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Ameliorative Potential of Aqueous Leaf Extract of Vernonia Amygdalina (Bitter Leaf) on Selected Renal and Hematological Parameters of Rats Exposed to Premium Motor Spirit (PMS)

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ABSTRACT

The ameliorative potential of aqueous leaf extract of *Vernonia amygdalina* (bitter leaf) on selected renal and hematological parameters of rats exposed to premium motor spirit (PMS) was investigated using standard analytical methods. Thirty-six healthy albino rats weighing 75-200 g was purchased and housed in plastic cages of six groups with six rats each. Improvised method of nose inhalation was adopted for exposure to PMS. Rats fed with normal rat chow constituted the group A (negative control). Group B (positive control) was exposed to PMS vapour for 5 hours daily. After five hours of daily exposure to PMS, the remaining groups (C–F) received 0.3 mg/kg of vitamin C, 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Vernonia amygdalina* aqueous leaf extract, respectively. Treatment lasted for 28 days during which rats were exposed daily and after each daily exposure, the cages housing the rats were transferred to a PMS vapour-free environment. The quantitative phytochemical composition of *Vernonia amygdalina* leaves extract showed that it is rich in catechin (192.06 ppm) followed by kaempferol (71.97 ppm) and genistein (39.85 ppm). The renal parameters: urea, creatinine, chloride ion (Cl^-), potassium ion (K^+), and calcium ion (Ca^+) were significantly elevated ($p<0.05$) in comparison to the normal control rats. However, there was a significant decrease ($p<0.05$) in the hematological indices of rats in group B when compared with group A. The administration of *Vernonia amygdalina* aqueous leaf extract reversed the effect in every biomarker. Histopathological examination of the kidney showed histological normal liver in normal control rats and distortion of kidney sections in rats exposed to PMS vapour which was corrected by aqueous leaves extract of *Vernonia amygdalina* at two higher concentrations (200 and 400) mg/kg. Finally, it can be advised to prevent PMS damage because the aqueous leaf extract of *Vernonia amygdalina* clearly showed a beneficial modulatory effect on the renal and hematological parameters of albino rats subjected to PMS.

Keywords: *Premium motor spirit, Vernonia amygdalina, renal parameter, hematology, wistar rats.*

INTRODUCTION

Since the discovery of crude oil/petroleum in Nigeria in 1956 at a community in Bayelsa State, Nigeria, it has been Nigeria's major source of income as these products affects virtually every aspect of the economy and institutions (Ogbuigwe, 2018; Chukwurah, 2006). One of the products of fractional distillation of crude oil is premium motor spirit (PMS). PMS can be utilized for several purposes such as petrol for generators, automobiles, some factory machines and some machines used for domestic purposes thus making PMS an essential commodity for many Nigerians (Chukwurah et al., 2020). Unfortunately, the environment and end users particularly petrol station workers (PSW) who are constantly exposed to PMS are at risk of several toxicological effect because

of uncontrolled exposure. Some studies have highlighted the negative impact of PMS to man and environment (Udonwa et al., 2009), therefore caution need to be applied while utilizing this product.

PMS (petrol) is a highly inflammable and volatile product that evaporates on exposure releasing chemical contaminants that are harmful to man and his environment (Xu et al., 2018). According to Chukwurah et al. (2020), individual exposed to PMS at work place suffers greater complications than persons that sparingly utilize it. The major constituents of PMS include hydrocarbons (aromatic and cyclic), xylene, toluene, benzene, pentane, hexane, octane, 2, 2, 4-trimehtylbenzene, heptane, e.t.c. Some of these constituents like benzene and toluene are said to be carcinogenic as a result of free radicals they release (Chukwurah et al., 2020).

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More so, studies have shown that exposure to benzene at work place can make the bone marrow to degenerate, trigger leukaemia and anemia as a result of obstruction of cell cycle via p53-mediated overexpression of p21 during kinase cascade reactions in the body. The resultant effect is alteration of erythropoiesis process in the system (Synder, 2012). Also, studies stated that lower levels of saturated hydrocarbons and high concentrations of unsaturated hydrocarbons accumulate in human blood and animals when exposed to petroleum vapour (Chukwurah et al., 2020). Therefore, there is need to assess the effect of PMS exposure on experimental animals in order to obtain clue on the potential side effects on man.

Earlier animal studies on rats with chronic exposure to gasoline vapor revealed that the animals developed tumors in their liver cells, kidneys and nephrons (Johnson et al., 2005). Similarly, Agbogidi et al. (2009) stated that stress and poor health quality of aquatic animals are associated with pollution from crude oil spill. This report was strongly backed by later studies which reported negative effects on fishes and aquatic environment by crude oil pollution (Mohsen, 2012; Chukwurah, 2006). Therefore, there is need for remedy from natural sources to prevent creating more problems while trying to solve an existing issue. *Vernonia amygdalina* (bitter leaf) is an essential medicinal plant with several pharmacological importance such as, cholesterol lowering, anti-diabetic, hypoglycemic, e.t.c (Achuba, 2018). In addition to this, bitter leaf plant has shown ability to ameliorate chemical toxicity with minimal side effects.

METHODOLOGY

Sample collection

Okpiabhele and Aihiokhai's (2022) approach was used for sample preparation and collection. Prof. F.N. Mbagwu, a qualified taxanomist from the Department of Plant Science and Biotechnology at Imo State University Owerri (IMSU), Owerri, identified and verified fresh mature leaves of

Vernonia amygdalina that were collected from a garden there. With voucher number IMSUH 454, the voucher specimens were made and placed at the IMSU Herbarium (IMSUH).

Preparation of plant extract

The leaves of *Vernonia amygdalina* were cleaned, sorted, sun-dried, and then ground into a fine powder in a milling machine. After that, 500 milliliters of distilled water were used to dissolve 100, 200, and 400 grams of pulverized *Vernonia amygdalina* leaves apiece, and the mixture was allowed to stand for a full day. A muslin bag was then used to filter the mixture, which was then stored at room temperature (Okpiabhele & Aihiokhai, 2022).

Exposure to Premium Motor Spirit (PMS)

Premium motor spirit was acquired for the study from MRS filling stations along Okigwe road, Orji in Owerri, Imo State, Nigeria, following a week of acclimatization. For this investigation, the improvised nasal inhalation technique used by Uboh *et al.* (2005) was used. To allow PMS to freely evaporate and inhale, precisely 500 cc was metered into highly perforated containers and placed next to each test rat cage for five hours. To enable appropriate PMS inhalation, the experiment was set up in a closed chamber. The rats were exposed every day for 28 days, and following each exposure, their cages were moved to an area devoid of PMS vapor. PMS was not administered to the control rats.

Experimental Animals

Albino strains (total of 36 healthy albino rats of both sexes, 12 weeks old, weighing 75-200 g were used for this study. Albino rats were individually housed at room temperature controlled (27±3 ^{o}C) in cages with a 12; 12 light dark cycle and wire lid to allow proper ventilation. Albino rats were allowed access to standard laboratory chow and filtered water *ad libitum*.

Acclimatization: Prior to experimentation, the animals were given a week to get used to the facilities. Following acclimation, the rats' initial weights were noted, along with the animals' weekly feed consumption and body weight.

Ethical approach: Imo State University's Institutional Animal Care and Use Committee (IACUC) authorized all of the animals generated in accordance with the Natural Institutes of Health's guidelines for the care and use of laboratory animals, and they were all assigned the ethical number (IMSU/FBS/2024/0018).

Experimental groups: The albino rats were divided into six groups, each consisting of six rats (one control and five treatments). The distribution of men and women among the different categories was done at random. The test rats were inhaled 500 cc of PMS each day for five hours every morning for four weeks, while the control rats were not exposed to PMS. Below is a summary of the therapies:

Group A (Negative control group that received only normal rats chow and clean water, not exposed to PMS),
Group B (positive control rats exposed to PMS for 5 hrs daily without treatment),
Group C (rats exposed to PMS for 5 hrs daily followed by treatment with 0.3 mg/kg of vitamin C as antioxidant),
Group D (rats exposed to PMS for 5 hrs daily followed by treatment with 100 mg/kg aqueous leaves extract of *V. amygdalina*),
Group E (rats exposed to PMS for 5 hrs daily followed by treatment with 200 mg/kg aqueous leaves extract of *V. amygdalina*), and

Group F (rats exposed to PMS for 5 hrs daily followed by treatment with 400 mg/kg aqueous leaves extract of *V. amygdalina*).

Six albino rats were used in each treatment, and the experiment was set up using a completely randomized design. The albino rats were kept in similar hygienic settings throughout the trial, therefore the only things that differed were the kind of extract and cholesterol oil mixture that the rats were given.

Weight Gain

The animals' original weights were determined by weighing them at the conclusion of the acclimation phase. Following acclimation, weekly weight measurements were made of the animals. The following formula was used to calculate the rats' weight growth (Oke *et al.,* 2016): Body weight at the conclusion of the period minus body weight at the start of the preceding period is the weight gain.

Collection of blood samples

After being fed for 28 days, the six rats in each group were given a 12-hour fast, weighed, and exposed to formalin to induce anesthesia. After the rats were humanely killed, a 5 ml hypodermic needle and syringe were used to puncture each rat's heart and draw blood, which was then placed into bottles containing heparin and ethylene diamine tetra acetic acid (EDTA) sample. The anticoagulated blood in heparin sample vials was spun for 10 minutes at 1000 rpm to extract plasma for the assessment of kidney function markers. EDTA sample bottles were used to acquire hematological indicators. Samples were carefully stored in a refrigerator for analysis (Iwu *et al.,* 2020).

Measurement of kidney function parameters

Creatinine Determination was carried out according to Bartels and Bolmer (1971), serum urea concentration was determined by Weatherburn (1967), calcium, potassium and sodium electrolyte were determined by Henry, 1974, chloride electrolyte was determined by Young, 1995, while magnesium electrolyte was dones according to Young (2001).

Measurements of Hematological Parameters

Micro-haematocrit was used to measure packed cell volume (PCV), and capillary tubes containing blood were centrifuged at 3000 g for five minutes. While the red blood cell (RBC) counts were visually estimated, the DTH Haemoglobinometer TM was used to determine the plasma hemoglobin (Hb) content (Cheesbrough, 2006). A BC-2600 model of haematology autoanalyzer (Bio-medical Electronics, UK) was used to measure the platelet count (PLT) and the white blood cell (WBC) count and its differentials (monocytes, neutrophils, eosinophils, basophils, and lymphocytes).

Histopathology of the kidney

The kidneys were extracted from the rats' chest region and longitudinally dissected after being freed from the surrounding tissues. They were kept in a 10% neutral formalin solution for fixing. After a week, the organs were dehydrated using progressively higher-grade alcohol, cleansed with tap water for 24 hours, clarified in xylene, and blocked in paraffin. The sample was stained with eosin and hematoxylin and inspected under a microscope once it had reached a thickness of 4-5 µm. The prepared slides were examined at 400X magnification (Achuba, 2018b).

Data analysis

The software utilized was the Statistical Package for Biological and Social Sciences (SPSS) Inc. 27.0. One-way analysis of variance (ANOVA) was used for multiple comparisons, and mean values (M) ± SD were computed. Data points (p) below 0.05 ($p<0.05$) were deemed statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of *Vernonia amygdalina* leaves

Tables 1 and 2 showed the qualitative and quantitative phytochemical analysis of *Vernonia amygdalina* leaves respectively. Results showed that leave extract of *Vernonia amygdalina* was most abundant in flavonoids followed by phenols, glycosides, polyphenols and isoflavones. The qualitative analysis further revealed that sterols, saponins, polysaccharides, lectins and terpenoids were absent. Results of Table 2 equally showed that *Vernonia amygdalina* leaves extract is rich in catechin (192.06 ppm) followed by kaempferol (71.97 ppm) and genistein (39.85 ppm).

Table 1: Qualitative Phytochemical Composition of *Vernonia amygdalina* Leave Extract

S/N	Phytochemical class	Results
1	Flavonoids	+++
2	Phenolic compound	+++
3	Glycosides	++
4	Polyphenols	+
5	Isoflavines	+
6	Sterols	-
7	Saponins	-
8	Polysaccharides	-
9	Lectins	-
10	Terpenoids	-

Key for qualitative data:

(+) weak presence; (++) moderate presence; (+++) strong or intense presence; (-) absent

Table 2: Quantitative Phytochemical Composition of *Vernonia amygdalina* Leave Extract

S/N	Retention time	Area	Amount (ppm)	Phytochemical name	Class of phytochemical
1	3.836	440.64	71.97	Kaempferol	Flavonoids
2	4.267	1366.32	192.06	Catechin	Flavonoids
3	5.176	19.70	4.76	Quercetin	Flavonoids
4	5.951	155.79	23.89	Hesperidin	Phenolic compound
5	6.358	79.56	11.18	Luteolin	Flavonoids stuitbens
6	6.936	81.53	12.44	Resveratrol	Phenolic compounds
7	7.405	15.37	4.15	Artemetin	Flavonoid methylated
8	7.687	29.87	4.18	Naringin	Glycosides
9	8.287	8.48	3.18	Retusin	Isoflavones
10	8.700	11.83	1.65	Myricetin	Flavonoids
11	9.283	91.03	12.80	Ellagic acid	Polyphenols
12	9.467	211.1	29.67	Vanillic	Phenolic
13	10.309	12.30	3.72	Isorhamnetin	Flavonoids
14	10.680	1.39	1.86	Naringenin	Flavonoids
15	11.661	1.83	2.36	Apigenin	Flavonoids
16	13.007	2.55	3.49	Maricetin	Flavonoids
17	13.444	182.53	25.66	Epicatechin	Flavonoids

18	14.061	205.02	28.82	Daidzein	Flavonoids
19	14.723	284.40	39.85	Genistein	Flavonoids
20	15.064	43.13	6.05	Apigenin	Flavonoids
21	15.730	1.70	2.28	Lunamarin	Glycosides

Weight gain of rats

Table 3 shows the weight gain of albino rats given an aqueous leaf extract of *Vernonia amygdalina* after they were exposed to PMS. The albino rats in group B gained the most weight (34.50±2.10 g), while group A gained the least (13.00±2.25 g), according to the results. The weight gain of rats in group B was significantly higher ($p<0.05$) than that of rats in other groups, according to the results. When compared to group B, the weight gain was substantially reversed in groups C, D, E, and F.

Table 3: Weight gain of albino wistar rats exposed to PMS and treated with aqueous leaves extract of *Vernonia amygdalina* for 28 days

Weeks	Group A (negative control)	Group B (positive control)	Group C (standard)	Group D	Group E	Group F
Initial weight	90.5±1.80	94.0±1.76	97.5±0.66	97.0±0.38	83.5±1.18	78.5±0.75
Final weight (Day 28)	103.5±2.15	128.5±1.15	122.5±0.25	120.0±1.25	106.0±1.45	99.5±0.86
Weight gain (g)	**13.00±2.25^{a}**	**34.50±2.10^{b}**	**25.00±0.68^{c}**	**23.00±2.36^{c}**	**22.50±1.95^{c}**	**21.00±1.06^{c}**

Data are (M±S.D) of six determinations (n=6). Superscript alphabets "a, b, c" show significant difference ($p<0.05$) across the row when compared to one another.

The effects of aqueous leaves extract of *Vernonia amygdalina* on the renal function parameters of rats exposed to premium motor spirit

Table 4 displays the impact of *Vernonia amygdalina* aqueous leaf extract on the renal function metrics of rats given premium motor spirit. When comparing group B rats to group A, the results revealed a significant rise ($p<0.05$) in all renal parameters, with the exception of Na^{+} and Mg^{2+}. Rats in groups D, E, and F had significantly lower creatinine and Ca+ values ($p<0.05$) than rats in group B. The urea concentrations of groups E and F were significantly lower ($p<0.05$) than those of group B.

Table 4: Effects of aqueous leaves extract of *Vernonia amygdalina* on the renal function parameters of rats exposed to premium motor spirit

Groups	Creatinine (µmol/l)	Urea (mg/dl)	Cl^{-} (mmol/l)	Na^{+} (mEq/l)	K^{+} (mmol/l)	Mg^{2+} (mg/dl)	Ca^{+} (mmol/l)
Group A (negative control)	2.18±0.06^{a}	21.95±0.84^{a}	7.16±1.40^{a}	88.20±2.40^{a}	6.40±0.60^{a}	86.40±4.70^{a}	6.11±0.15^{a}
Group B	4.36±0.84^{b}	28.16±1.76^{b}	14.47±0.98^{b}	102.40±3.60^{a}	10.15±1.47^{b}	93.20±9.60^{a}	9.64±0.85^{b}

(positive control)							
Group C (Standard)	3.87±0.08^{c}	26.32±1.14^{b}	11.60±1.70^{b}	94.61±2.50^{a}	8.96±0.92^{ab}	90.80±9.80^{a}	7.30±0.24^{a}
Group D	3.35±0.19^{c}	26.94±1.22^{b}	8.52±1.10^{a}	95.62±4.10^{a}	8.45±0.66^{ab}	91.91±5.50^{a}	7.20±0.43^{a}
Group E	3.31±0.12^{c}	24.07±1.08^{a}	8.14±1.05^{a}	93.52±3.20^{a}	7.25±0.70^{a}	89.62±6.21^{a}	7.18±0.65^{a}
Group F	3.32±0.14^{c}	23.11±1.10^{a}	7.10±1.08^{a}	92.43±2.40^{a}	7.18±0.54^{a}	8a.54±4.14^{a}	6.74±0.55^{a}

Data are (M±S.D) of six determinations (n=6). Values bearing different superscript letters (a, b,c) down the column are significantly different ($p<0.05$) when compared to groups A and B.

Effects of aqueous leaves extract of *Vernonia amygdalina* on the hematological indices of rats exposed to premium motor spirit

Table 5 displays the impact of *Vernonia amygdalina* aqueous leaf extract on the hematological indicators of rats given premium motor spirit.

All of the hematological indices of the rats in group B were significantly lower ($p<0.05$) than those in group A, according to the data. However, when compared to group B, the administration of *Vernonia amygdalina* aqueous leaf extract significantly ($p<0.05$) reversed the negative effect of PMS on the exposed mice in groups D, E, and F.

Table 5: Effects of aqueous leaves extract of *Vernonia amygdalina* on the hematological indices of rats exposed to premium motor spirit

Parameters	Group A (negative control)	Group B (positive control)	Group C	Group D	Group E	Group F
RBC (×10^{6}/µL)	7.87±0.90^{a}	6.96±0.42^{b}	7.50±0.73^{a}	7.68±0.51^{a}	7.60±0.85^{a}	7.72±0.65^{a}
Hb (g/dl)	14.80±1.10^{a}	12.20±1.13^{b}	13.65±1.02^{a}	13.70±0.85^{a}	13.38±0.96^{a}	13.96±1.04^{a}
PCV (%)	45.60±0.75^{a}	36.70±0.30^{b}	41.20±0.50^{a}	42.12±1.10^{a}	41.07±0.80^{a}	42.50±0.94^{a}
WBC (×10^{3}/µL)	6.60±0.80^{a}	2.31±0.35^{b}	4.82±0.18^{a}	4.86±0.17^{a}	5.40±0.78^{a}	5.80±0.18^{a}
Platelet (10^{3}/µL)	287.60±9.20^{a}	120.56±7.20^{b}	192±5.30^{a}	195.40±4.60^{a}	210.50±4.50^{a}	224.50±7.18^{a}
MON (%)	1.60±0.14^{a}	0.50±0.03^{b}	0.80±0.08^{a}	0.94±0.10^{a}	1.10±0.40^{a}	1.19±0.16^{a}
NEU (%)	18.60±0.22^{a}	14.60±0.40^{b}	14.82±0.16^{b}	16.82±0.17^{a}	16.40±0.66^{a}	16.70±0.24^{a}
EOS (%)	2.60±0.40^{a}	1.12±0.13^{b}	2.41±0.16^{a}	2.42±0.50^{a}	2.42±0.26^{a}	2.47±0.51^{a}
BAS (%)	8.20±0.50^{a}	4.10±0.46^{b}	5.60±0.14^{a}	5.90±0.20^{a}	6.10±0.35^{a}	6.40±0.42^{a}

LYM (10^3/µl)	5.20±0.40[a]	1.68±0.30[a]	4.60±0.52[a]	4.80±0.60[a]	4.10±0.51[a]	4.32±0.12[a]
MCV (µM^3)	68.20±4.50[a]	57.87±2.62[b]	58.60±2.14[b]	57.80±4.24[b]	54.10±2.92[a]	55.20±3.60[a]
MCH (pg)	21.10±1.85[a]	18.87±0.62[b]	18.60±1.64[b]	18.80±2.50[b]	19.80±0.80[a]	19.82±0.54[a]
MCHC (g/dl)	33.50±2.40[a]	30.76±2.50[b]	32.60±2.30[a]	32.40±2.10[a]	32.50±2.80[a]	32.30±2.07[a]

Data are (M±S.D) of six determinations (n=6). Values bearing different superscript letters (a, b,c) across the rows are significantly different ($p<0.05$) when compared to groups A and B.

Histopathology of the kidney of rats exposed to premium motor spirit and treated with aqueous leaf extract of *Vernonia amygdalina*

To determine how *Vernonia amygdalina's* aqueous leaf extract affected the kidneys of wistar rats, renal histopathology was performed. According to the groupings, the analysis's findings are shown in plates 1–6.

Plate 1: Photomicrograph of kidney section of a normal control rat showed normal renal cortex with its features well aligned in its normal kidney architecture and the features very conspicuous (Group A having histological normal kidney).

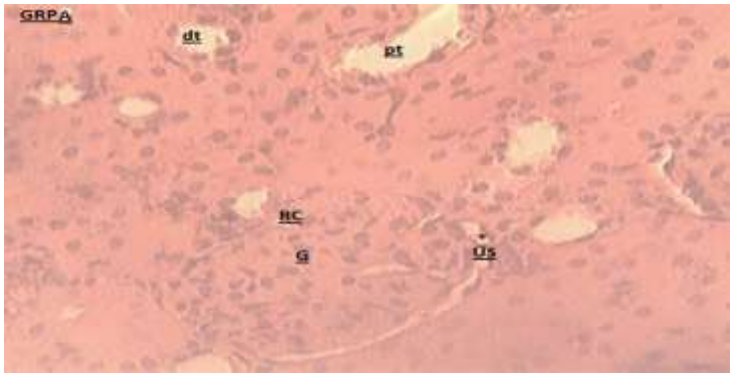
Plate 2: Photomicrograph of kidney section of a wistar rat exposed to PMS showed histological distortion. It have two renal corpuscle (RC); the first has its contents intact while the second has splitting glomeruli (g). The duo have urinary spaces (US) with slight fat deposits on the lubules (proximal and distal tubules) (Group B having histological distortion).

Plate 3: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with vitamin C standard drug showed regeneration. This group showed double renal corpuscles with wider urinary spaces. The tubules are more conspicuous than group B (Group C having regeneration).

Plate 4: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with 100 mg/kg *Vernonia amygdalina* leaf extract showed kidney with normal architecture but renal corpuscle somehow dilated with striking glomeruli. Tubules are not too conspicuous (Group D having slight distortion)

Plate 5: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with 200 mg/kg *Vernonia amygdalina* leaf extract showed histologically normal kidney with some degree of improvement as the renal corpuscle is restored although it had slight distortion at the upper left side (Group E having histologically normal kidney with certain degree of improvement).

Plate 6: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with 400 mg/kg *Vernonia amygdalina* leaf extract showed histologically normal kidney with well regenerated kidney lessions as features are seen intact (Group F having histologically normal kidney).



H&E staining; magnification X400
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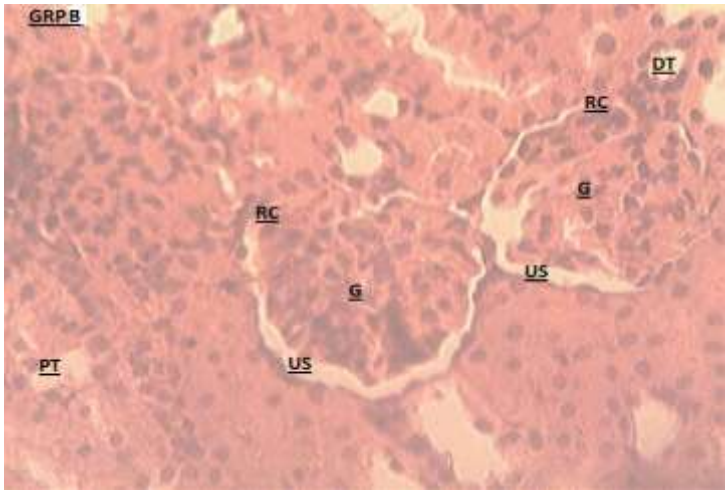
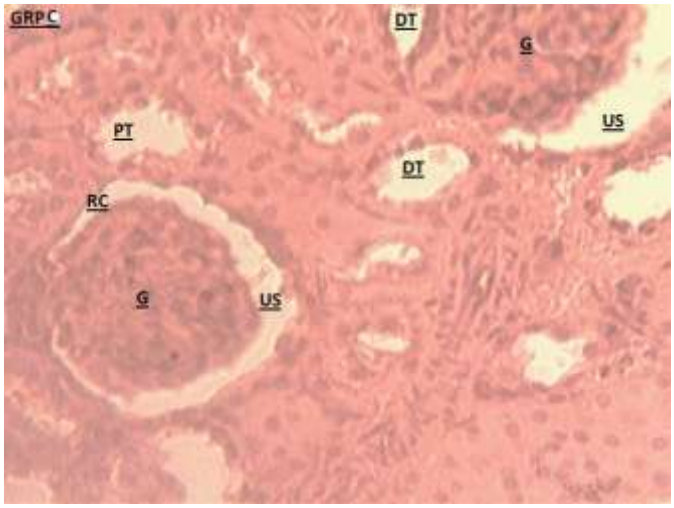


H&E staining; magnification X400

Plate 2: Photomicrograph of kidney section of a wistar rat exposed to PMS showed histological distortion. It have two renal corpuscle (RC); the first has its contents intact while the second has splitting glomeruli (g). The duo have urinary spaces (US) with slight fat deposits on the lubules (proximal and distal tubules).



H&E staining; magnification X400

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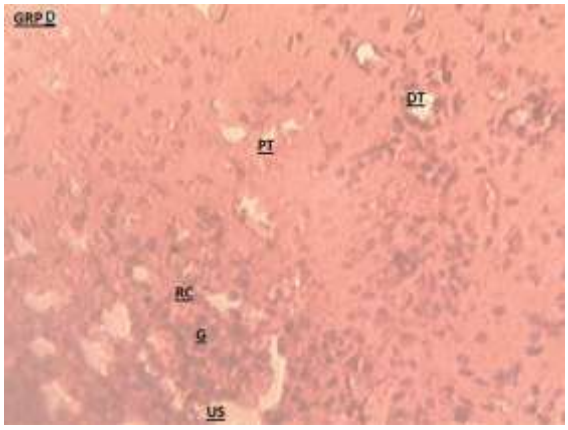

H&E staining; magnification X400

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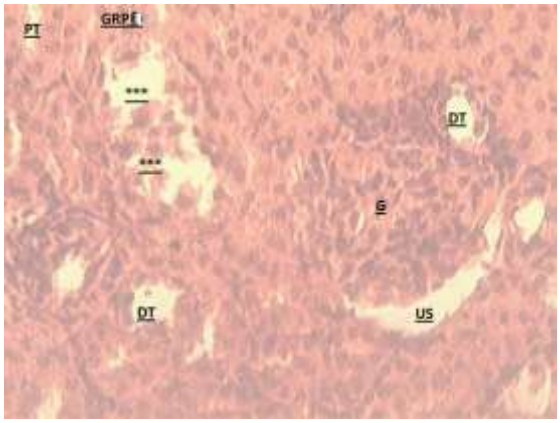
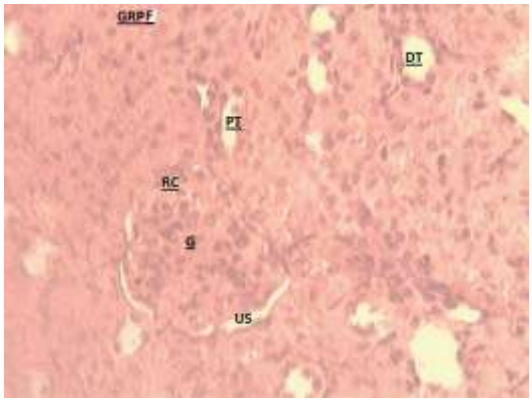


H&E staining; magnification X400

Plate 5: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with 200 mg/kg *Vernonia amygdalina* leaf extract showed histologically normal kidney with some degree of improvement as the renal corpuscle is restored although it had slight distortion at the upper left side.



H&E staining; magnification X400

Plate 6: Photomicrograph of kidney section of an albino rat exposed to PMS and treated with 400 mg/kg *Vernonia amygdalina* leaf extract showed histologically normal kidney with well regenerated kidney lessions as features are seen intact.

Discussions

The deleterious e*ff*ects of crude petroleum to the kidney had been well elucidated by earlier studies (Abebe & Gebru, 2015; Achuba & Nwokogba, 2015). Likewise, the distortion of metabolic stability through the consumption of petroleum tainted diets has been reported by Okpoghono et al. (2018). This study reports similar dysfunction of the kidney owing to exposure to PMS vapour evidenced by rising serum creatinine and urea in rats not treated with any dose of aqueous leaves extract of *Vernonia amygdalina* but exposed to PMS (Table 4). Rising serum creatinine and urea is an established indicator of poor glomerular *fi*ltration and has been established as a signi*fi*cant clinical marker for kidney dysfunction and loss of kidney integrity (Ogbeke et al., 2016; Achuba & Nwokogba, 2015). The present study, although observed no significant rise in serum sodium ion concentrations, there were signi*fi*cant increases in both serum potassium ion concentration and calcium ion concentration owing to exposure to PMS vapour (Table 4). These results are in agreement with the studies of Orisakwe et al. (2004) and Uboh et al. (2009). The observed high levels of calcium and potassium ions have been reported to be linked with the disruption of certain ion pumps and transmembrane ATPases owing to increased lyses within the kidney and the liver (Ita et al., 2013; Gowda et al., 2010). Furthermore, the study equally observed a significant increase in chloride ion concentration in rats owing to crude petroleum inhalation relative to the control which was in agreement with those reported in the study of Ita and Edagha (2016) which reported increased serum chloride ions due to oral consumption of crude petroleum. Although the treatment of rats with aqueous leaves extract of *Vernonia amygdalina* indicated observed reduction in the serum calcium, potassium and chloride ion concentration relative to those not treated especially at 200 mg/kg and 400 mg/kg, it is indicative that the aqueous leaves extract of *Vernonia amygdalina* had some level of e*ffi*cacy in balancing the observed distortion in electrolyte derangement.

The renal improvement potential possessed by aqueous leaves extract of *Vernonia amygdalina* in the presence of the toxicological e*ff*ects of crude petroleum inhalation may have been conferred on the leaves extract of *Vernonia amygdalina* due to its high antioxidant defense capacities as reported by their abundant antioxidants such as flavonoids, polyphenols, phenols and

isoflavones (Tables 1 and 2). According to Ijeh and Ejike (2011), plant extracts rich in flavonoids, polyphenols and alkaloids antioxidants have the tendency to enhance renal function.

It is reported that reduction in haematological index is an indication of anemic state of an animal (Christain et al., 2016). In addition, the increase in WBC indicates response of the immune system to disease stimulating toxicants (Okoye et al., 2014; Ita et al., 2013). This explains why there is alteration in immune cells in this study (Table 5), which agrees with the report of Okonkwo et al. (2016). Previous studies indicated that certain organic and inorganic substances can mitigate the toxic effect of petroleum hydrocarbon (Achuba & Nwokogba, 2015; Achuba *et al.,* 2016). This is consistent with the result of the present study. Administration of aqueous leaves extracts of bitter leaf to rats exposed to PMS vapour reduced the deleterious effect of PMS on the exposed animals. As a matter of fact, the wound healing property of bitter leaf is previously documented (Oguwike *et al*. 2014).

It is no surprise that exposure of rats to petroleum hydrocarbon could elicit disease process (Ita *et al.,* 2015). This agrees with the present investigation that showed alterations in white blood cell profile (Table 5). Similarly, administration of aqueous leaves extracts of bitter leaf to rats exposed to PMS vapour maintained white blood cell types relative to the level in control animals. However, the white blood types were enhanced by bitter leaf in diet close to values in control rats. The immunostimulant property of bitter leaf was earlier reported (Osho *et al.,* 2014).

The significant increase in RBC, Hb, PCV, WBC and platelet levels in rats administered aqueous leaves extract of *Vernonia amygdalina* strongly suggest positive hematological potential that could reduce risk of complications like anemia (Agomuo *et al.,* 2017). Precisely, the significant increase in the white blood cell count showed that the white blood cells differentials were not compromised or destroyed by the extracts in line with the report by Haratym-Maj (2002). This further implies intake of leaves extract of *Vernonia amygdalina* will not trigger immune-suppression observed from the reduction in white blood cell differential indices like monocyte levels (Amadi *et al.,* 2018).

The histopathology of the kidney revealed that rats exposed to PMS vapour without treatment showed clear distortion of kidney tissues which further supports the results of the renal parameters. As presented by the histopathological examination (Plates 1-6), rats treated with aqueous leaves extract of *Vernonia amygdalina* at 200 mg/kg and 400 mg/kg were observed to show visible regeneration and clearance of the observed tissue necrosis than rats that were exposed to PMS but not treated with aqueous leaves extract of bitter leaf. Similar findings were reported by Achuba and Ichipi-Ifukor (2020) on the histopathology of liver of rats administered petroleum contaminated diet and treated with methanolic extract of bitter leaf.

CONCLUSIONS

Conclusively, this study has clearly demonstrated the protective tendency of aqueous extract of *Vernonia amygdalina* on weight gain, renal, liver, hematological parameters of albino rats exposed to premium motor spirit, hence can be recommended against toxicity to PMS damage. Further studies should be undertaken to ascertain the mechanism of positive modulation of aqueous leaves extract of *Vernonia amygdalina*. We equally recommend further studies that will require varying the duration of treatment with aqueous leaves extract of *Vernonia amygdalina* at uniform extract concentration to know if it will have any effect on biomarkers.

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