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Protection Effects of Vernonia Amygdalina Methanolic Extracts Against Hepatocellular Damage Induced by Petroleum Contaminated Diets in Male Rats

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Abstract

Recently, bitter leaf (*Vernonia amygdalina*) was found to prevent petroleum – induced toxicities on the kidney whereas it potentiates the toxic effect of petroleum adulterated diet on the testes of animal model. This differential action has elicited further inquest into the role of bitter leaf extract in other organs in the midst of petroleum affronts. The hepatoprotective ability of *Vernonia amygdalina* methanol extract (VAME) is the objective of this investigation. Administration of VAME significantly ($P < 0.05$) reduced serum liver function indices relative to the control. In addition, the activities of liver oxidative enzymes, energy metabolizing enzymes and oxidative stress indices altered by crude oil adulterated diet were significantly ($P < 0.05$) reversed near control values. Similarly, VAME injection restored the histopathological indices caused by crude oil adulterated diet. It is obvious that all the liver damage indices induced by crude oil contaminated feed were prevented by the intake of VAME, indicating the hepatoprotective ability of bitter leaf

Key words: Bitter leaf, liver, lipid panel, oxidative stress

1. Introduction

It is an unhidden truth that petroleum- related resource is a major source of income in some countries of the world. However, as it generates revenue for the country, its deleterious effect to the unbundling of our environment continues to be a serious case for worry. Release of environmental petroleum-related substances occurs in several ways and manners that range from industrial oil spills in pipelines and tankers, mechanic workshops and even in some cases domestic accidents [1-4].

Today, man stands a greater risk of being consciously or unconsciously exposed to several petroleum products and its allied constituent chemicals [5,6].Several compounds related to petroleum are broken down to free radicals or highly activated metabolites that often results in several illnesses such as cancer, neurodegenerative and metabolic syndromes[7-9] . The noxiousness of environmental petroleum is highly documented as numerous reports also indicate that food borne allied exposure of petroleum to man remains a serious challenge that must be undaunted [10, 11]. Although several plant-related materials such as *Monodora myristica* [12]; *Moringa oleifera* [13]; Honey [14]; Palm oil [15,16] have been explored for controlling of food borne petroleum toxic consequences, there remains scarcity of reports on the beneficial role of bitter leaf on ameliorating food borne petroleum noxiousness in the liver.

With the understanding that the liver is the primary site of xenobiotic metabolism [17], it is justifiable to state that it will be most vulnerable to the toxic effects of food borne petroleum toxicity in man. Earlier, the beneficial role of *Vernonia amygdalina* had been published on kidney and haematological indices of rat [18, 19]. This report presents the ameliorative role of *Vernonia*

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amygdalina methanolic extract (VAME) on petroleum contaminated diet induced hepatocellular changes in rats.

2. Materials and Methods

The bitter leaf (*Vernonia amygdalina*) was obtained from a farm at Abraka, treated and the methanolic extract was prepared and administered(at a concentration of 100 mg/ pbwt and 200 mg/pbwt) as reported previously [18].This research is a continuation to a previous study. Therefore, animal mobilisation and experimental design followed a previously published experimental design [18] .The feed composition and feeding regimen were published earlier [18].

2.1 Sample Collection and treatment

The male (*Rattus norvegicus*) rats after treatment period were sacrificed by cervical decapitation under chloroform sedation on the 31st day after an overnight fasting. The Blood and liver samples were collected and prepared for serum and tissue homogenate analysis as previously reported [18]

2.2 Biochemical Analysis

Determination of serum AST, ALT, ALP, ACP, Glucose, Total Protein, Albumin, Globin and Lipid Profile (Cholesterol, TAG, HDL and LDL) were done using commercial Randox diagnostic kits. While Glucose-6- phosphate dehydrogenase activity was determined following the method of Henry [21] All other reagents used for biochemical assay were of analytical grades and employed the use of the experimental procedures such as Gutteridge and Wilkins [20] method for lipid peroxidation (MDA) ,Omarov et al. [22], D'Errico et al [23] and McEwen [24] for aldehyde oxidase (AO) , sulphite oxidase; xanthine oxidase (XO) and monoamine oxidase (MO) activities respectively. The level of reduced glutathione in the liver was determined using the procedures of Ellman [25] while vitamin C estimation employed the method reported previously [26]. The following methods were adopted during the determination of enzymatic antioxidants activities , which were Misra and Fredorich [27], Cohen et al. [28] Habig et al. [29], Khan et al [30] for superoxide dismutase (SOD), Catalase (CAT), gluthatione-s-transferases (GSTs) and glutathione peroxidase (GPx) activities, respectively.

2.3 Histological Analysis

The examination of the liver tissues followed the method of Al-Attar et al.[31] which has been described in detail by Achuba [18]

2.4 Statistical Analysis

The Statistical Package for Social Sciences (SPSS 17) was employed to perform analysis of variance (ANOVA) and Post hoc examination (multiple groups comparisons) performed with Bonferroni at $p<0.05$ significance point.

Results and Discussion

The continual search for antidote for petroleum toxicity remains an onus task for scientists as people living in oil producing areas of the world contend with several illnesses arising from petroleum pollution and cross contaminations [32, 33]. Evidences from this present study indicates that the consumption of petroleum contaminated diets elevated the various liver function enzymes in the serum (AST, ALT, ACP, ALP) while reducing serum glucose, total protein, albumin and globulin levels compared to rats that did not consume petroleum tainted diets (Table-1).

Table 1-Effect of VAME on serumBiochemical liver function parameters of rats fed petroleum contaminated diet

	A	B	C	D	E	F
AST(UL^{-1})	58.98±0.59^{a}	53.47±1.03^{ab}	56.05±0.57^{b}	69.94±1.61^{c}	54.17±10.35^{c}	57.09±7.76^{d}
ALT(UL^{-1})	41.62±0.94^{a}	40.97±1.48^{b}	44.99±1.21^{b}	59.33±3.92^{c}	43.70±2.89^{d}	48.09±4.65^{d}
ACP(UL^{-1})	4.48±0.36^{a}	5.87±0.56^{b}	5.07±0.88^{c}	11.88±0.61^{d}	7.99±1.62^{e}	7.82±1.27^{f}
ALP(UL^{-1})	112.63±0.83^{a}	126.77±2.61^{b}	130.94±1.00^{c}	149.15±5.03^{d}	142.01±5.51^{e}	146.55±5.47^{f}
Glucose(gdl^{-1})	137.50±1.29^{a}	133.00±0.82^{a}	132.25±1.71^{b}	121.50±5.20^{b}	123.67±0.57^{b}	119.50±1.29^{b}
TotalProtein (gdl^{-1})	6.30±0.37^{ac}	6.45±0.13^{ac}	6.25±0.13^{ac}	4.70.0.18^{ac}	5.77±0.15^{b}	5.65±0.33^{b}
Albumin(gdl^{-1})	3.55±0.13^{a}	3.70±0.14^{a}	3.18±0.13^{b}	2.50±0.22^{ab}	3.10±0.10^{b}	2.66±0.13^{c}
Globin(gdl^{-1})	2.70±0.08^{a}	2.55±0.34^{a}	3.00±0.18^{ab}	1.40±0.37^{b}	2.77±0.15^{ab}	2.93±0.22^{ab}

Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME.

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference

There is no doubt that petroleum contamination has been severely implicated in the up regulation of liver function enzymes such as the tansferases and phosphatases [14, 34]. The observed up-regulation of the liver enzymes (transferases and phosphatases) and the concomitant reduction in serum protein levels may be indicative of critical threats to the liver integrity and is similar to the once reported by Okpoghono et al.[35], Achuba and Ogwumu [16] who reported on the consumption of petroleum contaminated cat fish meal and petroleum tainted diet an upsurge in these enzymes and reduction in the serum proteins. Treatment with both doses (100mKg^{-1} and 200$mgKg^{-1}$) body weight of Bitter leaf methanolic extract was observed to mitigate the liver enzymes while modulating the total protein, albumin and serum glucose levels. This observation is consequential with reports made in previous studies as to the ability of plant materials to mitigate the upsurge in serum liver enzymes. It is important to note that the high phytonutrients and phytochemicals inherent in plant materials have been previously reported to contribute to the eventual mitigation and reversal of petroleum induced upsurge in AST, ALT, ACP, ALP as reported by Achuba *et al.* [13], Okpoghono et al.,[35] and Achuba and Ogwumu [16].

Serum lipid profiling has been identified as a very significant marker for possible cardiovascular disorders [13, 36]. Likewise serum lipid profile has also been used as a significant marker for the integrity of liver cells [35]. The observed drop in total cholesterol, triglyceride, HDL-cholesterol and concomitant rise in LDL-cholesterol in rats consuming only petroleum contaminated diets and rats treated with both doses of VAME after consumption of petroleum tainted diets compared to normal control (Table-2) is in line with the report of Achuba and Otuya [37] who reported the ability of antioxidant vitamins to modulate positively the lipid panel of rats fed with petroleum tainted diet.

Table 2-Effect of VAME on serum lipid profile of rats fed petroleum contaminated diets

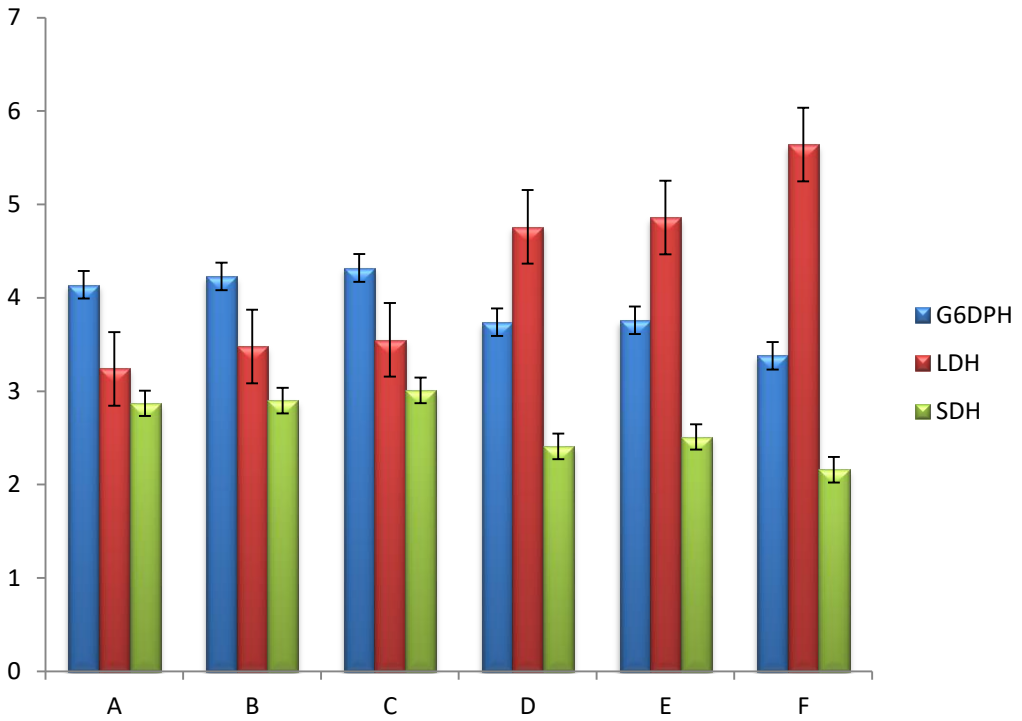
	A	B	C	D	E	F
Cholesterol(mg /dl)	104.25±0.96^{a}	101.50±1.29^{b}	101.50±1.29^{b}	115.25±0.96^{a}	111.00±1.00^{c}	105.00±0.82^{d}
TAG(mg/dl)	130.50±1.91^{a}	123.00±2.58^{b}	108.25±2.06^{c}	126.25±2.36^{b}	127.00±1.00^{c}	124.25±1.25^{d}
HDL(mg/dl)	18.25±0.96^{a}	13.00±0.82^{bc}	12.75±1.25^{b}	14.00±0.82^{ac}	18.00±1.00^{ac}	15.50±1.29^{c}
LDL(mg/dl)	40.03±24.96^{a}	33.33±0.73^{b}	35.05±1.10^{b}	43.50±0.51^{b}	36.17±2.86^{b}	37.27±1.96^{b}

Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME.

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference

Several plant materials have been reported to contribute to eventual reversal of such trends near control values [13, 38, 39]. The trend in the observations made in this study further gives credence to an earlier implication of extracts of bitter leaf on lipid profiles [40].

Another significant observation in this study was the fluctuations in key enzymes of energy metabolism (Figure-1). The observed drop in glucose 6 phosphate dehydrogenase and increased lactate dehydrogenase activities in rats fed only petroleum contaminated diets and those treated with both doses of VAME after consumption of petroleum polluted diets relative to the control group may be indicative of a starve in energy demand owing to the toxicity impact of petroleum diet consumption and the eventual counter-effective functions of the VAME.



Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME.

Figure 1-Effect of VAME on key enzymes of energy metabolism in the liver of rat fed crude oil contaminated diet

It is important to note that the G6DPH is a significant enzyme catalyzing the oxidation of glucose 6 phosphate in the pentose phosphate partway while contributing to the supply of reducing energy potentials to cells for the continuation of the energy cycle and to tissues involved in the biosynthesis of fatty acids[41-43]. This drop in the G6DPH activity could be the justification of the increased activities of LDH which could be said to have been triggered for the promotion of anaerobic respiration for replenishing the increased depletion of NADPH within the cells which has been cut-short by possible inhibition of the G6DPH. A further drop in the SDH activities also gives credence to our earlier claim and suggestion of possible drop in energy supply owing to shortfall of certain metabolites. Succinate dehydrogenase has been reported to be a very significant enzyme in the citric acid cycle (TCA) and the electron transport chain (ETC). In the TCA cycle, it catalyses the oxidation of succinate to fumarate which eventually supplies the $FADH_2$ needed for the reduction of ubiquinone to ubiquinol [44- 46]. By implication therefore, the alternative promotion of LDH activities within the metabolic cycle could be said to have not been effective enough for the supply of the needed substrates that promotes the TCA cycle hence drop in the SDH activities.

Increase in lipid peroxidation (MDA) and activities of the oxidative enzymes (AO, SO, MO and XO) has been noted as very significant indicators of metabolic stress (Table-3).

Table 3-Effect of VAME on lipid peroxidation and oxidative enzyme activities in the liver

Groups	MDA $\mu molml^{-1}$	AOUnitsg^{-1}tissue	SO Unitsg^{-1}tissue	MO Unitsg^{-1}tissue	XO Unitsg^{-1}tissue
A	40.19±0.49^{a}	76.00±3.65^{a}	666.75±28.55^{a}	157.75±12.55^{a}	62.25±1.71^{a}
B	40.58±0.67^{a}	85.75±5.38^{b}	705.25±32.55^{ab}	166.00±11.17^{ab}	66.70±1.71^{ab}
C	44.96±0.96^{b}	89.50±5.44^{b}	747.25±19.24^{b}	182.75±2.98^{b}	70.50±2.08^{bc}
D	43.65±1.16^{bc}	93.00±2.94^{b}	772.25±13.67^{bc}	189.75±2.74^{bc}	73.50±2.38^{c}
E	42.38±1.78^{ab}	106.00±7.70^{c}	790.25±12.71^{bc}	193.75±4.35^{bc}	76.00±2.16^{d}
F	41.37±1.51^{ac}	119.00±3.56^{d}	804.75±5.37^{c}	203.00±5.10^{c}	80.00±2.16^{d}

Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference

The increased MDA levels in rats only fed petroleum tainted diets is in response to the generation of reactive oxygen species while the rise in the oxidative enzymes occurred as a result of a second messenger response for the clearance of the oxidative radicals generated within the tissues so as to help reduce the rising MDA and is in agreement with the studies of Okpoghono *et al.* [35]; Achuba *et al.* [13]. Although treatment with both doses of VAME further led to a rise in MDA levels and increase in oxidative enzyme activities this must have occurred as a result of the abilities of VAME to promote or induce the oxidative enzyme activities to rise up to the increasing challenge of petroleum induced oxidative stress. On the other hand rising lipid peroxidation and increase in ROS has been reported to be a significant inducer of several antioxidant enzymes to aid the clearance of the oxidative radicals. Such enzymes as SOD, catalase, GPx and GSTs are known buffers for oxidative stress defense systems. Other non-enzymatic antioxidants such as vitamin C and GSH are also said to contribute to this oxidative buffering system but may be depleted when overpowered by a high degree of ROS generation. As in other studies, there were observed depletion in non-enzymatic antioxidants Vitamin C and GSH as well as catalase, and GSTs activities while GPx increased in rats consuming only petroleum polluted diets compared to control (Table-4).

Table 4-Effect of VAME on some enzymatic and non-enzymatic antioxidant profiles in liver

	Vit.C mgg^{-1}Fwt	GSH µmolmg^{-1} protein	CAT µmolmg^{-1} protein	GPx µmolmg^{-1} protein	GSTs µmolmg^{-1} protein
A	4.13±0.44^{a}	0.37±0.84^{a}	168.06±1.99^{a}	0.39±0.03^{a}	390.75±29.48^{a}
B	4.83±0.17^{a}	0.41±0.72^{a}	170.20±1.76^{a}	0.41±0.03^{ac}	418.25±39.28^{ab}
C	4.93±0.30^{a}	1.57±2.29^{a}	213.04±1.46^{b}	0.92±0.04^{b}	455.25±21.75^{b}
D	3.55±0.34^{a}	0.31±0.06^{a}	162.99±0.70^{c}	0.78±0.07^{b}	343.00±8.04^{ac}
E	3.45±0.31^{a}	0.29±0.05^{a}	113.57±1.10^{d}	0.80±0.07^{b}	359.00±18.31^{c}
F	2.18±0.66^{c}	0.23±0.05^{a}	112.32±0.51^{d}	0.55±0.09^{c}	316.00±10.86^{c}

Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference

Treatment with both doses of VAME alone was also observed to have boosted the levels of these antioxidants, post treatment of VAME after petroleum diet consumption was observed to have further declined compared to control and the rats not treated after petroleum diet consumption (Figure-2).

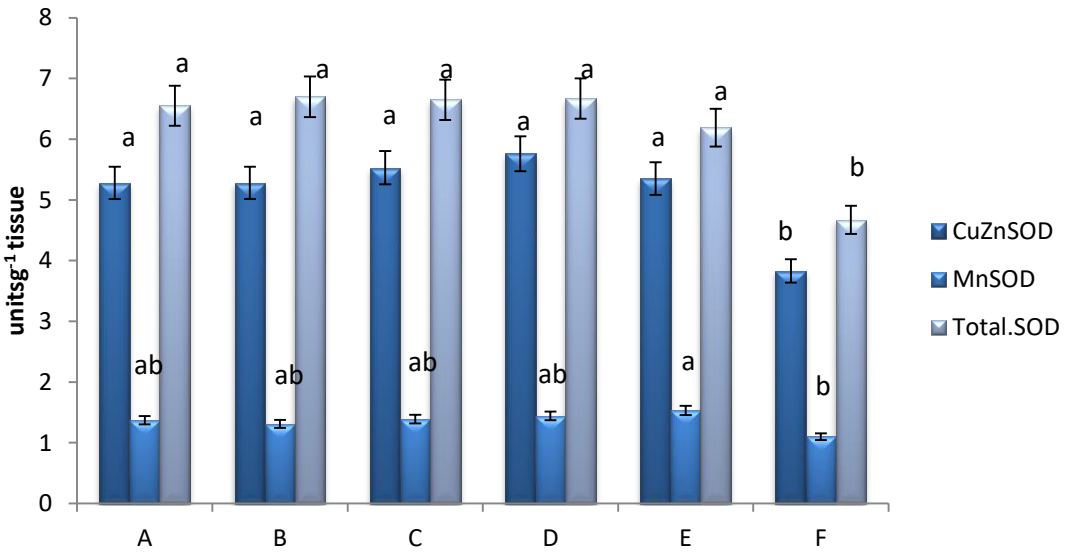


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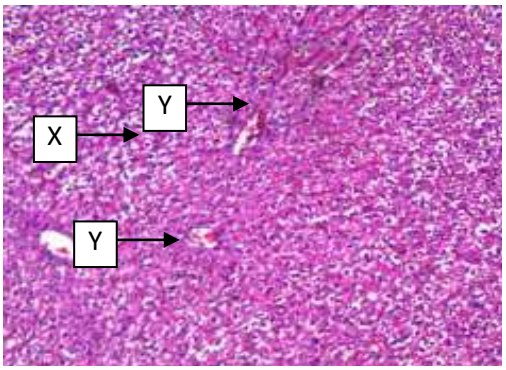
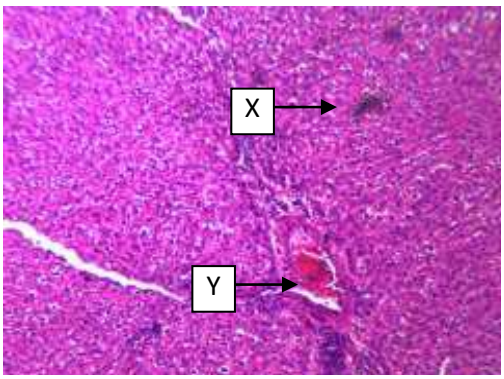
Figure 2- Effect of VAME on Liver CuZnSOD, MnSoD and total SOD activities. Different alphabet different superscripts on bars of the colour designate a significant difference.

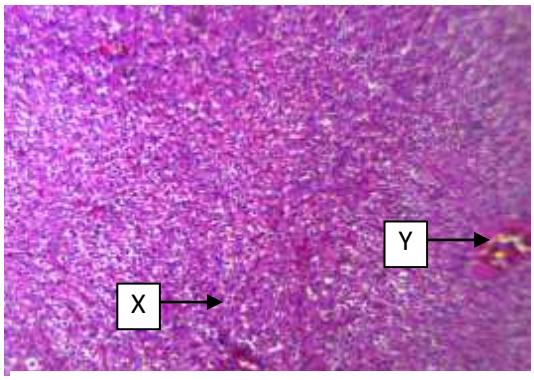
The possible justification for this trend is that although VAME contributed to antioxidant defense, the veracity of ROS generation due to petroleum metabolism within the liver tissue may have overpowered the continual upsurge in the antioxidant defense hence the eventual depletion. In giving further explanation to this phenomenon various authors submitted that metabolic stress inducers are also significant inducers of several antioxidant and oxidative enzymes which act competitively until either of them (stressor or antioxidant) is overpowered to achieve metabolic stability or instability[47-49].



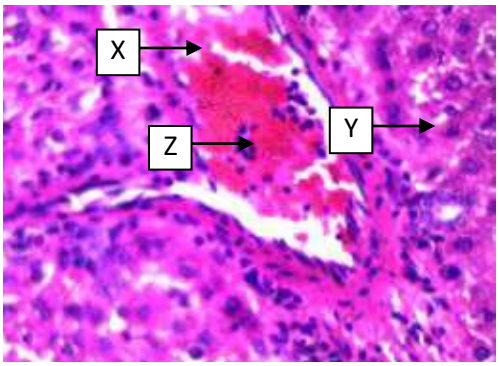
A Normal hepatic cells showing visible nucleus (x) and hepatic vein (Y)



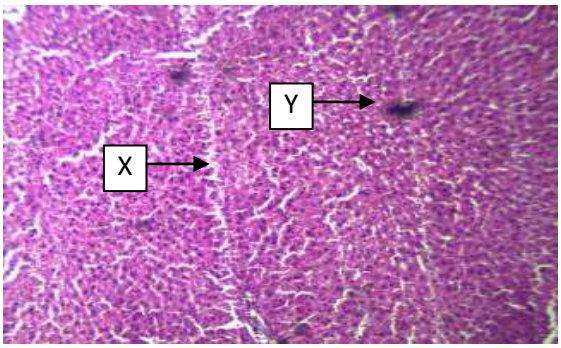
B Normal hepatic cells showing round nucleus (X) and congested veins (Y)

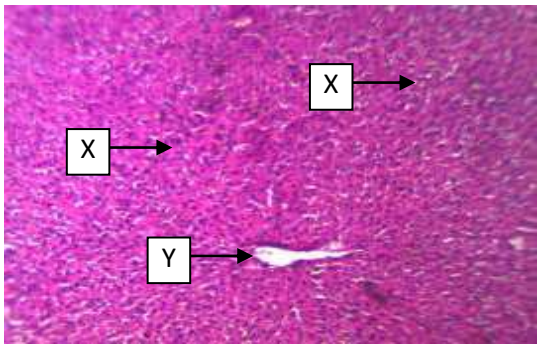


C Normal hepatic cells showing slight congestion of hepatic vein (X) and round nucleus (Y)



D ballooning of hepatic veins (X) and Inflammation and congestion of nucleus (Y) Hepatic Necrosis (Z)





E Reduced necrosis showing very clear nucleus (X) and slight inflammation and congestion of central

Reduced hepatic necrosis showing very clear nucleus (X) Hepatic sinusoid (Y)

Group A=Feed; Group B=Feed+100 mg kg^{-1} body weight of VAME; Group C=Feed+200 mg kg^{-1} body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg^{-1} body weight of VAME; Group F= Tainted feed +200 mg kg−1 body weight of VAME.

Figure 3A-F: Photomicrographs of rat liver fed petroleum contaminated diets and treated with bitter leaf extract. Haematoxylin and Eosin x 100 magnification

The liver histology revealed that rats administered only petroleum contaminated diet indicates a very visible distortion of the liver architecture which gives further credence to the observed depletion in antioxidant defence systems (Figure-3A-F). Treatment with both doses of BLME conferred protection on the liver integrity; this is in agreement with similar observations made in the kidney by Achuba [18] which also reported the prevention of petroleum induced kidney necrosis by BLME. This further gives credence to earlier report by Kambizi and Afolanya [50] on the efficacy of bitter leaf.

Conclusions

The present study further established the role of petroleum contaminated diet consumption in the induction of several metabolic anomalies; it also showed the ability of VAME to confer certain levels of protection to the liver on the outcomes of these anomalies.

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