REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Toxicopathological Effect Of Benzene, Toluene, Ethylbenzene, And Xylenes (BTEX) As A Mixture And The Protective Effect Of Citicoline In Male Rats Followings 90-Day Oral Exposure

Mohammed. E. Abduljalel[1] and Raghad. N. Al-Saadi[2*]
1,2 Department of Pathology and poultry disease, College of Veterinary Medicine, University of Baghdad, Iraq
raghad.n@covm.uobaghdad.edu.iq

Abstract
The chemicals benzene, toluene, ethylbenzene, and xylene (BTEX) are neurotoxic. Although health effects of the individual (BTEX) chemicals are generally well described, relatively less is known on the toxicity following the exposures to "whole" mixtures. In the present study, the chronic toxicity of BTEX mixture following 90 days of consecutive oral exposure and the protective effect of citicoline was investigated in male rats. Doses for the BTEX mixture were selected based on the no observed adverse effect level NOAEL, as determined in previous animal toxicity studies for each compound. A total of 64 rats were divided in four equal groups of 16 male rats each. Rats in G1were the control. Rats in G2 were gavaged with BTEX mixture at doses of 600 mg/kg. Rats in G3 were exposed to BTEX mixture at 600 mg/kg in combination with citicoline 500 mg/kg orally. Rats in G4 were treated with citicoline at 500 mg/kg only. All treatments were conducted 7 days/per week for 90-day oral exposure. Histopathological changes in the brain, liver and kidney and caspase-3-activity in brain stained slides were evaluated in this study. Rats treated with BTEX showed histopathological alteration such as microgliosis in the brain with sever degenerative changes in the liver and kidney and marked increase of caspase-3 activity in the brain. Rats treated with BTEX and citicoline showed improvement in brain, liver architecture and kidney and marked decrease in the brain positive caspase-3 activity.

In conclusion, the results showed that the safe level (NOAEL) of BTEX compound individually, can produce a significant adverse effect when administrated as a mixture in male rats. The results also indicated that citicoline treatment can protect against BTEX induced toxicity in male rats.

Keywords: Volatile organic compounds -Groundwater-Neurotoxic-Neurotransmitter

Introduction

Exposure to the volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, and xylene (BTEX) in the environment poses the greatest risk on the health of human and animals (18) . The volatile organic chemicals (VOCs) benzene, toluene, ethylbenzene, and three isomers of xylene (p-xylene, m-xylene, and o-xylene), commonly known as BTEX, are found in almost every contaminated environment (6,31). These chemically similar molecules are the main elements of crude oil, ingredients of petroleum products including gasoline, jet fuels, coal and kerosene and can also be found in automotive exhaust, cigarette smoke, solvents in paints and coatings. Oil spills, discharges from petroleum businesses, and engine combustion are frequently linked to extensive contamination of BTEX compounds in the environment (31,4).The BTEX compounds are all highly flammable (16). The BTEX group is well-recognized for its poisonous, mutagenic, and carcinogenic effects, particularly in an indoor work setting where these compounds disposed frequently (34). The US Environmental Protection Agency (U.S. EPA) has classed BTEX, as a major pollutant. BTEX mixture exposure has the potential to cause intensive hazard to public health in any region (37). BTEX (benzene, toluene, ethylbenzene, and xylene) and other aromatic hydrocarbons which are the

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

most common components of gasoline and diesel engine emissions (23), have also been found to be persistent in the air and easily transferred into natural water bodies during rainfalls (20,29). At hazardous waste sites, benzene, toluene, ethylbenzene, and xylenes are regularly mixed. Exposure to each of the constituent compounds has been studied, but there are no studies that directly address exposure to "total" BTEX mixture. The primary objective of this study is to evaluate data on the toxicity of the "whole" mixture in order to recommend approaches for assessing the potential environmental hazard of benzene, toluene, ethylbenzene, and xylenes (BTEX). Contamination of groundwater and subsurface soil with BTEX might result in these chemicals entering basements as soil gas. Benzene is a well-known industrial chemical and contaminant that enters the environment through the combustion of fossil fuels, motor vehicle exhaust, forest fires, and other combustion processes. Long-term benzene exposure causes a decrease in bone marrow cellularity, which looks to eventually lead to aplastic anemia and acute myelogenous leukemia development and cognitive damage. Toluene is a volatile organic compound (VOC) that is commonly employed in industrial settings and has been linked to a number of health problems, Acute exposure to toluene vapors and other VOCs has been demonstrated to be particularly harmful to the neurological and cardiovascular systems (10). Chronic ethylbenzene exposure has been linked to negative effects on the respiratory system and kidneys (7). Xylene, or dimethyl benzene C_6H_4 $(CH_3)_2$ including the three isomers of xylene (p-xylene, m-xylene, and o-xylene) is one of the BTEX compounds that is widely used for the manufacture of ethyl benzene, polyester, and plastics as well as can be used as a solvent in paints or varnishes. Toxicity studies showed that exposure to mixed xylene or their individual isomers for short period can cause irritation of the nose, eyes and throat and can lead to neurological, gastrointestinal and reproductive damage. Moreover, exposure to xylene for long period may cause harmful effects on respiratory system, central nervous system, cardio-vascular system, and renal system. The toxicity of each BTEX compound including the three isomers of xylene are well-known in animals and human (38). However, there were no studies that looked specifically at the effects of joint toxicity on the neurological system or discussed the non or carcinogenicity of entire BTEX mixture. Citicoline (cytidine diphosphocholine, CDP-choline) is neuroprotective medication that used to treat acute ischemic stroke, Alzheimer's and Parkinson's disease to promote learning and memory and improve cognitive impairment. Clinical studies suggest that citicoline is well absorbed and highly bioavailable via oral treatment. Further, citicoline is an exogenous source for acetylcholine, a neurotransmitter and a member of the group of molecules that involve in cellular metabolism known as nucleotide (25). In several animal models, citicoline has been shown to minimize the toxic effects of volatile organic compounds (VOCs) such as toluene (44). The purpose of this paper is to study the histopathological alterations following chronic oral administration of BETX mixture (benzene, toluene, ethylbenzene, and xylene) on male rat tissues (brain, liver and kidney) and to investigate the protective effects of citicoline against the mixture toxicity.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

MATERIALS & METHODS

Preparation of BTEX and citicoline dosages:

Benzene, toluene, ethylbenzene and Xylene (m-xylene, o-xylene, p-xylene) (BTEX) chemicals with high purity [≥99.5%] were purchased from Thomas Baker (Chemicals) Pvt. Ltd/India. BTEX were prepared individually, then concentrations of each BTEX compound mixed with the solvent dimethyl sulfoxide (obtained from Chem-Lab NV/Belgium) to prepare the final mixture. The dosing solution of BTEX compound was prepared freshly at the time of the experiment and stored in clean amber glass. The BETX dose was calculated depending on the no-observed-adverse-effect level (NOAEL) that have been reported in the litterateur previously as shown in (Table 1). The final concentration of BTEX solution was 600 mg/kg/B.W. Cytidine diphosphocholine (citicoline, somazina, 1000 mg/ml oral solution; Ferrer international SA, Barcelona, Spain) was used in this study. The Citicoline dose was calculated as 500 mg/kg/BW, administrated for animals once a day for 12 weeks.

Table (1): The no-observed-adverse-effect level (NOAEL) in rats for BTEX mixture

Material	week	NOAEL mg/kg	Reference
Benzene	17	100	(39)
Toluene	13	225	(40)
Ethylbenzene	13	75	(35)
Xylene	12	200	(47)
Total		**600 mg/kg BW**	

Design of the experiment

Total of (64) male rats were divided randomly into four equal groups, (16) rats in each group (all treatments were done orally via stomach gavage once in a day). Animals in control group (G1) were dosed with distal water. The second group (G2) rats dosed with BTEX mixture (600 mg/kg/BW), while the animals in the third group (G3) were dosed with BTEX mixture and citicoline (500 mg/kg/BW). Animals in the fourth group G4 were dosed with citicoline (500 mg/kg/BW) only. All treatments were done 7 days/per week for 90-day oral exposure. The study Approved for Care and Use of Laboratory animals from the Ethics Committee in the Pathology Department/ Veterinary Medicine/ Baghdad University.

Histological assessment

For histopathological examination, brain, liver and kidney samples of all treated animals were dissected immediately after scarified. The tissues then fixed in 10% neutral-buffered formalin saline. All the organs after washing with tap water were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Cut sections were stained with hematoxylin and eosin (H&E) for histopathological study (33).

Immunohistochemistry for caspase-3 activity

Immunohistochemistry study was performed with a mouse monoclonal caspase-3 antibody for

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

detection of the caspase-3 activity in the brain tissues. The paraffin sections of the selected specimen were heated in a microwave oven (25 min at 720W) to retrieval the antigen then incubated with anti-caspase (1: 50 dilution) overnight at 4°C, then washed with PBS, and incubated with biotinylated goat-anti-rabbit-immunoglobulin G secondary antibodies (1: 200 dilution) and streptavidin/alkaline phosphatase complex
(1: 200 dilution) for 30 min at room temperature. DAB was used to visualize the antibody binding locations. The samples were dehydrated by moving them in ascending grades of ethanol solutions (30, 50, 70, 80, 95, and 100 percent ethanol) after being washed with PBS and counterstained with H&E for 2–3 minutes. After dehydration, the slices were soaked twice in xylene at room temperature for 5 minutes. The slices assessed evaluated by using a high-powered light microscope.

THE RESULTS

To investigate histopathological effects of BTEX mixture on rat's and the prevention effect of citicoline against the mixture toxicity, tissues sections from brain, liver and kidney were examined microscopically to determine any changes following BTEX toxicity. The histopathological sections of the brain showed that treated rats with distilled water (G1) served as a negative control have no abnormal structures or noticeable lesions (Figure 1- A). However, animals in G2 that administrated BTEX mixture 600 mg/kg/B.W. showed remarkable lesions in comparison with the control group G1. Congestion of the meninges in rats treated with BTEX mixture (12 weeks by oral gavage) (Figure 1-B) with mild focal inflammation within the meninges. All animals that treated with BTEX mixtures 600 mg/kg/B.W. for 12 weeks showed remarkable microgliosis (increase number of the cells) in response to the BTEX toxicity (Figure 2-A) next to perineural edema (Figure 2-B). Figure 2-A showed perivascular spaces (Virchow–Robin spaces (VRS) and perineural edema in all brain sections from animals dosed with BTEX mixture. Moreover, (Figure 3-A) indicated neuron necrosis, neuronal edema and vacuolated neuropil (Figure 3-B). Figure 4 illustrated neuron swelling (neuronal body balloons), the degenerating neurons shows swollen cytoplasm and peripheral chromotalysis (Figure 4-A, B). Perineural satellitosis also seen represented by cluster of glial cells surrounding the cell body of neurons near degenerating neuron cell body (Figure 5-A, B). On the other hand, rats treated with citicoline following BTEX mixture (G3) showed lesions which can be described as less severity comparing the lesions that seen in BTEX group G2. The proliferation of the glial cells and chromatolysis of neuron cells noticeably decreased in comparison with G2 (Figure 6-A). Animals in G4 that treated with citicoline 500 mg/kg/B.W. showed no clear lesion comparing with G1, G2 and G3 (Figure 6-B).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

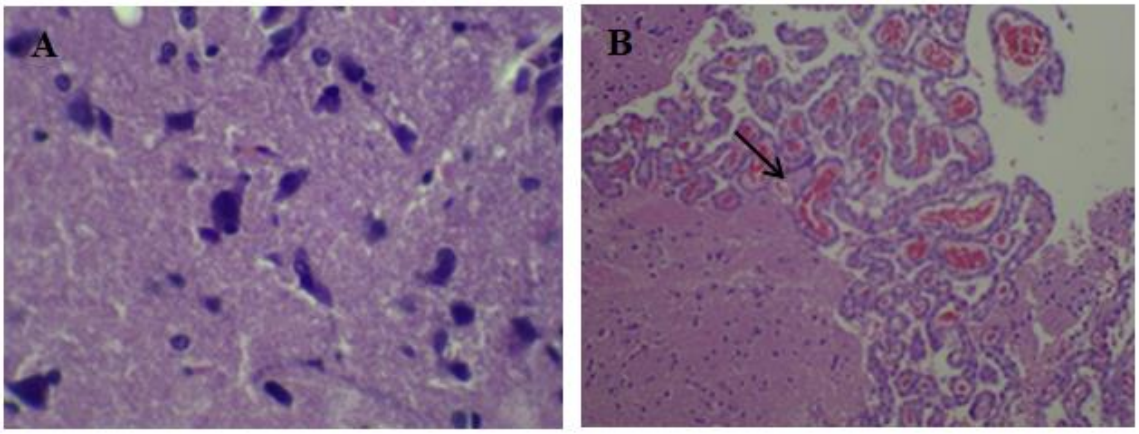

Figure (1): Histopathological sections of brain tissues in male rats from control group G1 (A) and treated group with BTEX mixtures 600 mg/kg/B.W. for 12 weeks G2 (B). (A): brain tissue shows normal structure of neuron cells. (B): congestion of the meninges with mild focal inflammation. H & E (A x40, B x40).

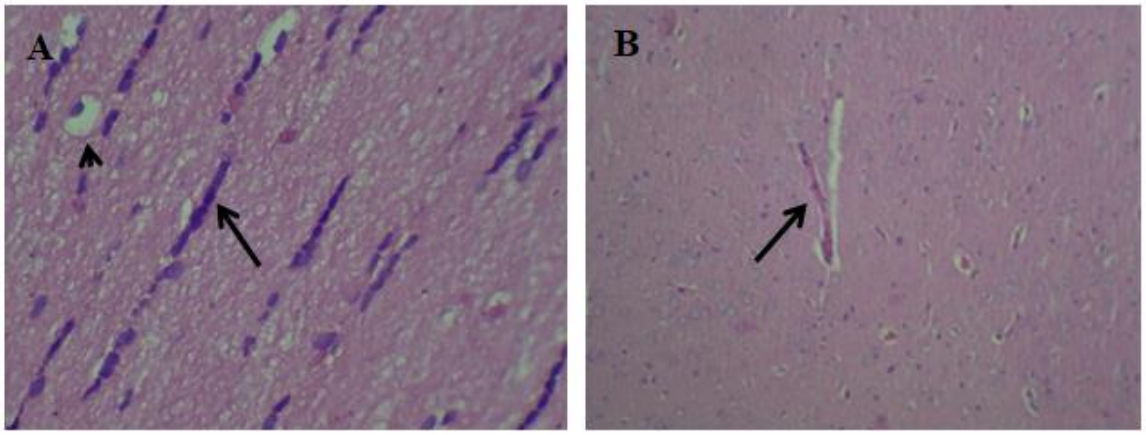

Figure (2) : Histopathological sections of brain tissues in male rats treated with BTEX mixtures 600 mg/kg/B.W. for 12 weeks G2 (A and B) (A): microgliosis (arrow) next to perineural edema (arrow head). Note the increase the number of elongated, irregular nuclei of typical microglial cells. (B): perivascular spaces (Virchow–Robin spaces (VRS) (arrow). H & E (A x40, B x10).

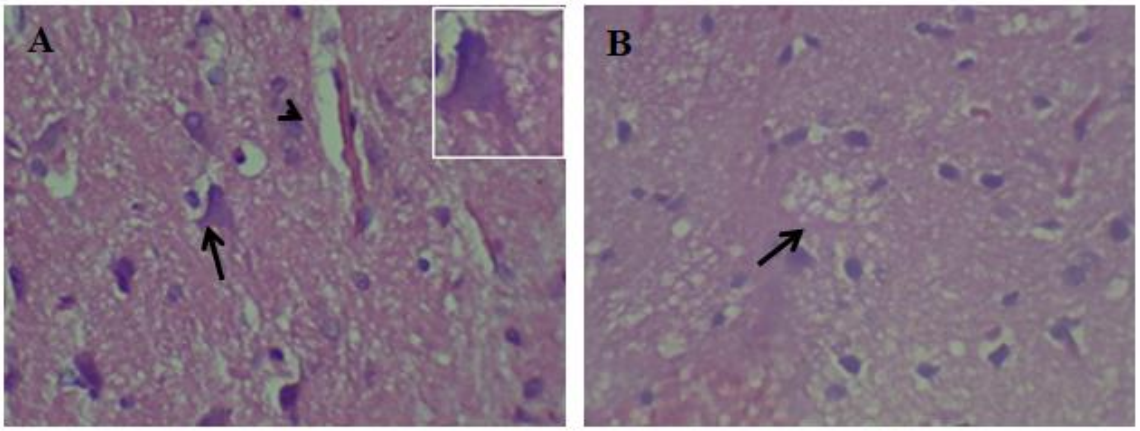

Figure (3) : Histopathological sections of brain tissues in male rats treated with BTEX mixtures 600 mg/kg/B.W. for 12 weeks G2 (A and B) (A): neuron necrosis (arrow), and perivascular space (VRS) (arrow head). (B): : vacuolated neuropil (arrow) H & E (A x40, B x40).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

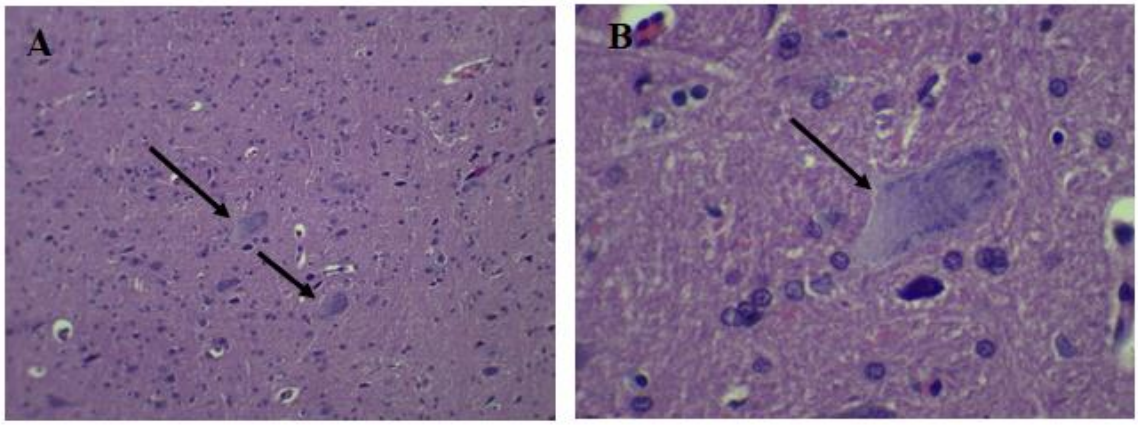

Figure (4): Histopathological sections of brain tissues treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (A and B): shows neuron swelling (neuronal body balloons), the degenerating neurons shows swollen cytoplasm and peripheral chromotalysis (arrow) H & E, (A x10, B x40).

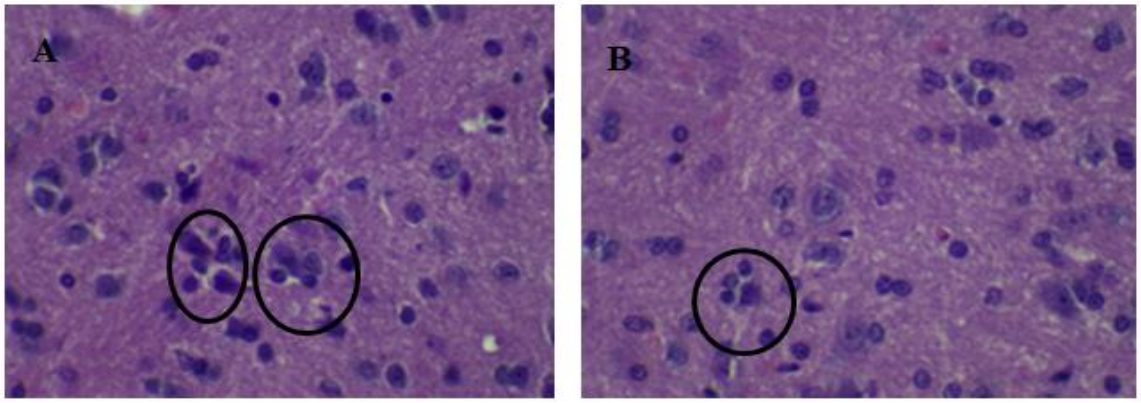

Figure (5): Histopathological sections of brain tissues treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (A and B): shows perineural satellitosis represented by cluster of glial cells surrounding the cell body of neurons near degenerating neuron cell body (circles) H & E, (A x40, B x40).

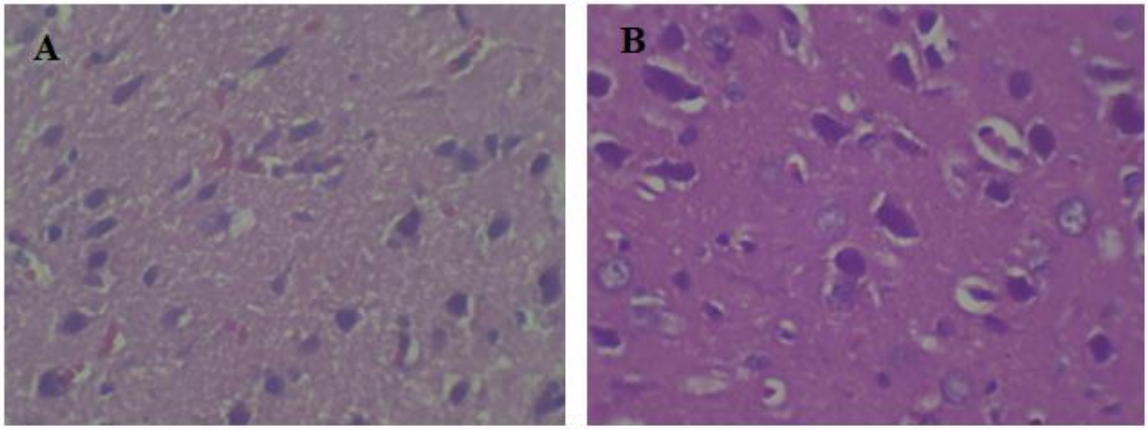

Figure (6): Histopathological sections of brain tissues treated with (A) BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 and (B) from rat treated with citicoline 500 mg/kg/B.W. G4 for 12 weeks (A and B): (A) shows remarkable decrease in the proliferation of the glial cells (B) normal neuron cells H & E, (A x40& B x40).

The histopathological sections of the liver showed that treated rats with distilled water (G1) served as a negative control have no abnormal structures or noticeable lesions (Figure 3-A). The liver loss its normal architecture following BTEX mixture 600 mg/kg/B.W.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

administration orally after 12 weeks leading to significant lesions in comparison with the control group G1. (Figure 7-B) showed focal inflammation with marked vacuolation of the hepatocytes (Figure 8- A) (circle). Furthermore, severe congestion of portal vein (arrow) and degeneration of hepatocytes were seen (Figure 8-B). Significant disruption of the liver architecture hepatocyte with sinosidal diltation and congestion in rat treated with BTEX mixture G2. On the other hands, histopathological sections of liver tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 showed remarkable improvement in liver histological structure (Figure 9- A). Normal hepatocytes were seen without any degenerative changes. Central vein and sinusoids appears normal (Figure 9-A). Animals treated with citicoline 500 mg/kg/B.W. G4 alone revealed normal histological structure of the central vein and surrounding hepatocytes (Figure 9-B).

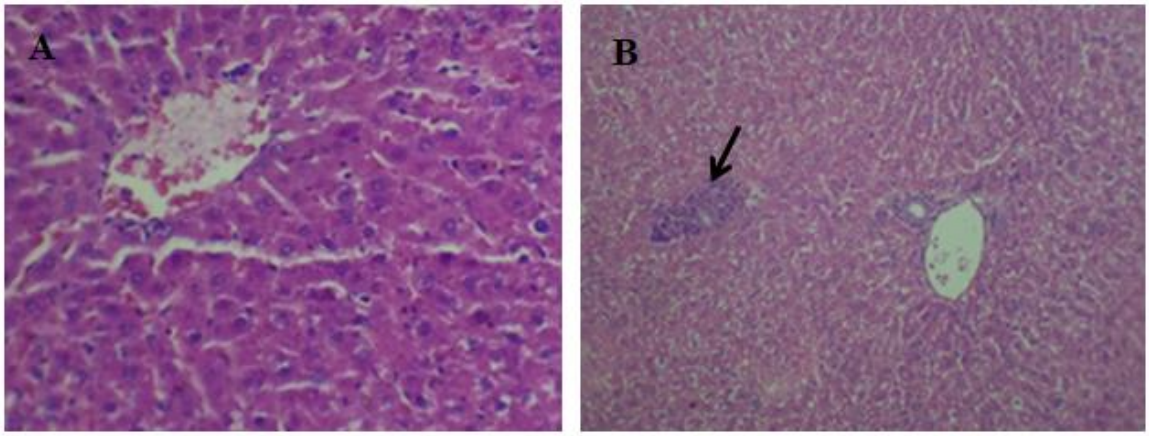

Figure (7): Histopathological sections of liver tissue in male rats in control group G1 (A), histopathological sections of liver tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (B). A: shows normal histological structure of the central vein and surrounding hepatocytes, B: focal area of inflammatory cells infiltration (arrow) H & E (A x20, B x10).

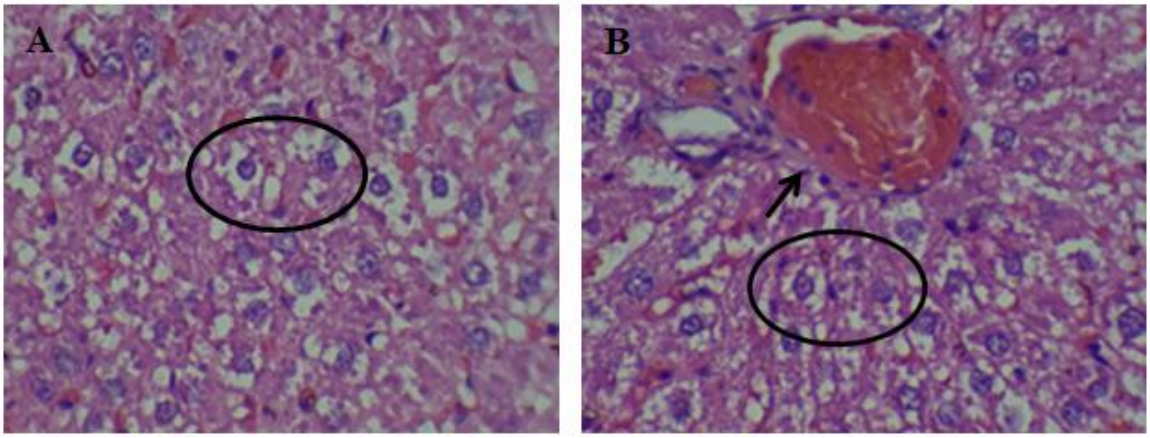

Figure (8): Histopathological sections of liver tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (A and B), (A): showed multipal areas shows cytoplasmic vaculation of hepatocytes represent sever degenerative changes (circle) and necrosis. (B): showed Blood vessel congestion (arrow head) with marked hepatocytic degeneratration (circle) H & E (A x40, B x40).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

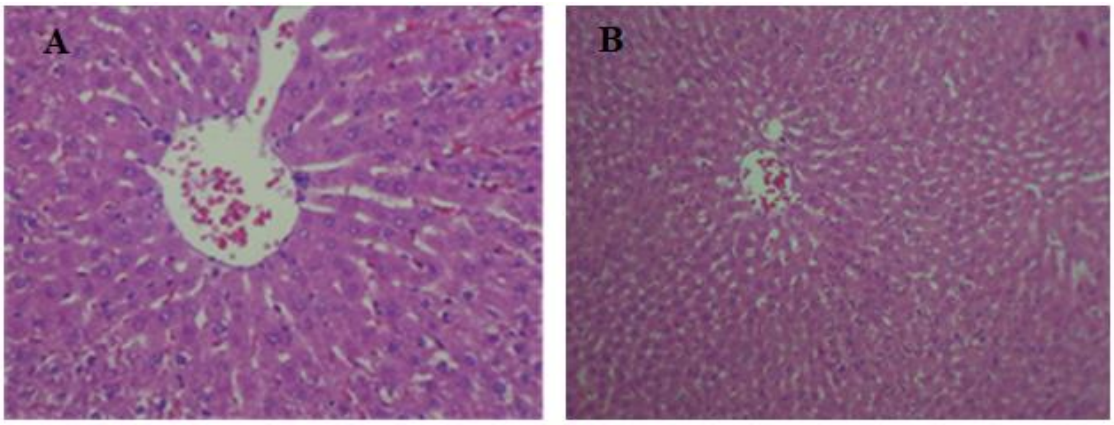

Figure (9): Histopathological sections of liver tissue in male rats BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 (A and B) in male rats treated with citicoline 500 mg/kg/B.W. G4 alone. (A): shows improvement in liver architecture, the hepatocyte appears normal, (B): shows normal histological structure of the central vein and surrounding hepatocytes H & E (A x20 and B x10).

The histopathological sections of the kidney showed that treated rats with distilled water (G1) served as a negative control have no abnormal structures or noticeable lesions (Figure 10-A). Histopathological sections of kidney tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks indicated significant lesions. Interstitial hemorrhage, congested blood vessels, renal tubules cloudy swelling and segmented glomeruli were detected mostly in all experimental rats with BTEX dosed. Detached epithelium lining, increase Bowman space and retraction of capillary tufts with segmented glomeruli were noted in designated animals in G2 (Figure 10-B). Moreover, hyaline cast formation in the renal tubules was observed (Figure 11-A). In addition, focal areas of mononuclear cells infiltrations with tubules desquamation of its epithelial cells were seen (Figure 11-B).

However, the histopathological sections of kidney tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 after 12 weeks revealed normal glomeruli and renal tubules structure (Figure 12-A). Furthermore, animals treated with 500 mg/kg/B.W. of citicoline G4 (Figure 12-B) showed no clear lesion with normal morphological structure of kidney tissue.

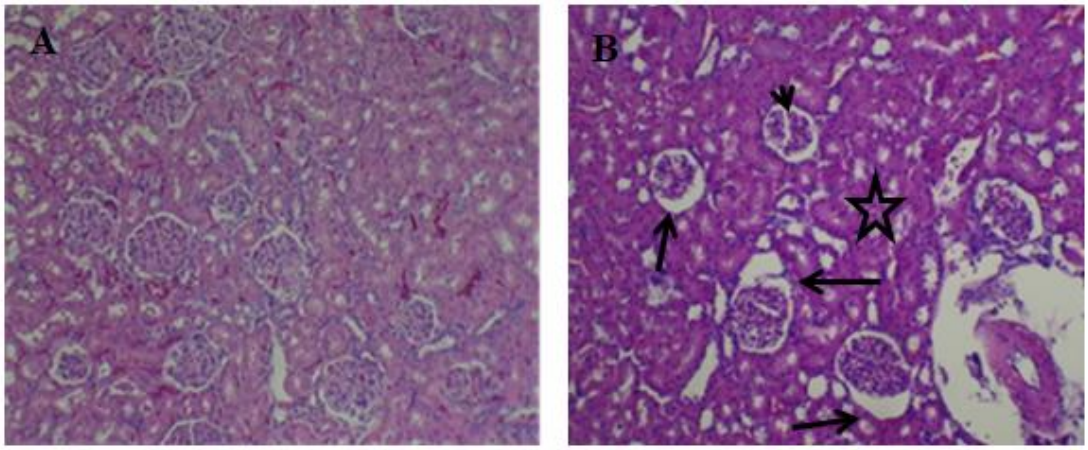

Figure (10): Histopathological sections of kidney tissue in male rats in control group G1 (A), histopathological sections of kidney tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (B). (A): histopathological sections of kideny tissue in male rats in control group G1 shows normal histological structure of the renal tubules and glomeruli. (B): increase Bowman space and retraction of capillary tufts (arrow), renal tubules shows cloudy swelling (star) and segmented glomeruli (arrow head) H & E,
(A x20, B x20).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

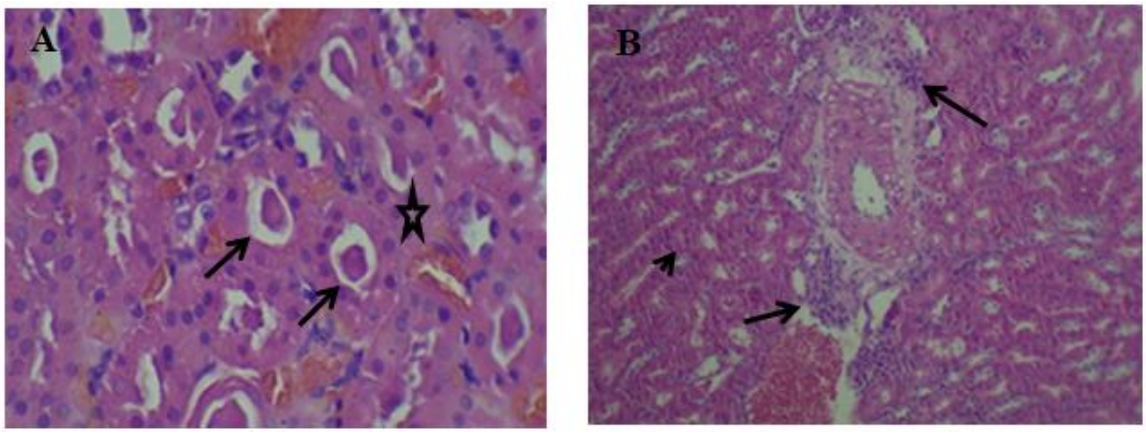

Figure (11): Histopathological sections of kidney tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. G2 for 12 weeks (A and B). (A): hyaline cast formation in the renal tubules (arrow), (B): focal areas of mononuclear cells infiltrations (arrow), some tubules shows desquamation of its epithelial cells (arrow head) H & E, (A x40, B x40).

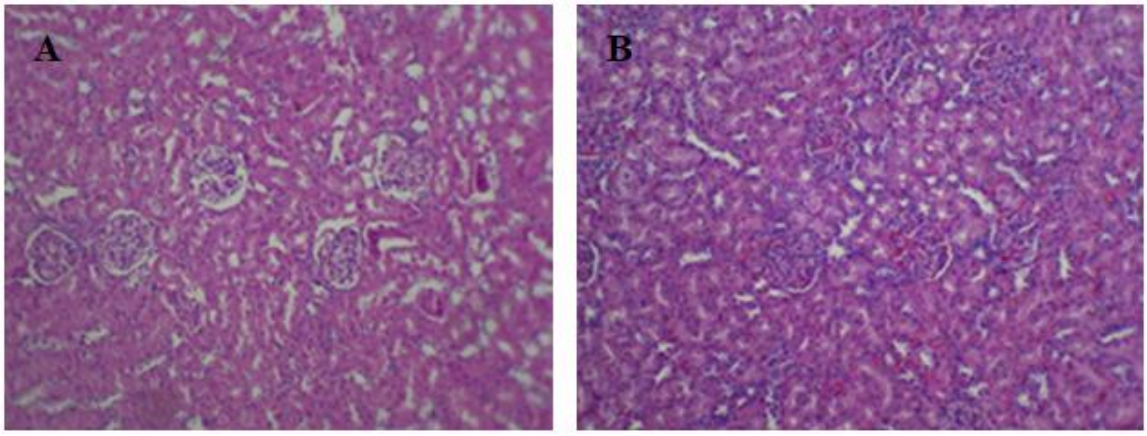

Figure (12): Histopathological sections of kidney tissue in male rats treated with BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 (A) and histopathological sections of kidney tissue in male rat treated with citicoline 500 mg/kg/B.W. only G4 (B), (A): shows no clear lesion with normal morphological structure of kidney tissue (B): shows normal glomeruli and renal tubules structure. H & E, (A x20, B x20).

Caspase-3 area activity in studied groups

A photomicrograph of brain tissue sections stained immunohistochemically with caspase-3 antibody revealed normal caspase-3 activity in control group G1(Figure 13-A-B). Rats treated with BTEX compounds 600 mg/kg B.W. G2 showed marked increase of caspase-3 activity when compared with normal reaction in control group G1(Figure 14 A-B). However, rats treated with BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 showed that positive caspase-3 activity is markedly decreased when compared with G2 (Figure 15-A). Rats treated with citicoline 500 mg/kg/B.W. G4 showed normal caspase-3 activity when compared with normal reaction in control group G1(Figure 15-B).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

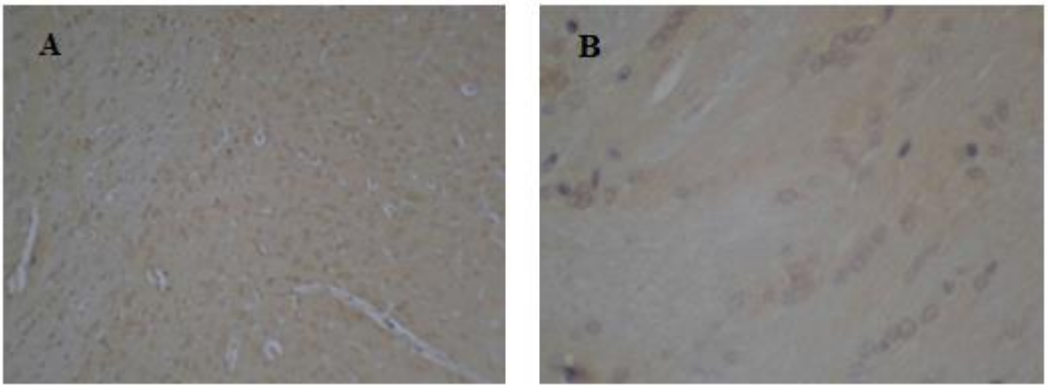

Figure (13): A photomicrograph of brain tissue sections stained immunohistochemically with caspase-3 antibody (A-B control group), (A-B): normal caspase-3 activity in control group

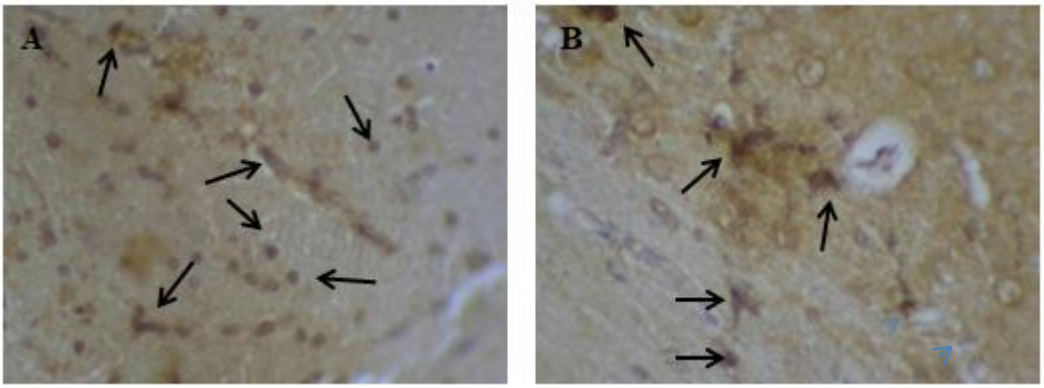

Figure (14): A photomicrograph of brain tissue sections stained immunohistochemically with caspase-3 antibody in rat treated with BTEX compounds 600 mg/kg B.W. G2. (A-B): shows marked increase of caspase-3 activity (arrow) (A x40, B x40).

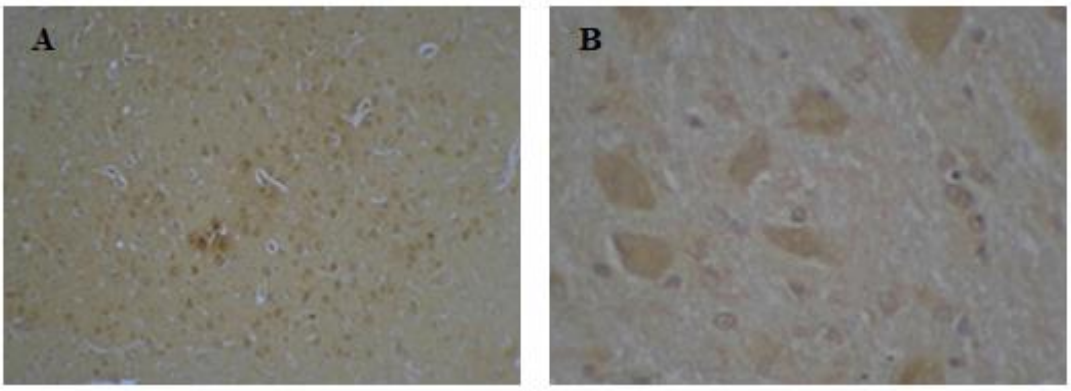

Figure (15): A photomicrograph of brain tissue sections stained immunohistochemically with caspase-3 antibody (A) in rats treated with BTEX mixture 600 mg/kg/B.W. and citicoline 500 mg/kg/B.W. G3 (B) in rat treated with citicoline 500 mg/kg/B.W. (A-B): (A): shows that positive caspase-3 activity is markedly decreased, (B): shows normal caspase-3 activity (A x40, B x40).

DISCUSSION

In this study, we investigated the toxic effect of benzene, toluene, ethylbenzene, and xylene (BTEX), on brain, liver, and renal tissues. In addition, we investigated the protective effect of

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

citicoline against BTEX toxicity. The BTEX compounds can accumulate in the body through direct intake of contaminated crops, inhalation of vapour from the soil, intake of contaminated drinking water and skin exposure (12). All of the BTEX compounds particularly after chronic exposure has been associated with damage to the central nervous system, liver and kidney damage while exposure to benzene can additionally cause haematological effects including aplastic anaemia and acute myelogenous leukaemia, impairment in foetal development, male reproductive function, respiratory system, metabolic function and immune system (11,48). As a common volatile chemical, benzene is easily absorbed and metabolized. Although benzene is not commonly thought of as an acute toxin, it has been linked to neurological impairment, hematotoxicity, and the development of aplastic anemia and leukemia (15). Benzene is transformed to a range of hydroxylated and ring-opened compounds in the liver, which are then transferred to the peripheral tissues for secondary metabolism. The covalent binding of reactive benzene metabolites to cellular macromolecules may cause toxicity from benzene and its metabolites (45).

Two enzymes, cytochrome P450 2E1 (CYP2E1) and quinone oxidoreductase, have been implicated in the mechanism of benzene toxicity. Toluene is a cyclic benzene ring with a methyl group attached. It is produced from mono-substituted benzene. Furthermore, toluene is a lipophilic and non-polar molecule, allowing it to rapidly distribute to organs with high lipid content (13).

Toluene inhalation causes neuronal apoptosis in the central nervous system in rats (32). Most inhalation toluene is converted to benzoic acid in the liver by two enzymes: alcohol dehydrogenase and aldehyde dehydrogenase (22).

Inhalation of ethylbenzene at high concentration may cause dizziness and throat and eye irritation in human. Furthermore, exposure to low concentration of ethylbenzene via inhalation, has caused hearing problems and renal damage in animals (8).

Exposure to xylene has a harmful effect on the neurological system, respiratory tract, liver, kidneys and reproductive system (30,38).

Oral exposure of BTEX in rats showed significant damage to the brain, liver and kidney tissues.

These changes include gliosis, neurons shrank and darkened (karyorrhectic) in the cerebral cortex in brain tissue (44). Kanter (27) suggested that chronic exposure of toluene in rats caused significant degenerative alterations, reduced cytoplasm, and dark picnotic nuclei in neurons of the frontal cortex. Furthermore, neuronal apoptosis in the central nervous system was seen in rats following toluene inhalation (32). Moreover, vaculation and gliosis of myelined nerve fibers, as well as vaculation perkanji nerve cells in the cerebrilum were seen in mice exposed to benzene via inhalation (41). In another study, vacuolar degeneration, gliosis, perivascular demyelination, and numerous pyknotic cells and necrosis were seen in rabbits administrated toluene (19).

These findings are in agreement with the result of our study, which demonstrated that BTEX mixture can cause causes brain toxicity. All animals that treated with BTEX mixtures 600 mg/kg/B.W. for 12 weeks showed remarkable microgliosis (increase number of the cells), perineural edema, perivascular spaces (Virchow–Robin spaces (VRS), neuron necrosis,

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

vacuolated neuropil and perineural satellitosis.

Rats and mice exposed to benzene at different concentrations showed pathological lesions in the liver tissues such as blood vessels congestion, hemorrhage, vacuolar degeneration, necrosis, with infiltration of inflammatory cells and dilatation of the central vein (24,28).

These results are in accordance with our findings in the liver of rats that exposed to 600 mg/kg/B.W. of BTEX for 12 weeks. Histopathological alterations showed dilatation and congestion of blood sinusoids with vacuolar degeneration of the hepatocytes.

In another study, the pathological effect of benzene on mice renal tissue showed increase interstitial tissue and cellular hyperplasia suggested as a preneoplastic response to benzene toxicity beside fibrosis, glomerular enlargement, and necrosis of the renal tubules (5). Meydan, et al.(36) indicated that toluene exposure in rats causes glomerular tuft reduction and increase in the connective tissue at the interstitial area. Moreover, El-Sheikh, et al. (21) found that toluene can induce nephrotoxicity in rats. Furthermore, rat exposed to 750 ppm ethylbenzene via inhalation showed an increase in the number and size of hyaline droplets in cells of the proximal convoluted tubules of the kidney (46). These results strongly supported our results that BTEX mixture is toxic to kidney. All rats that treated with 600 mg/kg/B.W. of BTEX mixture showed blood vessels congestion, degeneration renal tubules, segmented glomeruli, increase Bowman space and formation of hyaline cast in the renal tubules. In addition, mononuclear cells infiltrations and desquamation of epithelial lining cells.

Citicoline is the generic name for cytidine-5'-diphosphocholine (CDP-choline), an endogenous molecule that interacts with the formation of cellular membrane phospholipids, particularly phosphatidylcholine, to enhance neurotransmitter levels in the central nervous system (14). Citicoline from exogenous sources is hydrolyzed and absorbed as cytidine and choline (43). Citicoline, as an exogenous choline source for acetylcholine synthesis, re-synthesis membrane phospholipids (particularly phosphatidylcholine) that leads to inhibit neuronal cell death (2). Citicoline has the ability to ameliorate the disruption of glucose metabolism, increased brain choline levels and stimulated acetylcholine synthesis (26). In animal models of cerebral ischemia, citicoline was observed to minimize brain edema and the damage of the blood-brain barrier in rats (9). Citicoline appears to enhance dopamine release in the brain in rats, by enhance acetylcholine release (3). Many studies have shown that citicoline improves impairments caused by a variety of diseases in humans and in induced animals (1). Our results showed that treatment with 500 mg/kg B.W. citicoline can protect against BTEX toxicity through reducing the pathological changes in the brain, liver and kidney. Also, examination of caspase-3 activity proved the ability of citicoline to ameliorate BTEX toxicity by deceased apoptosis via inhibiting caspase 3 activity. Inappropriate apoptosis has been linked with neurodegenerative disease, ischemic damage, development of cancer and autoimmune diseases (42), as caspase-3 is responsible for chromatin condensation, DNA disintegration and laddering, and membrane protein degradation (17). In this study, administration of BTEX was found to induce strong caspase-3 immunostaining, suggesting the involvement of caspase-3 activation. Our findings also indicated the ability of citicoline to inhibit the activation of caspase-3 in the brain of rats treated with BTEX.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Conclusion:

In conclusion, the results showed that the safe level (NOAEL) of BTEX compound individually, can produce a significant health adverse effect when administrated as a mixture. It's important to say that at hazardous waste sites, benzene, toluene, ethylbenzene, and xylenes are regularly mixed. Also our findings shown a protective action of citicoline against BTEX toxicity. In this study, administration of BTEX was found to induce histopathological alteration in the brain, liver and kidney in male rats following 90 days of administration and strong caspase-3 immunostaining in the brain sections. Obviously, treatment rats with 500 mg/kg BW. of citicoline showed significant improvement in the brain, liver and kidney tissues and marked decrease in the activation of caspase-3 in the brain-treated rats. In this context, citicoline has the ability to reduce the harm effects of BTEX chemicals and inhibit caspase-3 activity in the brain tissues.

REFERENCES:

[1]. Abdel-Zaher, A.O. Protective effect of citicoline against aluminum-induced cognitive impairments in rats. Toxicology and Industrial Health. 2017;**33**(4): 308-317.

[2]. Adibhatla, R. M., Hatcher, J., and Dempsey, R. Citicoline: neuroprotective mechanisms in cerebral ischemia. J. Neurochemistry. 2002;80(1), 12-23.

[3]. Agut, J., and Wurtman, R. Cytidine (5') diphosphocholine enhances the ability of haloperidol to increase dopamine metabolites in the striatum of the rat and to diminish stereotyped behavior induced by apomorphine. Neuropharmacology. 1984;23(12), 1403-1406.

[4]. Ali, M. F., M-Ridha, M. J., & Taly, A. H. Phytotoxicity Test Of Kerosene-Contaminated Soil Using Barley. The Iraqi Journal of Agricultural Science.2020; 51(1), 376-391.

[5]. Al-Shammari, A.M. 2017. Benzene histotoxic and teratogenic Effects in exposed Mice. Kufa J. For Vete. Med. Sci.2020;8(2), 143-151.

[6]. Alwan, S.W. Efficiency of the phragmites australis and typha domingensis roots in remediation of polycyclic aromatic hydrocarbons (PAHS) from freshwater sediments. Iraqi J. of Agric. Sci.2016; 47(2).

[7]. Agency for Toxic Substances and Disease Registry (ATSDR). Case Studies in environmental medicine. http://www. atsdr. cdc. gov/HEC/CSEM/csem. html. 2004.

[8]. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Ethylbenzene. Atlanta, GA:U.S. Department of Health and Human Services,Public Health Service. Agency for Toxic Substances and Disease Registry. 2010.

[9]. Başkaya, M. K., Doğan, A., Rao, A. M., and Dempsey, R. J. Neuroprotective effects of citicoline on brain edema and blood—brain barrier breakdown after traumatic brain injury. J. of neurosurgery.2000; 92(3), 448-452.

[10]. Benignus, V. Health effects of toluene: a review. Neurotoxicology.1981; 2(3): 567-588.

[11]. Bolden, A.L., C.F. Kwiatkowski, and T. Colborn. New look at BTEX: are ambient levels a problem? Environmental sci. and techn.2015; 49(9):5261-5276.

Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

[12]. Carlson, T.N., W.J. Capehart, and R.R. Gillies. A new look at the simplified method for remote sensing of daily evapotranspiration. Remote sensing of Environment.1995; 54(2): 161-167.

[13]. Chambers, D.M. An improved approach for accurate quantitation of benzene, toluene, ethylbenzene, xylene, and styrene in blood. Analytical chemistry.2006;78(15): 5375-5383.

[14]. Chitu, I., Tudosescu, R., Leasu-Branet, C., & Voinea, L. Citicoline – a neuroprotector with proven effects on glaucomatous disease. Romanian J. of Ophthalm.2017; 61, 152-158. https://doi.org/10.22336/rjo.2017.29

[15]. Costantini, A.S. Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian multicenter case–control study. American J. of industrial med.2008; 51(11): 803-811.

[16]. Cozzarelli, I.M. and A. Baehr. Volatile fuel hydrocarbons and MTBE in the environment. Treatise on geochemistry.2003; 9: 612.

[17]. D'amelio, M., V. Cavallucci, and F. Cecconi. Neuronal caspase-3 signaling: not only cell death. Cell Death & Differentiation.2010; 17(7): 1104-1114.

[18]. Davidson, C., J. Hannigan, and S. Bowen. Effects of inhaled combined Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX): Toward an environmental exposure model. Environ. Toxicology and Pharmacology.2021; 81: 103518.

[19]. Demır, M. 2017. Effects of acute toluene toxicity on different regions of rabbit brain. Analytical Cellular Pathology.

[20]. Dutta, C. Mixing ratios of carbonyls and BTEX in ambient air of Kolkata, India and their associated health risk. Enviro. monitoring and assessment.2009; 148(1): 97-107.

[21]. El-Sheikh, A.A. and A.M. Abdelbary. Evaluation of The Role of Buffalo Milk on Acute Toluene Induced Hepatotoxicity and Nephrotoxicity in Adult Male Albino Rats. Zagazig J. of Forensic Med.2021; 19(2): 31-48.

[22]. Filley, C.M., W. Halliday, and B. Kleinschmidt-DeMasters. The effects of toluene on the central nervous system. J. of Neuropathology & Exp. Neurology. 2004;63(1): 1-12.

[23]. Friedrich, R. and A. Obermeier. Anthropogenic emissions of volatile organic compounds, in Reactive hydrocarbons in the atmosphere. Elsevier. 1999; 1-39.

[24]. Golabi-Habashi, N., A. Salimi, and H. Malekinejad. Quercetin attenuated the Benzene-induced hemato-and hepatotoxicity in mice. Toxicology reports. 2021;8: 1569-1575.

[25]. Nouby M. Ghazaly, A. H. H. . (2022). A Review of Using Natural Gas in Internal Combustion Engines. International Journal on Recent Technologies in Mechanical and Electrical Engineering, 9(2), 07–12. https://doi.org/10.17762/ijrmee.v9i2.365

[26]. Iulia, C. Citicoline–a neuroprotector with proven effects on glaucomatous disease. Romanian j. of ophthalmology. 2017; 61(3): 152.

[27]. Kakihana M., Fukuda N., Suno M. and Nagaoka A. Effects of CDP-choline on neurologic de®cits and cerebral glucose metabolism in a rat model of cerebral ischemia. Stroke. 1988; 19, 217±222

[28]. Kanter, M. Protective effects of quercetine on the neuronal injury in frontal cortex after chronic toluene exposure. Toxicology and industrial health. 2013; 29(7): 643-651.

[29]. Karim, H.T. and D.Q.S. Al-Derawi. Histopathological study of the effect of super benzene inhalation on Laboratory rats Rattus norvigicus. Basrah J. of sci. 2020; 28(1B arabic).

[30]. Chaudhary, D. S. . (2022). Analysis of Concept of Big Data Process, Strategies, Adoption and Implementation. International Journal on Future Revolution in Computer Science &Amp; Communication Engineering, 8(1), 05–08. https://doi.org/10.17762/ijfrcsce.v8i1.2065

[31]. Khalaf S. M.; Hassan F. M.and Al-Obaidy, A.H.J. Detection of polycyclic aromatic hydrocarbons compounds concentrations and their fate in Tigris river within Baghdad city -Iraq. Iraqi J. of Agric. Sci. 2019; 50(Special).

[32]. Korsak, Z. Toxic effects of acute exposure to particular xylene isomers in animals. Pol. J. Occup. MED. 1990; 3(2): 221-226.

[33]. Li, L.Pollution characteristics and health risk assessment of benzene homologues in ambient air in the northeastern urban area of Beijing, China. J. of Enviro. Sci. 2014;26(1): 214-223.

[34]. Lin, H. Acute exposure of toluene transiently potentiates p42/44 mitogen-activated protein kinase (MAPK) activity in cultured rat cortical astrocytes. Neuroscience letters. 2002;332(2): 103-106.

[35]. Luna, L. G. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 1968; 3rd Edition, McGraw- Hill, New York.

[36]. Martins, E.M. BTEX in an occupational environment. International J. of Environmental Impacts. 2019;2(2): 174-191.

[37]. Mellert, W. Ethylbenzene: 4-and 13-week rat oral toxicity. Archives of toxicology. 2007; 81(5): 361-370.

[38]. Meydan, S. The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats. Toxicology and Industrial Health. 2016;32(1): 15-21.

[39]. Montero-Montoya, R., R. López-Vargas, and O. Arellano-Aguilar. Volatile organic compounds in air: sources, distribution, exposure and associated illnesses in children. Annals of global health. 2018;84(2): p. 225.

[40]. Gill, R. . (2022). Hulls for a Partially Right-Prime, Orthogonal Random Variable. International Journal on Recent Trends in Life Science and Mathematics, 9(2), 47–57. https://doi.org/10.17762/ijlsm.v9i2.135

[41]. Niaz, K. A review of enviro. and occupational exposure to xylene and its health concerns. EXCLI J. 2015; 14: 1167.

[42]. Program, N.T. NTP toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2) in F344/N rats and B6C3F1 mice (Gavage Studies). National Toxicology Program technical report series. 1986; 289: 1-277.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

[43]. Program, N.T. NTP toxicology and carcinogenesis studies of toluene (CAS No. 108-88-3) in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program technical report series. 1990;371: 1-253.

[44]. Salman, H.A., M.K. Ebraheem, and S.K. Majeed. the effect of ordinary benzene in the brain of adults female of laboratory mice (mus muscululus l.) 2015; Volume 3.

[45]. Saraste, A. and K. Pulkki. Morphologic and biochemical hallmarks of apoptosis. Cardiovascular research. 2000;45(3): 528-537.

[46]. Secades, J. J., & Frontera, G. CDP-choline: pharmacological and clinical review. Methods and findings in experimental and clinical pharmacology, 1995;17, 1-54.

[47]. Shaffie, N. and M.E. Shabana.Role of citicoline as a protective agent on toluene-induced toxicity in rats. J. of the Arab Society for Med.Research.2019;14(1): 14.

[48]. Snyder, R. and C.C. Hedli. An overview of benzene metabolism. Enviro. health perspectives. 1996;104(suppl 6): 1165-1171.

[49]. Palagan Senopati Sewoyo, Anak Agung Ayu Mirah Adi, Ni Luh Eka Setiasih, I Gusti Ayu Mirah Afsari Dewi. (2022). Haematological Profile of Rat Fibrosarcoma Models After Virulent Newcastle Disease Virus Virotherapy: A Pilot Study. Revista Electronica De Veterinaria, 47 - 55. Retrieved from https://www.veterinaria.org/index.php/REDVET/article/view/138

[50]. Stott, W.T. Evaluation of potential modes of action of inhaled ethylbenzene in rats and mice. Toxicological Sciences. 2003;71(1): 53-66.

[51]. Wolfe, G.W. Subchronic toxicity study in rats with m-xylene. Report by Hazletion Laboratories America, Inc., Rockville MD. Sponsored by Dynamac Corporation.1988; Rockville, MD..

[52]. Zhang, J., M. Tang, and L. Viikari. Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases. Bioresource technology. 2012;121: 8-12.