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Comparison of Antioxidant Levels among Petroleum Hawkers and Non-Petroleum Hawkers in Bauchi Metropolis

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Abstract

Background: The major components of petroleum are hydrocarbons which are toxic and have been implicated in a number of human diseases. Petroleum hawkers are exposed to these hydrocarbons continuously by inhalation. This study aimed to determine the effects of petroleum exposure on malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSSH) among petroleum hawkers in Bauchi metropolis. This was a descriptive cross-sectional study carried out among 120 consenting petroleum hawkers in Bauchi metropolis.

Method: Venous blood samples were collected from the subjects and analyzed using spectrophotometric technique, data was also collected using structured interviewer-administered questionnaire and analyzed using SPSS version 23 software. Analysis employed descriptive and inferential statistics. Level of significance was set at 0.05.

Result: The result showed statistically significant mean difference in superoxide dismutase (SOD) in exposed group as compared to unexposed group ($p<0.001$). There were also differences in mean of malondialdehyde (MDA), catalase (CAT) and reduced glutathione (GSSH) though this differences were not statistically significant with p- values 0.100, 0.127 and 0.057 respectively. In addition, petroleum hawkers who were exposed to petroleum products for greater than 8hrs had higher malondialdehyde (MDA) levels compared to those exposed for less than 8hrs and this difference was statistically significant (p-value=0.003).

Conclusion: The results of this study showed that petroleum hawkers are at risk of adverse effects on human health especially diseases of liver and kidney.

Keywords: Antioxidant, Petroleum hawkers, Petroleum products, Bauchi state.

INTRODUCTION

Petroleum is an extremely complex mixture of a wide variety of low and high molecular weight hydrocarbons. This complex mixture contains saturated alkanes, branched alkanes, alkenes,

Vol. 5, No. 03; 2021

ISSN: 2581-3366

naphthenes (homo-cyclics and hetero-cyclics), aromatics (including aromatics containing hetero atoms like sulfur, oxygen, nitrogen, and other heavy metal complexes), naphtheno-aromatics, large aromatic molecules like resins, asphaltenes, and hydrocarbon containing different functional groups like carboxylic acids, ethers, etc. (Shukla and Cameotra, 2012).

Petroleum has mixture of volatile hydrocarbons which is readily available in the atmosphere and dispenses easily during hawking, at petrol filling stations and depots. Hence inhalation 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). If the Petroleum hawkers are routinely exposed to hydrocarbon fuel vapor that has been reported to increase the risks for acute and chronic health problems (Hegazy and Kamel, 2014). They are more prone to respiratory tract ailments due to the interruption by the particulate matter (PM) and fuel constituents (Malini and Maithily, 2017). Exposure to higher concentrations of vapor may affect the central nervous system (CNS) resulting in staggered gait, slurred speech and confusion and very high concentrations may result in rapid unconsciousness and death due to respiratory failure (Chilcott, 2007).

Benzene occupies the major composition of petroleum constituents and is a class I human carcinogen (Sumathi and Neelambikai, 2016). Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which leads to lipid peroxidation and damages DNA, RNA, leading to genetic modification and alterations in the functions of important enzymes and proteins (Mohammed *et al.,* 2020). Chronic benzene exposure leads to decrease in antioxidant enzymes activity and hematologic disorders. Benzene affects many enzyme activities in the liver, tissues, and peripheral blood and this can lead to a decrease in the activity of antioxidants enzymes and may result in oxidative stress (Poljsak *et al.,* 2013). Oxidative stress occurs as a consequence of the imbalance between pro-oxidants and antioxidants. This imbalance is due to excessive accumulation of reactive oxygen species or antioxidant depletion or both together resulting in cellular damages (Domej *et al*., 2014). Increased levels of hydrogen peroxide, hypochlorous acid (HClO) and free radicals including hydroxyl radical (OH) and superoxide anion (O_2^-) enhances the production of ROS in those organisms which are exposed to petroleum compounds (Birben *et al*., 2012).

Antioxidants act as a defence mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system (Kurutas, 2015). Reactive oxygen species not only are produced naturally in cell following stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol, and psychological or emotional stress. Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process (Kirkeleit *et al*., 2008, Tan *et al*., 2018). Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Mohammed *et al.,* 2020). The antioxidants in biological system can be either enzymatic or non-

Vol. 5, No. 03; 2021

ISSN: 2581-3366

enzymatic. The enzymatic antioxidants include catalase, superoxide dismutase, and glutathione which catalyse neutralization of many types of free radicals (Xiang *et al.*, 2014), while the non-enzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and neurological disorder (Mohammed *et al.*, 2020).

MATERIALS AND METHODS

Study Area

The study was conducted in Bauchi metropolis, Bauchi Local Government Area, Bauchi. State, Nigeria. The area is located within the North-eastern part of Nigeria. It is situated between latitude 10^0 20' N and longitude 10^0 10' E. The area has a population of 341,748 people.

Study population

1. Male Petroleum hawkers in Bauchi metropolis
2. Non- petroleum male hawkers in Bauchi metropolis

Inclusion criteria

Male petroleum hawkers aged 17-50 years
Male non-petroleum hawkers aged 17-50 years

Exclusion criteria

1. Petroleum and non-petroleum hawkers who have spent less than six months in the profession

Study design

A descriptive comparative cross sectional study design was used

Sample size estimation

The sample size was calculated according to Cochran (1975) and a sample size of 60 was obtained for each comparison group after correction.

Sampling technique

Multistage sampling technique was used

Stage one (Selection of wards)

Of the 12 wards in Bauchi metropolis, 2 were selected using simple random sampling technique by balloting, and these were considered as clusters.

Stage two (Selection of study participants)

Using cluster sampling, all the petroleum hawkers in the selected wards who gave consent were selected and studied. This was done until the sample size was reached. For the comparison

Vol. 5, No. 03; 2021

ISSN: 2581-3366

group, cluster sampling was also used to select those who fulfilled the inclusion criteria and hawking other items in the same selected wards.

Data Collection

A structured interviewer administered questionnaire was used and obtained data on socio-demographic characteristics such as age, level of education and socio-economic status. Data on smoking history and occupational history was also obtained. The questionnaire was pretested in Alkaleri LGA of Bauchi and relevant modifications were effected afterwards.

Procedure for Sample collection

Venous 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 sera which were used for the biochemical analysis.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 23. Quantitative results such as age were expressed as mean ± standard deviation. T test was used to determine the mean difrence in antioxidant levels between the comparison groups, Mann whitney U test was used the test for the mean difference between skewed data in both comparison groups. Also ANOVA test was used to determine the mean difference of antioxidants and number of years of exposure in both comparison groups. A p- value of < 0.05 was considered statistically significant.

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.

RESULTS

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 comparison group, 53.5% is between the age of 18-24 and 55% are tertiary school students.

Vol. 5, No. 03; 2021

ISSN: 2581-3366

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

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

More of the respondents are aged 25-34 years and have secondary school educational qualification 53.3% and 55% respectively.

Table 2: Socio-Demographic Characteristics of Respondents who are Un-Exposed to Petroleum Products.

VARIABLE	N=60 F (%)
Age respondents (years)	
<18	1(1.7)
18-24	32(53.3)
25-34	24(40.0)
44-45	2(3.3)
>45	1(1.7)
Level of education	
None	1(1.7)
Primary	1(1.7)
Secondary	33(55.0)
Tertiary	25(41.7)

Vol. 5, No. 03; 2021

ISSN: 2581-3366

There was a statistically significant mean difference in Superoxide Dismutase levels in between those exposed and unexposed individuals with a P-value < 0.001.

Table 3: Mean Difference in Antioxidant Parameters between those Exposed to Petroleum Products and those Un- Exposed to Petroleum Products.

VARIABLE	Exposed Mean ± SD	Un-Exposed Mean ± SD	T-test	p-value
Reduced Glutathione	6.986±2.735	14.826±3.501	13.668	0.087
Superoxide Dismutase	1075.374±1653.633	1048.377±1721.154	3.550	**<0.001***
Malondialdehyde	65.720	55.280	1487.000**	0.100
Catalase	65.350	55.650	1509.000**	0.127

**** Mann-Whitney**

There is a statistically significant difference in the mean Malondialdehyde level between those exposed for<8 hours and those exposed for >8hrs to petroleum products.

Table 4: Mean Difference in Antioxidant Parameters between those Exposed to Petroleum Products for >8hrs Daily and those Exposed to Petroleum Products for >8hrs Daily.

VARIABLE	Exposed for <8hrs Mean ± SD	Exposed for >8 hrs Mean ± SD	T-test	p-value
Reduced Glutathione	7.213±2.457	6.911±2.843	0.368	0.714
Superoxide Dismutase	1156.363±1484.688	1048.377±1721.154	0.217	0.778
Malondialdehyde	18.730	34.420	161.000**	**0.003***
Catalase	26.400	31.870	281.000**	0.294

**** Mann-Whitney**

There was not statistically significant mean difference in the antioxidant levels based on number of years of exposure to petroleum products

Vol. 5, No. 03; 2021

ISSN: 2581-3366

Table 5: Anova Showing Mean Difference in Antioxidant Parameters between those Exposed to Petroleum Products by number of years.

VARIABLE	N=60	Mean ± SD	F-Test	P-value
Reduced Glutathione				
<1 year	4	7.300 ± 2.247	0.205	0.893
1-3 years	11	6.745 ± 2.653		
3-5 years	10	6.480 ± 1.764		
>5 years	35	7.171 ± 3.082		
Superoxide Dismutase				
<1 year	4	567.590 ± 469.202	0.913	0.440
1-3 years	11	1170.698 ± 1571.549		
3-5 years	10	1801.301 ± 2471.968		
>5 years	35	896.039 ± 1469.846		
Malondialdehyde				
<1 year	4	44.397 ± 11.260		
1-3 years	11	66.440 ± 48.802	0.426**	0.514**
3-5 years	10	97.522 ± 70.675		
>5 years	35	90.908 ± 52.250		
Catalase				
<1 year	4	2268.385 ± 1223.153	0.614**	0.433**
1-3 years	11	5954.606 ± 8534.535		
3-5 years	10	4498.047 ± 3703.297		
>5 years	35	3467.184 ± 2183.272		

****Kruskal-Wallis test**

DISCUSSION

Benzene occupies the major composition of petroleum constituents and is a class I human carcinogen (Sumathi and Neelambikai, 2016). Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which leads to lipid peroxidation and damages DNA, RNA, leading to genetic modification and alterations in the functions of important enzymes and proteins (Mohammed *et al*., 2020). Therefore, individuals exposed to these constituents from petroleum products are at risks of many health implications. The results of this study show that most (43.3%) of the petroleum hawkers were within the age of 25-34years and most of them were exposed for >5years. About 41.7% of them are cigarette smokers and 60% attended secondary school. The result also showed significant increase in antioxidant activities in those exposed to petroleum products compared to those un-exposed. This is in agreement with Aida *et al*., (2015), who worked on petrol attendants exposed to benzene at Zagazig city which showed that there is a high statistically significant difference regarding the level of (ttMA) & (MDA) which is higher among exposed compared to control group, while the level of (SOD) is statistically lower among exposed group, the result also showed that there is a high statistical significant difference concerning the level of (MDA)

Vol. 5, No. 03; 2021

ISSN: 2581-3366

among smokers and non-smokers in exposed group as it is higher among smokers compared to non-smokers. In the same vein Owagboriaye *et al.,* (2016) assessed the effect of gasoline fumes on antioxidant status and lipid peroxidation in albino rats and reported significantly lower concentration of serum GSH and significantly higher concentration of serum MDA. Also, Odewabi *et al.,* (2014), reported significantly lower levels of serum GSH accompanied by a significantly higher serum MDA levels among petrol attendants in Ogun, Nigeria.

The results however, is not in concordance with work of Malini and Maithily, (2017) who reported finding showed no significant difference in superoxide dismutase and levels of Malondialdehyde and total antioxidant capacity among 165 males divided into three groups were the petrol fillers, tanker drivers and the controls. However, there was significant changes observed for total antioxidant capacity and vitamin A when exposed group is compared with control subject. The discrepancy as to the activities of these oxidant enzymes and the levels of other oxidative markers in petrol-induced toxicity reports depends on the type of tissue examined as well as study protocols among other factors (Georgieva *et al.,* 2002; Li and Han 2006; Kinawy 2009; Reckhadevi *et al.,* 2010; Uzma *et al.,* 2010). However, the findings from this research work showed no significant difference in antioxidant status within individuals exposured to petroleum products over time.

CONCLUSION

The result of this study showed that prolong exposure to petroleum products could lead to increased activities of antioxidant enzymes. The effect of exposure to this petroleum products increases with increasing frequency of exposure and duration of exposure. This can result in so many health implications affecting various body systems.

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Vol. 5, No. 03; 2021

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Vol. 5, No. 03; 2021

ISSN: 2581-3366

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Vol. 5, No. 03; 2021

ISSN: 2581-3366

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,
Bayaro 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

Vol. 5, No. 03; 2021

ISSN: 2581-3366

APPENDIX II

QUESTIONNAIRE

1. **Age**

a) < 18 b) 18-24 c) 25-34 d) 35-44 e) above 45

2. **Sex**
   a) Male b) Female
3. **Tribe**
   a) Hausa/ Fulani b) Igbo c) Yoruba d) Others
4. **Religion**
   a) Islam b) Christianity c) Others
5. **Educational qualification**
   a) None b) Primary c) Secondary d) Tertiary
6. **Number of hours of work**
   a) < 8 hours b) > 8 hours c) 0hours
7. **How long have you been doing this current work (with petroleum products)**
   a) < 1 year b) 1-3 years c) 3-5 years d) >5years e) 0 years
8. **Have you ever smoked cigarette?**
   a) Yes b) No
9. **Do you currently smoke cigarette?**
   a) Yes b) No
10. **How many cigarettes do you smoke a day?**

a) 1-5 b) 6-10 c)11-15 d) 16-20 e) above 20

11. **Have you ever been told that you have kidney problem?**

a) Yes b) No

12. **Have you ever been told you have liver problem?**
    a) Yes b) No