Asian Journal of Advanced Research and Reports

7(3): 1-8, 2019; Article no.AJARR.52702
ISSN: 2582-3248

Variation in Leaf Constituents and Biochemical Indices of Rats given *Psidium guajava* from Two Different 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.

Article Information

DOI: 10.9734/AJARR/2019/v7i330177
Editor(s):
(1) Dr. Maria Luisa Kennedy Rolon, Professor, Facultad de Ciencias Quimicas, University of North Alabama, USA.
Reviewers:
(1) Abubakar Kabeer, Federal University of Lafia, Nigeria.
(2) S. Parasuraman, AIMST University, Malaysia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52702

Original Research Article

Received 14 September 2019
Accepted 19 November 2019
Published 05 December 2019

ABSTRACT

Variation in leaf (heavy metal) constituents and biochemical indices of rats given leaf samples of *Psidium guajava* from two different areas were evaluated. Results obtained for heavy metal constituents the leaf samples showed the presence of mercury (0.14±0.01 mg/100 g), lead (2.90±0.10 mg/100 g), cadmium (0.05±0.01 mg/100 g), copper (5.01±0.17 mg/100 g), chromium (0.40±0.01 mg/100 g), and cobalt (5.64±0.64 mg/100 g) in *P. guajava* leaf sample from crude oil polluted area. Only copper (0.80±0.20 mg/100 g) was observed in *P. guajava* leaf sample from non-crude oil polluted area. The biochemical studies on the leaf samples were carried out using standard methods. Thirty-six rats were distributed in six subgroups with six rats each, under three main groups (I-III). Three of the subgroups were placed on *P. guajava* leaves from crude oil polluted area (designated Ia, IIa and IIIa) while the other three subgroups were placed on *P.*

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guajava leaves from non-crude oil polluted area (designated Ib, IIb and IIIb). The haematological parameters of rats placed on *P. guajava* from crude oil polluted area such as RBC, Hb, PCV, MCV, and MCH were significantly affected ($p<0.05$) when compared to those of rats placed on *P. guajava* from non-crude oil polluted area. AST and ALT liver enzymes significantly increased in rats placed on *P. guajava* leaves from crude oil polluted area against rats placed on *P. guajava* leaves from non-crude oil pollute area. Since data obtained with animals become more severe when translated to humans, it therefore becomes pertinent for those that use medicinal plants from crude oil polluted areas to become aware of the possible effects of using such plants. This study has evaluated the variation in leaf constituents and biochemical indices of rats given leaf samples of *Psidium guajava* from two different areas were evaluated.

Keywords: Biochemical studies; heavy metals; polluted areas; Psidum guajava.

1. INTRODUCTION

Plants and their products are noted for their usefulness as food materials [1-6], ornamental materials or as medicinal materials [7-13]. They also play a big role in environmental protection [14]. A lot has been reported on medicinal potential of plants [15-16]. The potency of plants against disease pathogens is no more in doubt because different researchers have demonstrated plants` potency with different animals induced with different disease conditions [17-27]. According to Sofowora [7] and Duru, et al. [28], plants with confirmed potency against pathogens and diseases are known as medicinal plants. Medicinal plant is any plant used for the extraction of pure substances either for direct medicinal compounds which can be used for the synthesis of useful drugs [7]. In recent years, the potency of *Psidum guajava,* popularly known as guava as a medicinal plant cannot be doubted because of many studies on the plant and its different parts. *P. guajava*`s lipidaemic [29], liver protective, haemapoetic, anti-diarrheal, antihypertensive, antioxidant, antimicrob- ial, hypoglycemic and antimutagenic potency have been reported by different authors [30-32]. *P. guajava* belongs to the family *myrtaceae* [33]. Different parts of the plant are extensively being used in the preparation of syrups and concoctions used against diseases in traditional medicine. As a known medicinal plant, any of its needed part is indiscriminately harvested, and used without taking into consideration the type of environment where the plant is grown or gotten from and as well the possible implications in a biological system.

Environmental degradation due to oil pollution is associated with the Niger Delta area of Nigeria. The Niger Delta area of Nigeria is saddled with the production of crude oil for which the country is known for. Traditional healers in this area rely on medicinal plants grown within for herbalism. *P. guajava* is among such plants that are commonly employed in such area against diseases in traditional medicine. It is on record that not much has been done to comparatively look at constituents and the possible effects of a medicinal plants from to two different areas on the biological system when used.

This study addressed such issue and evaluated the variation in heavy metal constituents and biochemical indices of rats given leaves of *Psidium guajava* from two different areas were evaluated.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Sample Materials

The plant materials used in this study were collected from a crude oil polluted site in Okirika, 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 in the 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 feeds for further studies.

2.2 Preparation of Plants for Heavy Metal Analysis

The samples of *P. guajava* from the considered sites were prepared for heavy metal analysis following the method as described by Okwu [34]. Heavy metals in the samples were determined using atomic absorption spectrophotometer (Model: Unicam 9939/959) method. Heavy metals evaluated were mercury, lead, cadmium, copper, chromium and cobalt.

2.3 Laboratory Animals

Thirty-six male albino rats of Wistar strains weighing between 90-110 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. The rats were divided into three major groups of I-III, with each group having two subgroups designated "a" and "b". Each of the subgroup consist of six rats. The rats were fed with 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 [35]. The treatment lasted for 28 days.

2.4 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 heparin treated tubes for haematology analysis, while the blood for creatinine, urea and liver enzyme studies were collected in anticoagulant free tubes. The tubes were properly labeled for analysis. Haematology indices such as Packed Cell Volume (PCV) was estimated using micro-haematocrit method as described by Alexandar and Griffths [36], haemoglobin level (Hb) was determined using cynomethaemoglobin as described Alexandar and Griffths [36], whereas white blood cells count (WBC) was estimated by visual means using the new improved Neubauer counting chamber as described by Dacie and Lewis [37]. Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were estimated using the methods as described by Jain [38]. Urea, creatinine, as well as the liver enzymes considered such as aspartate aminotransferase (AST), alanine aminotrans-ferase (ALT), and alkaline phosphatase (ALP) were spectrophotometrically determined using the standard ready to use kits from Rondox Laboratory Ltd. Co. Antrim, United Kingdom.

2.5 Statistical Analysis

Results were presented as the mean ± 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

Table 1 reveals the presence of mercury (0.14 ±0.01 mg/100 g), lead (2.90±0.10 mg/100 g), cadmium (0.05±0.01 mg/100 g), copper (5.01±0.17 mg/100 g), chromium (0.40±0.01 mg/100 g), and cobalt (5.64±0.64 mg/100 g) in leaves of *P. guajava* from crude oil polluted site whereas only copper (0.80±0.20 mg/100 g) was observed in leaves from non-crude oil polluted site. The biological significance as well as the toxicity of the heavy metals observed have been reported by many authors.

The biological significance of haematological assessment has been noted by Duru et al. [3], Yakubu et al. [27] and Alexander and Griffths [36]. The haematology of rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted sites as presented in Table 2 shows that red blood cell (RBC) ranged from 5.22×10^{12}/L to 7.43×10^{12}/L. RBC significantly reduced ($p<0.05$) in rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa), when compared to rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, and IIIb). The reduction in RBC of rats placed on *P. guajava* leaves from crude oil polluted site could be indication of

imbalance between the rate of production (erythropoiesis) and destruction of the blood corpuscles. Hb ranged from 13.54 g/dl to 16.44 g/dl. Hb of rats given *P. guajava* leaves from crude oil polluted sites significantly reduced ($p<0.05$) against rats placed on *P. guajava* leaves from non-crude oil polluted site. The relationship between packed cell volume (PCV) and Hb was observed in the present study for all the subgroups. PCV ranged from 40.76±1.85% to 49.81%. PCV of rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, IIIa) significantly reduced ($p<0.05$) when compared to those placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb and IIb). It has been noted that RBC, Hb, and PCV are associated with the total population of red blood cells [39]. Adebayo, et al. [39] noted that increase in number of white blood cell (WBC) signals normal reaction of rats to foreign substances. It has been reported that leucocytosis could be directly proportional to the severity of the causative stress condition [2,40]. The significant increase ($p<0.05$) in WBC of rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, IIIa) against rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, IIIb) as observed in the present study, could be indication of severity of causative stress condition induced by constituents of *P. guajava* leaves from crude oil polluted site. The volume of the average red cells is associated to mean cell volume (MCV) while mean corpuscular haemoglobin (MCH) represents the absolute amount of haemoglobin in the average red cells. MCV ranged from 64.23 to 83.96 fl while MCH range from 21.06 to 27.99 pg. MCV and MCH of rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa) significantly increased ($p<0.05$) when compared to MCV and MCH of rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, and IIIb). Mean corpuscular haemoglobin concentration (MCHC) ranged from 32.79 to 33.01%. MCHC of rats on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa) were insignificantly ($p>0.05$) effected, when compared to rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, and IIIb). The significant effects observed in RBC, Hb, PCV, MCV, and MCH of rats placed on *P. guajava* leaves from crude oil polluted site against those of rats placed on *P. guajava* leaves from non-crude oil polluted site, could suggest alteration in incorporation of haemoglobin into red blood cells and the morphology as well as the osmotic fragility of the red blood cells [41].

Table 1. Heavy metals constituents in leaf samples of *P. guajava* from crude oil polluted and non-crude oil polluted sites

Heavy metal (mg/100 g)	Leaves from crude oil polluted site	Leaves from non-crude oil polluted site
Mercury	0.14 ±0.01	ND
Lead	2.90±0.10	ND
Cadmium	0.05±0.01	ND
Copper	5.01±0.17	0.80±0.20
Chromium	0.40±0.01	ND
Cobalt	5.64±0.64	ND

Results are presented as mean ± standard deviation of triplicate determination

Table 2. Haematology of rats given *P. guajava* leaf samples from crude oil polluted and non-crude oil polluted sites

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
RBC($\times10^{12}$/L)	5.22±0.70	7.09±0.21*	4.96±0.78	7.64±0.13*	4.87±0.54	7.43±0.90*
Hb (g/dl)	13.54±1.23	15.98±0.82*	13.76±0.23	16.09±0.18*	13.63±0.59	16.44±0.53*
PCV (%)	40.76±1.85	48.26±1.03*	41.42±1.58	49.07±0.22*	40.89±2.19	49.81±1.20*
WBC($\times10^{9}$/L)	6.30±0.10	4.00±0.11*	6.27±0.31	4.23±0.40*	6.94±0.42	4.86 ±0.38*
MCV (fl)	78.08±0.34	68.07±1.00*	83.51±2.20	64.23±2.00*	83.96±1.18	67.04±1.85*
MCH (pg)	25.94±1.11	22.54±0.43*	27.74±1.31	21.06±0.26*	27.99±0.57	22.13±0.90*
MCHC (%)	33.22±2.67	33.11±1.65	33.22±2.90	32.79±1.10	33.33±1.47	33.01±0.57

Results are presented as mean ± 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 3. Liver enzyme studies of rats given *P. guajava* leaf samples from crude oil polluted and non-crude oil polluted sites

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
AST (U/L)	36.17±1.80	40.32±2.93*	38.12±0.82	44.1±0.53*	37.99±1.35	45.98±0.64*
ALT (U/L)	43.65±2.16	49.00±0.10*	41.74±1.90	47.37±1.05*	42.14±1.10	53.31±1.16*
ALP (U/L)	10.12±0.15	15.22±0.73*	13.09±0.11	20.28±2.14*	14.19±1.81	23.21±2.10*

Results are presented as mean ± 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 4. Urea and creatinine in rats given leaves of *P. guajava* leaf samples from crude oil polluted and non-crude oil polluted sites

Parameters	Group I		Group II		Group III	
	Ia	Ib	IIa	IIb	IIIa	IIIb
Creatinine (mg/dl)	0.60±0.01	0.90±0.02	0.63.±0.03	1.18±0.04*	0.47±1.10	1.30±0.03*
Urea (mg/dl)	45.13±0.13	51.33±0.30*	41.70±0.20	55.95±0.18*	47.±0.23	57.76±0.48*

Results are presented as mean ± 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

Liver damage is pertinent when it is exposed to toxic substances [42-43]. Aspartate aminotrans-ferase (AST) and alanine aminotransferase (ALT) are known makers of liver damage, though ALT is more specific marker of liver damage than AST. Alkaline phosphatase (ALP) also leaks in the same manner as AST and ALT [42]. From Table 3, AST and ALT in rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa) increased significantly ($p<0.05$), when compared to those of rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib,IIb and IIIb). The same order was followed by ALP in rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa) against those of rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, and IIIb).

Creatinine is a major catabolic product of the muscle [39]. It is excreted in kidneys [39]. Creatinine is a major indicator of kidney failure [39]. Its retention in the body is a sign of kidney failure [39,44]. From Table 4, creatinine ranged from 0.47 to 1.30 mg/dl. Rats placed on *P. guajava* leaves from crude oil polluted site had reduced creatinine against rats place on *P. guajava* leaves from non-crude oil polluted site. The observed reduction became significant ($p<0.05$) in rats of subgroups IIa and IIIa against those of IIb and IIIb. Diminished urea excretion and excess protein breakdown result in high blood urea in blood [44-46]. Urea reduced significantly ($p<0.05$) in rats placed on *P. guajava* leaves from crude oil polluted site (Ia, IIa, and IIIa) when compared to rats placed on *P. guajava* leaves from non-crude oil polluted site (Ib, IIb, and IIIb). The significant reduction could be indication of retention of urea in the blood of rats given *P. guajava* leaves from crude oil polluted site.

4. CONCLUSION

This study has shown the variation in heavy metal constituents and biochemical indices of rats given leaves of *Psidium guajava* from two different areas. The study as well ascertained the status of the plants in biological system of rats. Since data obtained with animals become more severe when translated to humans, therefore it becomes pertinent for those that use medicinal plants from crude oil polluted areas to become very aware of the possible effects of using such plants.

ETHICAL APPROVAL

This study was approved by University of Calabar and Rhema University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/52702