستيك والمخففات والجلود والأصباغ ويؤدي التعرض للتولوين الي تلف العديد من الاعضاء مثل المخ ،القلب ،الرئه ،الكبد والكلي وحيث يعتبر الكبد والكلي عضوي التمثيل الغذائي واخراج التولوين فان ذلك يجعلهما اكثر الاعضاء عرضه للتسمم به . كان الهدف من هذه الدراسة هوفحص التغيرات النسيجية المرضية في الكبد والكلي الناجمة عن التعرض الحاد للتولوين والآليات الكامنة المحتملة وتقييم دور الحليب الجاموسي في تخفيف الآثار السامة للتولوين في ذكور الفئران البيضاء البالغة. اجريت هذه الدراسه علي ٧٢ من ذكور الجرذان البيضاء البالغه مقسمه الي خمس مجموعات كالتالي ١. المجموعه الاولي (مجموعة ضابطة سالبة):-تتكون من ١٢ جرذ وتم اعطاء كل جرذ من هذه المجموعة غذاءً وماءً منتظمًا بدون اي علاج لمدة سبعه ايام للحصول على معاملاتها المختبرية والتشريحية كقيم مرجعية قابلة للمقارنة ٢.المجموعة الثانيه (مجموعة ضابطة موجبة) : وتتكون من ٢٤جرذ مقسمة بطريقة عشوائية ومتساوية(١٢ فى كل مجموعة) الى:مجموعة ضابطة موجبة أ:(مجموعه الماء المقطر) :تم اعطاء كل جرذ الماء المقطر ١ ملليلتريوميا عن طريق الفم لمدة سبعه ايام. مجموعة ضابطة موجبة ب: (مجموعه زيت الذره):تم اعطاء كل جرذ المادة المذيبة للتولوين وهي ١مل من زيت الذره مرة واحدة يوميا لمده سبعه ايام .المجموعه الثالثه (مجموعه الحليب الجاموسي المبستر): تتكون من ١٢ جرذ و تم إعطاء كل جرذ الحليب الجاموسي المبستر ١مل يوميا لمده سبعه ايام.المجموعه الرابعه (مجموعه التولوين): تتكون من ١٢ جرذ وتم اعطاء كل جرذ التولوين بتركيز٩٠٠ مجم /كجم يوميا لمده سبعه ايام .المجموعه الخامسه(التولوين +الحليب الجمسي المبستر): تتكون من ١٢ جرذ و تم اعطاء كل جرذ التولوين بتركيز ٩٠٠ مجم /كجم يوميا لمده سبعه ايام + الحليب الجمسي المبستر امل يوميا لمده سبعه ايام.واوضحت النتائج ان التسمم الحاد بالتولوين ادي الي ارتفاع ذو دلاله احصائيه في نسبه انزيمات الكبد والكرياتينين وانخفاض ذو دلاله احصائيه في نسبه الالبيومين وسعه مضادات الاكسده الكليه في الدم كما تسبب في احداث بعض التغيرات الباثولوجيه وزياده النشاط المناعي لكاسباس٣ في خلايا الكبد والكلي .وعند اعطاء الحليب الجمسي المبستر مع التولوين احدث تحسنا جزئيا في نسبه الانزيمات والتغيرات المورفولوجيه وقلل من النشاط المناعي لكاسباس ٣ في انسجه الكبد والكلي .الخلاصه: التعرض الحاد للتولوين لمده ٧ ايام احدث تاثيرات سميه علي الكبد والكلي في ذكور الجرذان البيضاء البالغه كما ادي الي تغيرات هستوباثولوجيه وموت مبرمج للخلايا .كما اثبتت الدراسة الحالية أن إعطاء الحليب الجمسي مع التولوين أدى إلي تحسن جزئى ملحوظ فى الآثار السميه الناجمه عن التسمم الحاد بالتولوين علي الكبد والكلي لذا ينصح باخذ احتياطات صارمة أثناء استخدام التولوين والتخلص منه لمنع الآثار البيئية غير المرغوب فيها كما ينصح باجراء فحوصات دورية للعمال الذين يتعرضون للتولوين لفترات طويلة لمتابعة حدوث اى خلل فى وظائف الكبد والكلى .الحليب الجاموسي المبستر هو عامل تحسين للوقاية من التسمم الكبدي والكلوي الناجم عن التولوين وذلك بسبب خواصه المضادة للأكسدة ، لذلك هناك حاجة إلى مزيد من الدراسات لاستقصاء دور الحليب الجاموسي المبستر ضد الآثار السامة المختلفة للتولوين .