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ASSESSMENT OF THE IMPACT OF INHALED TOLUENE IN VAPOUR EXPOSURE CHAMBER ON RENAL AND LIVER FUNCTIONS AND OXIDATIVE STRESS IN RATS

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ABSTRACT
This study assessed the effect of toluene inhalation on kidney and liver functions and markers of oxidative stress in rats. Twenty-four rats were grouped randomly into four groups – each group having six rats. Group 1 (control), group 2 (exposed to toluene vapour at concentration of 8,200 ppm for 14 days), group 3 (exposed to toluene vapour at concentration of 4,100 ppm for 28 days), and group 4 (exposed to toluene vapour at concentration of 2,050 ppm for 56 days). Using a whole-body vapour exposure chamber, animals in groups 2 – 4 were put through toluene vapour exposure challenge. Blood samples from rats in all groups were examined to determine the concentration of urea, creatinine, and electrolytes in serum. Likewise examined were the levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) enzymes, alongside total protein and albumin in serum. The concentration in serum of markers of oxidative stress; malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)were estimated. Data showed that when compared with the control, in the 14-day group; inhaled toluene significantly increased ($p \leq 0.05$) potassium ion concentration, chloride ion concentration, and the level of urea and ALT enzyme. Also, the level of GSH and MDA (11.16±2.66) significantly increased ($p \leq 0.05$) while albumin level significantly decreased ($p \leq 0.05$). In the 28-day group, chloride ion concentration, urea level and AST enzyme level significantly increased ($p \leq 0.05$). Additionally, the level of GSH and MDA (10.59±0.58) significantly increased ($p \leq 0.05$) while ALP enzyme level significantly decreased ($p \leq 0.05$).In the 56-day group, in comparison with the control, albumin level significantly decreased ($p \leq 0.05$). However, no statistically significant change was observed in the concentration of potassium and chloride ions, urea, ALT, AST, and ALP levels, and in the level of GSH and MDA (1.63±0.35). These outcomes thus demonstrate a strong connection between observed concentration and duration dependent toluene vapour toxicity in the kidneys and liver, and the existence of severe oxidative stress-related tissue injury following exposure to toluene vapour.

KEYWORDS: Toluene, Inhalation, Oxidative Stress, Vapour Toxicity, Tissue Injury.

INTRODUCTION
In the sphere of substance abuse in Nigeria, inhalants as substances of abuse evoke minute interest – even though as far back. In abusing inhalants, the individual breathes in vapour from the abused products. He/she could achieve such through: **Sniffing or snorting** - direct inhalation of vapour from product container, Baydala (2010). **Bagging** - inhaling vapour from substances introduced into a paper bag or plastic bag, National Institute of Drug Abuse (2012). **Huffing** - inhaling vapour emanating from a rag or cloth soaked in the substance and held over the mouth or nose, Baydala (2010). **Dusting** - direct introduction of the contents of aerosols into the mouth, Baydala (2010).

Two multiple endpoints inhalation works (acute and sub chronic) utilizing Adult male Long-Evans rats, were carried out by Kodavantia, *et al*. (2015) to decide the effect of toluene, and to make correlations between the two investigations. Bae and Yoon (2002) completed a research to decide the impact of vitamin C and E in improving toluene hepatotoxicity in rats. Exposure to toluene was accomplished by abdominal infusion, and oral application of vitamin C and E proceeded after.

MATERIALS AND METHODS
Animals
The experimental subjects were rats (Wistar strain). Twenty-four healthy, adult rats were procured for this study.

An improvised whole-body vapour exposure chamber, of a very simplistic design (with the inside space measuring 0.425 m x 0.20 m x 0.265 m) was employed for the toluene vapour exposure challenge. The vapour exposure chamber's build material is a combination of aluminium, glass, and plastic. The access door of the vapour chamber is sited on the chamber's upside with a single vapour inlet sited at the rear side of the chamber. after construction, sealant was used on spaces between edges, all joints and even screws.

The access door was made to require a significant amount of pressure in a downward direction to achieve a lock.

Also a patch of rubber, was adhered to the vapour inlet from inside the chamber, much like a membrane. This patch with a central slit allowed connection of a delivery tube. When in use, an end of the delivery tube connects to the vapour inlet and another is fixed to a conical flask.

After each exposure session, the delivery tube and conical flask is pulled out from the vapour chamber.

Miniature cages

Two miniature wire mesh cages, each measuring 0.17 m x 0.155 m x 0.245 m (each cage capable of holding six rats) were constructed to be used together with the improvised vapour exposure chamber. Both miniature cages fit easily into the interior of the chamber, allowing an upper limit of twelve rats to be exposed in one exposure session.

However, only one of these cages was required because no more than six rats were exposed at a time throughout the study.

The miniature wire mesh cage ensured that, even though each cell was spacious enough to permit change of position during an exposure session, each animal was relatively confined so that the animals were unable to move around the chamber and cluster together, as is very common with rats. Hence, every animal, while in the chamber, is exposed to vapour evenly.

Toluene

Toluene was procured from a local chemical retail store – Joechem Chemicals. China.

Animal Grouping and Care

Immediately after procurement, the animals were sorted according to sex and mean weight (g). Six rats, with a mean weight of 110 g made up Group 1 (14-day exposure), another six rats, also having a mean weight of 110 g were categorized as Group 2 (28-day exposure). Group 3 (56-day exposure) comprised of six animals, having a mean weight of 98 g while Group 4 (Control) had six rats with a mean weight of 112 g.

The animals were housed in the housing section of the Animal House, University of Port Harcourt, which was properly illuminated and well aerated. Wooden cages were used, with cells having ample space to allow the six rats contained in each cell to move around freely.

The front and rear end of each cell had wire mesh covering, to ensure proper ventilation. The floor of each cell was covered with dry saw dust to serve as beddings, providing warmth. Introduction of the sorted animals into their respective cage cells marked the commencement of a two-week acclimatization period.

Standard rat feed procured from a local animal feeds outlet and clean water were given to all animals *ad libitum* bar exposure sessions. Feed and water were renewed every other day, while the saw dust beddings were renewed once every week to maintain good hygiene levels and clean surroundings. The improvised vapour exposure chamber during operation, was strategically situated in a place which was relatively cool irrespective of time, or the weather condition. Also, crucially, all vapour challenges began no later than 8:00 a. m. Together, these measures helped to ensure that the ambient temperature in which the operation of the vapour chamber took place in, remained more or less, within the desired range (standard temperature, 25 ºC).

A lookup of the Lethal Concentration (LC 50) of toluene, allowed the researcher to establish the LC 50 of toluene for rats, via inhalation, as 26,700 ppm/1 hr – 8,800 ppm/4 hr – 6,000 ppm/6 hr, indicating a decrease in concentration with increasing exposure duration. Data from Safety Data Sheets of toluene from different manufacturing companies, informed this find.

The Safety Data Sheets (SDS) of toluene, prepared by Taiwan SM Corporation, in view of toxicity, lists toluene as having an LC 50 value of 6,000 ppm/6 h (rat, inhalation), Taiwan SM Corporation (2008). For 4 hours, 8,800 ppm is indicated as the LC 50 (exposure via inhalation) for rat by British Drug Houses Chemicals in the Material Safety Data Sheet for toluene, BDH Analytical Chemicals (2015). Total Petrochemicals and Refining, under toxicological information in their Safety Data Sheet for toluene, stated that toluene had an LC 50 value of >26,700 ppm/1 h (inhalation, rat), Total Petrochemicals and Refining (2015).

With information on LC 50 of toluene for rats (inhalation), and the solution proffered by the formula above, the vapour exposure challenge for each experimental group was thus:

- **Group 1: Control** (No exposure)
- **Group 2: 14-Day Exposure**
  - Number of animals: n = 6.
  - Volume of liquid toluene vapourized: 0.8 mL.
  - Concentration of toluene vapour in vapour exposure chamber: 8,200 ppm.
  - Frequency of exposure: 4 hr/day, 5 days/week.
  - Duration of exposure: 14 days.
- **Group 3: 28-Day Exposure**
  - Number of animals: n = 6.
  - Volume of liquid toluene vapourized: 0.4 mL.
  - Concentration of toluene vapour in vapour exposure chamber: 4,100 ppm.
  - Frequency of exposure: 2 hr/day, 5 days/week.
  - Duration of exposure: 28 days.
- **Group 4: 56-Day Exposure**
  - Number of animals: n = 6.
  - Volume of liquid toluene vapourized: 0.2 mL.

- Concentration of toluene vapour in vapour exposure chamber: 2,050 ppm.
- Frequency of exposure: 1 hr/day, 5 days/week.
- Duration of exposure: 56 days.

Preparation for an exposure session began with the placement of six animals of a particular experimental group into the six cells of the miniature wire mesh cage, and placement of the miniature cage in the vapour exposure chamber, ensuring the access door remains open. Following this, the required volume of liquid toluene is collected using the syringe, this volume is introduced into the conical flask (collecting vessel). Immediately, the access door is shut, delivery tube attached to collecting vessel is attached to the vapour chamber via the vapour inlet, and the countdown timer is started – signalling the commencement of an exposure session.

Directly after the concluding vapour exposure session for a particular group, the rats were sacrificed and samples of blood obtained for biochemical investigation.

Statistical Analysis

Data from this research were stated as mean ± SEM. They were put through statistical analysis employing one-way analysis of variance (ANOVA), employing SPSS (Statistical Package for Social Sciences) software - version 20.

PRESENTATION OF RESULTS

Table 1: Serum Concentration of Electrolytes and Relative Percentage Change Compared to Control following Toluene Vapour Exposure.

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Groups	Na^+ (mmol/L)	Relative Change (%)	K^+ (mmol/L)	Relative Change (%)	Cl^- (mmol/L)	Relative Change (%)	HCO_3^- (mmol/L)	Relative Change (%)
Group 1	140.88 ± 1.18	_	4.07 ± 0.03	_	99.67 ± 0.88	_	21.33 ± 0.67	_
Group 2	141.00 ± 2.74	0.09	8.78 ± 1.42*****	115.72	107.75 ± 1.93*****	8.11	19.50 ± 0.96	-8.58
Group 3	137.20 ± 2.92	-2.61	4.08 ± 0.15	0.25	94.20 ± 1.28*****	-5.49	25.00 ± 2.02	17.21
Group 4	143.60 ± 0.24	1.93	4.24 ± 0.07	4.18	99.80 ± 0.37	0.13	21.20 ± 0.37	-0.61

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalatiosure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week)

Table 2: Serum Concentration of Creatinine and Urea and Relative Percentage Change Compared to Control following Toluene Vapour Exposure.

Groups	Creatinine (mg/dL)	Relative Change (%)	Urea (mg/dL)	Relative Change (%)
Group 1	1.43 ± 0.34	_	22.51 ± 3.11	_
Group 2	1.41 ± 0.39	-1.40	81.54 ± 15.12*****	262.24
Group 3	1.77 ± 0.56	23.78	90.44 ± 5.94*****	301.78
Group 4	0.51 ± 0.16	-64.34	18.45 ± 0.97	-18.04

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalation exposure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week)

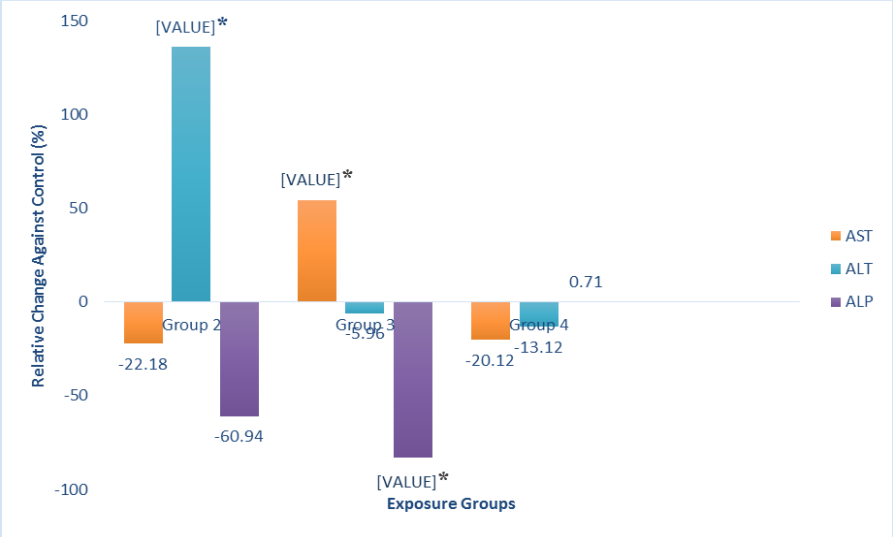

Figure 1: Liver Function Investigation– Enzymes.

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalation exposure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week)

Relative Percentage Change Compared to Control in the Level of Aspartate Aminotransferase(AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) Enzymes following Toluene Vapour Exposure

Table 3: Level of Albumin and Total Protein and Relative Percentage Change Compared to Control following Toluene Vapour Exposure.

Groups	Albumin (g/dL)	Relative Change (%)	Total Protein (g/dL)	Relative Change (%)
Group 1	5.07 ± 0.75	_	8.08 ± 0.66	_
Group 2	3.62 ± 0.11*	-28.60	5.95 ± 0.39	-26.36
Group 3	5.61 ± 0.26	10.65	8.45 ± 0.85	4.58
Group 4	3.46 ± 0.13*	-31.76	7.24 ± 1.27	-10.40

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalation exposure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week)

Table 4: Level of Antioxidants and Relative Percentage Change Compared to Control following Toluene Vapour Exposure.

Groups	SOD (U/mL)	Relative Change (%)	CAT (U/g)	Relative Change (%)	GSH (µg/mg)	Relative Change (%)	GPx (µg/mg)	Relative Change (%)
Group 1	10.87 ± 1.27	_	0.08 ± 0.039	_	12.33 ± 2.48	_	42.00 ± 18.08	_
Group 2	10.25 ± 2.13	-5.70	0.01 ± 0.003	-87.50	130.00 ± 23.53*	954.34	16.53 ± 3.09	-60.64
Group 3	10.83 ± 1.67	-0.37	0.11 ± 0.038	37.50	107.40 ± 3.44*	771.05	96.40 ± 1.60*	129.52
Group 4	9.68 ± 0.66	-10.95	0.02 ± 0.009	-75	15.92 ± 1.15	29.12	49.60 ± 11.91	18.10

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalation exposure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week)

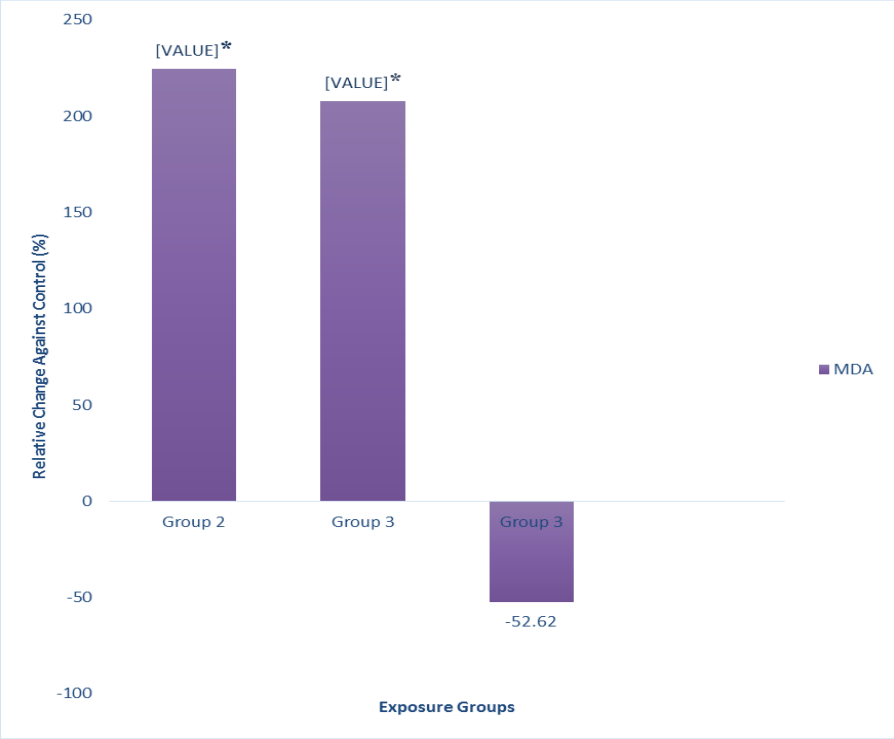

Figure 2: Relative Percentage Change Compared to Control in the Level of Malondialdehyde following Toluene Vapour Exposure.

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control, values in brackets represent percentage change.

Key: **group 1** (control; 0 ppm- no exposure); **group 2** (14 - day toluene vapour inhalation exposure; 8,200 pp - 4 hours/day, 5 days/ week); **group 3** (28 - day toluene vapour inhalation exposure; 4,100 ppm - 2 hours/day, 5 days/ week); **group 4** (56 - day toluene vapour inhalation exposure; 2,050 ppm - 1 hour/day, 5 days/ week).

DISCUSSION OF FINDINGS

Toluene vapor inhalation at a concentration of 8,200 ppm, exposure frequency of 4 hours/day, for 14 days significantlyincreased($p \leq 0.05$) ALT level with no relating increment in AST level while toluene vapor inhalation at 4,100 ppm, 2 hours/day, for a 28-day time span altogether raised ($p \leq 0.05$) AST serum concentration without a corresponding rise in ALT level.

ALP level was decreased significantly ($p \leq 0.05$) in the 4,100 ppm (2 hr/day, 28 days) toluene exposure group while in the trial group exposed to toluene vapor at 8,200 ppm (2 hr/day, 14 days), however not statistically significant, a decrease in ALP level which contrasted with the non- exposure group's ALP level was additionally taken note of.

In contrast with the non-exposure group, 8,200 ppm and 2,050 ppm toluene-exposure groups exhibited decreased serum albumin concentration, notwithstanding, 4,100 ppm toluene-treated group demonstrated no such change. The diminished albumin levels in the above-mentioned test groups concur with the perception of Tas *et al*. (2011) who reported toluene inhalation to have decreased albumin levels in rats subjected to 3000 ppm toluene for 21 days, at an exposure frequency of 1 hour for each day.

Examination concerning the impact of inhaled toluene on the kidneys' capacity to function optimally presented no statistically significant contrast in sodium ion concentration in serum of exposed animals against sodium ion level in the experimental groups. Be that as it may, dissimilar to the previously mentioned experimental groups where the levels marginally increased, concentration of sodium ion in the 4,100 ppm (2 hr/day, 28 days) group decreased slightly.

Potassium ion concentration was seen to significantly increase ($p \leq 0.05$) in the 8,200 ppm (4 hr/day, 14 days) toluene exposure group, but no statistically significant changes were seen in the 4,100 ppm (2 hr/day, 28 days) toluene exposure group and 2,050 ppm (1 hr/day, 56 days) toluene exposure group, as against level of potassium ion concentration in the non-exposure group.

The increased potassium ion level seen in 8,200 ppm (4 hr/day, 14 days) toluene exposure group is suggestive of a high level of injury to renal cells.

Chloride ion concentration, which increased significantly ($p \leq 0.05$) against the non-exposure group in the 8,200 ppm (4 hr/day, 14 days) toluene exposure group points to possible abnormal ion retention due to reduced renal function. Conversely, the 4,100 ppm (2 hr/day, 28 days) toluene exposure group expressed a chloride ion

concentration level which was significantly decreased ($p \leq 0.05$) against the non- exposure group, such could be attributable to the expansion of extracellular fluid volume following the abnormal retention of chloride and sodium ions.

This present investigation's information demonstrated inhaled toluene to significantly raise ($p \leq 0.05$) urea concentration in the 8,200 ppm (4 hr/day, 14 days) toluene exposure group and 4,100 ppm (2 hr/day, 28 days) toluene exposure group, somewhat more so in the 4,100 ppm group. The result was substantiated with the reports of Bhat, & Anand (2010).

in comparison to creatinine level in the non-exposure group, concentration of creatinine in the 4,100 ppm (2 hr/day, 28 days) toluene exposure group was higher than that seen in the non-exposure group, the 8,200 ppm (4 hr/day, 14 days) toluene exposure group, and the 2,050 ppm (1 hr/day, 56 days) toluene exposure group.

Investigation into the serum oxidative status of rats subjected to vapour produced results that showed significant rises ($p \leq 0.05$) in MDA level in the 8,200 ppm (4 hr/day, 14 days) toluene exposure group and 4,100 ppm (2 hr/day, 28 days) toluene exposure group, when compared with level of MDA in the non-exposure group. These outcomes are consistent with the documentation of Bae and Yoon (2002).

CONCLUSION

This study's outcome shows inhalation exposure to toluene to markedly affect renal and hepatic physiology adversely, especially at high concentrations, and thus, if unchecked, could prompt renal or hepatic pathologies.

Furthermore, disturbance of oxidative balance and extreme oxidative damage to tissues observed following exposure to high concentrations of vapor from toluene points to a strong connection between the generation and action of oxygen radicals and the negative changes to renal and hepatic capacities determined.

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