Original Research Article

HEPATIC AND RENAL BIOCHEMICAL PROFILE OF WISTAR RATS EXPOSED TO TOLUENE

Abstract

Toluene is an organic solvent that is widely used in many industrial processes and commerce. It is one of the environmental pollutants frequently associated with occupational hazards. In recent times, young people are becoming more interested in toluene addiction as a result of intentional inhalation of toluene-containing compounds, which can result in high levels of toluene exposure that can be damaging to their health. This study was carried out to investigate the effect of subacute oral exposure of toluene on hepatic and renal biochemical profile (an aspect of health indices) of wistar rats. Fifteen wistar rats were randomly divided into 3 groups of 5 rats each. Group A (Control) received 0.5 ml of olive oil (vehicle) while groups B and C received 63.6 and 127.2 mg/kg respectively of toluene for 21 days by oral gavage. At the end of the treatments, blood samples were taken and the sera were used for analyses of serum biochemical parameters such as alanine aminotransferase - ALT, alkaline phosphatase – ALP, aspartate aminotransferase – AST, total protein, albumin, bilirubin, urea, creatinine and blood electrolytes (potassium, sodium, bicarbonate and chloride ions). Sections of liver and kidney samples were taken for histopathological examination. There was no significant ($p > 0.05$) change in the hepatic and renal biochemical parameters of toluene-treated rats relative to the control. The liver and kidney histomorphology of the exposed rats were not different from those of the control. Oral administration of toluene, at the doses and duration used in this study, did not show any toxicity in the liver and kidney of exposed animals.

Keywords: toluene, blood, liver, kidney, toxicity, serum

Introduction

Toluene, an organic solvent commonly known as methylbenzene or phenylmethane (Ware, 1988), is one of the most common dangerous causes of pollution. It is widely utilized as an industrial solvent in the production of vehicle fuels, chemical medications, and a variety of consumer and commercial products like ink, glue, paint, rubber, cements, and other adhesives (ASTDR, 2015). Toluene enters the body in three ways: through the skin, inhalation, and ingestion, with inhalation being the most common (Faust, 1994).
It is rapidly absorbed through the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin. Exposure frequently occurs through contaminated air, food and drinking water as well as various consumer products (ASTDR, 2015).

Toluene toxicity as a result of solvent addiction, occupational dangers, and pollution has been a source of worry in recent years. Young people are becoming more interested in toluene addiction by intentionally inhaling toluene-containing substances, which can result in high amounts of toluene exposure. This misuse could be harmful to their health.

The liver and kidneys are two of the body's major internal organs, performing a variety of functions such as metabolism and detoxification. The assessment of liver and kidney functions, which are components of

serum / plasma biochemical assays, is therefore critical in determining the toxicity of compounds that enter the body.

The aim of this research is to see if subacute toluene exposure has a deleterious effect on liver and kidney function using wistar rat as animal model. The study is particularly important since, as a result of an increase in both juvenile addiction and industrial use, individuals are becoming increasingly exposed to this organic solvent.

Materials and Methods

Chemicals and Reagents

Analytical grade of toluene which is a clear colourless liquid was purchased from Joechem Ventures Nigeria. The experimental doses were reconstituted in Goya® olive oil bought from the supermarket.

Animals and Treatment

Fifteen mature male wistar rats with an average weight of an average of 190g were purchased from the Animal House of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt. The rats were acclimatized for two weeks prior the study and were allowed free access to commercially sourced feed and clean water throughout the study. After acclimatization, the animals were randomly allotted to three groups – A, B and C. Group A served as the control and was given 0.5ml of olive oil (vehicle) while the treatment groups B and C were administered with toluene at the doses - 63.6mg/kg and 127.2 mg/kg respectively which corresponded to 1/10 and 1/5 of the LD_{50} which is 636mg/kg according to Doro-on (2014). The rats were treated by oral gavage daily for 21 consecutive days.

Ethics Approval

The rats for the study were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

Sample Collection and Analysis

On day 22, the animals were anaesthetized and blood samples were collected from the retro-orbital plexus into plain bottles. Liver and kidney samples were excised and fixed in 10% formalin and later processed for histopathological study according to the method of Lillie (1965) and stained with Haematoxylin and Eosin blue. The processed tissues were examined under a standard light microscope and the photomicrographs were captured using Olympus® CX31 digital camera.

The collected blood samples were allowed to stand for 30-45 mins in order to coagulate and then centrifuged for 15 mins at 3000 rev/min to obtain the sera which were used for the estimation of Hepatic biochemical parameters - alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein, albumin and bilirubin (total & conjugated), and Renal biochemical parameters - Urea, creatinine and blood electrolyte levels – sodium, bicarbonate, sodium and chloride ions). The hepatic and renal serum biochemistry determinations were done using commercial test kits. The activities of ALT and AST were measured according to Reitman and Frankel (1957) while ALP activity was determined by the thymolphthalein monophosphate method according to Roy (1970). Total bilirubin and conjugated bilirubin were determined by the Jendrassik-Grof method (Jendrassik and Grof, 1938) while the total protein was

assayed by the direct Biuret method (1995). The albumin, urea and creatinine were determined by the bromocresol green method (Doumas *et al*., 1971), the Urease-Berthelot method (Fawcett and Scott, 1960) and the modified Jaffe method (Blass *et al*., 1974) respectively. The serum potassium, sodium, bicarbonate and chloride ions were determined by colorimetric method (Henry *et. al*., 1974)

Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the Tukey post-test. The significance level was set at $p<0.05$.

Results

The effect of oral exposure of toluene on hepatic and renal biochemical profile of wistar rats are summarized in Tables 1 and 2. Ingestion of toluene by wistar rats at the doses of 63.6 and 127.2 mg/kg for 21days had no significant ($p > 0.05$) effect on both the hepatic and renal biochemical parameters in comparison with the control as shown in Tables 1 and 2 respectively.

There is no significant variation ($p > 0.05$) in the mean serum activities of the liver enzymes – ALT, ALP and ASP of toluene-treated rats relative to the control (table 1). The mean concentration of the total protein, albumin and bilirubin (total and conjugated) of exposed rats did not differ significantly ($p > 0.05$) from the control (table 1). There is no significant difference in the mean urea, creatinine and blood electrolytes (sodium, chloride, potassium and bicarbonate ions) of toluene-treated rats when compared with the control (table 2).

The photomicrographs of liver and kidney sections of toluene-treated rats did not show any abnormality in the historachitecture in comparison with the control (plates 1 and 2).

Table 1: Effect of Toluene on Hepatic Biochemical parameters of rats exposed for 21 days

Groups	AST (U/L)	ALP (U/L)	ALT (U/L)	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)
Group A (Control)	27.00±2.61	10.40±1.11	27.00±2.30	58.80±1.91	41.60±0.93	5.50±0.39	3.20±0.31
Group B (63.6mg/kg Toluene)	27.80±3.90	11.60±1.72	28.40±3.12	65.40±4.57	41.20±2.29	5.54±0.77	3.38±0.63
Group C (127.2mg/kg Toluene)	17.00±2.42	7.30±0.29	17.75±0.85	50.00±6.75	39.50±1.32	4.00±0.21	2.58±0.11

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p >0.05$). Groups A, B and C represent the control (given 0.5 ml olive oil), 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively

Table 2: Effect of Toluene on Renal Biochemical parameters of rats exposed for 21 days

Groups	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)	Sodium (mmol/L)
Group A (Control)	3.92±0.15	70.40±1.03	27.80±1.50	3.54±0.21	72.60±3.83	122.4±4.50
Group B (63.6mg/kg Toluene)	3.82±0.12	66.80±4.26	24.60±2.11	3.50±0.34	73.20±6.67	120.20±3.15
Group C (127.2mg/kg Toluene)	3.70±0.11	68.50±1.55	27.75±1.70	3.23±0.20	66.50±3.28	117.50±1.94

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B and C represent the control (given 0.5 ml olive oil), 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively

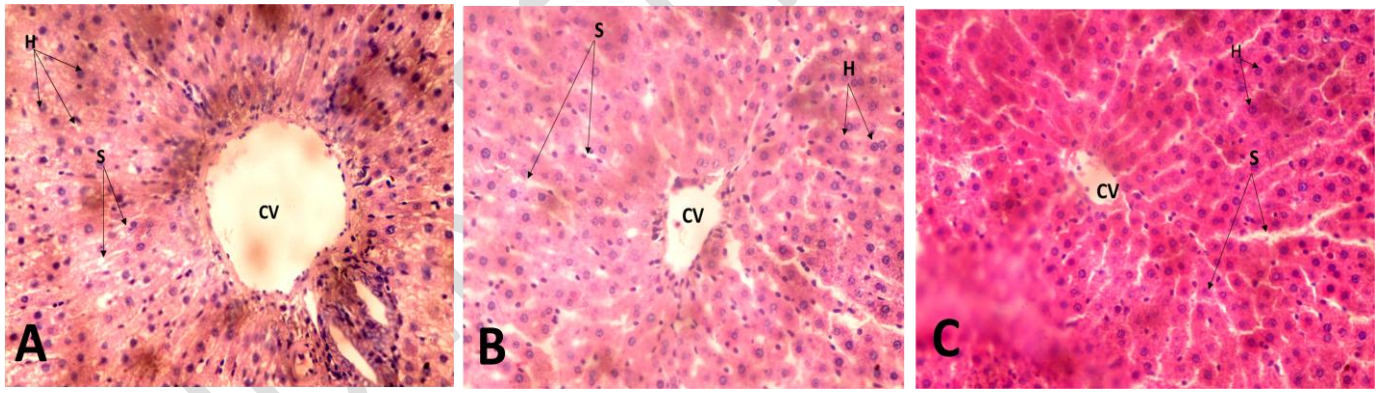

Plate 1: Photomicrographs of liver sections of rats from groups A (Control), B and C (toluene doses of 63.6 and 127.2 mg/kg respectively) treated for 21days; stained with H&E (×400). No obvious histological change in the liver of toluene-treated rats relative to the control. No obvious change in the histoarchitecture of the liver sections of toluene-treated rats when compared with the control. The sinusoids (S) are seen radiating away from the central vein (CV) with very prominent hepatocytes (H).

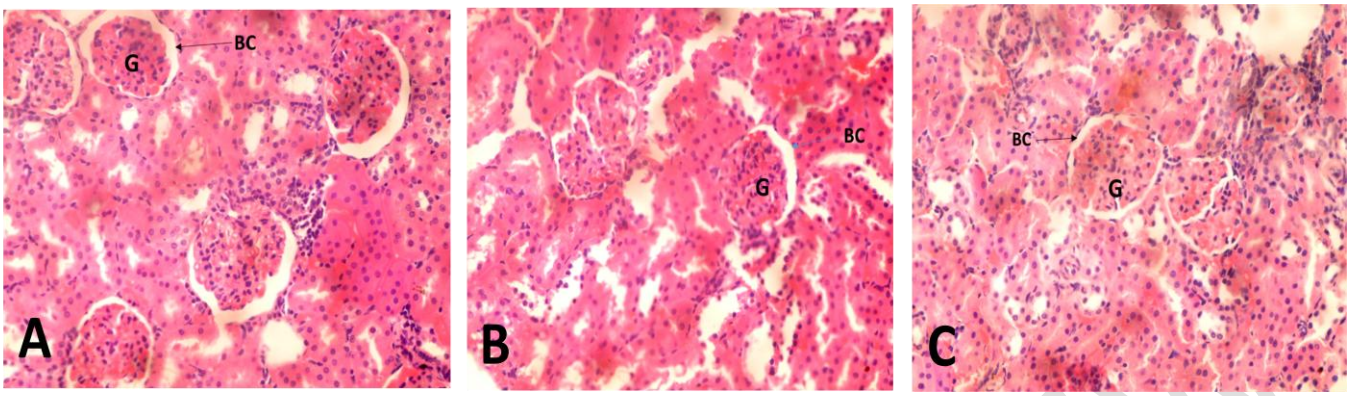

Plate 2: Photomicrographs of kidney sections of rats from groups A (Control), B and C (toluene doses of 63.6 and 127.2 mg/kg respectively) treated for 21days stained with H&E (×400). No obvious histological change in the kidney of toluene treated rats relative to the control. The photomicrograph shows the glomeruli (G) with normal tuffs, surrounded by patent Bowman's capsules (BC).

Discussion

From this study, oral administration of toluene to wistar rats for 21 consecutive days did not alter the serum concentration of total protein, albumin, bilirubin and the enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). ALT and AST, which are found inside hepatocytes, are the most sensitive indicators of liver injury (Dasgupta, 2015). These enzymes which are usually present in low concentration in the blood, leak into circulation to cause a rise in the blood concentration, as a result of cellular injury or changes in cell membrane permeability. ALT is more sensitive and specific test for liver injury as AST concentration can also be increased in the cases of cardiac arrest or muscle injury. ALP, found in the cell lining of the biliary duct of the liver, are elevated in hepatobiliary disease (Jeschke, 2007).

The result also shows that the renal biochemical parameters such as the mean levels of urea, creatinine and blood electrolytes were not affected by toluene. This result suggests that toluene at the doses and duration used in the study did not cause injury to the liver and kidney. Contrary to our findings, Tas *et al*., (2011) demonstrated that exposure to toluene by inhalation at the dose of 3000 ppm/1hour/day for 4 weeks, caused significant toxicity to liver of rats by increasing the serum concentration of ALT and AST as well as massive hepatic degeneration. The reason for the disparity in the findings could be attributed to the route of exposure. It has been reported that toluene is readily absorbed via inhalation route than other routes (Molhave and Pedersen, 1984). Although absorption following oral exposure is complete, pulmonary absorption is faster (Shaffie and Shabana, 2019). According to Carlsson (1982), the average percentage of toluene retained by the body after inhalation is estimated to be 36–85%.

Exposure to vapours of toluene once a day at the dose of 1000ppm for 15 minutes for 45 days resulted in a significant reduction in plasma level of Growth hormone, thyroid stimulating hormone, follicle stimulating hormone and luteinizing hormone in male albino rats (Salem and Kelada, 2020). Intraperitoneal exposure of rats to toluene at the dose of 900mg/kg for 6 days caused vacuolar degeneration of hepatocytes and epithelial lining of renal tubules, neuronal damage, and neurodegeneration (Shaffie and Shabana, 2019). Alrezaki, et

al. (2021) found that exposure of non pregnant female rats to toluene inhalation at the doses of 2000, 4000 and 8000 ppm for the duration of 28 days at the rate of 30 min per day increased the peripheral progesterone and testosterone levels, decreased ovarian weight and increased the number of abnormally growing follicles.

From our previous studies on toluene at the doses and duration used in this study, it was found that toluene was toxic to blood parameters evidenced by a significant ($p<0.05$) increase in lymphocyte count and a highly significant ($p<0.01$) rise in neutrophil count (Obinna and Agu, 2019). In the same vein, toluene was shown to be noxious to male reproductive functions due to the reduction in testosterone level at the doses of 63.6 and 127.2 mg/kg for 21days (Obinna and Agu, 2021).

Conclusion

Oral exposure to toluene, at the doses and duration of exposure used in this study, did not alter the hepatic and renal biochemical profile of wistar rats as well as their histomorphology of the liver and kidney.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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8

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