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Variation in Body Weight, Lipid Profile and Selected Reproduction Hormones in Rats Given *Psidium guajava* Leaves from Crude Oil Polluted and Non-Crude Oil Polluted Areas

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GE, AE and AB designed the study. Authors OM and OIS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OM, OIS and DP managed the analyses of the study. Author DP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Variation in body weight, lipid profile and selected reproduction hormones in rats given *Psidium guajava* leaf samples from crude oil polluted and non-crude oil polluted areas was evaluated. Thirty-six albino rats of Wistar strain weighing between 90-120 g were divided into three major groups of I-III, with each group having two subgroups designated "a" and "b". Each of the subgroup housed six rats and they were given different concentrations of the compounded feed of the leaf samples. Rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area had significantly ($p<0.05$) increased weight when compared to rat groups placed on *P. guajava* leaf sample from crude oil polluted area. Triglyceride, cholesterol and low density lipoprotein cholesterol (LDL-C) increased significantly ($p<0.05$) in rat groups placed on *P. guajava* leaf sample from crude

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oil polluted area against rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. Atherogenic indices of rat groups placed on *P. guajava* leaf sample from crude oil polluted area showed increased risk to cardiovascular diseases when compared to rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. The evaluated reproductive hormones increased significantly ($p<0.05$) in rats placed on *P. guajava* leaf sample from crude oil polluted area against those groups placed on *P. guajava* leaf sample from non-crude oil polluted area. The constituents of *P. guajava* leaf sample from crude oil polluted area could be behind the observed risk while the increase in hormones could be linked to increased cholesterol in rats groups placed on the leaf sample from crude oil polluted area. There is to sensitise those in the act of herbalism to be aware of where they harvest the plants they use as raw materials. This study has shown the variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas.

Keywords: Body weight; lipid profile; Psidium guajava; crude oil polluted; hormones.

1. INTRODUCTION

Herbalism, the act of using plants to remedy disease conditions is as old as mankind on this planet Earth [1]. The act solely makes use of plant materials addressed as medicinal plants [2]. According to Sofowora [2], medicinal plant is one whose one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The substances that could be responsible for the potency of medicinal plants have been identified as phytochemicals and phytonutrients [2-9]. These substances are biologically active in nature, and are physiologically active against disease pathogens [10-18].

Psidium guajava commonly called guava, is among those plants with phytochemicals and phytonutrients, which are biologically and physiologically active against disease pathogens [19]. The plant has a confirmed potency against different disease conditions [20]. *P. guajava* belongs to the family *myrtaceae* [11-19]. Parts of *P. guajava* are use in the act of herbalism for preparation of syrups and concoctions used against diseases in traditional medicine. Studies have shown the lipidaemic, liver protective, haemapoetic, anti-diarrheal, antihypertensive, antioxidant, antimicrobal, hypoglycemic and antimutagenic potency of *P. guajava* [21,22]. Due to the position occupy by *P.guajava* in the practice of herbalism, any of its parts is indiscriminately collected when needed without taking into consideration the nature of the area or site where the tree is found. Considerations should be taken against collecting medicinal plants found in polluted environments to avoid taking any poison that comes which such pollution into the body [23].

The Niger Delta area of Nigeria harbours polluted environment where medicinal plants used in the act of herbalism are found [23]. The area is known for crude oil production which Nigeria is associated with. The environmental degradation associated with crude oil and refined crude oil products are synonymous with this area. Okrika, a port town in Rivers State is one of such towns found in Niger Delta area of Nigeria, which is associated with environmental degradation of crude oil and refined crude oil products [23]. It is on record that medicinal plants found within Okrika are employed in the act of herbalism. Since Okrika town harbours crude oil degraded environment on which medicinal plants grow, there is need to consider the possible effect of a known medicinal plant from such degraded environment in the body.

This study looked into that area and comparatively established variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted area such as Okrika and non-crude oil polluted areas.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The plant materials used in this study were collected from a crude oil polluted site in Okrika Rivers State, and a botanical garden (Non-crude oil polluted site) found in Owerri, Imo State, both in Nigeria. The plant materials were identified by Professor Ferdinand Nkem Mbagwu of Department of Plant Science and Biotechnology, Imo State, University Owerri, Nigeria as *P. guajava*. Their leaves were collected, air dried and crushed with pestle and mortar, then sieved to obtain the coarse powder, which was used to compound the feed used for further studies.

2.2 Experimental Animals

Thirty-six albino rats of Wistar strains weighing between 90-120 g were purchased from the animal colony of Department of Biochemistry, Gregory University, Uturu, Nigeria. The rats were allowed to acclimatize in their new environment for five days before they were used for studies. They were separated into three major groups of I-III, with each group having two subgroups designated "a" and "b". Each of the subgroup housed six rats. The rats were given compounded feed of *P. guajava* and rat feed. The rat feed was a brand of commercial grower freshly obtained from a feed dealer along Abayi road, Aba.

Treatment given to the rats are as follows

Group Ia: 5% of *P. guajava* (crude oil polluted area) + 95% normal feed + potable water.
Group Ib: 5% of *P. guajava* (non-crude oil polluted area) + 95% normal feed + potable water.

Group IIa: 25% of *P. guajava* (crude oil polluted area) + 75% normal feed + potable water.
Group IIb: 25% of *P. guajava* (non-crude oil polluted area) + 75% normal feed + potable water.

Group IIIa: 50% of *P. guajava* (crude oil polluted area) + 50% normal feed + potable water.
Group IIIb: 50% of *P. guajava* (non-crude oil polluted area) + 50% normal feed + potable water.

The treatments of experimental rats were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [24]. The treatment lasted for 28 days.

2.3 Biochemical Studies

Rats from the various groups were weighed and sacrificed while under chloroform anesthesia after the treatment period. Blood was collected by direct cardiac puncture into tubes for lipid and hormonal studies. The tubes were properly labeled for analysis[25] Aside very low density lipoproteins, VLDL-cholesterol, the assays were performed according to their manufacturers' instructions using diagnostic test kits for the lipid profile parameters purchased from BioSystems® (S.A. Costa Brava of Barcelona, Spain). VLDL-cholesterol concentration was estimated using the methods of Burnstein and Sammaille [26]. LDL- cholesterol/ HDL-cholesterol ratio was estimated using simple mathematical method as reported by Duru et al. [27]. The atherogenic indices were calculated as follows Cardiac Risk Ratio (CRR) = TC / HDL-C [28]; Atherogenic Coefficient (AC) = (TC – HDL-C)/ HDL-C [22]. Atherogenic Index of Plasma (AIP) = log (TG / HDL-C) [29].The instruction found in the kit for luteinizing hormone was adhered to for its estimation. Serum testosterone assay was carry out using tube based enzyme immunoassay (EIA) method [30].

2.4 Statistical Analysis

Results were presented as mean and standard deviation of triplicate determinations using Tables. Significant difference was established using students t-tests between two subgroups "a" and "b" of a main group at $p<0.05$.

3. RESULTS AND DISCUSSION

Body weight change for rats placed on leaves of *P. guajava* ranged from 108.90 to 109.10 g (Table 1). Rats placed on leaves of *P. guajava* from oil polluted site (Ia, IIa and IIIa) had significantly ($p<0.05$) reduced body weight when compared to rats placed on leaves of *P. guajava* from non-crude oil polluted site (Ib, IIb and IIIb). The reduction in weight could be attributed to the contents of leaves of *P. guajava* from oil polluted site.

Lipid profile as present in Table 2 shows that triglyceride ranged from 91.13 to 119.90 mg/dl; cholesterol ranged from 75.78 to 97.45 mg/dl; LDL-C ranged from 6.44 to 35.31 mg/dl; HDL-C ranged from 39.10 to 50.19 mg/dl; Non-HDL-C ranged from 25.59 to 58.35 mg/dl; and VLDL-C ranged from 15.08 to 19.16 mg/dl. Triglyceride and cholesterol are both needed for the maintenance of healthy cells in the body [31-33]. However, their high levels have been associated with coronary artery disease [32,33]. Higher risk of heart and blood vessel disease has been linked to high level of triglyceride [31-33]. Triglyceride ranged from 91.13 to 119.90 mg/dl [Table 2], and significantly increased ($p<0.05$) in rats placed on leaves of *P. guajava* from crude oil polluted site (Ia, IIa and IIIa) when compared to rats placed on leaves of *P. guajava* from non-crude oil polluted site (Ib, IIb and IIIb respectively). Rats in groups Ia, IIa and IIIa had significantly increased cholesterol ($p<0.05$) when compared to rats of Ib, IIb and IIIb groups respectively. LDL-C is regarded as bad cholesterol. High level of LDL-C has been linked to an increased risk of heart and blood vessel disease [26]. LDL-C increased significantly ($p<0.05$) in groups Ia, IIa, and IIIa against their respective Ib, IIb and IIIb in this study. The good cholesterol of the body is high density lipoprotein (HDL-Cholesterol) [33]. Levels of HDL-C reduced significantly ($p<0.05$) in rat groups (Ia, IIa, and IIIa) placed on leaves of *P. guajava* from crude oil polluted site when compared to respective rat groups (Ib, IIb and IIIb) placed on *P. guajava* from non-crude oil polluted site. This observation could imply that rats placed on leaves of *P. guajava* from crude oil polluted site may be exposed to increased risk of heart and blood vessel disease than those placed on leaves of *P. guajava* from non-crude oil polluted site. It has been reported that non-HDL cholesterol (Non-HDL-C) is a better predictor of cardiovascular risk than LDL-C. Non-HDL-C levels of rats groups (Ia,IIa and IIIa) placed on leaves of *P. guajava* from crude oil polluted site increased significantly ($p<0.05$) when compared respectively to rats groups (Ib, IIb and IIIb) placed on leaves of *P.guajava* from non-crude oil polluted site. Very low-density lipoprotein (VLDL) is another blood fat that is as bad as LDL-C. It is one of the four major lipoprotein particles. It is considered a bad cholesterol which contains triglycerides [33,34]. The observed values of VLDL increased significantly in rats groups (Ia, IIa and IIIa) placed on leaves of *P. guajava* from crude oil polluted site against the respective rats groups (Ib, IIb and IIIb) placed on leaves of *P. guajava* from non-crude oil polluted site. High VLDL-C and triglyceride simply mean a very high risk of cardiovascular disease [33,34]. The high values of VLDL as observed in the present study could imply a very high risk of cardiovascular disease for rats placed on leaves of *P. guajava* from crude oil polluted site.

LDL-C/HDL-C ratio is an important parameter of risk assessment for dyslipidaemia [35]. Christine et al. [35] noted that LDL-C/HDL-C ratio is a stronger predictor of coronary heart disease. Total cholesterol /HDL-C and LDL-C/HDL-C ratios have been used as an indices of ischemic heart disease in men [36]. Both AC and AIP have been found to indicate atherogenic risk and are better predictors of cardiovascular risk than lipids alone [37]. AIP which is a mathematical relationship of TG and HDL-C, has been used effectively as an index for assessment of cardiovascular risk [38]. Atherogenic indices presented in Table 3 had LDL-C/HDL-C ratio ranged from 0.13 to 0.90; CRR ranged from 1.51 to 2.29; AC ranged from 0.51 to 1.49; and AIP ranged from 0.27 to 0.46. All the indices were high in rats placed on leave of *P. guajava* from crude oil polluted site against those placed on leaves of *P. guajava* from non-crude oil polluted site. This could imply higher exposure to risk of cardiovascular and heart diseases.

Luteinizing hormone (LH) is one the gonadotropins that stimulate the gonads both in testes of male and ovaries of female [39,40]. Luteinizing hormone stimulates the synthesis as well as the secretion of testosterone with the help of its receptors that bind to leydig cells [40,41]. Both hormones are important tools of reproduction and have steroid nucleus [41]. Luteinizing hormone ranged from 0.12 to 0.58 miu/ml while testosterone ranged from 0.29 to 1.89 ng/ml (Table 4). Levels of luteinizing and testosterone hormones as observed in the present study, increased significantly ($p<0.05$) in rat groups Ia, IIa and IIIa when compared to those of rat groups Ib, IIb and IIIb. The observed increase in the hormones could be linked to increased cholesterol in rats placed on *P. guajava* leaf sample from crude oil polluted.

Table 1. Change in weight of rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
Final weight (g)	159.31±1.90	183.88±1.22	155.23±3.18	177.27±2.96	139.70±134	170.80±2.14
Initial weight (g)	109.13±3.11	108.98±1.67	109.10±1.40	108.97±1.60	109.03±1.30	108.90±1.65
Weight change (g)	50.18±2.70	74.90±0.19*	46.13±1.91	68.30±2.54*	30.67±2.80	61.90±1.50*

Results are presented as mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at p<0.05

Table 2. Lipid profile (mg/dl) of rats placed on leaves of *P. guajava* from crude oil polluted and non-crude oil polluted areas

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
Triglyceride	115.19±2.90	97.33±1.80*	102.12±4.30	91.13±2.10*	119.90±1.60	95.73±2.08*
Cholesterol	97.45±1.06	83.90±2.81*	94.05±0.57	79.32±1.13*	93.37±0.80	75.78±1.40*
LDL-C	35.31± 1.43	21.33±2.15*	32.54±1.32	12.14±2.64*	25.46±1.82	6.44±0.82*
HDL-C	39.10±0.87	43.10±1.23*	41.09±1.50	48.95±1.46*	43.96±1.54	50.19±1.32*
Non-HDL-C	58.35±2.63	40.80±1.94*	52.96±0.87	30.37±1.98*	49.41±1.22	25.59±1.50*
VLDL-C	19.16±1.90	16.20±0.73*	18.64±046	15.64±1.23*	18.47±1.54	15.08±1.22*

Results are presented as mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at p<0.05.
LDL-C= Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol; Non-HDL-C= Non-High density lipoprotein cholesterol; and VLDL-C-Very low density lipoprotein cholesterol

Table 3. Atherogenic indices of rats placed on *P. guajava* from polluted and non-polluted areas

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
LDL-C/HDL-C	0.90	0.49	0.79	0.24	0.58	0.13
CRR	2.49	1.95	2.29	1.62	1.89	1.51
AC	1.49	0.95	1.29	0.62	1.12	0.51
AIP	0.46	0.35	0.40	0.27	0.44	0.28

LDL-C= Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol; CRR= Cardiac risk ratio; AC= Atherogenic coefficient; AIP= Atherogenic index of plasma

Table 4. Level of reproductive hormones of rats placed on *P. guajava* from polluted and non-polluted areas

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
Luteinizing hormone (miu/ml)	0.40±0.02	0.12±0.01*	0.47±0.03	0.26±0.01*	0.58±0.06	0.28±0.04*
Testosterone (ng/ml)	1.56±0.13	0.29±0.14*	1.78±0.17	0.54±0.18*	1.89±0.12	0.98±0.10*

Results are mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at p<0.05

4. CONCLUSION

Rats groups placed on *P. guajava* leaf sample from crude oil polluted area showed marked degeneration in lipid profile and atherogenic indices against rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. There is to sensitise those in the act of herbalism to be aware of where they harvest the plants they use as raw materials. This study has shown the variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas.

ETHICAL APPROVAL

This work was approved by the Ethical Committee in the Department of Biochemistry, Rhema University, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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