Protective Influence of *Elaesis Guineensis* Leaf in Diet on Petroleum-Mediated Kidney Damage in Rat

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ABSTRACT

Herbal medicine is gaining ground these days and the search for simple antidote for crude oil toxicity is ongoing. This was the reason for this study. The study was comprised ninety six female rats divided into six groups of sixteen rats each. Rats in control group were fed with diet without any treatment while rats in groups 2 and 3 were fed with diets treated with known amount of *Elaesis guineensis* leaf. Rats in group 4 were fed with crude oil contaminated diet. Rats in groups 5 and 6 were fed with contaminated diet mixed with known amount of ground *Elaesis guineensis* leaf. Biochemical and histological analysis were carried out after three and six months respectively. The results show that pretreatment of crude oil contaminated diet mixed with *Elaesis guineensis* leaf tend to restore values of lipid peroxidation, xanthine oxidase (XO) activity, superoxide dismutase (SOD) activity and catalase (CAT) activity close to control values. Histological examination indicates protective effect of Elaesis guineensis leaf against deleterious effect of crude oil on the kidney. It is obvious that the leaves of *Elaesis guineensis* could be used in the treatment of crude oil toxicity. And indeed setting a fresh agenda for further scientific investigations

Keywords: Catalase, Crude oil, Kidney, Lipid peroxidation, *Elaesis guineensis*, Superoxide dismutase .Xanthine oxidase, Nephrotoxicity

INTRODUCTION

Humans and animals get exposed to crude oil or its byproducts when these chemicals are released into the surroundings during oil exploration activities, equipment failures, corrosion, illegal bunkering, usage, oil theft and illicit refining (Ovuru and Ekweozor 2004; Otitoju *et. al.,* 2007; Ogudu and Esemuede, 2013). Crude oil stimulates oxidative stress in animals (Achuba and Osakwe, 2003; Anozie and Onwurah, 2001). Lipid peroxidation, xanthine oxidase (XO), superoxide dismutase (SOD) and catalase (CAT) activities are part of oxidative stress indices (Achuba, 2014). Lipid peroxidation elicits oxidative damage in plants and animals and its value in conjunction with alterations in the level of antioxidants represent a measure of oxidative stress. Similarly, the activity of XO is a defense mechanism as well as measure of oxidative stress in animals (Achuba, 2014). Report has it that the deleterious action of crude oil on the kidney is based on oxidative stress (Azeez *et. al.,* 2013).

Byproducts of the *Elaesis guineensis* tree are important medicinally. This is because the leaf juice has wound healing property while the sap is used as laxative (Sasidharan *et. al.,* 2012).This is due to compounds rich in medicinal and antioxidant properties inherent in *Elaesis guineensis* leaf (Chong, 2008; Rout, 2009). The antioxidant action is attributed to the presence of phytochemicals (flavonoid, tannin and phenols) in the leaves of *Elaesis guineensis* tree (Yin *et. al.,* 2013). In fact, *Elaesis guineensis* leaf extract contains more antioxidative phenolic compounds than various extracts of green tea (Runnie *et. al.,* 2003). Therefore, *Elaesis guineensis* leaf extract is a potential source of functional food ingredient, based on reports of its health benefit (Mohamed, 2014) .This study is aimed at evaluating the protective potentials of *Elaesis guineensis* leaf against crude oil contaminated diet induced nephrotoxicity in rats.

MATERIALS AND METHODS

The crude oil used for this study was obtained from Nigeria National Petroleum Corporation (NNPC) Warri, Delta State, Nigeria. The palm leaf used was obtained from *Elaeis guineensis* tree in Obiaruku, Delta state, Nigeria Ninety six female albino wistar rats with weights ranging from 178 g to 182 g obtained from the animal house of Department of Anatomy, Delta State University, Abraka, Nigeria were used for this study.

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The rats were housed in a standard wooden cage made up of wire gauze, net and solid woods and left to acclimatize for one week on grower's marsh and tap water at laboratory temperature of 28^{o} C and 12 hour day/ night regime. After the acclimatization period, the rats were weighed and grouped.

Preparation of leaf powder

The leaves of *Elaeis guineensis* were isolated from the stock and sun- dried. The dried leaf was then ground with domestic kitchen blender into a fine powder and stored in a clean and sealed plastic container

Treatment of animals

The ninety six female albino wistar rats were assigned to six groups according to their weights, with eight rats in each group. Rats in the control, Group 1 were fed with grower's marsh only. Rats in Group 2 were fed with grower's marsh treated with 5g of powdered *Elaeis guineensis* leaf. Group 3 rats were fed with grower's marsh treated 10g of powdered Elaeis *guineensis* leaf. Group 4 rats were fed with grower's marsh contaminated with crude oil (4ml per 100g of feed).This concentration of crude oil in diet was established to be tolerated by the rats over a long period in a preliminary study. Rats in Group 5 were fed grower's marsh contaminated with crude oil (4ml per 100g of feed) plus 5g of powdered palm fronds. While rats in Group 6 were fed with crude oil contaminated marsh (4ml per 100g of feed) plus 10g of powdered palm leaves. The rats in each group were allowed access to clean drinking water while the experiment lasted. The feeds were prepared fresh daily and stale feed remnants were discarded regularly. This was done every morning between the hours of 8 am – 9 am and each group provided with 400 g of the respective diet.. The animals in each group were exposed to their respective diets for three and six months respectively. The National Institute of health guide for the care and use of laboratory animals was adhered to in the course of the experiment (NIH, 1985).

Collection of samples

After three months, eight rats were sacrificed in each group and the kidneys collected. One gram of the kidneys were weighed in 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^{o} C in the refrigerator before used for analysis within forty eight hours. This same procedure was adopted after six months exposure period.

Determination of biochemical parameters and histological analysis

The activity of xanthine oxidase in the kidney of rats was measured using the method of Bergmeyer *et. al.* (1974), a reaction based on the oxidation of xanthine to uric acid, a molecule that absorbs light maximally at 290 nm. A unit of activity is that forming one micromole of uric acid per minute at 25^{o}C. Lipid peroxidation in the kidney of rats was measured by the thiobarbituric acid reacting substances (TBARS) method of Guttridge and Wilkins (1982).Total superoxide dismutase activity was assayed using the method of Misra and Fredorich (1972). Catalase was assayed as reported by Rani *et. al* (2004). The of method of Al-Attar *et. al*. (2017) was adopted for the histological study.

Statistical Analysis

Analysis of variance (ANOVA) and post Hoc Fisher's tests 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 effects of *Elaeis guineensis* leaf on kidney lipid peroxidation and activities of oxidative stress marker enzymes against crude oil induced nephrotoxicity in rats after three and six months are shown in tables 1 and 2. Lipid peroxidation in the kidney of rats exposed to crude oil contaminated diet (group 4) was significantly (P<0.05) higher in comparison with the control (group 1). Rats fed palm leaf pretreated diets (Group 2 and 3) showed significantly lower kidney levels of lipid peroxidation when compared with the control (group 4). Moreover, rats fed crude oil contaminated diets that was pretreated with various amounts of *Elaesis guineensis* leaf (Group 5 and 6) exhibited significantly lower kidney lipid peroxidation level when compared with rats fed crude oil contaminated diet alone (group 4) .

Table 1: The effect of *Elaeis guineensis* leaf on the level of oxidative stress indicators in the kidney of rats after three months of exposure to crude oil contaminated diet.

Groups	Lipid peroxidation (nmol/g tissue)	Xanthine oxidase activity (units/g tissue)	SOD activity (units/g tissue)	Catalase activity (nmol/g tissue)
Group 1	0.35± 0.05 [a]	60.04 ± 4.28 [a]	26.75 ± 2.21 [a]	54.53± 2.55 [a]
Group 2	0.14 ± 0.02 [b]	60.83 ± 1.76 [a]	28.63 ± 3.62 [a]	51.33± 3.61 [b]
Group 3	0.10 ± 0.03 [b]	69.28 ± 3.34 [b]	29.44 ± 1.47 [b]	52.1 2± 1.15 [b]
Group 4	0.76 ± 0.10 [c]	42.43 ± 1.78 [c]	20.10 ± 1.66 [c]	46.42± 2.11 [c]
Group 5	0.52 ± 0.01 [d]	51.09 ± 2.70 [d]	22.22 ± 1.80 [d]	49.44± 1.52 [d]
Group 6	0.34 ± 0.01 [a]	57.05 ± 5.89 [a]	24.52 ± 1.33 [a]	50. 33± 1.66 [b]

Each value represents mean ± standard deviation. n = 4 in each group. Values not sharing a common superscript letter in the same column differ significantly at $P < 0.05$.
Group 1: ((Normal Control). Group 2: feed mixed with 5.0g *Elaesis guineensis* leaf. Group 3: feed mixed with 10.0g *Elaesis guineensis* leaf. Group 4: Feed mixed with 4ml crude oil (Crude oil Control). Group 5: Contaminated diet mixed with 5.0 g of *Elaesis guineensis* leaf. Group 6: contaminated diet mixed with 10.0 g of *Elaesis guineensis* leaf.

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

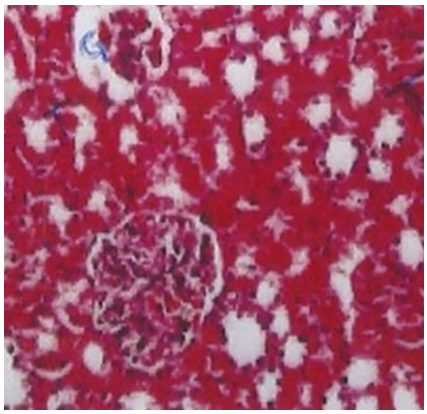
Groups	Lipid peroxidation (nmol/g tissue)	Xanthine oxidase activity (units/g tissue)	SOD activity (units/g tissue)	Catalase activity (nmol/g tissue)
Group 1	0.42± 0.08 [a]	62.04 ± 3.80 [a]	28.88 ± 1.11 [a]	53.97± 1.45 [a]
Group 2	0.22 ± 0.01 [b]	61.41 ± 2.64 [a]	27.96 ± 3.62 [a]	52.36± 2.55 [a]
Group 3	0.11 ± 0.04 [b]	68.24 ± 2.22 [b]	29.55 ± 2.81 [a]	52.66± 1.22 [a]
Group 4	0.89 ± 0.11 [c]	38.43 ± 2.66 [c]	18.33 ± 1.88 [c]	43.31± 1.53 [c]
Group 5	0.66 ± 0.12 [d]	54.11 ± 3.50 [d]	23.43 ± 1.92 [d]	50.02± 1.68 [b]
Group 6	0.53 ± 0.06 [a]	55.44 ± 6.70 [a]	24.99 ± 1.63 [a]	50. 91± 1.74 [b]

Each value represents mean ± standard deviation. n = 4 in each group. Values not sharing a common superscript letter in the same column differ significantly at $P < 0.05$.
Group 1: ((Normal Control). Group 2: feed mixed with 5.0g *Elaesis guineensis* leaf. Group 3: feed mixed with 10.0g *Elaesis guineensis* leaf. Group 4: Feed mixed with 4ml crude oil (Crude oil Control). Group 5: Contaminated diet mixed with 5.0 g of *Elaesis guineensis* leaf. Group 6: contaminated diet mixed with 10.0 g of *Elaesis guineensis leaf*.

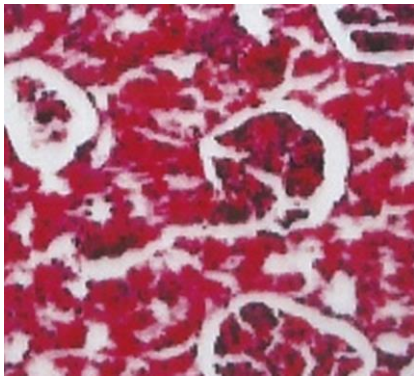
DISCUSSION

Lipid peroxidation is an index of oxidative stress, it induces functional loss of biomembranes, that results in inactivation of membrane bound receptors and enzymes (Halliwell, 1994; Niki, 2008; Greenberg et. al. 2008). This study showed that exposure to crude oil leads to oxidative damage of the kidney as evident by the rise in renal level of lipid peroxidation. This is based on the premise that metabolism of hydrocarbons generates free radicals, that is in line with earlier studies (Anozie and Onwurah, 2001; Achuba, 2014; Azeez et. al., 2013; Achuba, 2010; Alisi, 2011). *Elaesis guineensis* leaf is rich in bioactive phytochemicals whose antioxidant activity is several folds higher than that of vitamins C and E (Cowan, 1999; Lee et. al., 2008; Jaffri et. al., 2011). This may be the basis for the decreased level of lipid peroxidation in the kidney of rats exposed to crude oil that was treated with *Elaesis guineensis* leaf.

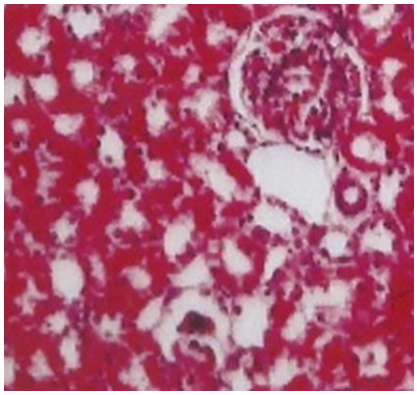
The kidney oxidative stress enzyme (XO, SOD and CAT) activities were significantly ($P<0.05$) lower in rats fed crude oil contaminated diets (group 4) in comparison with all the experimental groups (Tables 1 and 2). XO is involved in phase one process in the inactivation of xenobiotic in animals (Ezedom and Asagba, 2016). The increase in the activity of XO in rats exposed to *Elaesis guineensis* leaf treated diet indicates response of the enzyme to enhance the metabolism of endogenous xanthine. This is in a bid to increase the production of uric acid, a potent antioxidant (Cowan, 1999; Achuba, 2008; Azeez et. al. 2013). The decrease in activity of XO in rats exposed to crude oil contaminated diet alone shows that the metabolism of crude oil leads to a reduced ability to produce uric acid. Nevertheless, the alteration in the activity of oxidative enzymes had been reported as a measure of oxidative stress (Jaffri *et. al.*, 2011).



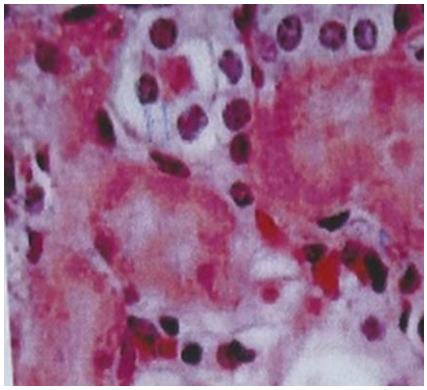
Group 1: Normal tubular brush border and intact glomeruli (H and E x 400)



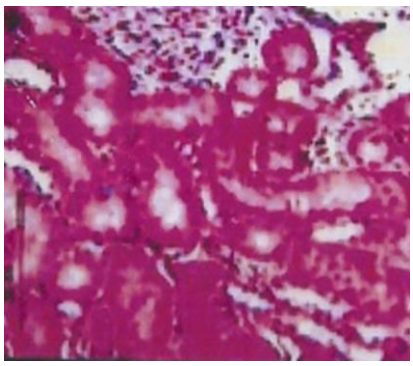
Group 2: Normal tubular brush border and intact glomeruli. (H and E x 400)



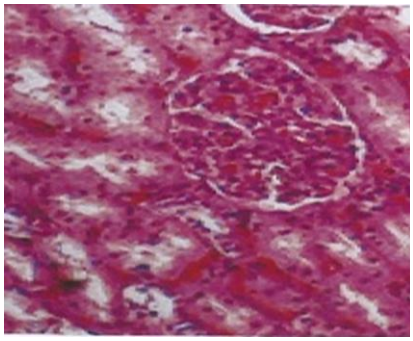
Group 3: well intact glomeruli and tubular brush border and intact glomeruli (H and E x 400)



Group 4: glomerular hypercellularity, epithelium necrosis, cells inflammation and brush borders loss. (H and E x 400)



Group 5: Reduced inflammatory cells and attenuated necrosis
(H and E x 400)



Group 6: Reduced inflammatory cells and attenuated necrosis
(H and E x 400)

Figure 1**:** Photomicrographs of kidney section of rats fed crude oil contaminated diet and diets pretreated with different amount of ground *Elaesis guineensis* leaf.
Group 1: ((Normal Control). Group 2: feed mixed with 5.0g *Elaesis guineensis* leaf. Group 3: feed mixed with 10.0g *Elaesis guineensis* leaf. Group 4: Feed mixed with 4ml crude oil (Crude oil Control). Group 5: Contaminated diet mixed with 5.0 g of *Elaesis guineensis* leaf. Group 6: contaminated diet mixed with 10.0 g of *Elaesis guineensis* leaf.

However, addition of ground *Elaesis guineensis* leaf resulted in decrease in toxic effects of crude oil. This is exhibited in the increase in activities of oxidative stress marker enzymes towards control values in rats fed with crude oil contaminated diets that were pretreated with Elaesis guineensis leaf. This is due to the ability of *Elaesis guineensis* leaf to act as an antioxidant, protecting endothelial cells of the kidney against reactive free radicals thereby restoring the level of antioxidant enzymes (Yin *et. al.,* 2013; Mohamed, 2014). This is attributed to the presence of substances with antioxidant potentials and health promoting properties, which quench free radicals that are involved in many diseases processes(Achuba, 2008; Mohamed, 2014; Ezedom and Asagba, 2016: Forstermann *et. al.,* 2017; Galli *et. al.,* 2005).

Generally, the deleterious action of crude oil on kidney tissue and the protective influence of the *Elaesis guineensis* leaf are further highlighted by histological examination of the kidney tissue (Figure 1). Previous study had shown that plant materials with antioxidant properties can attenuate the negative effect of crude oil on animals (Achuba *et. al.,* 2016).

CONCLUSION

This study has indicated that the ingestion of crude oil treated diet can result in increase in oxidative stress and consequent kidney damage. However, the crude oil toxicities were reversed by the consumption of diets that were pretreated with *Elaesis guineensis* leaf. This study, therefore, shows possible protective role of *Elaesis guineensis* leaf against crude oil induced nephrotoxicity.

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