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Methyl tertiary butyl ether inhalation induced biochemical and Histological alterations in rabbits

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ABSTRACT

The present work studied the effect of inhalation of methyl tertiary butyl ether (MTBE) on biochemical parameters and histology of liver of rabbits. Animals were divided into 2 groups. Group 1 served as controls and group 2 were inhaled (MTBE). Animals were sacrificed after 10, 15 and 20 days of treatment. The results showed that exposing animals to MTBE induced significant decrease in RBCs count, hemoglobin, hematocrit percentage and blood platelets. On the other hand, the WBCs count increase. Triglycerides, cholesterol and transaminases (ALT and AST) were increased in the sera of treated rabbits. Histological examination of liver of treated rabbits showed leucocytic infiltrations, congestion of blood vessels, degeneration of hepatocytes and fatty degeneration. It is concluded from these results that that the toxicity of MTBE in rabbits may be attributed to induction of oxidative stress.

INTRODUCTION

Methyl tertiary butyl ether (MTBE) is a synthetic organic chemical produced primarily for the petroleum industry and is blended into gasoline to increase the octane rating. It is blended into gasoline at about 15% by volume (Johnson *et al.,* 2000). By 1988, it was among the top 50 chemicals made in the USA, and by 1993 it was the second most produced chemical in the USA, essentially reaching 'commodity' status (Borghoff and Williams, 2000). People have been exposed to MTBE through skin contact, inhalation and ingestion and MTBE has been detected in blood, breath and urine specimens (Buckley *et al.,* 1997).

Exposure to MTBE was accompanied by several side effects. There have been some reports of acute symptoms, such as headaches, nausea, dizziness, and difficulty breathing, from people exposed during refueling to gasoline with higher levels of MTBE, such as in reformulated gasoline (White *et al*., 1995). MTBE leads to an increase in liver and kidney weights and increased severity of spontaneous renal lesions in female rats, as well as increased prostration in females and swollen periocular tissue in male and female rats (IRIS, 1993). It was reported that MTBE induced renal damage in the proximal tubules of male rats through interaction with α- 2u-globulin (Prescott-Mathews *et al*., 1999). Long term exposure to MTBE was found to induce testicular interstitial cell adenomas and renal tubular cell tumors in male Fischer 344 rats at 10,800 and 28,800 mg/m^3 (Bird *et al.*, 1997). It also caused increased incidence of hepatocellular adenomas in female CD-1 mice at 28,800 mg/m^3 (Burleigh-Flayer et al.1992). In Sprague Dawley rats given MTBE in olive oil by gavage at 0, 250, or 1000 mg/kg bw, females had an increased incidence of lymphomas and leukemia, and males had an increased incidence of testicular interstitial cell adenomas. Some evaluations (California EPA, 1999; Interagency Oxygenated Fuels Assessment Steering Committee, 1997; U.S. EPA, 1994) have concluded that MTBE could pose a possible or potential cancer risk to humans, whereas other public health bodies (WHO 1998) have concluded that there is not enough information to classify MTBE with regard to human carcinogenicity under their classification schemes. The aim of the present work was to investigate the possible biochemical and histological effects of MTBE on rabbits.

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MATERIALS AND METHODS

The experiment was performed on 25 adult male New Zealand rabbits weighing 1.5± 0.2 kg. Animals were fed on pellets of standard rabbit ration and free access to water *ad libitum*. They were divided into two groups:

Group 1: animals of this group (5 rabbits) served as controls.

Group 2: animals of this group (20 rabbits) were inhaled methyl tertiary butyl ether (MTBE).Animals were kept individually in a closed cage containing 80 ppm MTBE for 5 minutes daily for 20 days.

Biochemical assays

The treated animals and their controls were sacrificed by decapitation after 10, 15 and 20 days of treatment. For hematological study, blood was collected from control and treated animals. The hematological parameters: red blood cells count (RBCS), hemoglobin value (Hb), hematocrit value (HCT %), white blood cells count (WBCS) and blood platelets number were measured by a fully automated Coulter counter (Coulter Electronics Limited, England). For biochemical assays, blood samples were collected from animals and sera were obtained by centrifugation of the blood sample and stored at -20°C. Triglycerides, cholesterol, aspartate aminotransferase (AST) and alanin aminotransferase (ALT) were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (Randox Laboratories, LTD, Ardomre, Crumlin, United Kingdom) were used in these analysis.

Histological examination

The histological effects of MTBE on the liver of rabbits were examined after 20 days of treatment. After dissection, Livers of control and treated animals were removed and fixed in Bouin's fluid. Fixed materials were embedded in paraffin wax and sections of 5 micrometers thickness were cut. Slides were stained with haematoxylin and eosin for histological examination.

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, P.A).

RESULTS

Biochemical results

Data in table (**1**) showed that the number of erythrocytes was significantly decreased in sera of rabbits after 20 days of treatment with MTBE. Similarly, haemoglobin content was significantly decreased after 20 days. The haematocrit percentage was significantly decreased after 15 and 20 days. The number of platelets showed a significant decrease after 15 and 20 days of treatment with MTBE. On the other hand, the leucocyte counts were found to increase and this increase became significant after 15 and 20 days. Results in table (2) showed that triglycerides was increased in sera of MTBE -treated rabbits and this increase was significant ($P<0.05$) after 15 and 20 days. A significant increase in cholesterol was recorded in animals treated with MTBE for 10, 15 and 20 days. Concerning the effect of MTBE on transaminases, data in Figure 1 and 2 revealed that ALT and AST were gradually increased in the sera of MTBE –treated rabbits and this increase became significant ($P<0.05$) after 15 and 20 days of treatment.

Table. 1: Effect of MTBE on blood parameters of male rabbits .

Parameters \ Treatment	Control	10 days	15 days	20 days
RBCs 10^6/mm^3	5.43±0.35	4.98±0.3	4.25±0.6	4.23±0.8*
Hb gm/dl	12±1.2	11.2±1.1	10.2±0.9	8.5±1.3*
HCT %	27.2±1.3	31.5±1.8	33.4±1.4*	38.2±2.1*
Platelets 10^3/L	775±3.1	562±2.2	387±5.3*	352±4.6*
WBCs10^6/mm^3	5.1 ±0.5	8.8±1.3	11.2±0.5*	13.1±2.1*

-Values are expressed as mean ± SD
- (*) Significant at $p<0.05$

Table. 2: Effect of MTBE on triglycerides and cholesterol in male rbbits.

Parameters \ Treatment	Control	10 days	15 days	20 days
Triglycerides(mg/dl)	90±3.7	98±2.2	109±4.6*	139±3.6*
Cholesterol (mg/dl)	36±2.1	57±2.1*	87±3.5*	98±2.3*

-Values are expressed as mean ± SD
- (*) Significant at $p<0.05$

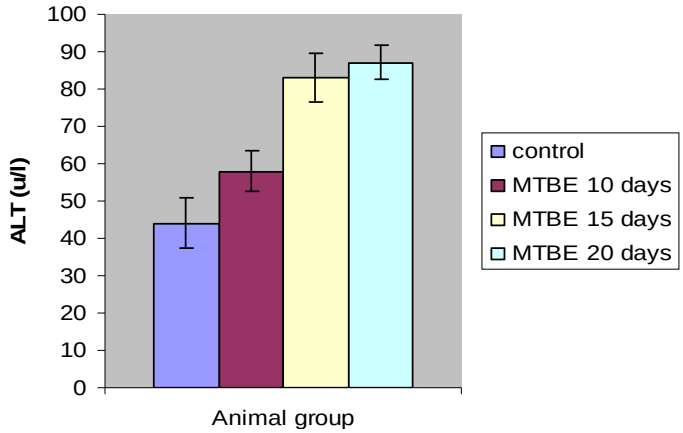

Fig.1: change in ALT in different animal groups.

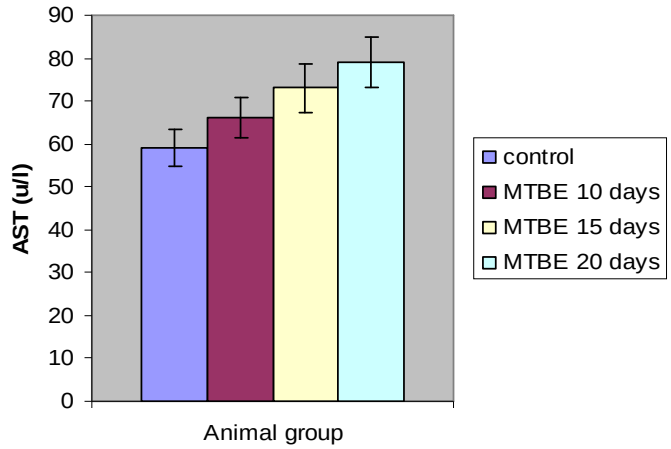

Fig. 2: change in AST in different animal groups.

Histological observations

Histological examination of liver of the untreated rabbits (control) showed normal architecture. The parenchyma appears as regular cords arranged radially from the hepatic cells with eosinophilic, finely granular cytoplasm containing one or two prominent nuclei. Treating rabbits with MTBE for 20 days caused many histopathological alterations In treated animals, the blood vessels (central and portal veins) were enlarged and congested, the sinusoidal spaces were filled with blood (Fig.3).

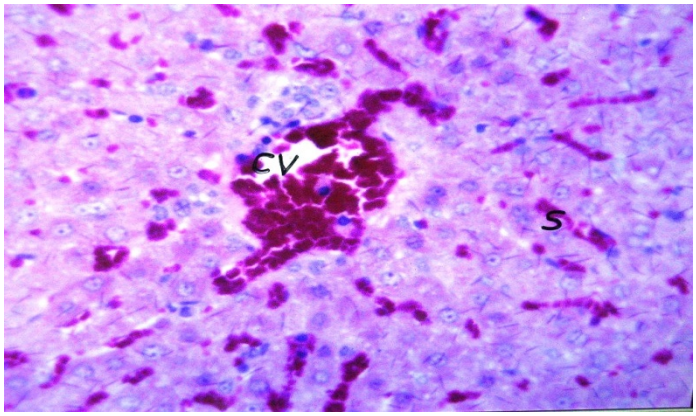

Fig. :3 Section in the liver of a treated rabbit showing congestion in central vein (cv) and sinusoids (s). H & E, X 80.

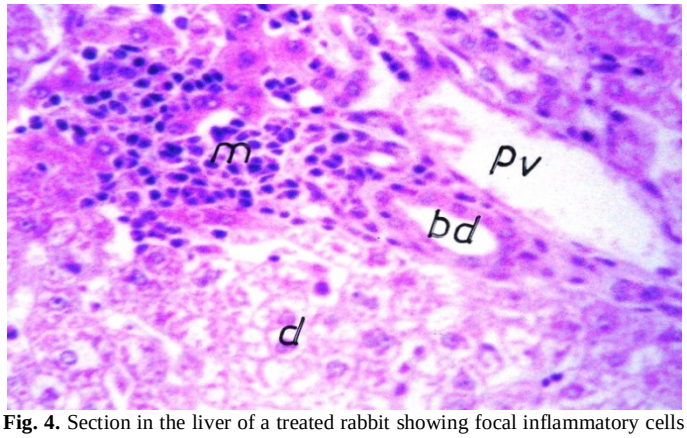

Fig. 4. Section in the liver of a treated rabbit showing focal inflammatory cells infiltration (m) in degenerated parenchyma (d) with dilated portal vein (PV) and bile duct (bd). H & E, X 80.

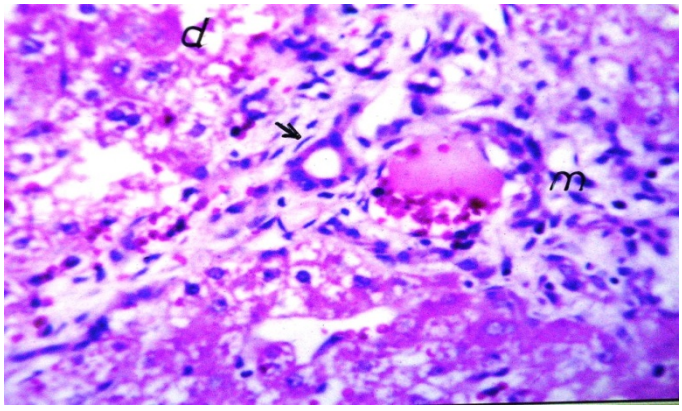

Fig. 5: Section in the liver of a treated rabbit showing inflammatory cells infiltration (m) and fibrosis (arrow) in portal area. H & E, X 80.

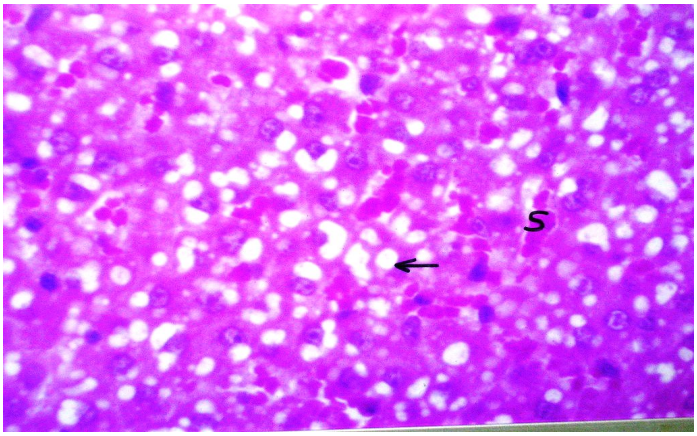

Fig. 6: Section in the liver of a treated rabbit showing congestion in the sinusoids (s) with fatty change in the hepatocytes (arrow). H & E, X 160

Additionally, in MTBE - treated animals, masses of leucocytic infiltrative cells were abundant in many areas of the liver tissue which showed degenerated hepatocytes (Fig.4). The portal area showed inflammatory cells infiltration and fibrosis (Fig.5). Fatty infiltrations of different size of fat droplets were observed in large areas of the liver (Fig.6).

DISCUSSION

The present results showed that MTBE caused significant decrease in the number of erythrocytes, haemoglobin content and haematocrit percentage. Gill *et al.*(1990) reported that exposure of male rats for 13 weeks to 800, 4,000, or 8,000 ppm MTBE caused mild decreases (2-4%) in erythrocyte count and mean corpuscular hemoglobin concentration and mild increases (2-5%) in mean corpuscular volume, mean corpuscular hemoglobin and reticulocytes. At the end of the treatment period, female rats of the 8,000 ppm group had increased hematocrit and segmented neutrophil count. Costantini (1993) reported that MTBE may cause alterations of red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) values. On the other hand, chronic-duration inhalation studies in rats (Chun *et al.*, 1992) and mice (Burleigh-Flayer *et al.*, 1992) exposed intermittently to 400, 3,000, or 8,000 ppm MTBE, showed no changes in hematological parameters. The number of leucocytes was significantly increased in the treated rabbits. This means that the defense mechanism represented in the leucocytes could compensate the toxic effect of MTBE Hutcheon *et al.*(1996) recorded a biphasic pattern of increase and decrease of white blood cells in rats according to MTBE concentration. Thus, at lower MTBE concentrations (1,000, and 1,500 ppm) WBC count was reduced significantly while at higher concentrations (2,000, and 2,500 ppm) their counts were significantly elevated. This biphasic pattern of WBC count was positively correlated with a similar pattern of decrease and increase exhibited by neutrophils and lymphocytes.

Significant increase in triglycerids and cholesterol were recorded in sera of MTBE treated animals. In agreement with these results, Ubani *et al.* (2009) reported that gasoline which is a mixture of hydrocarbons and additives such as methyl tert-butyl

ether induced significant increase in triglycerids and cholesterol in sera of rats. They added that gasoline inducing cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation. Male rats given high dose of MTBE (1428 mg/kg) displayed significantly increased AST, LDH and cholesterol, and males gavaged with 1071 mg/kg exhibited significantly increased AST and LDH (Robinson *et al*. 1990). The obtained results showed that MTBE caused a significant increase in transaminases (ALT, AST) in sera of rabbits. Similarly, Elovaara *et al*.(2007) reported that exposing rats to the gasoline additives methyl *tert*-butyl ether (MTBE) and *tert*-amyl methyl ether (TAME) resulted in hepatomegaly (13–30%) and induction of cytochrome P450 (CYP) activity. Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were elevated in blood plasma after administration of the ethers.Liver microsomal uridine diphosphate glucuronosyl transferase (UDPGT) activity was increased in a dose-related manner in rats exposed to 50, 100, and 300 ppm MTBE for 2 weeks, but not at other times (Savolainen *et al*. 1985).

Histological examination of liver of rabbits treated with MTBE revealed many alterations include congestion of blood vessels, leucocytic infiltrations and fatty degeneration. These results are similar to those of Medinsky *et al*. (1999) who reported that MTBE caused cenerilobular hypertrophy, necrosis and increase of rate of cell proliferation in liver of rats. These effects are consistent with a mitogenic response of the liver to MTBE (Moser *et al*.1996). Bird *et al.*, (1997) reported that female and male rats exposed to MTBE experienced a dose-related increase in mortality from chronic progressive nephropathy. All treated males had increases in the severity of mineralization and interstitial fibrosis of the kidney, while increases in mild to moderate glomerulosclerosis, interstitial fibrosis, and tubular proteinosis were observed in females.

Oxidative stress due to abnormal production of reactive oxygen species (ROS) is believed to be involved in the etiology of toxicities of many xenobiotics. Oxidative stress has been related to lipid peroxidation and membrane damage, oxidation of glutathione and consequently, ATP and NADPH depletion that would cause marked disruption in lipid synthesis and transport. Membrane peroxidation may alter the activity of liver enzymes involved in cholesterol metabolism and lipoprotein formation resulting in a higher total serum cholesterol concentration (Tarama *et al.*,2003). Assessment of the MTBE-induced oxidative stress revealed that MTBE increased the production of reactive oxygen species (ROS) and enhanced lipid peroxidation. In addition, cytosolic SOD activity decreased in isolated rat spermatogenic cells (*Li et al*..,2009). It is concluded from the results of the present work that the toxicity of MTBE in rabbits may be attributed to induction of oxidative stress.

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