Original Article

Effects of *Phyllanthus amarus* on Garlic-Induced Hepatotoxicity

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ABSTRACT

Background: One of the most important organs that regulate various physiological processes in the body is the liver. The liver, if diseased or damaged by toxic agent (s) could contribute to the cause of morbidity and mortality globally. *Phyllanthus amarus* is highly valued in African traditional medicine for its hepatoprotective, anti-inflammatory and several related ailments.
Aim: The aim of the present study was to investigate the effects of the administration of *P. amarus* on garlic-induced hepatotoxicity in rats.
Settings and Design: Albino rats ($n = 30$) weighing 170–200 g were randomly divided into five Groups (I-V) of six. Group I (control) received orally 1 ml/kg body weight of distilled water while Groups II, III, IV and V were administered orally same volume of garlic homogenate corresponding to 5 g/kg body weight on a daily basis for 14 days to induce liver toxicity. Similarly, Groups III, IV and V were orally administered with 100, 200 and 400 mg/kg body weight of *P. amarus* aqueous leaf extract, respectively, for another 7 days.
Materials and Methods: The levels of serum alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin (TB) were determined by standard methods. Histopathological analysis of the liver tissue was carried out as described by Druby and Wallington.
Statistical Analysis: Results were expressed as mean ± standard errors of means. Statistical analysis was performed using one-way ANOVA and Bonferroni *post hoc* test was used to determine the difference between means at 95% level of significance.
Results: The results showed that all the doses of the extract of *P. amarus* significantly decreased ($P < 0.05$) the levels of ALP, ALT and AST and TB relative to those administered 5 g/kg body weight of garlic only (Group II). The histopathological analysis of the liver samples also confirmed the hepatocurative potential of *P. amarus* against the hepatotoxicity caused by garlic. The ameriorative effect of *P. amarus* is suspected to be due to the presence of some antioxidants (phenols and flavonoids) as determined and quantified in the present work.
Conclusion: It was concluded that *P. amarus* showed hepatocurative effect having ameliorated the lobular necrosis and inflammation of the liver induced by garlic homogenate in albino rats.

Key words: And antioxidants, garlic, hepatoprotection, hepatotoxicity, *Phyllanthus amarus,* Schum and Thonn

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INTRODUCTION

The liver although plays a central role in biotransformation and clearing chemicals, it is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ.[1] Every chemical agent/drug is known to be associated with hepatotoxicity largely due to its ability to generate free radicals and to cause a disturbance in cell biochemically.[2] The fact that herbs are natural does not mean that they are harmless. In fact, there have been many reports of people suffering from serious health problems or even dying due to herbal remedies. Since substances consumed are metabolised through the liver, the liver is a prime target for the toxic effects of some herbs. People with normal liver function and have no history of prior liver disease have suffered adverse consequences to the liver as a result of taking certain herbs.[3]

Garlic *(Allium sativum*) is one of the wonder herbs from the lily family,[4] and it is the oldest known medicinal plant variety, or spice in existence and mankind recognised the curative qualities of this magic herb for over 3000 years. Meanwhile,

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it must not be assumed that since an herb has been used for thousands of years that the herb is possibly safe and truly effective for its claimed indication (s).[4]

Garlic has been an integral part of the diet for centuries, but it is taken for granted that garlic is safe in a wide range of doses. Garlic in high dose has the potential to induce liver damage while at low doses are considered safe.[5] Garlic is popularly consumed as a food condiment and has an important role in the treatment and management of several diseases. Nevertheless, unregulated and chronic consumption of garlic can cause damage to hepatocytes through the production of free radicals.[6]

Phyllanthus amarus (Family: *Euphorbiaceae*) is a widely distributed small, erect and tropical annual herb. This herb is commonly called stonebreaker, or is popularly called 'Chanka peidra' which translates to 'stone breaker'.[7,8] *P. amarus* is, however, highly valued in African traditional medicine for its hepatoprotective, antidiabetic, anti-hypertensive, analgesic, anti-inflammatory and antimicrobial properties.[9]

However, in an extensive review of related literature, documented evidence to show that *P. amarus* can protect animal livers against garlic-induced hepatatoxicity is scarce. Against this background, the present study was designed to show the hepatocurative potentials of this plant extract against garlic-induced hepatotoxicity.

MATERIALS AND METHODS

Source and identification of plant materials

P. amarus herbs and dried cloves of *A. sativum* (Garlic) were obtained from the Botanical Garden of University of Maiduguri road and Maiduguri Monday Market, Nigeria, respectively. These plants were authenticated by a plant taxonomist in the Botanical Unit, Faculty of Science, University of Maiduguri, Borno State and the voucher number BCH#0080 of the herb deposited in the herbarium of Biochemistry Department of the University.

Preparation of garlic homogenate

Cold maceration (soaking) method was used to prepare the homogenate.[10] The garlic was peeled off, washed and weighed using a digital weighing balance. The weighed garlic (20 g) was pounded in a clean mortar with pestle into a paste. The pounded garlic was soaked in distilled water for 48 h (steering at intervals for proper mixing). After 48 h, it was sieved into a conical flask using a clean sieve and a Whatman No. 1 filter paper. The filtrate was taken to the rotary evaporator to remove the excess amount of water, at a temperature of 60°C–80°C leaving behind the crude extract in the flask. The crude extract was further concentrated using the oven at a temperature of 30°C–40°C. A stock solution of 5 g/ml was prepared from the concentrated paste-like garlic extract.[11] The stock solution was kept in the refrigerator at a temperature of 4°C until required.

Preparation of *Phyllanthus amarus* extract

The plant (*P. amarus*) leaves were gently sorted, air-dried at room temperature (28°C ± 2°C) for 2 weeks until a constant weight was obtained. The dried leaves (40 g) were pulverised into dry powder. The powder was extracted with distilled water using Soxhlet apparatus and concentrated by rotary evaporator at 65°C. It was then transferred into a suitable container and freeze-dried before further analysis.

Experimental animals

Matured Albino Wistar rats weighing (170–200 g) of both sexes were obtained from Animal House of the Department of Biochemistry, Faculty of Science, University of Maiduguri, Borno State, Nigeria. Before the experiment, these animals were allowed to acclimatise for 14 days in the animal house and maintained under standard conditions of temperature, light and relative humidity. Drinking water and standard pellet diet (Grand Cereal Nig. Ltd.,) were provided *ad libitum*. All experimental procedures were conducted in strict compliance with the guidelines for the care and use of laboratory animals.

Experimental procedures

Acute toxicity study (LD_{50})

The lethal dose in 50% of the total population (LD_{50}) was interpolated using Lorke method.[12]

$$LD_{50} = \sqrt{D_o \times D_{100}}$$

Where D_o = highest dose that gave no mortality, D_{100} = lowest dose that produced mortality.

Phytochemical analysis

Qualitative phytochemical tests were carried out on the aqueous leaf extract using standard procedures to identify the constituents as described by Sofowara,[13] Trease and Evans[14] and Harborne.[15] However, quantitative phytochemical analysis of total tannin content was determined by Van-Burden and Robinson[16] method, total saponin by the method of Obadoni and Ochuko,[17] total flavonoid content was determined by the Method of Bohm and Kocipai-Abyazan[18] and alkaloids using Harbone method.[19]

Hepatotoxicity induction and hepatocurative study

From the result of the LD_{50} test of the experimental animals, 30 albino rats of the Wistar strain weighing 170–200 g were randomised into five groups of six rats such that the differences in the average weights between and within groups do not exceed ± 20% of the average weights of all the rats. Group I, which served as the control was orally administered l. 0 ml/kg/day of distilled water, while Groups II-V were orally administered 5 g/kg/day of fresh garlic homogenate for 14 days to induce hepatotoxicity.

Following garlic hepatotoxicity induction by day 14, Group II served as garlic control (negative control) while Groups III, IV and V were orally administered graded doses of 100, 200 and 400 mg/kg/day, respectively, of *P. amarus* aqueous leaf extract for another 7 days.

Chemical pathological assay of serum

At the end of 21 days of treatment, the rats were sacrificed under diethyl ether anaesthesia (Sigma chemical Co; St Louis,

USA) and the blood sample of each rat was collected separately into sterilised dried centrifuge tubes. The blood samples were allowed to clot and then spun. The supernatants (sera) were separated from the cell debris and decanted into new test tubes for some biochemical analysis, such as serum alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) and total bilirubin (TB).

Histopathological study of the liver

The liver organ for each rat was cut to about 5 mm thickness and fixed in 10% formalin. It was later processed and embedded in paraffin wax and then cut and stained with haematoxylin and eosin dye.[20] Photomicrographs were obtained using photographic microscope from the Department of Histology, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria.

Statistical analysis

Results were expressed as mean ± standard errors of means. Statistical analysis was performed using one-way ANOVA, and Bonferroni *post hoc* test was used to determine the difference between means at 95% level of significance.

RESULTS

Acute toxicity study of *P. amarus* leaf extract showed no significant toxic effect in the albino rats [Table I]. The phytochemical investigations of the leaf extract showed the presence of flavonoids, tannins, alkaloids, phenols, saponins and terpenoids [Table II]. The animals were weak; eyes were red and showed decreased water and food intake after the administration of garlic. However, there was no vomiting and no death recorded. Garlic treatment (Group II) as depicted in Table III induced statistically significant ($P < 0.05$) elevation in serum ALP, ALT, AST and TB when compared to the control group (Group I). However, the increase in the levels of serum ALP, ALT, AST and TB in Group II was significantly ($P < 0.05$) decreased by the oral administration of *P. amarus* for 7 days in a dose-related fashion. The tissue sections were examined under a light microscope, and the extent of necrosis was represented in photomicrographs of the histopathological examination in the liver tissue of Groups I to V are presented as Plates 1-5.

DISCUSSION

Garlic (*A. sativum)* is popularly consumed in Nigeria and a major part of West Africa because of its health benefit in the treatment and management of several disease conditions.[21] However, excessive intake of garlic may cause liver damage[22] and haemolytic anaemia[23] as reported to have a significant increase in serum levels of ALT, AST and ALP and decrease in PCV, respectively. The release of these enzymes and their increased activity in the rats treated and observed in the present work was also reported earlier Buratai *et al*., (2000)[24] and Yahya *et al*., (2013).[37] The observed increase in the level of these enzymes was attributed to the excess garlic homogenate (5 g/kg/day) administered which may have caused injury and alteration in their permeability [Table III]. Garlic oil

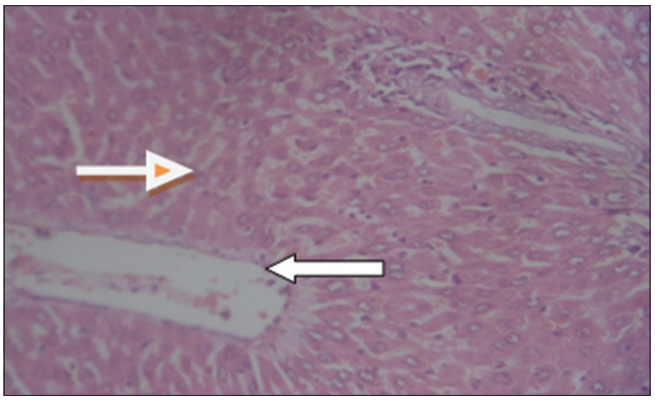

Plate 1: Represents the control group. The photomicrograph of the liver shows a normal liver with the white arrow showing the central vein while the red arrow showing the bile duct Mg × 100 Control group

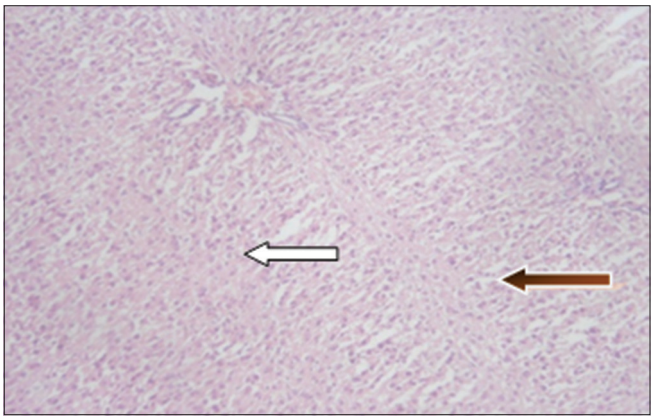

Plate 2: Represents Group II with the white arrow showing confluent lobular necrosis while the black arrow shows the portal-central bridging necrosis. It also shows periportal inflammation with piece meal necrosis ×100 Group II (Garlic control)

Table I: Effect of aqueous leaf extract of *Phyllanthus amarus* on albino rats

Phase	Group	Number of animals	Dosage (mg/kg BW)	Mortality within 24 h	Delayed symptoms for 7-14 days
I	I	3	10	Nil	No abnormalities detected
	II	3	100	Nil	No abnormalities detected
	III	3	1000	Nil	No abnormalities detected
II	I	1	1600	Nil	No abnormalities detected
	II	1	2900	Nil	No abnormalities detected
	III	1	5000	Nil	No abnormalities detected

BW: Body weight

feeding (100 mg/100 g bw intragastrically) after 24 h fasting was found lethal; and loss in body weight is highly due to increased toxicity which probably led to decreased food and water intake as reported by Joseph *et al*.[22] However, similar feeding of garlic oil was well tolerated by rats in the fed state.[22]

The administration of *P. amarus* aqueous leaf extract for 7 days significantly alleviated or prevented the increased serum enzymes in garlic-induced liver injury. *P. amarus* plant has been reported to possess a membrane stabilising properties which consequently ameliorated the induced increases.[25] It, therefore, implies that the effect of any hepatocurative drug is dependent on its ability of reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a known hepatotoxin. [25] Changes in the blood parameters such as TB can also be used to explain blood-relating functions of the plant extract or its products(2008).[26]

The present study has shown that aqueous extract of *P. amarus* is relatively safe in rats even at the concentration of 5000 mg/kg body weight [Table I]. However, phytochemical screening of *P. amarus* demonstrated the presence of flavonoids, saponins and tannins as well as the existence of phenolic compounds as indicated by high phenolic content value.[27] Garlic is a commonly used spice in medicine that can exert adverse effects when given at a high dose.[28] Unregulated and chronic consumption of garlic can cause damage to hepatocytes through the production of free radicals.[6] However, 5 g/kg body weight of garlic homogenate which is a toxic dose[5] showed significant elevation of serum level of hepatic enzymes ALT, AST, ALP as well as serum TB as compared to the normal control group. Therefore, this study demonstrates that garlic homogenate administered at 5 g/kg body weight to rats provoked a marked elevation in serum AST, ALT and ALP activities which indicates hepatocellular damage. The results of this corroborate with those of Rana *et al*.,[5] who reported elevated levels in serum levels of hepatic damage markers with marked histological damage in the rats liver administered 5 g/kg/day of garlic homogenate. Meanwhile, the administration of 100 mg, 200 mg and 400 mg of *P. amarus aqueous* leaf extract to the rats administered a high dosage of garlic successfully lowered the level of these enzymes as compared to Group II (negative control) as shown in Table III. The hepatocurative potential of *P. amarus* can be explained based on the respective phytoconstituents detected in the extract. For example, flavonoids have been reported to exert antioxidant[29,30] and anti-inflammatory[31] activities. The inhibitory activity of flavonoids on free radical release could be related to their hepatocurative effect since exogenous anti-oxidants may counteract the pathological effects of oxidative stress associated with chemical-induced hepatotoxicity, co-operating with natural systems such as glutathione, tocopherol or protective enzymes.[32] Moreover, saponins have been reported to exert hepatoprotective activity through the modulation of its antioxidant[33] and inflammatory activities[34] while tannins have been suggested to possess

Table II: Qualitative and quantitative phytochemical components of aqueous leaf extract of *Phyllanthus amarus*

Phytochemical	Result	
	Qualitative	Quantitative (mg/g)
Phenols	+	0.101±0.02
Flavonoids	+	0.119±0.01
Cardiac glycoside	-	-
Saponins	+	0.119±0.02
Tannins	+	0.110±0.01
Terpenoid	+	0.081±0.01
Alkaloid	+	0.117±0.01
Phlobatannin	-	-

Result are presented as mean±SEM of three determinations. -: Absent, +: Present, SEM: Standard error of mean

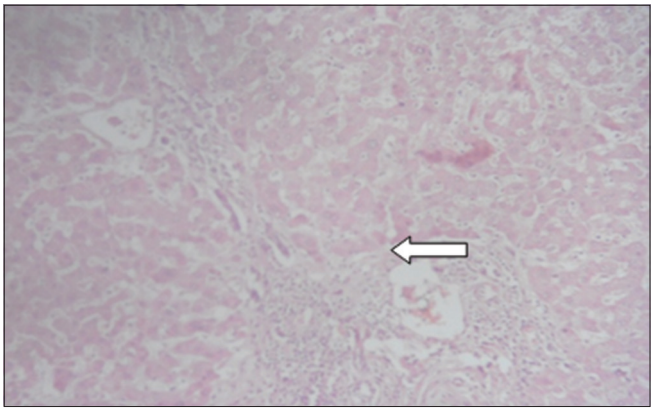

Plate 3: Represents Group III showing mild portal, periportal and lobular inflammation with associated portal-central bridging inflammation and piecemeal necrosis (interface hepatitis) Mg × 100 Group III (Treatment)

Table III: Effects of aqueous leaf extract of *Phyllanthus amarus* aqueous on garlic induced hepatotoxicity in albino rats

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TB (g/L)
Control (I)	64.36±4.12	18.60±2.09	85.87±5.09	41.04±1.15
5 g/kg GH (II)	276.78±11.81[a]	75.00±27.39[a]	170.50±10.11[a]	46.08±2.48
GH +100 mg/kg of PA (III)	101.19±2.31[a,b]	24.58±4.30[b]	59.08±3.59[a,b]	29.51±3.44[a,b]
GH +200 mg/kg of PA (IV)	99.79±5.74[a,b]	26.25±3.42[b]	73.09±1.75[b]	44.33±0.99
GH + 400 mg/kg of PA (V)	112.86±1.9[a,b]	22.56±1.35[b]	55.78±6.85[a,b]	40.37±3.56

[a]Statistically significant to control (I) at $P<0.05$, [b]Statistically significant to GH at $P<0.05$. Results are presented as mean±SEM; $n=6$; PA: *Phyllanthus amarus*, GH: Garlic homogenate

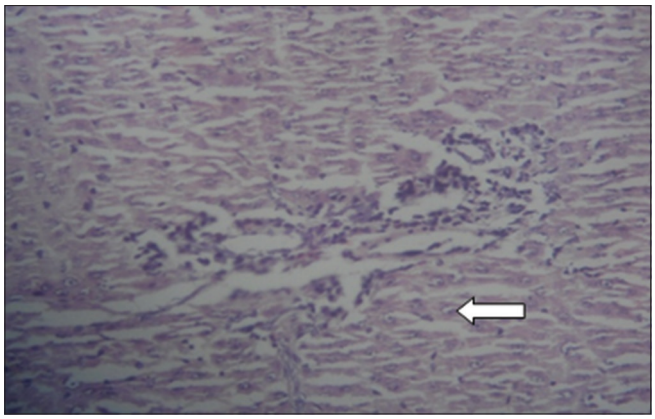

Plate 4: Represents Group IV which shows moderate lobular inflammation with the white arrow showing infiltration by mononuclear cells mainly the lymphocytes Mg × 100 Group IV (Treatment)

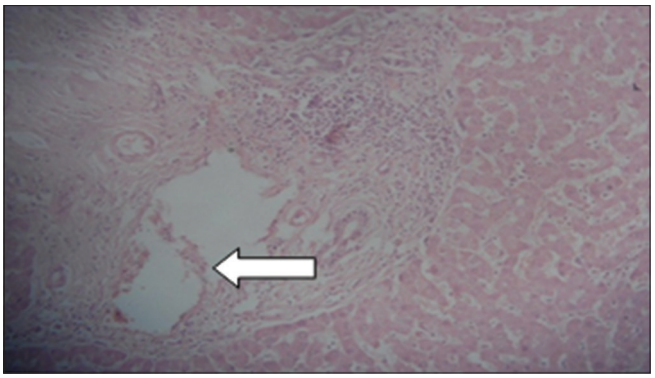

Plate 5: This is the representative of Group V shows predominantly portal inflammation (white arrow). However, neither necrosis nor interface hepatitis was observed Mg × 100 Group V (Treatment)

free radical scavenging, antioxidant, anti-inflammatory and hepatoprotective activities.[35]

Liver sections from excess garlic (5 g/kg body weight) treated rats demonstrated the destruction of architectural pattern, moderate inflammation of the portal area and severe distortion of the liver parenchyma [Plate 2] when compared to the liver section of control rats (Group I). This shows normal hepatocytes and normal architecture (normal portal tract, central vein and normal hepatocytes) [Plate 1]. The administration of *P. amarus* aqueous leaf extract in the dosage of 100, 200 and 400 mg apparently restored the liver against garlic-induced damage [Plates 3-5, respectively]. The hepatocurative or healing effect of the crude extract of *P. amarus* was thought to be due to the normalisation of impaired membrane function activity of the liver.[36] This healing or normalisation process as well as the improved structural integrity of some histopathological changes against the hepatotoxicity induced by garlic might also be associated with the presence of some antioxidative phytochemicals in *P. amarus* aqueous leaf extract.

CONCLUSION

We thus concluded that the administration of aqueous extract of *P. amarus* to albino rats ameliorates garlic-induced liver toxicity. The data further lend credence to the prevention of garlic-induced increases in the hepatic biomarkers.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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