EFFECTS OF AQUEOUS LEAF EXTRACT OF *PHYLLANTHUS AMARUS* ON LIVER FUNCTION AND BLOOD PARAMETERS IN MALE WISTAR RATS.

Ajiboye Kolawole[1]* I., Ndubusi Blessing[1], Ajiboye Oyebimpe F[2] and Oluwole Francis S[3]

1 Department of Physiology, Benjamin S Carson Snr School of Medicine, Babcock University, Nigeria

2 Radiodiagnosis Department, Babcock University Teaching Hospital, Babcock University, Nigeria

3 Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

(Received on Date: 23 June 2019 Date of Acceptance: 22st July 2019)

ABSTRACT

Liver damage is a clinical syndrome that is characterized by the disturbances of liver function thus compromising its integrity and the capacity to meet the needs of the body. One of the most common sources of function-assault on the liver is via the ingestion of uncertified herbal products in the name of medicinal plant. *Phyllanthus amarus* (P. *amarus*) is one such herbal plant that has varied use in ethno-medicine but with minimal toxicological studies or report backing its use. This study investigated the effect of *P. amarus* on haematological parameters and on liver function in male Wistar rats. Thirty – six adult male rats weighing 120- 140 grams were randomly distributed into six groups of six animals each. Rats were fed with regular chow and given water ad libitum. Liver injury was induced by administration of acetaminophen (800mg/kg BW). Graded doses of *P. amarus* (50mg/kg and 500mg/kg BW) was administered post-liver damage induction. Silymarin (200mg/kg) was administered as the standard drug. Data was reported as mean ± SEM and analysed by One Way ANOVA, followed Newman Keul's post hoc test. $p<0.05$ was taken as statistical significant. Results showed there was a significant increase in RBC count secondary to *P. amarus* administration when compared to control group. The same response was observed for the haematocrit index. Alanine phosphate (ALP) level was significantly raised in sham-operated animals when compared to control thus demonstrating a successful liver damage induction. The different graded doses of *P. amarus* produced significantly lower ALP when compared to the sham-operated rats. These observations are supported by the liver histo-architecture study. The sham-operated animals have lobular necrosis as evidenced by appearance of apoptotic bodies. The *Phyllanthus amarus*-treated rats showed mildly inflamed and degenerating hepatocytes in what looks likes the early stages of repair. All these point to the ameliorative effects of *P. amarus* in acetaminophen-induced hepatoxicity. It may be concluded that *P. amarus* possesses mitigating effects on liver injury in acetaminophen-treated rats. Its consumption as a local herbal remedy may therefore be considered safe with regards to blood parameters and liver function.

Key words: *Phyllanthus amarus*, Acetaminophen, liver damage, hepatotoxicity.

No: of Figures: 7 **No: of Referenes: 9**

INTRODUCTION

The liver is one of the most metabolically active organs in the body. Hepatocytes (liver parenchymal cells) are the basic unit of the liver structure and perform the liver's metabolic functions. The liver is involved in nutrient metabolism and it processes the various digestive products (glucose, amino acids, glycerol and fatty acids) via the hepatic portal vein. The hepatocytes deal with nutrient availability and alter the metabolic pathways of these energy substrates accordingly. For liver damage assessment, enzymes involved in intermediary metabolic are commonly assayed in the serum. Among them are alanine aminotransferase, aspartate transaminaseand alkaline phosphatase.

Plants are not only a staple source of nutrition but an affordable medicinal source especially for populations in low income countries. More than 60% of drugs today are of natural plant-product origin. Hence, plants are now an essential part of drug development programs in the pharmaceutical industry (Burton *et al*., 1983). Like the synthetic therapeutic agents, there is the danger of drug overdose or abuse with the potential to induce adverse effects when it comes to phytotherapy. P. amarus is a broad spectrum medicinal plant with a variety of applications across many cultures world-wide (Srividya and Periwal, 1995). It has different names among the various tribal groups in Nigeria. It is called "Oyomokeisoamankedem" in Efik, "eyin Olobe" in Yoruba and "Ebebenizo" in Benin, "ngwu" in Igbo and "geeron-tsuntsaayee" or bird's millet in Hausa. *P. amarus* is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, used as anti-helminthic to expel intestinal worms, as a mild laxative and has also been shown to have a mitigating effect on chronic carriers of hepatitis B virus (Thyagarajan *et al.,* 1988). Despite the varied use of *P. amarus* in ethno-medicine, there is a dearth of toxicological studies or report backing its use. Given that the liver is the organ tasked with detoxification of ingested food or drink in the body, this study investigated was designed to investigate the effect of *P. amarus* on haematological parameters and on liver function in male Wistar rats.

METHODOLOGY

Plant preparation and extraction

A quantity of 255g of healthy and shade-dried leaves of P. amarus was reduced to fine powder and was extracted using 70% ethanol via soxhlet hot extraction procedure. It yielded 25.7g of P. amarus extract, representing 10.01% yield.

Animals

Thirty six adult male rats weighing 120- 140 grams were randomly distributed into six groups of six animals each. All rats were rats fed with regular rat chow and water and were kept in a temperature controlled facility with a 12 hour light/dark cycle; food was withdrawn 12–15 hours prior to treatment with acetaminophen. Blood was drawn from the retro-orbital sinus and then centrifuged to obtain serum. The animals were thereafter sacrificed by cervical dislocation under anaesthesia. The

liver was excised and portions were flash frozen for determination of liver function biomarker content. The remaining portion was fixed in 10% phosphate-buffered formalin for histological assessment (H&E staining). Experiments on animals were performed in accordance with the guidelines and regulations set by the Babcock University Health Research and Ethics Committee, Nigeria.

Induction of Liver Damage

Liver damage was induced by oral administration of 800 mg/kg body weight acetaminophen in what was a modified method of the standard protocol (McGill *et al.* 2012). The drug was administered p.o. at the indicated doses in metabolically inert vehicle (20% Tween-80) which is commonly used for mouse and rat models and have been shown to not interfere with acetaminophen toxicity or mechanism of action (Kelava et al., 2010). Graded doses of the extract of *P. amarus* were administered after 5 days to test group animals. The reference group was administered silymarin, a standard liver injury drug. Administration of various treatments was continued for 2 weeks post liver damage induction. Liver function biomarkers, Aspartate Transaminase(AST), Alanine Transaminase(ALT) and Alkaline Phosphatase (ALP) were assayed for using Randox kits.

Table 1: Animal Grouping and Treatment Schedule

Group 1 (control)	Normal rat chow and water ad libitum
Group 2 (negative control)	Acetaminophen 800mg/kg
Group 3 (low dose)	Acetaminophen 800mg/kg + 50mg/kg *P. amarus* Extract
Group 4 (high dose)	Acetaminophen 800mg/kg + 500mg/kg of *P. amarus* Extract
Group 5 (reference group)	Acetaminophen 800mg/kg + Silymarin 200mg/kg
Group 6 (curative group)	*P. amarus* 500mg/kg + Acetaminophen (APAP) 800mg/kg

RESULTS

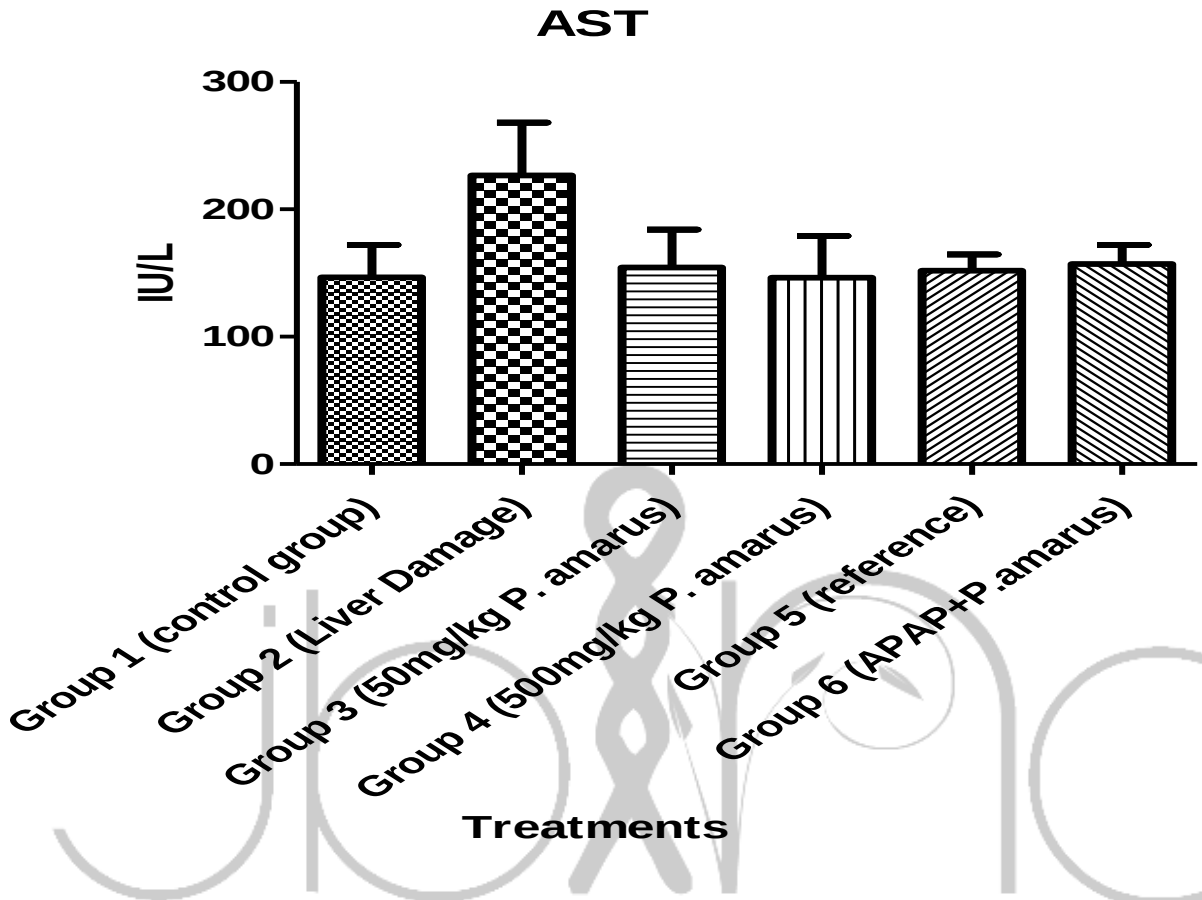


Figure 1: serum concentration of aspartate transaminase in the control and test groups

Data is presented as Mean ± SEM, $p<0.05$

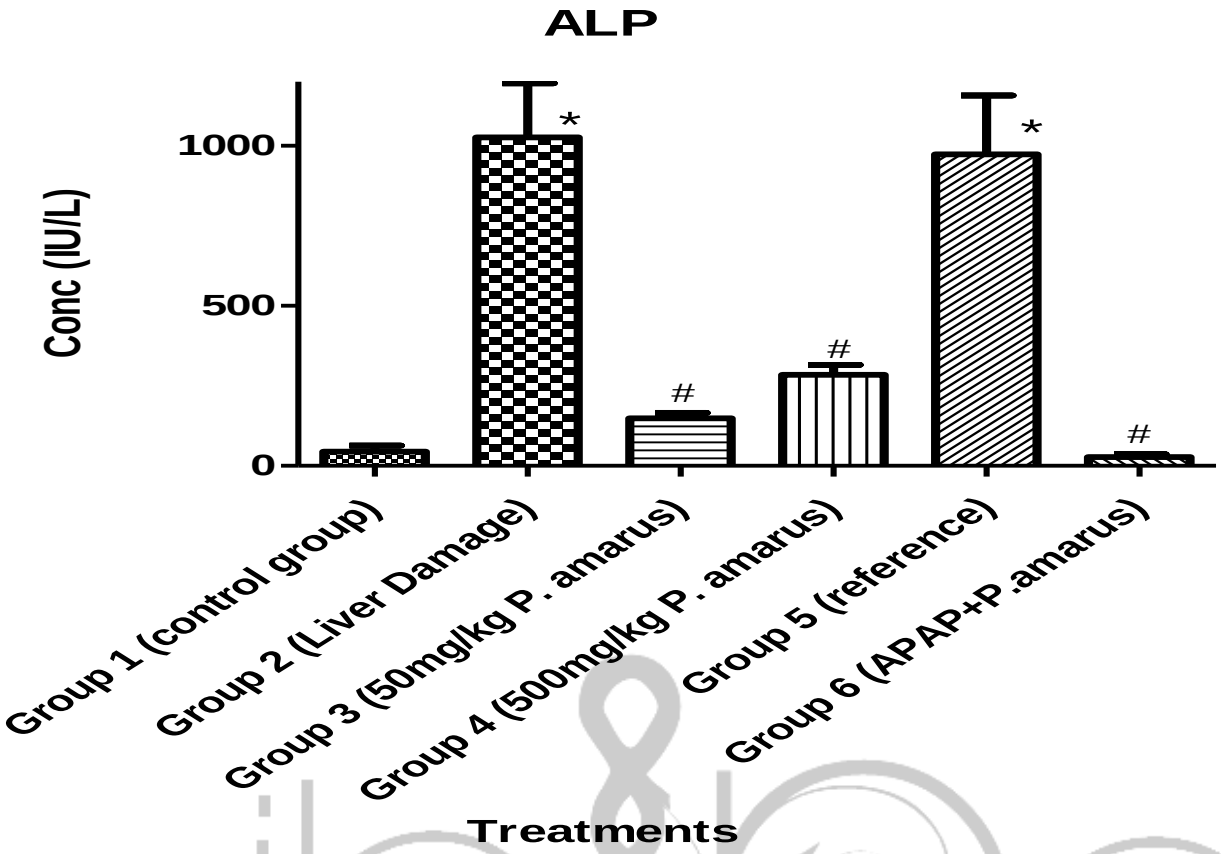


Figure 2: Serum concentration of alkaline phosphatase in the control and test groups

Data is presented as Mean±SEM,

*- significant when compared with Control, $p<0.05$

#- significant when compared with sham-operated, $p<0.05$

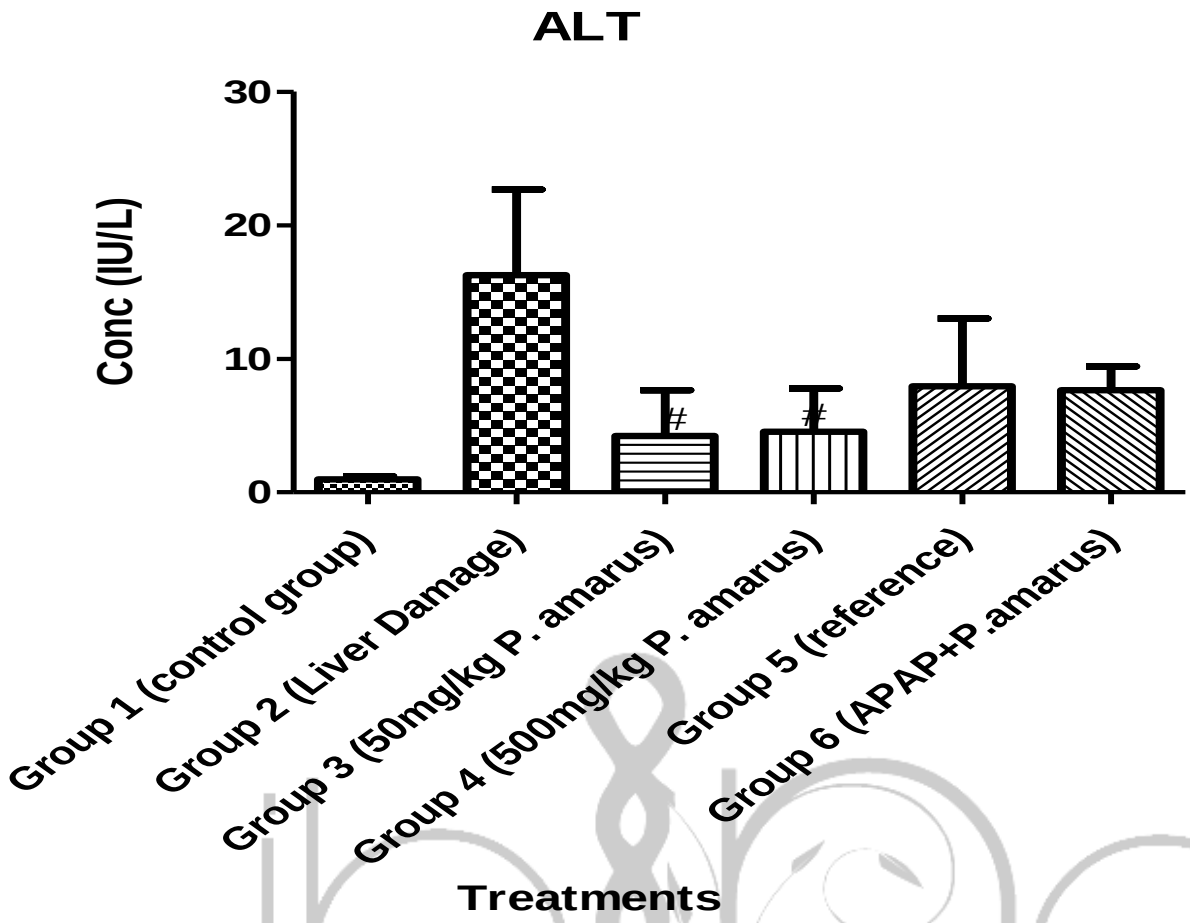


Figure 3: Serum concentration of alanine transaminase in the control and test groups

Data is presented as Mean±SEM,

*- significant when compared with Control, $p<0.05$

#- significant when compared with sham-operated, $p<0.05$

BLOOD PARAMETERS

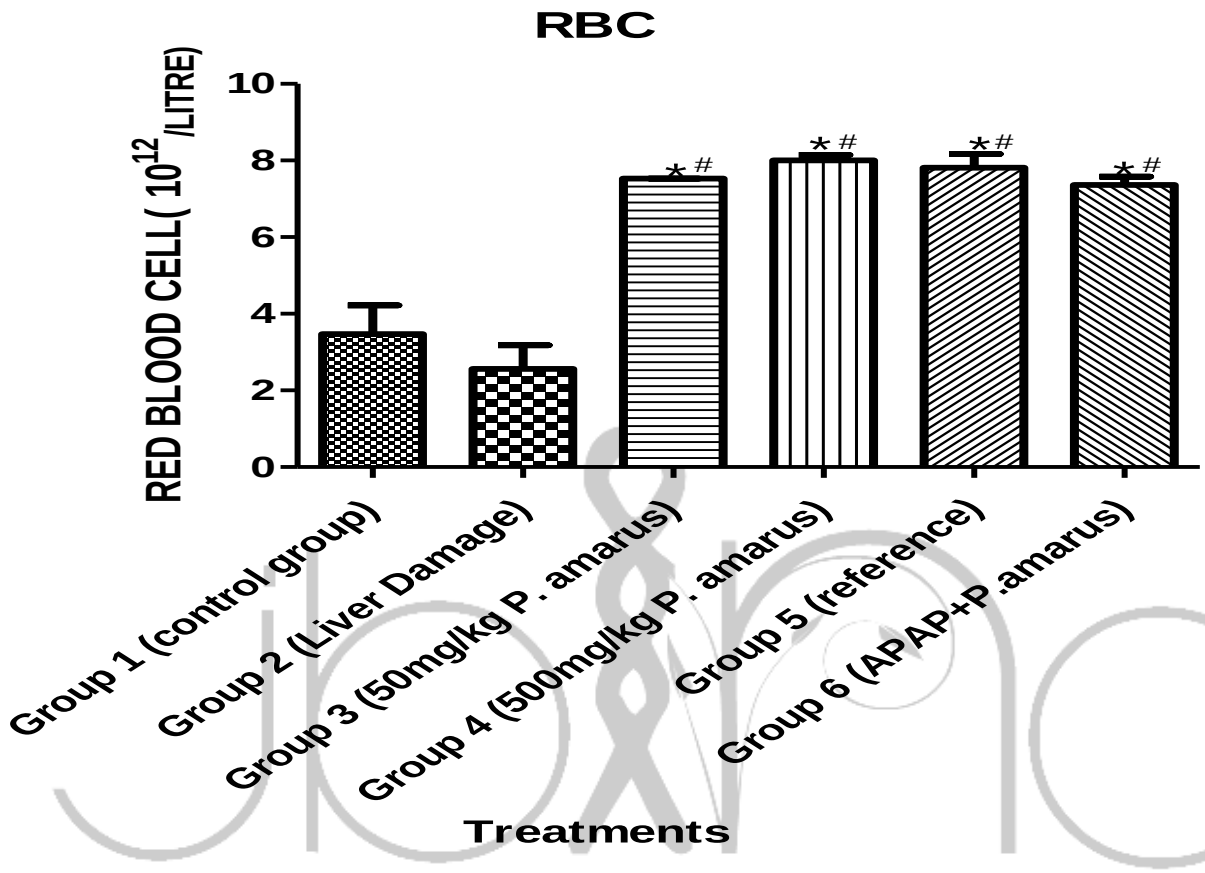


Fig 4: Red Blood Cell Count in Control and test groups

Data is presented as Mean±SEM,

*- significant when compared with Control, $p<0.05$

#- significant when compared with sham-operated, $p<0.05$

There was an elevated increase in red blood cell count in P. amarus treated animals when compared to both the control group and the sham-operated group

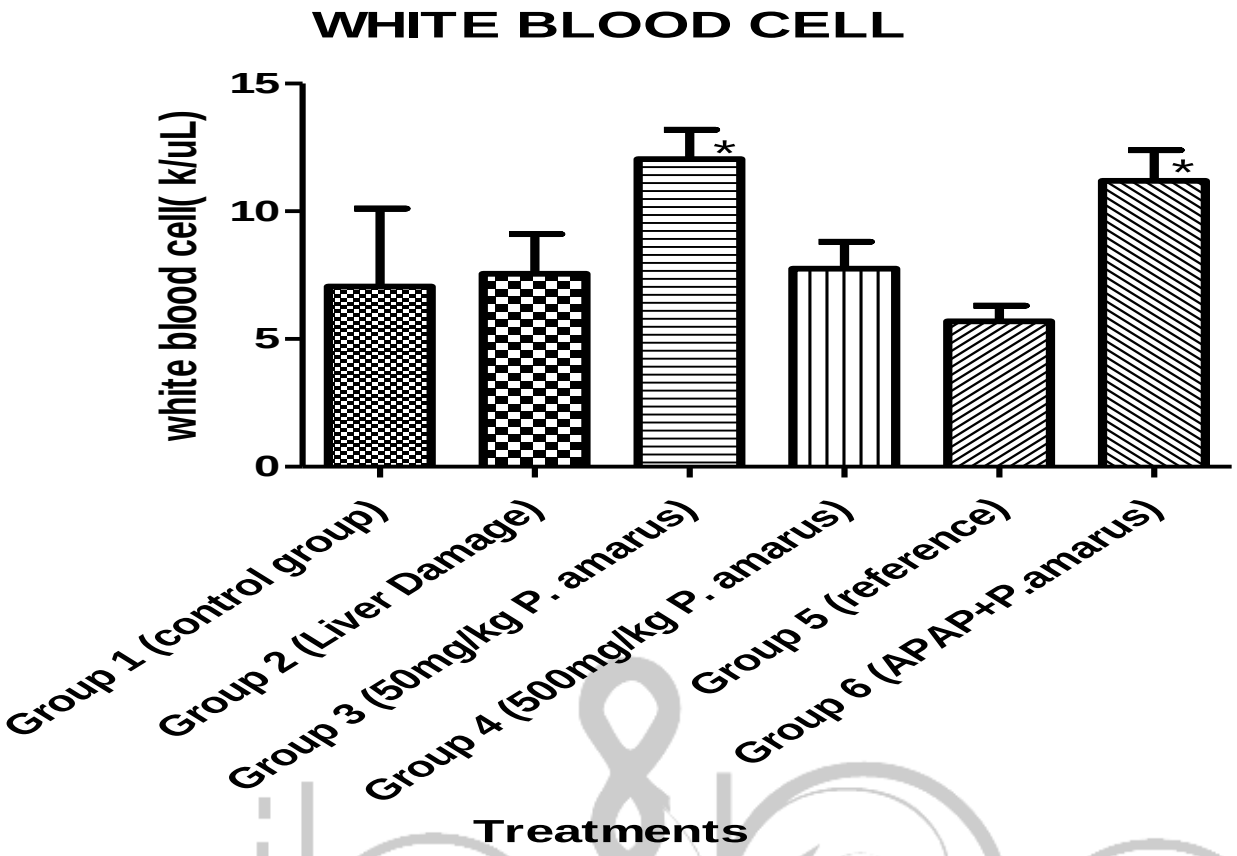


FIG 5: white blood cell count of *Phyllanthus amarus*-treated rats.

Data is presented as Mean±SEM, $p<0.05$

There was no significant increase in white blood cell when compared to control group across all groups.

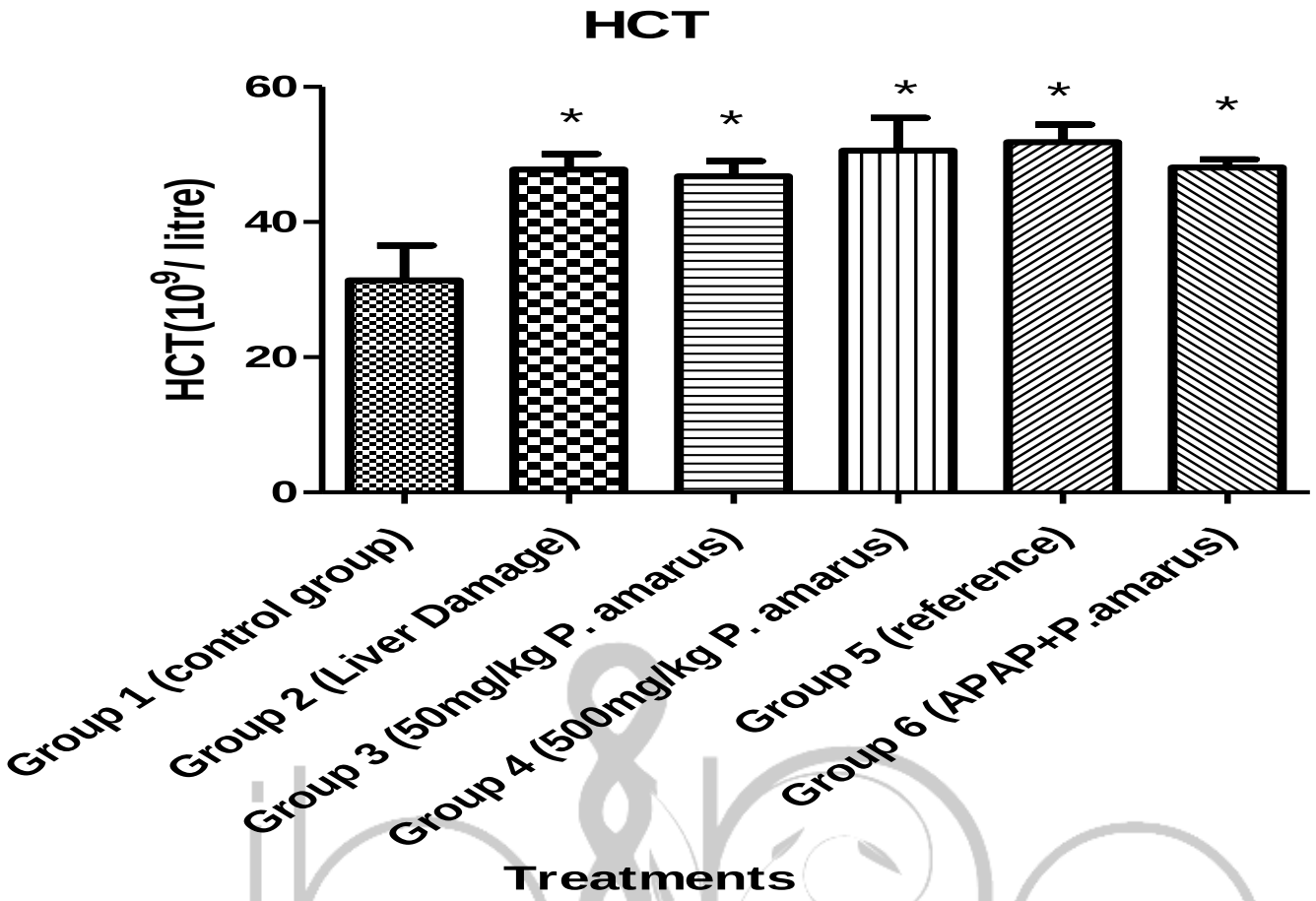
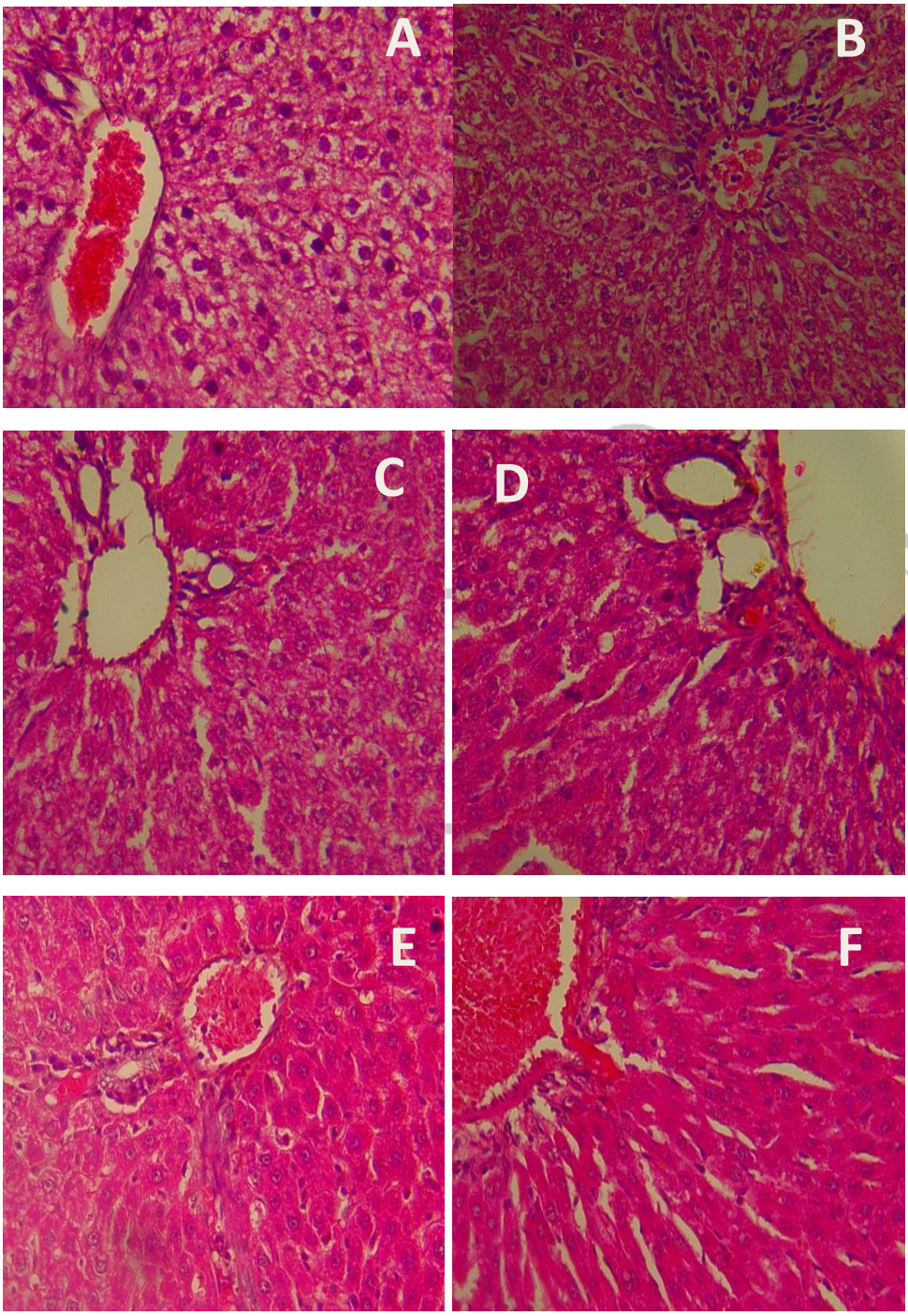


Fig 6: HCT count in Phyllanthus-*amarus* treated rats.

Data is presented as Mean±SEM

All groups shows significant elevation in total haematocrit when compared to control ($p < 0.05$)

Figure 7: Histological slides



The representative Histopathology

A- Normal liver histology in group 1

B- Severe infiltration of centri-lobular necrosis and appearance of apoptotic bodies in group 2.

C- Infiltration with inflammatory cells in group 3

D- Appearance of fatty cysts coalescing to form fatty vacuoles in degenerating hepatocytes

E- Slightly normal liver histology with spotted apoptotic bodies

F- Normal liver cells showing inflammation and slight vascular disintegration.

DISCUSSION

The liver due to its metabolic activity is one of the most susceptible organ in the body and very much prone to derangement of function. Hepatocytes perform the liver's metabolic functions and they are the most affected by assaults coming from ingested substances. One of the most common sources of function assault for the liver is via the ingestion of uncertified herbal products commonly used as medicinal substitutes. P. amarus is one such herbal plant that has varied use in ethno-medicine but with minimal toxicological report backing its use. This study investigated the effect of *P. amarus* on haematological parameters and on liver function in male Wistar rats.

Blood is the special circulatory tissue that is composed of various cellular elements suspended in plasma with the major function of maintaining general cell and electrolyte homeostasis in addition to its respiratory gases delivery function. In this study, there was a significant increase in RBC count ($p<0.05$) across all groups secondary to *P. amarus* administration when compared to control (sham operated 28%; Low dose 38.6%; high dose 46.3%; reference 42.8%; Curative group 34.6%). This finding is buttressed by the work of Nwankpa *et al*., (2014) who reported that *P. amarus* increased RBC count in *Salmonellae typhi*-infested Wistar albino rats. *P. amarus* extract effects seem to be dose dependent as the values observed are incremental by dosage. A similar pattern of increment in response was observed for the haematocrit index. The white blood cell count was significant only in the low dose (70.9%) and curative group (58.9%) when compared with control. The white blood cell count of the rest groups were not significantly affected by the *P. amarus* administration. This may probably be due to the fact that there is absence of infection despite the liver damage induced even though there are studies in which P. amarus was demonstrated to have WBC boosting capacity (Kumar and Kuttan, 2004).

Acetaminophen overuse is one of the most common causes of acute liver damage and failure in the world. Acetaminophen hepatotoxicity can be rapidly induced by a single overdose of the drug. Alanine transaminase (ALT) level has been shown to increase significantly 24 hours after acetaminophen (1g/kg BW) administration

(McGill et al., 2012). In the current study however, aspartate transaminase (AST) level was not significantly elevated. Alkaline phosphatase (ALP) and Alanine transaminase levels were however significantly raised in hepatotoxic Group 2 rats when compared to control ($P<0.05$); a clear demonstration of successful liver damage procedure. The 50mg and 500 mg/kg BW doses of *P. amarus* had significantly lower ALP and ALT levels when compared to the sham operated animals of Group 2. It may thus be argued that *P. amarus* extract has a repair effect on liver hepatocytes damaged by acetaminophen. Adomi *et al.,* (2017) reported a significant reduction in plasma alkaline phosphate while establishing the toxicity levels on various organs following *P. amarus* administration in rats. By contrast, in a study conducted on the effects of *P. amarus* in piglets fed with contaminated feed, it was observed that the P. amarus fed piglets had a significantly elevated alanine aminotransferase, alkaline phosphatase and aspartate transaminase values (Phuong *et al,* 2012). Pre-administration with *P. amarus* was effective in preventing over-expression of ALP secondary to liver damage by acetaminophen (APAP) (Figure 2). The Phyllanthus specie has been shown to possess significant levels of free scavenging activity resulting in inhibition of cell destroying processes such as lipid peroxidation (Manjrekar *et al.,* 2008)

These observations are also supported by the examination of the liver histo-architecture. The sham-operated animals have lobular necrosis as evidenced by appearance of apoptotic bodies. The *Phyllanthus amarus*-treated rats showed mildly inflamed and degenerating hepatocytes in what looks likes the early stages of repair while the Group 5 animals on the reference drug silymarin, showed fairly normal hepatocytes with minimal inflammation. All these point to the ameliorative effects of *P. amarus* in acetaminophen-induced hepatoxicity.

CONCLUSION

P. amarus may be said to possess mitigating effects on the liver in acetaminophen--treated rats. Its consumption as a local herbal remedy may therefore be considered safe with regards to blood parameters and liver function.

ACKNOWLEDGEMENT

Major contributors to this work are those listed in the list of authors.

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