

Powdered Oil Palm (*Elaeis guineensis* Jacq) Leaf as Remedy for Hydrocarbon induced Liver Damage in Rats

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ABSTRACT

Humans are faced with a number of diseases and chemical toxicity due to sustained pollution of environment. One of the management strategies adopted in recent times is the use of herbs. This is the driving force for this study. Ninety six female rats were mobilized for this study and were divided into six groups of sixteen rats each. Rats in group A served as control and were fed with diet devoid of any treatment while rats in groups B and C were fed with diets treated with varying amounts of ground *Elaeis guineensis* leaf. Rats in group D were fed with hydrocarbon adulterated diet. Rats in groups E and F were fed with adulterated diet mixed with the same amounts of ground *Elaeis guineensis* leaf as in groups B and C. Oxidative stress indicators and histological analysis were carried out on the rat's liver after exposure period of three and six months respectively. The results show that pretreatment of hydrocarbon adulterated diet with *Elaeis guineensis* leaf normalized values of lipid peroxidation (LP), superoxide dismutase activity (SOD), catalase activity (CAT) and xanthine oxidase activity (XO) relative to control values. Histological analyses indicate protective influence of *Elaeis guineensis* leaf against harmful consequence of hydrocarbon on the liver. It is apparent that the leaves of *Elaeis guineensis* could be used in the management of hydrocarbon linked liver damage.

Keywords: Antioxidants, Enzymes, Hydrocarbon, Liver, Oil palm

INTRODUCTION

Crude oil comprises of different constituents of hydrocarbons, metals, and nonmetallic substances (Azeez *et al.*, 2013; Almeda 2013a); and when refined, different fractions with household and industrial importance are produced (Achuba, 2015). This is why these fractions are in constant contact with man (Azeez *et al.*, 2013). The increase in the domestic and industrial utilization of products of crude oil due to urbanization and upsurge in human population has imposed its attendant hazardous effects on humans (Uboh *et al.*, 2013). In certain cases, petroleum find itself into the food chain and gradually builds up its negative effects in the tissues which culminates in tissue/organ damage (Achuba and Ogwumu, 2014; Achuba and Nwokogba, 2015). Oil palm (*Elaeis guineensis*) is said to have originated from West Africa with its commercial value being mainly in its oil (Obahiagbon, 2012). The oil palm tree is a major source of vegetable oil for human consumption (Rosalina *et al.*, 2011). However, the oil palm leaf constitutes a major waste from the oil palm industry because the fleshy part is discarded after sweeping broom production (Anyanji *et al.*, 2013; Yin *et al.*, 2013). It's however said to be seriously underutilized as studies have confirmed its richness in highly beneficial phytochemicals and oils. The oil palm leaf is said to be rich in nontoxic phenolic compounds such as glycosylated flavonoids, alkaloids, saponins and gallic acid (Ravajel *et al.*, 2012; Ibraheem *et al.*, 2012; Vijayarathna, *et al.*,

2012). The use of the leaf extracts of oil palm in folk lore medicine has also been documented. It is said to have potency for diabetes (Ravajel *et al.*, 2012); wound healing properties (Sasidharan *et al.*, 2012; Rajoo *et al.*, 2013); anticancer and rheumatism (Yin *et al.*, 2013), headaches and as an aphrodisiac (Mohamed, 2014); liniment and diuretic (Jafri *et al.*, 2011a; 2011b); anti hyperlipidemia (Mohamed 2014; Abdul-Razak, 2009) and enhancement of cognitivity (Mohamed *et al.*, 2013). Hydrocarbon has been implicated in the induction of oxidative stress which is responsible for tissue damage in animals (Achuba and Osakwe, 2003; Achuba and Otuya, 2006; Azeez *et al.*, 2013; Nwaogu and Onyeze, 2014). Moreover, the medicinal advantages of oil palm tree is hinged on its high antioxidant potential (Chong, 2008; Rout, 2009; Yin *et al.*, 2013). At the moment, there is no record on the ameliorative properties of oil palm leaf on crude oil contaminated diets induced hepatotoxicity in rats; this is the need for the present study.

MATERIALS AND METHODS

The Nigeria National Petroleum Corporation (NNPC) Warri, Delta State, Nigeria supplied crude oil while oil palm leaf used was obtained from *Elaeis guineensis* tree in Site III of Delta State University, Delta state, Nigeria. Physical identification of the leaf was done by Dr. Erhenhi A.H, Head of Department of Botany, Delta state University, Abraka, Nigeria.

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Ninety six female albino wistar rats weighing between 178 g to 182 g were obtained from the Animal house of Department of Anatomy, Delta State University, Abraka, Nigeria. The rats were kept in a wooden cage and left to acclimatize for one week on grower's feed and tap water at laboratory temperature of 28° C and 12 hour day/ night regime.

Preparation of oil palm leaf powder.

The leaves of *Elaeis guineensis* were isolated from the stock and air-dried (28°C) in the laboratory. The dried leaf was then ground with domestic kitchen blender and sieved with a 2 mm mesh into a fine powder. The leaf powder was then stored in a clean sealed plastic container for subsequent use.

Feeding regimen of experimental rats

The ninety six female albino wistar rats were assigned to six groups according to their weights, which consist of eight rats in each group. Group A, the control and fed with grower's marsh only. Rats in Group B and C were fed with grower's marsh treated with 5g and 10 g of powdered *Elaeis guineensis* leaf respectively. Group D rats were fed with grower's marsh contaminated with crude oil (4ml per 100g of feed) (Achuba, 2018b). Rats in Group E and F were fed grower's marsh contaminated with crude oil (4ml per 100g of feed) plus 5g and 10 g of powdered palm leaf respectively. All the rats had free access to clean drinking water during the experiment. The feeds for each treatment were prepared daily and stale feed discarded. The feeding regime was between the hours of 8 am – 9 am daily. The National Institute of health guide for the care and use of laboratory animals was adopted in the course of the experiment (NIH, 1985). Tests were carried out on the animals after three and six months respectively.

Preparation of liver homogenate

After three months, eight rats were sacrificed in each group and the liver collected. One gram of the liver was weighed under chilled conditions and homogenized with 5ml of normal saline in a mortar. The mixture was diluted with 5 ml of buffered saline (pH 7.4) before it was subjected to centrifugation at 2,500 rpm and the supernatant was transferred into

plastic tubes and stored at – 4° C in the refrigerator before used for assay within forty eight hours. This same procedure was adopted after six months exposure period.

Determination of biochemical parameters and histological analysis

The method of Bergmeyer *et al.* (1974) was adopted in measuring the activity of xanthine oxidase. Lipid peroxidation was measured by the thiobarbituric acid reacting substances (TBARS) method of Gutteridge and Wilkins (1982). Total superoxide dismutase activity was assayed using the method of Misra and Fredorich (1972). Catalase was assayed as previously described by Rani *et al.* (2004). The histological study was performed according to Al-Attar *et al.* (2017).

Statistical Analysis

Analysis of variance (ANOVA) and post Hoc Fisher's test for multiple comparison were carried out using version 20 of statistical package for social science (SPSS) to determine statistical significant differences between means. P values <0.05 were taken as being significantly different

RESULTS

The incorporation of *Elaeis guineensis* leaf powder in the feeds of the experimental rats prevented lipid peroxidation and positively modulated activities of enzymes associated oxidative stress. After three and six months, rats fed with palm leaf pretreated diets, without crude oil (Groups 2 and 3) and rats fed with crude oil contaminated diets that was pretreated with various amounts of *Elaeis guineensis* leaf (Groups 5 and 6) had significant (P < 0.05) lower liver lipid peroxidation relative to rats fed with crude oil contaminated diet (group 4) (Tables 1 and 2). Similarly, Significant (P <0.05) decreases in oxidative stress marker enzymes stimulated by crude oil treated diet were raised by incorporating *Elaeis guineensis* leaf powder in the feeds (Tables 1 and 2).. Moreso, alteration of liver ultrastructure was corrected by feeding *Elaeis guineensis* leaf powder pretreated contaminated diet (Figure 1).

Table 1: Effect of oil palm leaf treatment of diet on hydrocarbon induced alterations in liver oxidative stress markers

Sample	Liver MDA (nmol/g tissue)	Liver superoxide dismutase activity (Unit/g tissue)	Liver catalase activity (Unit/g tissue)	Liver xanthine oxidase (Unit/g tissue)
Group A	0.30 ± 0.07 ^a	66.14 ± 2.80 ^a	40.80 ± 1.24 ^a	79.01 ± 2.40 ^a
Group B	0.23 ± 0.07 ^a	64.44 ± 1.63 ^a	43.04 ± 2.73 ^a	82.50 ± 1.77 ^b
Group C	0.15 ± 0.04 ^b	68.28 ± 3.42 ^a	45.43 ± 1.51 ^a	93.29 ± 8.54 ^c
Group D	0.87 ± 0.44 ^c	44.22 ± 2.91 ^b	32.43 ± 1.88 ^b	57.67 ± 13.13 ^d
Group E	0.72 ± 0.15 ^d	57.93 ± 2.42 ^c	38.55 ± 2.20 ^c	62.93 ± 2.61 ^d
Group F	0.50 ± 0.15 ^a	57.05 ± 5.89 ^c	37.53 ± 1.22 ^c	67.36 ± 4.93 ^d

The results are expressed as mean ± standard deviation with n = 6. Values not sharing a common superscript on the same column differ significantly (P <0.05)

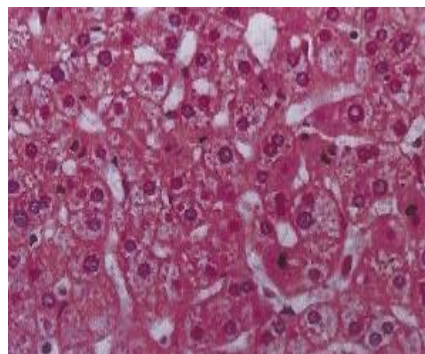
Group A: ((Control). Group B: feed mixed with 5.0g *Elaeis guineensis* leaf. Group C: feed mixed with 10.0g *Elaeis guineensis* leaf. Group D: Feed mixed with 4ml crude oil (Crude oil Control). Group E: Contaminated diet mixed with 5.0 g of *Elaeis guineensis* leaf. Group F: contaminated diet mixed with 10.0 g of *Elaeis guineensis* leaf

Table 2: The effect of *Elaeis guineensis* leaf on the level of oxidative stress indicators in the liver of rats after six months of exposure to crude oil contaminated diet..

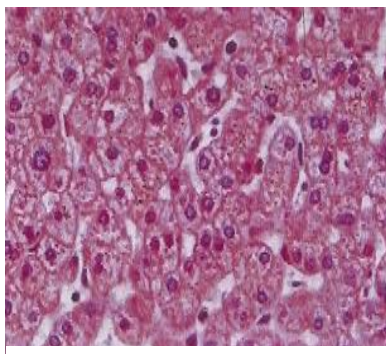
Groups	Lipid peroxidation (nmol/g tissue)	Liver SOD activity (units/g tissue)	Liver catalase activity (units/g tissue)	Liver xanthine oxidase activity (nmol/g tissue)
Group A	0.54± 0.15 ^a	62.14 ± 4.28 ^a	36.65 ± 1.25 ^a	54.53± 2.55 ^a
Group B	0.34 ± 0.11 ^b	61.43 ± 2.62 ^a	31.35 ± 1.52 ^a	56.33± 3.61 ^b
Group C	0.30 ± 0.13 ^b	64.22 ± 3.44 ^b	29.14 ± 1.48 ^b	57.12± 1.15 ^b
Group D	0.76 ± 0.15 ^c	42.33 ± 2.72 ^c	21.13 ± 1.16 ^c	46.42± 2.11 ^c
Group E	0.52 ± 0.11 ^d	51.54 ± 2.70 ^d	25.53 ± 1.80 ^d	49.44± 1.52 ^d
Group F	0.54 ± 0.12 ^a	57.25 ± 5.14 ^a	24.56 ± 1.43 ^a	50.33± 1.66 ^b

The results are expressed as mean ± standard deviation with n = 6. Values not sharing a common superscript on the same column differ significantly (P <0.05)

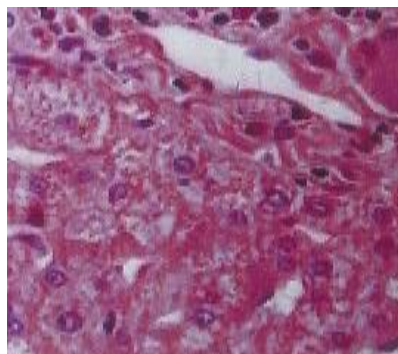
Group A: ((Control). Group B: feed mixed with 5.0g *Elaeis guineensis* leaf. Group C: feed mixed with 10.0g *Elaeis guineensis* leaf. Group D: Feed mixed with 4ml crude oil (Crude oil Control). Group E: Contaminated diet mixed with 5.0 g of *Elaeis guineensis* leaf. Group F: contaminated diet mixed with 10.0 g of *Elaeis guineensis* leaf



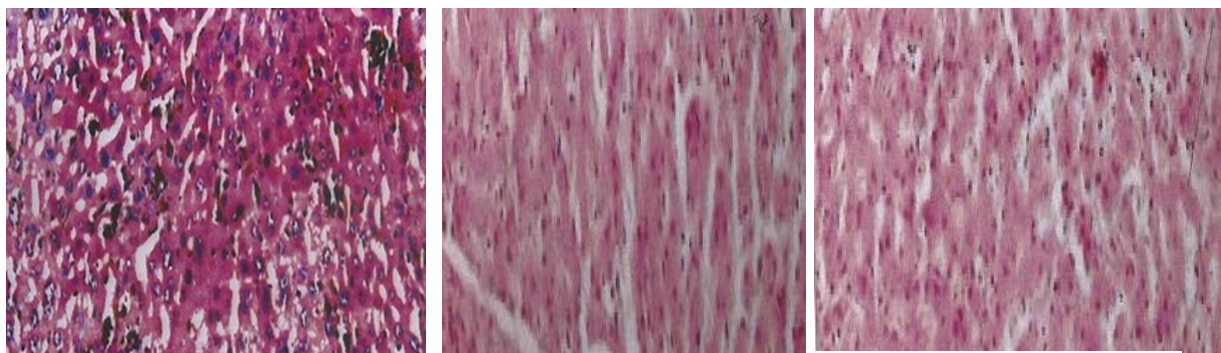
Group A: 100g feed, showing normal hepatic cells and central vein (CV) (H and E ×100)



Group B: 100g feed + 5g palm leaf, showing normal hepatic cells (H and E ×100)



Group C: 100 feed + 10g palm leaf, showing normal hepatic cells with large central vein (H and E ×100)



Group D: 100g feed + 4ml crude oil, showing degeneration of hepatic cells, necrosis (N) and Inflammation (H and E $\times 100$)

Group E: 100g feed and 4ml crude oil +5g palm leaf, showing slight reduction in necrosis and degeneration of hepatic cells (H and E $\times 100$)

Group F: 100g feed and 4ml crude oil +10g palm leaf, showing reduced necrosis and improvement in hepatocytes (H and E $\times 100$)

Figure 1: Photomicrographs of liver section of rats fed crude oil contaminated diet and diets pretreated with different levels of powdered *levels guineensis* leaf

DISCUSSION

Intake of hydrocarbon adulterated diet has been reported to cause a variety of metabolic and pathophysiological derangement in animals (Achuba and Osakwe, 2003; Achuba and Ogwumu, 2014b; Achuba and Nwokogba, 2015; Okpoghono *et al.*, 2018). This is the reason for the search for potential remedy for the negative health implication of hydrocarbon on animals and man (Ita *et al.*, 2016; Achuba, 2018a and b; Okpoghono *et al.*, 2018b). Lipid peroxidation products in rats open to hydrocarbon contaminated diet when compared to rats fed with normal diet was brought close to control values by oil palm leaf supplementation of diet (Table 1). This implies that supplementation of diet with oil palm leaf improved the antioxidative standing of hydrocarbon exposed animals. Previous report indicated that oil palm leaf improved metabolic and cellular integrity of the kidney of rats fed with hydrocarbon contaminated diet (Achuba, 2018a)..

Similarly, supplementation of diet with oil palm leaf improved the liver antioxidant standing (Table 2) as evidenced by the restoration of liver superoxide dismutase activity in hydrocarbon treated diet fed rats relative to the values in control rats. This observation is cogent because hydrocarbon had been linked with free radical generation in organs of animals (Achuba and Osakwe, 2003; Nwaogu and Onyeze, 2014). However, the protective ability of organic and inorganic materials with antioxidant properties against the harmful attributes of hydrocarbon had been reported (Achuba and Otuya, 2006; Achuba and Ahwin, 2008; Uboh *et al.*, 2009; Ita *et al.*, 2016, Achuba, 2018 a and b). This may be the basis why oil palm leaf, which has been reported to be rich in antioxidants, supplementation of diet attenuated hydrocarbon induced liver damage (Ravajel *et al.*, 2012; Ibraheem *et al.*, 2012; Vijayarathna, *et al.*, 2012).

Catalase, which acts synergistically with superoxide dismutase in removing super oxide anion from the system, has been reported as a measure of oxidative marker in animals (Achuba, 2018a). That oil palm leaf exhibited health promoting features is expressed in the positive alterations of hydrocarbon-induced changes in catalase activity. Hydrocarbon stimulated alteration in catalase activity has been documented (Achuba, 2018a). Oil palm leaf, bitter leaf, vitamins C and E and honey have been reported to reverse hydrocarbon induced changes in rats (Ita *et al.*, 2016, Achuba, 2018a and b). The potency of oil palm leaf is no surprise since hydrocarbon causes toxicity through the induction of free radicals (Achuba and Osakwe, 2003; Achuba, 2010); which is quenched by the antioxidant potentials of oil palm leaf.

Hydrocarbon can produce reactive anions such as singlet oxygen and peroxide radicals, that cause cellular toxicities in animals (Achuba and Osakwe, 2003; Achuba and Ogwumu, 2014a; Achuba and Nwokogba, 2015). Hence the inhibition of the liver enzymatic functions through production of reactive radicals that increased lipid peroxidation, which culminates in oxidative tissue damage, loss of membrane architecture/ functions and hepatic congestion due to hydrocarbon toxicity (Achuba and Osakwe 2003; Achuba, 2018; Okpoghono, *et al.*, 2018a). This is because antioxidant supplementation has been found to be beneficial in hydrocarbon toxicity and also oil palm leaf has been reported to have nephroprotective potentials (Achuba, 2018b). This may account for the hepatoprotective ability of oil palm leaf as exhibited by the histological study (Figure 1). Similar protective potentials of plants and plant extracts have been reported (Achuba *et al.*, 2016; Achuba, 2018a and b; Okpoghono *et al.*, 2018a and b).

Comparatively, lipid peroxidation, even in control rats increased while the activities of other oxidative stress marker enzymes decreased after six months relative to the levels in the rats at three months (Tables 1 and 2). This observation is in tandem with the report of Rikans *et al* (1991) which established a reciprocal relationship between lipid peroxidation and antioxidant levels during ageing in rat liver. However, sustained supplementation of diet with oil palm leaf reduced lipid peroxidation and improved the activities of oxidative marker enzymes in non-hydrocarbon exposed animals. It is very important to infer that intake of oil palm leaf has a protective role during age related oxidative insults. This protective attribute of oil palm leaf may be predicated on its rich antioxidants (Ravajel *et al.*, 2012; Ibraheem *et al.*, 2012; Vijayarathna, *et al.*, 2012).

The liver cytosol of various mammals contains xanthine oxidase which exhibits an oxidative activity towards many compounds both exogenous and endogenous which can be heterocyclic, aldehydes, nitrogen and sulphur containing substances (Ezedom and Asagba, 2016).

CONCLUSION

The data obtained from this study established that incorporation of powdered oil palm leaf in diet protect the liver against the toxic effect of crude oil and its intake should be encouraged among the inhabitants of hydrocarbon producing areas of the World.

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Therefore in a bid to increase the production of uric acid, one of the antioxidant in animals, there is a corresponding increase in endogenous xanthine oxidase activity (Achuba, 2008; Azeez *et. al.* 2013; Settle and Klandorf, 2014; Achuba, 2018a).The surge in the activity of xanthine oxidase in rats exposed to *Elaeis guineensis* leaf treated diet indicates response of the enzyme to enhance the metabolism of exogenous xanthine (Table 1). On the contrary, the decline in activity of the enzyme in rats fed with crude oil contaminated diet without any treatment implies that the metabolism of crude oil leads to a reduced ability of exposed animals to produce uric acid due to altered xanthine oxidase activity (Tables 1 and 2). Generally, alterations in oxidative enzymes activities depict measure of oxidative stress (Jaffri *et. al.*, 2011). However, pretreatment of crude oil contaminated with *Elaeis guineensis* leaf moved the value of xanthine oxidase activity close to the value in the control animals thereby enhancing the antioxidant status of the animal via uric acid production (Settle and Klandorf, 2014).

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