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BIOCHEMICAL ASSESSMENT OF LIVER AND KIDNEY PARAMETERS: A COMPARATIVE STUDY OF PETROLEUM EXPOSED AND PETROLEUM UN-EXPOSED ADULTS IN BAUCHI METROPOLIS

*ABDULRASHID MOHAMMED; *DANIEL HASSAN MHYA; **AMINA MOHAMMED; *SALAMATU YAU IBRAHIM; & ***MUKTARI BALA

*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria. **Department of Community Medicine, Faculty of Clinical Sciences, College of Medical Sciences, Abubakar Tafawa Balewa University, Bauchi Bauchi State, Nigeria. ***Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano, Kano State, Nigeria.

ABSTRACT

Hydrocarbons which are the main chemical components of petroleum are constantly inhaled by petroleum hawkers which may result in derangement of kidney and liver functions. This study aimed to compare the liver and kidney parameters in petroleum exposed compared to un-exposed individuals. This was a comparative cross-sectional study carried out among all consenting adults in Bauchi metropolis. Blood samples were collected from the subjects and analyzed using spectrophotometric technique while other data was collected using structured interviewer-administered questionnaire and analyzed using SPSS software. Analysis employed descriptive and

Introduction:

Petroleum has combination of impulsive hydrocarbons which is freely available in the atmosphere dispenses easily during hawking, at petrol stations and depots. Hence, the inhalation of these hydrocarbons is the most common form of exposure in those areas (Awasthi *et al.*, 2016). Even though exposure to supra lethal concentration of petroleum vapor is rare but possible in highly confined or poor ventilation areas (Malini and Maithily, 2017). It has

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inferential statistics. Level of significance was set at 5%. The result showed a statistically significant increase ($p<0.001$) in liver parameters like alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin and direct bilirubin. Also, total protein showed a statistically significant increase ($p=0.002$) in exposed group as compared to unexposed group, while albumin showed no statistical significant difference ($p=0.572$). There was a statistically significant increase in kidney parameters such as urea, sodium and chloride with a P-value <0.001 in all parameters and ($p<0.010$) for creatinine. In addition, there was no statistically significant difference in while bicarbonate and potassium levels in both comparison groups with p-values (0.207) and (0.807) respectively. The results of this study showed that petroleum hawkers are at risk of diseases of liver and kidney due to constant exposure and inhalation of these hydrocarbons.

Keywords: Hydrocarbons, Kidney parameters, Liver parameters, Petroleum hawkers

been reported to increase the possibilities of risks for acute and chronic health (Ibrahim *et al*., 2021). They are more likely to have respiratory tract ailments due to the interruption by the particulate matter (PM) and fuel constituents (Malini and Maithily, 2017). Exposure to higher level of vapor may affect the central nervous system (CNS) resulting in slurred speech and confusion and very high concentrations may result in rapid unconsciousness and death due to respiratory failure (Ibrahim *et al*., 2021). The highly toxic hydrocarbons contained in crude oil can damage any organ of the human body such as kidney and liver as well as the immune, nervous, respiratory, reproductive, and sensory systems. Subsequently, causing a wide-range of diseases and disorders (Curtis *et al.,* 2022). The chemical pollutants from petrol vapour are metabolically transformed like any other xenobiotic into different metabolites in the body (Adeniji *et al*., 2019). Some of these metabolites may be very reactive in countless ways, thereby interacting with the excreting and metabolizing tissues (mainly the liver and kidneys) in different ways to elicit toxic effects (Nygren, 1994).

The Liver organ plays an important role in metabolic functions and detoxification of drugs and environmental chemicals in the human body (Kalra *et al*., 2021). Other main functions of the liver include glucose storage and synthesis, plasma

protein synthesis, hormone production, and bile formation. Due to the blood flows from the stomach and intestine, the liver is the first internal organ to encounter a number of insults including ingested metals, drugs, and environmental toxicants (Kalra *et al.*, 2021). The consequence of these exposes the liver to significant concentrations of these chemicals, and liver functions can be adversely affected by the acute or chronic exposure (Chang *et al.*, 2013). The kidney on the other hand performs many excretory and regulatory functions necessary to sustain life. Under normal conditions, the kidney not only functions to maintain the constancy of the extracellular environment by excretion of the waste products of metabolism and the adjustment of urinary water and electrolyte excretion, but also is intricately involved in the regulation of blood pressure, red blood cell production, and bone mineral metabolism (Michael and Sushrut, 2012).

MATERIALS AND METHODS

Study Site/ Location

The study was conducted in Bauchi Metropolis a city in northeast Nigeria which covers an area of about 3,687km^2 and has a population of 493,810 in 2006. The people are mostly farmers and businessmen.

Study population

Adults exposed to and some unexposed to petroleum products within the study area

Inclusion and Exclusion Criteria

Inclusion criteria: Apparently healthy males aged of 17-50years, with a minimum of 6 months' exposure to petroleum products form the exposed group.

Exclusion criteria: Auto mechanics, and those working in cement and painting industries were excluded from the study.

Sample size Calculation

The sample size was calculated using the cochran's formular for comparative study, considering a confidemce level of 1.96 at 95% CI, a power of 80%, variance of aspartate amino transferase of 0.54 and 0.19 from petroleum fumes in exposed and non exposed in a study conducted in Ibadan (Ogunneye *et al.,* 2014) and minimum expected difference of 0.25, the minum sample size obtained was 41. However, this was incresead to 60 per group.

Sampling technique:
Simple random sampling technique was used to select one out of the twelve wards which make up Bauchi Metropolis while shops owners in the same environment were used as control.

Data Collection
A structured questionnaire was used to obtain information on age, socio-economic status, health status, occupational history. Blood (10ml) was taken from a peripheral vein on the arm of each participant using a sterile needle attached to a syringe for each participant and immediately transferred into sterile labeled EDTA anticoagulant bottles for haematological analysis and plain sample container for biochemical analysis. The samples in the plain containers were allowed to stand and clot for 30 minutes and then centrifuged at 5000rpm for 10 minutes to obtain the serum which were used for the biochemical analysis.

Ethical Consideration
The ethical committee of Bauchi State Ministry of Health approved the study protocol (NREC/12/05/2013/2018/33). Ethical consideration and confidentiality were respected. Informed consent was obtained from all participants of this study. The nature and purpose of the study was explained to the participants, following which they willingly consented to participation in the study.

Statistical Analysis
Statistical analysis was done using SPSS. Results were expressed as mean $\pm$ standard deviation. Statistical differences between groups were analyzed using independent T test for normally distributed data and Mann Whitney U test for skewed data. Differences at $P < 0.05$ were considered to be statistically significant.

RESULT
Table 1 presents Socio-demographic characteristics of respondents who were exposed and unexposed to petroleum. The highest age distribution of the exposed subjects was between 25-43 years, 75% of them work for greater than 8hours a day and 58.3% have been in the business for more than 5 years. Most of the exposed subjects have secondary school certificate as their highest educational qualification, 65% of them are non smokers. Among the controls, 53.5% is between the age of 18-24 and 55% are tertiary school students.

Table 1: Socio-Demographic Characteristics of Respondents who are exposed to Petroleum Products.

VARIABLE	EXPOSED N=60 F (%)	Un-EXPOSED N=60 F (%)
Age respondents		
<18	4(6.7)	1(1.7)
18-24	21(35.0)	32(53.3)
25-34	26(43.3)	24(40)
44-45	6(10.0)	2(3.3)
>45	3(5.0)	1(1.7)
Level of education		
None	4(6.7)	1(1.7)
Primary	11(18.3)	1(1.7)
Secondary	36(60.0)	33(55)
Tertiary	9(15.0)	25(41.7)
Number working hours		
<8 hours	15(25.0)	0
>8hours	45(75.0)	0
		60(100)
Years of exposure		
<1 year	4(6.7)	0
1-3 years	11(18.3)	0
3-5 years	10(16.7)	0
>5 years	35(58.3)	0
		60(100)

Table 2 shows the effect of Petroleum exposure on mean values of liver function parameters of Petroleum Hawkers. The mean values of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) for the exposed group are 12.467, 11.300 and 18.266 respectively while for the un-exposed group the mean values of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) are 6.700, 7.766 and 13.250 respectively.

Table 2: Mean of Liver Function Parameters between both comparison groups

VARIABLE	Petroleum exposed group Mean ± SD	Petroleum un-exposed group Mean ± SD
Protein	7.162 ± 1.010	6.667 ± 0.486
Alanine Amino Transferase (ALT)	12.467 ± 13.448	6.700 ± 2.631
Aspartate amino Transferase (AST)	11.300 ± 3.475	7.766 ± 2.714
Alkaline Phosphatase (ALP)	18.266 ± 5.757	13.250 ± 4.824
Albumin	3.525 ± 0.706	3.486 ± 0.486

Total Bilirubin	9.303 ± 2.505	6.667 ± 0.680
Direct Bilirubin	4.281 ± 5.292	2.981 ± 0.645

Table 3 shows the effect of Petroleum exposure on mean differences of liver function parameters of Petroleum Hawkers. There was Significant difference (P<0.001) in levels of serum Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP), Total Bilirubin and Direct Bilirubin between the exposed group as compared to the un-exposed group. While Total Protein showed Significant difference (P=0.002) between the exposed group as compared to the un-exposed group.

Table 3: Mann-Whitney and T- test showing the mean difference in Liver Function Parameters between those exposed to Petroleum Products and those un-exposed to Petroleum Products

VARIABLE	Petroleum Exposed Mean rank	Petroleum Unexposed Mean rank	Mann-Whitney test	p- value
Protein	7.16 ± 1.010	6.67 ± 0.680	3.148**	**0.002***
Alanine Amino Transferase (ALT)	78.01	42.99	749.500	**<0.001***
Aspartate amino Transferase (AST)	77.55	43.45	777.000	**<0.001***
Alkaline Phosphatase (ALP)	75.33	45.67	910.000	**<0.001***
Albumin	62.29	58.71	1692.500	0.572
Total Bilirubin	72.52	48.48	1079.000	**<0.001***
Direct Bilirubin	74.73	46.27	946.000	**<0.001***

**** t-test**

Table 4 shows the result of compared mean differences of liver function parameters based on the number of hours of exposure to petroleum vapours per day. There was Significant difference (P=0.049) in level of serum Alkaline phosphatase (ALP) between the exposed group as compared to the un-exposed group.

Table 4: Mean difference in Liver Function Parameters between those exposed to Petroleum Products based on number of exposure hours.

VARIABLE	Exposed for <8hrs Mean rank	Exposed for >8 hrs Mean rank	Mann-Whitney test	p-value
Protein	6.73 ± 1.049	7.304 ± 0.966	1.940**	0.057
Alanine Amino Transferase (ALT)	22.83	33.06	222.500	**0.049***
Aspartate amino Transferase (AST)	22.87	33.04	223.000	0.050
Alkaline Phosphatase (ALP)	26.97	31.68	284.500	0.365

Albumin	27.70	31.43	295.500	0.472
Total Bilirubin	26.90	31.70	283.500	0.365
Direct Bilirubin	25.13	32.29	257.000	0.168

** t-test

Table 5 shows the effect of Petroleum exposure on mean values of renal function parameters of Petroleum Hawkers. The mean values of Creatinine, Sodium and Chloride for the exposed group are 87.083, 163.966 and 105.336 respectively while for the un-exposed group the mean values of Creatinine, Sodium and Chloride are 79.800, 138.433 and 99.450 respectively.

Table 5: Mean of Renal Function Parameters between the comparison groups

VARIABLE	EXPOSED GROUP GROUP Mean ± SD	UN-EXPOSED Mean ± SD
Urea	4.580 ± 1.582	3.435±2.710
Creatinine	87.083 ± 14.159	79.800±7.451
Bicarbonate	24.950 ± 3.959	24.050±3.402
Sodium	163.966 ± 130.174	138.433±4.010
Potassium	3.870 ± 0.578	3.810±0.602
Chloride	105.366 ± 5.336	99.450±3.605

Table 6 shows the effect of Petroleum exposure on mean differences of renal function parameters of Petroleum Hawkers. There was Significant difference ($P<0.001$) in levels of serum Urea, Sodium and Chloride between the exposed group as compared to the un-exposed group. While Creatinine showed Significant difference ($P=0.010$) between the exposed group as compared to the un-exposed group.

Table 6: Mean difference in Renal Parameters between those exposed to Petroleum Products and those un-exposed to Petroleum Products

VARIABLE	Exposed Mean rank	Unexposed Mean rank	Mann-Whitney test	p- value
Urea	78.60	42.40	714.000	**<0.001***
Creatinine	68.68	52.32	1309.000	**0.010***
Bicarbonate	64.49	56.51	1560.500	0.207
Sodium	83.53	37.47	418.000	**<0.001***
Potassium	61.28	59.73	1753.500	0.807
Chloride	80.11	40.89	623.500	**<0.001***

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Table 7 shows the result of compared mean differences of renal function parameters based on the number of hours of exposure to petroleum vapours per day. There was no significant difference in levels of renal parameters with number of hours of exposure between the two groups.group.

Table 7: Mean difference in Renal Function Parameters between those exposed to Petroleum Products based on number of exposure hours.

VARIABLE	Exposed for <8hrs Mean rank	Exposed for >8 hrs Mean rank	Mann-Whitney test	p- value
Urea	24.97	32.34	254.500	0.156
Creatinine	25.17	32.28	257.500	0.171
Bicarbonate	36.83	28.39	242.500	0.103
Sodium	31.70	30.10	319.500	0.758
Potassium	28.33	31.22	305.000	0.578
Chloride	31.57	30.14	321.500	0.784

DISCUSSION

Petroleum products are known hepatotoxicants, causing increase in the levels of hepatic amino transferases (Patrick *et al.*, 2011). Following inhalation, benzene and the other hydrocarbons present in gasoline are readily absorbed from the lungs and get metabolized in the liver by CYP 450 2EI oxidative pathways which contribute to the production of free radicals and quinine metabolites such as phenol, hydroquinone, benzoquinone, 1,2,4-benzenetriol (Bahadar *et al.*, 2014). These free radicals and toxic metabolites cause lipid peroxidation and damage of hepatic cell membrane causing the release of liver enzymes in circulation (Uboh *et al.*, 2009; Bahadar *et al.*, 2014). The results of this study show that about (43%) of the petroleum hawkers were within the age of 25-34 years and most of them were exposed for >5years. About 42% of them are cigarette smokers and 60% attended secondary school.

Exposure to petroleum by hawkers results in a statistically significant increase ($p<0.001$) in liver parameters like alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin and direct bilirubin. Also, total protein showed a statistically significant increase ($p=0.002$) in exposed group as compared to unexposed group, while albumin showed no statistical significant difference ($p=0.572$). The elevation of the activities of

these enzymes may be likely due to accumulation of benzene constituents and their reactive metabolites in liver tissues which enhance peroxidation of lipids in biomembranes. Lipid peroxidation of biomembranes causes cellular leakage and loss of functional integrity of cell membrane in liver (Al-Olayan *et al.*, 2014). These results are in agreement with Rahul *et al* (2017) who reported that petrol pump workers were at greater risk of developing biochemical alterations in hepatic enzymes with time, Anakwue *et al* (2017) also confirmed that chronic exposure to petroleum products could cause increased level of liver aminotransferases. The findings of this work is also in accordance with the findings of Majid *et al* (2016) who reported that the levels of liver enzymes were significantly elevated among petrol station attendants in Basrah when compared with the control group. Similarly, Masoud *et al* (2015) reported that the level of activities of AST and ALT were significantly higher in groups exposed to petroleum than in unexposed groups in Iran. Moreover, Saadat and Ansari-lari (2005) reported significant increases in the serum activities of AST and ALT in oil industry workers. It is also in agreement with the work done by Mark and Kesava, (2014) who reported higher activities of ALT, AST and ALP in serum of residents exposed to benzene at the British petroleum plant in Texas City, USA. Nonetheless, this finding was not in agreement with that reported by Naza *et al.,* (2013) who worked with gasoline filling station workers in Sulaimani city and observed no significant changes in plasma protein profiles. The differences may be as a result of variation in their nature of exposure, duration of exposure and also the constituents of the petroleum vary from country to country. Albumin on the other hand showed statistically insignificant increase between the two groups. This is similar with the findings of Ogunneye *et al.,* (2014) who reported the hepatotoxic effect of petroleum fumes on petrol attendants in Ibadan Nigeria. Also, the result showed there was significantly ($P<0.05$) increase in activities ALT among petroleum hawkers expose to petroleum products for >8hrs daily as compared to those un-exposed to Petroleum Products for >8hrs daily.

The kidneys excrete waste products from the body and maintain homeostasis in the body by the reabsorption of important materials (Uhegbu *et al.,* 2015). The result showed statistically significant increase ($p<0.001$) in kidney parameters

such as urea, sodium and chloride while creatinine with a P-value <0.001 in all parameters and (p<0.010) for creatinine. In addition, there was no statistically significant difference in while bicarbonate and potassium levels in both comparison groups with p-values (0.207) and (0.807) respectively.

The increase in the level of these parametrs may be due to a fall in glomerular filtration rate (GFR), resulting in their retention in blood (Vander *et al.*, 2001). However, the mechanism(s) responsible for the nephrotoxic effect reported is not clear, but it is believed that reactive metabolites of the hydrocarbons and other constituents of the vapours must have interacted with the renal tissues to cause derangements in glomerular function (Uboh, *et al.*, 2009). This result is similar to the one reported by Nwanjo and Ojiako (2007) on potential health hazards of petrol station attendants in Owerri Nigeria, and closely related to the one reported by Awodele *et al* (2014) on petroleum tanker drivers in Lagos Nigeria. The result is also in agreement with the one reported by Uhegbu *et al.,* (2015) who found significant higher levels of serum creatinine and urea in albino rats exposed to different petroleum products. However, it is not in line with the work of Luay and Tareq (2014) who reported significantly lower levels of creatinine in workers exposed to petroleum pollutants in Mosul city compared with the control group. The variation may be possibly due to genetic differences, nutritional status and in the refining processes of the crude petroleum. The hydrocarbons present in Mosul petroleum might be significantly lower than the hydrocarbons present in Nigerian petroleum and the life style of the Nigerian petroleum hawkers differs from that of Mosul city.

Conclusion

The result of this study show that petroleum hawkers are at risk of developing liver and kidney diseases after prolong exposure. The effect may likely increase with increasing frequency of exposure and duration of exposure.

Reccomendation(s)

i. The need for work to be done on relationship of dietary intake and petroleum exposure

ii. Histopathological analysis should be conducted using experimental animals on liver and kidney organs to determine the level of the effect of exposure to the organs.

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APPENDIX I
ETHICAL APPROVAL

SECRET

GOVERNMENT OF BAUCHI STATE
MINISTRY OF HEALTH

Bello Kirfi Road, Off Murtala Mohammed Way,
P.M.B. 065, Bauchi
E-mail: bauchismoh@gmail.com

MOH/GEN/S/1409/I — 4th September 2018

Reference: _____ Date:- _____

PROTOCOL REG. N0: BSMOH/NREC/35/2018
PROTOCOL APPROVAL N0: NREC/12/05/2013/2018/33

Salamatu Ya'u Ibrahim
Department of Biochemistry,
Bayero University Kano,
Kano State.

ETHICAL CLEARANCE FOR SUBMITTED PROTOCOL:
"Epidemiological Risk Factors and Biochemical Impact of Petroleum Products Exposure Among Hawkers from Bauchi City."

The Bauchi State Health Research Ethics Committee (HREC) under the State Ministry of Health has received the above named protocol for ethical clearance and approval in line with the guidelines set by the Committee. The protocol was reviewed and the committee noted that that the research does not entails clinical trials or any invasive procedures.

2. Consequently, the Committee hereby granted expedited approval for the research to be conducted. However, you should share with us your workplan clearly indicating the start date, where and when to visit the research site(s) and also **the final results of your findings.**

3. The Committee requires you to comply with all Institutional Guidelines, Rules and Regulations and with the tenets of the National Health Research Ethics Committee Code including that all adverse events are reported promptly to the Committee. **No changes are permitted in the research without prior approval by the Committee** except in circumstances outlined in the Code. The Committee reserves the right to conduct compliance visit to your research site without prior notice.

4. Thank you.

(Usman U. Muhammad)
For: Hon. Commissioner.

SECRET