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ASSESSMENT OF THE EFFECT OF GASOLINE FUME ON STRESS HORMONES, ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN ALBINO RAT

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ABSTRACT

Gasoline fume has been considered a major air pollutant affecting the heart, lungs, brain, liver and kidneys. Therefore, this study aims at investigating the effect of inhalation exposure to gasoline fume on some endogenic stress hormones and oxidative enzymes of albino rats. Forty adult male albino rats were randomly assigned to five experimental treatments (T) with eight rats per treatment (T1, T2, T3, T4 and T5). The control treatment, T1 was housed in a section of experimental animal house free from gasoline fumes while T2, T3, T4 and T5 were exposed to gasoline fumes in exposure chambers for one, three, five and nine hours daily respectively for twelve weeks. The levels of Adrenocorticotropic hormone (ACTH), aldosterone and corticosterone were determined using Enzyme-Linked immunosorbent Assay (ELISA) kits. Concentrations of oxidative stress marker (GSH, CAT, MDA and BuChE) were assayed using standard method. Levels of ACTH were recorded to significantly reduce in the gasoline fume exposed rats when compared to control. Aldosterone and corticosterone significantly increase with increase in the daily period of gasoline fume exposure relative to the control. Values of ACTH negatively correlate with those of corticosterone and aldosterone in the exposed rats. The values of GSH, CAT and BuChE were significantly higher in the control rats and significantly reduce with increasing daily exposure time to gasoline fume. MDA concentration was lower in control rats but significantly increased with increasing daily exposure time to gasoline fume. Inhalation exposure to gasoline fume was observed to induce stress in the exposed animals.

Keywords: *Oxidative stress, gasoline fume, environmental pollution, adrenal hormones, inhalation*

1.0 INTRODUCTION

The volatile nature of petrol makes it readily available in the atmosphere any time it is dispensed, especially at petrol filling stations and depots. According to the Office of

Environmental Health Hazard Assessment (2014), gasoline fuel contains toxic substances that can enter the environment and cause adverse health effects in people. Some of these substances, such as benzene, toluene and xylenes, are found in crude oil and occur naturally in fuels and their vapours. Petrol contains mixture of volatile hydrocarbons and so inhalation is the most common form of exposure (Cecil et al. 1997). As reported by Takamiya et al. (2003), petrol vapour can reach supra-lethal concentrations especially in confined or poorly ventilated areas. Exposure to very high concentrations may result in rapid unconsciousness and death due to respiratory failure (Chilcott, 2007).

In Nigeria, there is an increase in the demand for petrol and other petroleum products which were used for various reasons at homes, in manufacturing and petrochemical industries. Some of these uses include fuel for vehicles, cooking and lighting fuel in homes and outside homes, as chemical feedstock for industries, therapeutic reasons (Hockabey et al. 1995) and as fuel for electricity generating machines at homes, offices and industries. This increasing daily use has increased the frequency at which individuals are exposed to its fume. Exposures to petroleum products both in and outside petroleum industries have been reported to have some effects on the users, with those who are occupationally exposed being more likely to be affected than their counterparts (Rothman et al. 1996; Carbello et al. 1994; Smith et al. 1993). Such effects include increased incidences of blood disorders and anaemia, higher cancer risk, renal function impairment and nephrotoxicity (Edokpolo et al. 2015; Riaz et al. 2014; Rothman et al. 1996; Festus et al. 2013).

Lipid peroxidation, the oxidative catabolism of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death, and has been implicated in diverse pathological conditions (Garcia-Souza and Oliveira, 2014; Maruyama et al. 2014). Also, superoxide dismutase (SOD) and catalase (CAT) were referred to as endogenous antioxidant enzymes that act as free-radical scavengers and hence prevent and repair damage done by reactive oxygen species (Wu et al. 2013; Wyse et al. 2004). The roles played by the hormones secreted by the adrenal cortex (cortisol/corticosterone and aldosterone) in the mediation of physiological stress have also been documented (Franklin et al. 2012; De Kloet and Rinne, 2007; Jacobson, 2005; Neil, 2004).

Organs such as the heart, lungs, skin and kidneys have been reported to be affected by the toxic effects of gasoline fume exposure, resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations (d'Azevedo et al. 1996; Smith et al. 1996; Rabble and Wong, 1996; Rothman et al. 1996). Specific effect of gasoline exposure on some organs of the body has been studied by several authors. Ahmed et al. (2011) reported that gasoline vapour inhalation induced lung tissue injury and cellular damage, increasing the activities of antioxidant enzymes such as glutathione- S- transferase, glutathione peroxidase and glutathione reductase. Similarly, Elsayed et al. (2015) observed a damaging effect of gasoline exposure on the brain tissue, causing a significant reduction in the activities of antioxidant enzymes and an increase in lipid and protein oxidation levels in brain tissue. Exposure to gasoline has also been shown to demonstrate some toxicity to the liver, significantly increasing malonildiahydehyde concentration and hepatic enzymes (aspartate amino transferase (AST) and alanine amino transferase (ALT) activities) (Bokolo and Ligha, 2013; Uboh et al. 2005). Exposure to petroleum hydrocarbon was also linked with renal dysfunction via oxidative stress, increasing lipid peroxidation and reducing the antioxidant defence mechanism (Oyebisi et al. 2013). Uboh et al. (2013) therefore concluded that exposure to diesel and gasoline may be a risk factor for nephrotoxicity.

Odewabi et al. (2014) evaluated the effect of petroleum fume exposure on the plasma antioxidant defence system using human subjects (petrol attendants). Enhanced lipid peroxidation was observed in the petrol attendants when compared with control subjects. Similarly, a decrease in the antioxidant defence system (oxidative enzymes) was recorded the blood of the petrol attendants. However, there is still paucity of information on the effect of different exposure time to gasoline fume on the antioxidant system of the blood. Also, previous studies on the stress effect of gasoline exposure have focused more on oxidative stress response in some specific organs of the body such as the lungs (Ahmed et al., 2011), liver (Bokolo and Ligha, 2013) and kidney (Azeez et al., 2013). In order to provide in-depth information on the status of stress-induction by exposure to gasoline fume, there is the need to study its effect on the hypothalamic-pituitary-adrenal (HPA) hormones which have also been reported to be involved in stress mediation (Neil, 2004; Jessica et al. 2006; Johnson and Grippo, 2006). Hence, the contribution of endogenic stress response hormones together with oxidative enzymes to the physiological effects of gasoline fume exposure needed to be evaluated, especially in the body circulating fluid. Therefore, this present study aims at evaluating the effect of varying daily period of gasoline fume exposure on the levels of stress mediated hormones; adrenocorticotropic hormone (ACTH), aldosterone and corticosterone; and oxidative enzymes; reduced glutathione (GSH), CAT, malondialdehyde (MDA) and butyryl-cholinesterase (BuChE) in the blood of Albino rats.

1.1 MATERIALS AND METHODS

1.1.1 Experimental Animal

Forty adult male albino rats aged 9 to 10 weeks (200 to 250g) were obtained from the breeding section of the animal house of the Department of Zoology, Olabisi Onabanjo University, Ago-Iwoye Nigeria and used for this study. The rats were divided into five groups (four test groups and a control group) consisting of eight rats each (Uboh et al. 2005; Uboh et al. 2007). The test groups were exposed to varying daily hourly rates of 1 (T2), 3 (T3), 5 (T4) and 9 hours (T5) of gasoline fumes in exposure chambers while the control group were housed in a gasoline fume free section of the animal house. The rats were individually kept in wooden cages (65cm × 35cm × 50cm) and housed in well ventilated animal house with free access to food and clean water throughout the 12 weeks period of the experiment.

Exposure to gasoline fume

The method of exposure earlier described by Uboh et al. (2005; Uboh et al. 2007) was adopted for this study. The animal cages housing the test groups were placed in an exposure chamber measuring 165cm x 95cm × 220cm. Two calibrated 1000ml cans containing 500ml of gasoline were placed in the chamber one hour prior to the commencement of the exposure to ensure that the exposure chamber was saturated with gasoline vapour. The exposed animals were later placed in the chamber and allowed to inhale the vapours generated from the direct evaporation of liquid gasoline from the cans at ambient humidity and temperature. At the end of each daily exposure period, the rats were removed from the exposure chamber. Exposure period of 1 (T2), 3 (T3), 5 (T4) and 9 hours (T5) daily was adopted for 12 weeks. The care of the animals was done in accordance with the U.S. public health service guidelines (National Research Council, 2011).

1.1.2 Sample collection

At the end of the experimental period, the animals were anaesthetized with chloroform and dissected for the collection of blood samples. Blood samples were collected 24 hours after the last exposure in baseline conditions between 8:00 a.m. and 10:00 a.m. by cardiac puncture using a 5ml hypodermic syringe and needle.

The blood samples were collected in a plain bottle to obtain the serum which was centrifuged at 2500 ×g for 10 min and the obtained sera samples were used for hormonal assay and oxidative enzyme activities estimation.

1.1.2.1 Stress hormonal assay

Serum samples obtained were analysed with commercially available ELISA kits for aldosterone, corticosterone (Enzo life sciences, PA) and ACTH (Eagle Biosciences Inc., Nashua). The sensitivity of aldosterone and corticosterone assay was 4.9 pg/mL and 0.027 ng/mL respectively with detection range of 3.9-250 pg/mL and 0.03-20 ng/mL respectively, while the limit of detection of ACTH assay was 0.4 pg/mL with detection range of 7.6-416 pg/mL. The protocols and procedures used for the assay were as described by the manufacturer.

1.1.2.2 Assay of oxidative stress marker

Catalase activity was estimated following the method of Aebi (1984). The method of Okhawa et al. (1979) was adopted for the estimation of lipid peroxidation. The rapid colorimetric method of Ellman et al. (1961) was also adopted in determining the activity of plasma cholinesterase or BuChE while the improved method of Beutler et al. (1963) was followed for the estimation of reduced glutathione activity.

1.2 DATA ANALYSIS

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0. Data were presented as Mean ± Standard error of mean (SEM). One way Analysis of Variance (ANOVA) was conducted to determine significant difference between parameters. Post hoc test was done using the Student-Newman-Keuls (SNK). P value less than 0.05 ($P < 0.05$) or 95% confidence interval was considered statistically significant. Correlation between the hormones and enzymes was also done using the Pearson correlation.

1.3 RESULTS

The concentrations of corticosterone, aldosterone, and ACTH recorded in the rats subjected to varying concentrations of gasoline fume exposure daily for 12 weeks is presented in Table 1. The concentration of ACTH was observed to significantly reduce ($P < 0.05$) in the gasoline fume exposed rats when compared with the control. On the other hand, the mean values of aldosterone and corticosterone in the rats subjected to the varying concentrations of gasoline fume exposure was observed to significantly increase ($P < 0.05$) with increasing concentrations of daily exposure to gasoline fume compared with the control. T5 had the highest level of aldosterone and corticosterone. However, there was no significant difference ($P > 0.05$) observed in the values of corticosterone in T1 and T2.

The levels of oxidative parameters recorded in the blood samples of rats daily exposed to gasoline fume at varying time duration is represented in Table 2. The values of GSH, CAT

and BuChE were significantly higher ($P < 0.05$) in T1 as compared to T2, T3, T4 and T5. The values of GSH, CAT and BuChE were observed to be significantly reducing ($P < 0.05$) with increasing daily exposure time to gasoline fume. On the other hand, the value of MDA was observed to be significantly lower in T1. This value was observed to increase significantly ($P < 0.05$) with increasing daily exposure time to gasoline fume.

Significantly strong positive correlation ($P < 0.01$) exists between the values of oxidative stress enzymes (GSH, CAT) and BuChE in the experimental rats (Table 3). However, the relationship between the values of these oxidative enzymes and lipid peroxidation (MDA) were significantly negative ($P < 0.01$). A strong positive significant ($P < 0.001$) relationship was observed between corticosterone and aldosterone concentrations recorded in the experimental rats. On the other hand, the ACTH relationship with corticosterone and aldosterone was observed to show a negatively strong ($P < 0.001$) correlation (Table 3).

Table 1: Serum concentrations of corticosterone, aldosterone and ACTH in rats subjected to varying concentrations of gasoline fume exposures for 12 weeks

	Corticosterone (ng/ml)	**Aldosterone (pg/ml)**	**ACTH (pg/ml)**
T1(Control)	5.52 ± 0.41^{d}	22.59 ± 0.70^{e}	72.90 ± 0.14^{a}
T2	5.82 ± 0.14^{d}	30.08 ± 0.77^{d}	72.21 ± 0.22^{a}
T3	9.35 ± 0.64^{c}	34.13 ± 0.24^{c}	71.51 ± 0.46^{ab}
T4	13.54 ± 0.71^{b}	37.73 ± 1.78^{b}	70.55 ± 0.53^{bc}
T5	18.11 ± 0.49^{a}	43.99 ± 0.74^{a}	69.69 ± 0.58^{c}

^{abcd}Mean values (±Standard error) of each of the enzymes in the same column having the same superscript are not significantly different at $P < 0.05$.

Table 2: Serum levels of oxidative parameters in rats subjected to varying concentrations of gasoline fume exposures for 12 weeks

	GSH (mg/dL)	**CAT (U/L)**	**BuChE (U/ L)**	**MDA (nmol/mL)**
T1(Control)	21.42 ± 0.40^{a}	501.70 ± 0.76^{a}	401.22 ± 0.56^{a}	22.74 ± 0.38^{e}
T2	19.72 ± 0.27^{b}	460.77 ± 1.80^{b}	392.72 ± 0.88^{b}	27.58 ± 1.21^{d}
T3	18.18 ± 0.54^{c}	412.56 ± 1.35^{c}	321.09 ± 0.61^{c}	36.22 ± 0.37^{c}
T4	16.98 ± 0.47^{c}	383.31 ± 2.11^{d}	299.10 ± 1.16^{d}	44.36 ± 0.58^{b}
T5	13.50 ± 0.20^{d}	306.51 ± 5.87^{e}	260.13 ± 2.11^{e}	63.87 ± 2.64^{a}

^{abcde}Mean (± Standard error) with same superscript in the same column are not significantly different ($P > 0.05$)

Table 3: Relationship between oxidative stress parameters and hormones in rats subjected to varying concentrations of gasoline fume exposures for 12 weeks

	GSH	CAT	BuChE	MDA	Corticosterone	Aldosterone	ACTH
GSH	1						
CAT	0.971**	1					
BuChE	0.935**	0.970**	1				
MDA	-0.976**	-0.972**	-0.941**	1			
Corticosterone	-0.051	-0.102	-0.24	-0.001	1		
Aldosterone	0.28	0.236	0.097	-0.324	0.923**	1	
ACTH	-0.167	-0.071	0.06	0.191	-0.814**	-0.862**	1

** Correlation (Pearson) is significant at the 0.01 level (2-tailed).

1.4 DISCUSSION

Exposure to gasoline fume significantly reduced the levels of antioxidant enzymes such as GSH and CAT in the experimental rats. Reactive oxygen species (ROS) are generated from molecular oxygen/nitrogen through Electron Transport Chain (ETC), cytochrome P450, and other cellular and sub-cellular functions (Noori, 2012). As reported by Damien et al. (2004), ROS which includes the superoxide radical ($^{\bullet}O^{2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) affect mainly lipids, proteins, carbohydrates and nucleic acid. In most cases, the abnormal generation of ROS, which results in significant damage to cell structure, is considered an important signal of oxidative damage (Barzilai and Yamamoto, 2004). Antioxidant enzymes have been reported to play major primary antioxidant defence roles in catalyzing the dismutation of superoxide radical (O^{2-}) to H_2O_2 and decomposition of H_2O_2 to H_2O, respectively (Cheng et al. 1981; Chelikani et al. 2004; Goodsell, 2010). Oxidative stress occurs when the presence of ROS in excess of the available antioxidant buffering capacity (Adly, 2010). The depletion of antioxidant enzymes predisposes the cell to the toxic actions of xenobiotics which could lead to cell injury or death. Hence, daily exposure to gasoline fumes has the potential to produce oxidative stress through the depletion of cellular activities of antioxidant defences in the body. Odewabi et al. (2014) also recorded significantly lower plasma levels of antioxidant enzymes in petrol attendants when compared with the control.

Although the exact physiological function of BuChE is unclear, it has been reported to substitute acetylcholinesterase in maintaining the structural and functional integrity of cholinergic pathways (Nordberg et al. 2013). It has also been shown that acetylcholine has a potential neuroprotective role as a scavenger of superoxide anion and is able to reduce lipid peroxidation, which suggests that a decrease in acetylcholine levels (or its BuChE substitute) could result in a decrease in neuroprotection and can lead to neurodegeneration (Bai et al. 2014). In this study, BuChE significantly reduced with increasing concentration of gasoline fume exposure. It could therefore be assumed that the toxic effect of gasoline fume is targeted at destroying protective enzymes in order to compromise normal physiological function of the body organs. This could also explain the reduction in antioxidant enzymes recorded in the gasoline exposed rats.

Environmental pollutants and petrol fumes have been identified as factors which can enhance peroxidative processes and oxidative stress within cells (Wright and Welbourne, 2002). As explained by Odewabi et al. (2014), lipid peroxidation results from release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes to form malondialdehyde. In this present study, the level of MDA significantly increased with increase in the concentration of gasoline fume exposure. This is in agreement with previous studies who affirmed that exposure to petroleum products including gasoline leads to increase lipid peroxidation (MDA) in rats (Bokolo and Ligha, 2013; Oyebisi et al. 2016; Uboh et al. 2013) and in human (Odewabi et al., 2014). Therefore, exposures to gasoline fume have a resultant lipid peroxidation effect, and lipid peroxidation is one of the known mechanisms of free radical generation in the biological systems (Uboh et al. 2013). This oxidative stress could have resulted from the build-up of ROS following the decrease in the activities of the enzymatic antioxidants (GSH and CAT) in the gasoline fume exposed rats.

Significant negative correlation was observed to exist between MDA and the oxidative enzymes. This was revealed in the higher lipid peroxidation which was observed in the gasoline fume exposed rats than the control and the decrease in the antioxidant enzymes (GSH and CAT) in the blood of the exposed rats than the control. Glutathione protects cells from the free radicals produced through oxidation (Pastore et al. 2003) while CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al. 2004). Several reports have also shown that inverse relationship exists between lipid peroxidation and glutathione activities during stress (Odewabi et al. 2014; Hill and Singal, 1996; Singal et al. 1993). McCord (1996) affirmed that under acute oxidative stress, the toxic effects of the pollutants may overwhelm the antioxidant defences. Gasoline fume could therefore exact acute oxidative stress on the physiology of the exposed rats, overwhelming their antioxidant defensive system.

The level of corticosterone was observed to significantly increase with increased daily gasoline fume exposure time in the experimental rats. Corticosterone (cortisol in human) is a stress related hormone secreted by the adrenal cortex and called glucocorticoid because of its effects on glucose metabolism. The roles of glucocorticoid have been associated to the breaking down of protein and converting it to glucose, making fats available for energy, increasing blood flow and stimulating behavioural responsiveness (Neil, 2004). Increased secretion of corticosterone (or cortisol) due to stress have been well documented (Franklin et al. 2012; De Kloet and Rinne, 2007; Jacobson, 2005). Rapid secretion of cortisol (corticosterone) was reported as the physiological response to acute stress (Sapolsky 1992). Increased and prolonged cortisol (corticosterone) in the body has also been reported to have negative effects on the brain. Such effects include hippocampal dysfunction which results in deficits in declarative memory function, reduced neural survival and plasticity, and promotion of inflammatory cascades (Franklin et al. 2012; Bremner, 1999; Diamond et al. 1999; Mesches et al. 1999). The hormone tends to destroy the neurons by decreasing the entry of glucose and decreasing the re-uptake of glutamate (Sapolskey, 1995; McEwen and Sapolskey, 1995; Sapolskey, 1992). Other effects of increased prolonged exposure to this hormone as reported by Neil (2004) includes increased blood pressure, damage to muscle tissue, steroid diabetes, infertility, inhibition to growth, inhibition of the inflammatory responses and suppressing the immune system. With the ability of daily gasoline fume exposure to increase the blood level of corticosterone as observed in this study, it is therefore imperative to avoid or minimize exposures to gasoline fume inhalation through the use of appropriate nose guide.

The levels of corticosterone and aldosterone were observed to be significantly positively correlated in the experimental rats. Hence, aldosterone level also increased in the rats with increased daily exposure to gasoline fume. The role of aldosterone in the body was explained in the area of maintaining the homeostasis of extracellular fluid (Miller and Harley, 1996). However, high levels of aldosterone can cause high blood pressure, muscle cramps and weakness (Yang and Ma, 2009). Also, Antonov et al. (2011) observed a pronounced stress-induced elevation of aldosterone secretion in rats and described it as an indication of the important contribution of aldosterone to the pathogenesis of stress dependent arterial hypertension. Higher level of aldosterone recorded in the gasoline fume exposed rats when compared with the control is therefore an indication of physiological stress which could lead to other physiological disorders.

ACTH is actively involved in the stimulation of the adrenal cortex of the adrenal gland to release cortisol (corticosterone) into the blood stream and also plays a minor role in the regulation of aldosterone production (Jovanovic et al. 2011; Yang and Ma, 2009). Similarly, Yang and Ma (2009) reported that ACTH stimulation test is sometimes used to stimulate the production of aldosterone along with cortisol to determine if primary or secondary adrenal insufficiency is present. The concentration of ACTH in the experimental rats as observed in this study significantly reduced with varying hours of gasoline fume exposure while corticosterone and aldosterone significantly increased when compared with the control. Also, the correlation between ACTH, corticisterone and aldosterone in gasoline fume exposed rats as recorded in this study were significantly negative. It is therefore possible that gasoline fume acts directly on the adrenal, increasing the levels of corticosterone and aldosterone while suppressing the activity of ACTH.

1.4.1 Conclusion

Based on the results of this study, exposure to gasoline fume may be harmful to the normal body physiology by increasing serum lipid peroxidation, corticosterone and aldosterone levels while reducing the activities of antioxidant enzymes such as GSH, CAT and BuChE. It is therefore imperative for individuals, especially people whose daily activities predisposes them to gasoline fume to be well enlightened and equipped against its inhalation.

1.5 ACKNOWLEDGEMENT

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1.6 CONFLICT OF INTEREST

The authors declare that, there are no conflicts of interest in this research.

1.7 DESIGNATION

OFO conceived the experiment. OFO and DGA designed and performed experiment.

BJA analyzed the data statistically. OFO drafted the article and critically revised by DGA, AAA and OWE. All authors gave final approval.

REFERENCES

Adly, A.A., 2010. Oxidative stress and disease: An updated review. Res. J. Immunol. 3, 129‑45.

Aebi, H., 1984. Catalse *in vitr* Methods, *Enzymol.* 105, 121-126

Ahmed, R. E., Nahed H.A., Nagui H. F., Hoda G. H. and Mabrouka A. A. (2011). Gasoline inhalation induces perturbation in the rat lung antioxidant defense system and tissue structure. International Journal of Environmental Science and Engineering (IJESE), 1, 1-14

Antonov, E.V., Markel, A.L., Yakobson, G.S., 2011. Aldosterone and stress-dependent arterial hypertension. Bulletin of Experimental Biology and Medicine. 152 (2), 188-191

Bai, H., Liu, R., Chen, H.L., Zhang, W., Wang, X., Zhang, X.D., 2014. Enhanced antioxidant effect of caffeic acid phenethyl ester and Trolox in combination against radiation induced-oxidative stress. Chem Biol Interact. 207, 7-15.

Barzilai, A., Yamamoto, K.I., 2004. DNA damage responses to oxidative stress, DNA Repair 3, 1109–1115.

Beutler, E.D., Duron, O., Kelly, M.B., 1963. Improved method for the determination of blood glutathione. J. Lab and Clin. Med. 61, 882-888.

Bokolo, B., Ligha, A.E., 2013. Antioxidative Effect of Zinc on Gasoline Induced Hepatotoxicity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 4 (3), 43 – 52

Bremner, J. D., 1999. Does stress damage the brain? Biological Psychiatry. 45(7), 797-805.

Carbello, M. A., Nigro, M. L., Fraga, I., Gadano, A., 1994. Ethylene oxide: cytogenic and biochemical studies in persons occupationally exposed. Environ. Mol. Mutagenesis. 23 (23), 7.

Cecil, R., Ellison, R.J., Larnimaa, K., Margary, S.A., Mata, J.M., Morcillo, L., Muller, J.M., Peterson, D.R., Short, D., 1997. Exposure profile: Gasoline. CONCAWE Report No. 97/52

Chelikani, P., Fita, I., Loewen, P.C., 2004. Diversity of structures and properties among catalases Cell. Mol. Life Sci. 61 (2), 192–208.

Cheng, L., Kellogg, E.W., Packer, L., 1981. Photo-inactivation of catalase. Photochem. Photobiol. 34, 124-129.

Chilcott, R.P., 2007. Petrol Toxicological Overview. Health Protection Agency Version 2, pp: 1-16.

Christian, M., Jean-Marie, M., Bernard, S., Claude, D., 1998. Myocardial Production of Aldosterone and Corticosterone in the Rat. The Journal of Biological Chemistry. 273 (9), 4883–4891

d'Azevedo, P. A., Tannhauser, A. L., Tannhauser, S. L., 1996. Haematological alternations in rats from xylene and benzene Vet. Human Toxicol. 38 (5), 340-344.

Damien, C., Chantal, V. H., Pirouz, S., Zerimech, F. H., Laurence, J., Jean, M. H., 2004. Cellular impact of metal trace elements in terricolous lichen Diploschistes muscorum (Scop.) R. Sant. –identification of oxidative stress biomarkers. Water Air and Soil Pollution. 152, 55-69.

De Kloet, E. R., Rinne, T., 2007. Neuroendocrine markers of early trauma: Implications for posttraumatic stress disorder. In E. Vermetten, M.J. Dorahy, & D. Spiegel (Eds.), Traumatic dissociation: Neurobiology and treatment. Washington, DC: American Psychiatric Press.

Diamond, D.M., Park, C.R., Heman, K.L., Rose, G.M., 1999. Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus. 9, 542-552

Edokpolo B., Yu, Q.J., Connell, D., 2015. Health Risk Assessment for Exposure to Benzene in Petroleum Refinery Environments. Int. J. Environ. Res. Public Health. 12, 595-610

Ellman, G.L., Coutney, K.D., Anders, V.J.R., Feartherstone, R.M., 1961. A new rapid colorimetric determination of acetyl cholinesterase activity, Biochem. Pharmacol. 7, 88-95

Festus, O.O., Dada, F.L., Iweka, F.K., Eyaufe, A.O., Osagie, R.N., Osagie, E.V., Akiyang, E. E., Fan-Osuala, C., 2013. Plasma renal functions amongst 'petrol station' attendants in owerri, south-east Nigeria. International Journal of Community Research. 2(2), 34-38.

Franklin, T. B., Saab, B. J., Mansuy, I. M., 2012. Neural mechanisms of stress resilience and vulnerability. Neuron. 75(5), 747-761.

Garcia-Souza, LF., Oliveira, M.F., 2014. Mitochondria: biological roles in platelet physiology and pathology. Int J Biochem Cell Biol. 50, 156- 60.

Goodsell, D.S., 2010. Catalase Molecule of the Month. Methods Mol Biol. 648, 269-77.

Hill, M.F., Singal, P.K., 1996. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infraction in rats. Am. J. Pathol. 148, 291-293.

Huckabay, P., Wendy, D., VanCleave, C., Ostrander, J., 1995. Petroleum sector notebook paper. Cameron University. J. Appl. Sci. Environ. Mgt. 6, 84 – 86.

Jacobson, L., 2005. Hypothalamic-pituitary-adrenocortical axis regulation. Endocrinol Metab Clin N Am 34, 271–292.

Jessica, L. M., Wendy, S., Fernando, R. G., Enrico, L. R., Daniel, R. J., Theodore, G., 2006. Baseline and Stress-Induced Plasma Corticosterone Concentrations of Mice Selectively Bred for High Voluntary Wheel Running. Physiological and Biochemical Zoology. 80(1), 146–156.

Johnson, A.K., Grippo, A.J., 2006. Sadness and broken hearts: neurohumoral mechanisms and co-morbidity of ischemic heart disease and psychological depression. J Physiol Pharmacol. 57 (Suppl 11), 5–29.

Jovanovic, T., Phifer, J. E., Sicking, K., Weiss, T., Norrholm, S. D., Bradley, B., Ressler, K. J., 2011. Cortisol suppression by dexamethasone reduces exaggerated fear responses in posttraumatic stress disorder. Psychoneuroendocrinology. 36, 1540-1552.

Maruyama, W., Shaomoto-Nagai, M., Kato, Y., Hisaka, S., Osawa, T., Naoi, M., 2014. Role of lipid peroxide in the neurodegenerative disorders. Subcell Biochem. 77, 127-36.

McCord, J.M., 1996. Effects of positive iron status at a cellular level. Nutr. Rev. 54, 85-88.

McEwen, B.S., Sapolskey, R.M., 1995. Stress and cognitive function. Current Biology. 5, 205-216

Mesches, M.H., Fleshner, M., Heman, K.L., Rose, G.M., Diamond, D., 1999. Exposing rats to a predator blocks primed burst potentiation in the hippocampus *in vitro*. Journal of Neuroscience. 19 (8), 1-5

Miller, A.M., Harley, J.P., 1996. Zoology. Wm. C. Brown Publishers, USA

National Research Council, 2011. Guide for the care and use of laboratory animals. 8th ed. Washington DC, National Academy Press, 41-81

Neil, R.C., 2004. Physiology of behaviour. Pearson Education Inc. U.S.A

Noori, S., 2012. An Overview of Oxidative Stress and Antioxidant Defensive System. Open Access Scientific Reports. 1(8), 1-9.

Nordberg, A., Ballard, C., Bullock, R., Darreh-Shori, T., Somogyi, M., 2013. A review of butyrylcholinesterase as a therapeutic target in the treatment of Alzheimer's disease. Prim Care Companion CNS Disord. 15(2).

Odewabi, A.O., Ogundahunsi, O.A., Oyalowo M., 2014. Effect of Exposure to Petroleum Fumes on Plasma Antioxidant Defense System in Petrol Attendants. British Journal of Pharmacology and Toxicology. 5(2), 83-87

Office of Environmental Health Hazard Assessment (OEHHA), 2014. Fuels and your health. Facts sheet of The California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) and The American Lung Association of California (ALAC). Sacramento, California

Okhawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95, 351-358.

Oyebisi, M. A., Roland, E. A., Chikodi N. A. (2013). Oxidative status in rat kidney exposed to petroleum hydrocarbons. Journal of Natural Science, Biology and Medicine, 4(1), 149-154

Pastore, A., Piemonte, F., Locatelli, M., Russo, A.L., Gaeta, L.M., Tozzi, G., Federici, G., 2003. Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. Clinical Chemistry. 47 (8), 1467–9.

Rabble, G. K., Wong, O., 1996. Leukemia mortality by cell type in petroleum workers with potential exposure to benzene. Environ. Health Perspect. 104, 1381 – 1392.

Riaz, M.A., Ijaz, B., Riaz, A., 2014. Overview of Occupational Exposure to Petroleum Derivatives and Risk of Anemia in Petrol Station Workers. Greener Journal of Environmental Management and Public Safety. 3 (1), 21-25

Rothman, N., Li, G. L., Dosemeci, M., Bechtold, W. E., Marti G. E., Wang, Y. Z., 1996. Haematotoxicity among Chinese Workers-heavily exposed to benzene from Am, J. ind. Med. 29 (3), 236-246.

Sapolskey, R., 1992. Stress, the ageing brain and the mechanisms of neuron death. MIT Press; Cambridge

Sapolskey, R.M., 1995. Social subordinance as a marker of hypercortisolism: Some unexpected subtleties. Annals of the New York Academy of Science. 771, 626-639

Singal, P.K., Dhalla, A.K., Hil, M., Thomas, T.P., 1993. Endogenesis antioxidant changes in the myocardium in response to acute and chronic stress conditions. Mol. Cell Biochem. 429, 179-186.

Smith, J. H., Mallet, A. K., Brantom, P. G., 1996. Ninety days feeding study in Fischer – 344 rats of highly refined petroleum-derived food grade white oils and waves. Toxicol Pathol. 24, 214-230.

Smith, T. J., Hammand, S. K., Wond, O., 1993. Health effect of gasoline exposure: Exposure assessment for US Distributions workers. Environ health perspectives. 101, 13-21.

Takamiya, M., H. Niitsu, K., Saigusa, J., Kanetake, Y., 2003. A case of acute gasoline intoxication at the scene of washing a petrol tank. Leg Med. 5, 165-169.

Uboh, F.E., Akpanabiatu, M.I., Atangwho, I.J., Ebong, P.E., Umoh, I.B., 2007. Effect of gasoline vapours on serum lipid profile and oxidative stress in hepatocyte of male and female rats. Acta Toxicol. 15, 13-18.

Uboh, F.E., Akpanabiatu, M.I., Eyong, U. E., Ebong, P.E., Eka, O.O., 2005. Evaluation of toxicological implications of inhalation exposure to kerosene fumes and petrol fumes in rats. Acta Biologica Szegediensis. 49(3-4), 19-22

Uboh, F.E., Ufot, S.U., Eyong, E.U., 2013. Comparative Effect of Withdrawal from Exposure on Gasoline and Diesel Induced Nephrotoxicity in Male Albino Wistar Rats. J. Clin. Toxicol. 3, 170. doi:10.4172/2161-0495.1000170

Wright, D.A., Welbourne, P., 2002. Environmental toxicology. Cambridge University Press, Cambridge.

Wu, J.Q., Kosten, T.R., Zhang, X.Y., 2013. Free radicals, antioxidant defense systems, and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 46, 200-6.

Wyse, A.T., Stefanello, F.M., Chiarani, F., Delwing, D., Wannmacher, C.M., Wajner, M., 2004. Arginine administration decreases cerebral cortex acetylcholinesterase and serum butyrylcholinesterase probably by oxidative stress induction. Neurochem Res. 29(2), 385-9.

Yang, Y., Ma, H., 2009. Aldosterone. Researcher. 1(5), 89-93