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Effect of dietary consumption of three Nigerian edible plants on the liver histology and enzymatic indices in induced anaemic adult wister rats

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ABSTRACT

Studies have demonstrated the apparent therapeutic and preventive nature of various plants consumed as food. Garlic, garden egg and groundnut are popularly consumed as diet or food supplement. The hepatoprotective potentials of combined consumption of these dietary plants on phenylhydrazine-induced anaemic rats were investigated. Twenty five Wister rats were divided equally into five groups. Group 1 served as the negative control while Group 2 was induced with anaemia and served as the positive control. Groups 3, 4 and five were anaemic; group 3 rats were fed with combined diet of garlic, garden egg and groundnut, group 4 rats fed with diet of garden egg and group 5 fed with diet of groundnut. The liver enzyme and tissue histology were determined using standard procedures. Histological alteration was observed in liver of rats in positive control as compared to negative control. Normal cytoarchitecture of liver was observed in rats in groups 3, 4 and 5. The activity of alkaline phosphatase (ALP) was not significantly ($p<0.05$) different among the five groups. Alanine aminotransferase (ALT) significantly ($p<0.05$) increased in Group 3, Group 4 and Group 5 as compared to the negative control and significantly ($p<0.05$) increased in Group 3 and Group 4 as compared to the positive control. Aspartate aminotransferase (AST) was significantly ($p<0.05$) decrease in Group 3, Group 4 and Group 5 as compared to the negative and positive controls. Data from the study suggests hepatoprotective potentials of garlic, garden egg and groundnut.

Key words: Anaemia, Garlic, Garden Egg, Groundnut, Phenylhydrazine, Serum Enzymes, Liver.

INTRODUCTION

It is well established that man consumes a wide variety of local crops and vegetables which are believed to contribute significantly to the improvement of human health in terms of disease prevention and therapy [1, 2]. Epidemiological, clinical and preclinical studies have shown the close relation between dietary habits, and the occurrence of disease, both in preventive and curative [3].

Allium sativum which is commonly called garlic belongs to the family *Liliaceae* and genus *Allium* [4]. Garlic is like onion made up of bulbs called cloves. It is cultivated in some parts of Nigeria and used as meat tenderizer and spice in many delicacies [5]. The traditional medical practitioners have considered garlic as an excellent medicinal plant that has a lot of therapeutic potentials. It has many local names. In Nigeria, it is ayo in Iboland, ayuu in Yorubaland, afemuwa in Hausaland [6], eyim makara in Efikland and ayi in Esan(Benin) [7]. It is used extensively as food and as ingredient in foods [8].

Garden egg or bitter tomato botanically called *Solanum melongena* is an economic flowering plant belonging to the family Solanaceae. Members are mostly herbaceous plant and the fruit is grown mainly for food and medicinal purposes. It is called Igba iyesu in Yorubaland, ayanra/afefea in Igboland, yalo/jauta in Hausaland [9] and nya in Efikland. It is popular in rural day to day cuisine [10].

Groundnut known also as peanut is an herbaceous plant with botanical name *Arachis hypogaea* and belongs to the Fabuceac family. Groundnut is widely grown as a food crop, provides an inexpensive source of high quality dietary protein and oil which is of benefit in reducing malnutrition in the developing countries [11]. The special taste and flavour of foods containing groundnut is important in the acceptance of these food preparation [12]. Groundnut is known as gyada in Hausaland, okpa ekele in Igboland [13], Isagwe in Delta [14], ekpa in Yorubaland and mbansang in Efikland.

The Phytochemical and nutritional constituents of garlic [3, 15, 16, 17, 18], garden egg[10, 19, 20] and groundnut [11,21, 22] have been well documented. Similarly, the effects of usage of the plants (via varied preparations) on the body have been previously reported. This study was designed to ascertain the effect of mixed diet of garlic, garden egg and groundnut on the liver, histologically and biochemically.

MATERIALS AND METHODS

Breeding of animals

Twenty five adult Wister rats of both sexes weighing between 150-200g were used. They were purchased from the animal house of Department of Physiology, University of Calabar. They were kept in the animal room of the department of Human Anatomy for a period of two weeks under standard conditions of temperature 27˚C - 30˚C, photo period of 12-hour natural light cycle and 12-hour dark to acclimatize. They were fed with pelleted chick mash manufactured by Agro Feed Mill Nigeria Ltd and drinking water given ad libitum. After the acclimatization period, they were randomly divided into five groups of five rats each; two controls and three experimental groups.

Preparation of the diet

Garlic, garden egg and groundnut were bought randomly from Watt Market located in Calabar, Cross River State, Nigeria and were identified by the botanist in the botanical garden of the University of Calabar. The plants were washed with water to remove impurities. Garlic was defoliated then minced while the garden egg was chopped. The garlic, garden egg and groundnuts were dried in carbolite moisture extraction drying oven (Grant Instruments, Cambridge England) at 50˚c. The groundnut was dried for an hour while the garden egg and garlic were dried for three hours. The now dried samples were blended into coarse powdered form using a kitchen Blender and kept in glass containers with plastic cover to keep them airtight.

Experimental protocol

After the two weeks of acclimatization, the rats were divided into five groups, each consisting of five rats and placed in a feeding regimen as follows: (Group 1-non-anaemic control, animals fed with normal rat chow; Group 2-anaemic control, anaemic and fed with normal rat chow; Group 3- anaemic, fed with 20g (75%ww) of garlic, garden egg and groundnut in the ratio of 1:1:1 with normal rat chow; Group 4-anaemic, fed with 10g of garden egg (50%ww) in the ratio 1:1 with normal rat chow and group 5-anaemic, fed with 10g of groundnut (50%ww) in the ratio 1:1 with normal rat chow). Each of these groups was fed for a period of fourteen days.

Induction of anaemia

Anaemia was induced by oral administration of phenylhydrazine (PHZ) given at 50mg/kg BW for the first two days and then at an interval of three days as maintenance dose. Anaemia was confirmed by test of haemoglobin (Hb) level using haemoglobemeter (Hemocue Hb 201$^+$, Ängelholm, Sweden).

Collection of blood samples

The blood samples from the experimental rats were collected by nipping of the rat tails during the course of the experiment and at the end of the fourteenth day of dietary regimen by puncturing the left ventricle the heart at and withdrawing blood using syringe and needle.

Histological study

The animals were sacrificed twenty four hours after the last administration from each group. The livers were excised and fixed in 10% formalin. Routine histological processing was carried out. The liver was stained with haemotoxylin and eosin.

Biochemical studies

The hepatic function was evaluated by the assay of the following biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatise (ALP) using clear serum which was separated at 2500rpm for 10minutes.

Alanine and aspartate aminotranferases determination

Plasma assays for tests on the function of liver vis-a-viz serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2,4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel (1957).

Alkaline phosphatase determination

Alkaline phosphatase (ALP) activity was determined with the Randox reagent kit using the p-nitrophenylphosphate as substrate according to the method described by Bassey et al. (1946).

Statistical analysis

This was done using analysis of variance (ANOVA) and post hoc test. All values is expressed as mean ± standard error of mean (SEM) and values are statistically significant at $p < 0.05$.

RESULTS

Histological study

The photomicrographs of liver sections of negative control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (fig 1). The liver sections of anaemic rats in the positive control showed liver with distorted cytoarchitecture, the central vein is dilated with lysed blood cells, hepatocytes not properly arranged and liver sinusoids enlarged (fig 2). The liver sections of rats (group 3) fed with combined diet of garlic, garden egg and groundnut exhibited normal histology of the liver, prominent central vein, hepatocytes radially arranged around the central vein and prominent sinusoids (fig 3). Group 4 rats fed with garden egg and Group 5 rats fed with groundnut showed normal histology of the liver, properly arranged hepatocytes, sinusoids and central vein prominent (fig 4 & fig 5).

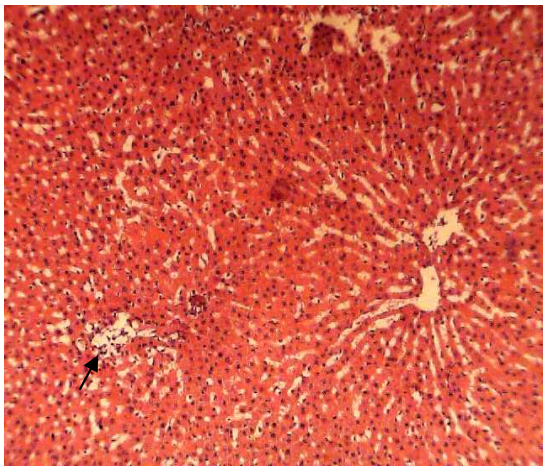

Fig. 1. Photomicrograph of the cross section of liver of rat in negative control
(H&E). Arrow shows normal central vein (X100).

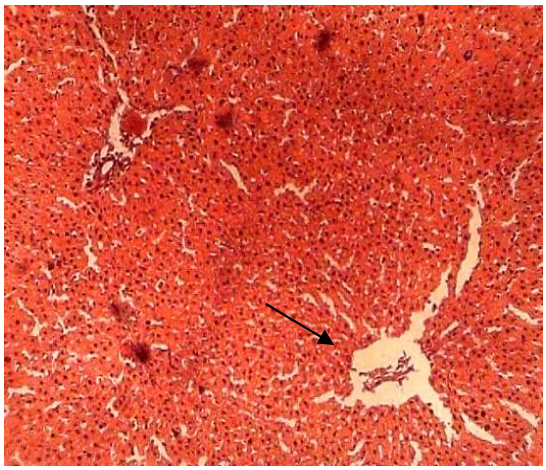

Fig. 2. photomicrograph of the cross section of liver from rat in positive control
(H&E). Arrow shows pronounced dilatation of the central vein with lysed blood cell(X100).

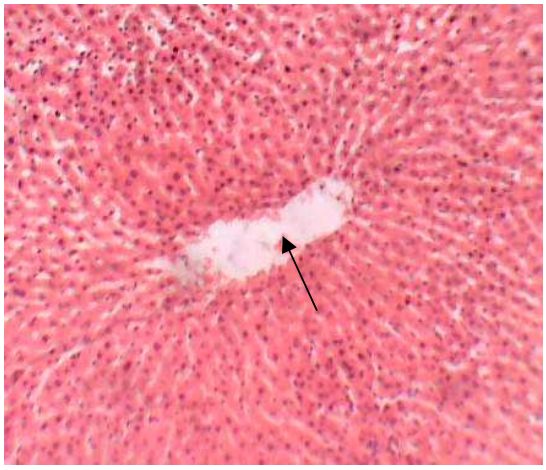

Fig. 3. photomicrograph of the cross section of liver from rat in group 3
(H&E). Arrow shows normal cytoarchitecture of the central vein(X100).

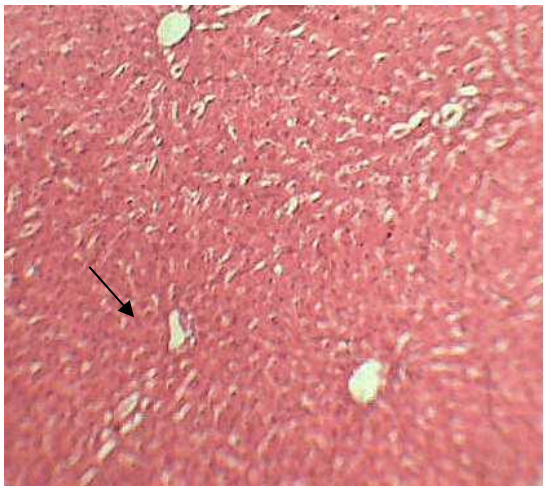

Fig. 4. photomicrograph of the cross section of liver from rat in group 4
(H&E). Arrow shows normal cytoarchitecture of the central vein(X100).

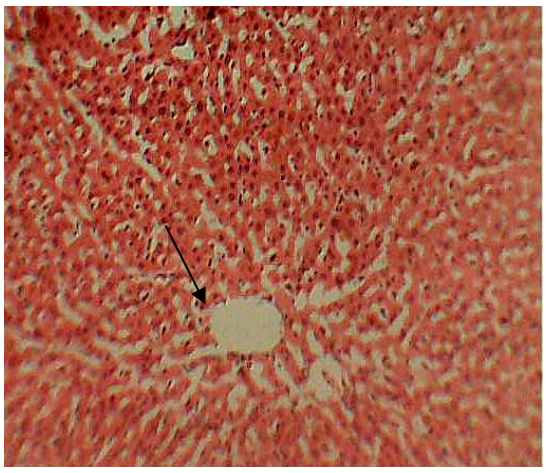

Fig. 5. photomicrograph of the cross section of liver from rat in group 5
(H&E). Arrow shows normal cytoarchitecture of the central vein(X100).

Biochemical Studies

The result from the liver function test (Table I) shows that in the positive control, there was increase in ALP, AST and ALT as compared to the negative control. A non significant ($p<0.05$) increase in ALP was observed in group 5 when compared with both the negative and positive control groups. In group 3, the ALP was slightly increased compared to the negative group and reduced compared to the positive control. Group 4 showed reduced ALP when

compared with the control groups. The ALT levels were significantly increased ($p<0.05$) in Groups 3, 4 and 5 when compared to negative control. A statistically significant increase ($p<0.05$) in ALT was also observed in rats in groups 3 and group 4 compared to rats in positive control. Statistically significant decreased ($p<0.05$) AST level was observed in groups 3, 4 and 5 when compared to negative control.

TABLE 1: Liver serum enzymes in the five experimental groups

Treatment	Serum enzyme levels (Iμ/L)		
	ALP	ALT	AST
Group 1	570.76±92.62	8.35±2.09	279.8±17.07
Group 2	603.73±187.38	17.57±2.29	283.80±9.88
Group 3	572.76±249.85	39.9±3.95*, a	101.45±9.90*, a
Group 4	395.98±24.32	52.08±3.79*, a	97.09±17.01*, a
Group 5	832.95±281.33	28.6±7.49*, c	50.49±10.71*, a, b, c

Values are mean±SEM, n=5.
**p<0.05 vs control; a=p<0.05 vs grp 2; b=p<0.05 vs grp 3; c=p<0.05 vs grp 4*

Group 1=normal rats fed normal rat chow(negative control).
Group 2=anaemic rats fed normal rat chow(positive control).
Group 3=anaemic rats fed normal rat chow with combined diet of garlic, garden egg and groundnut.
Group 4=anaemic rats fed normal rat chow with garden egg.
Group 5=anaemic rats fed normal rat chow with groundnut.

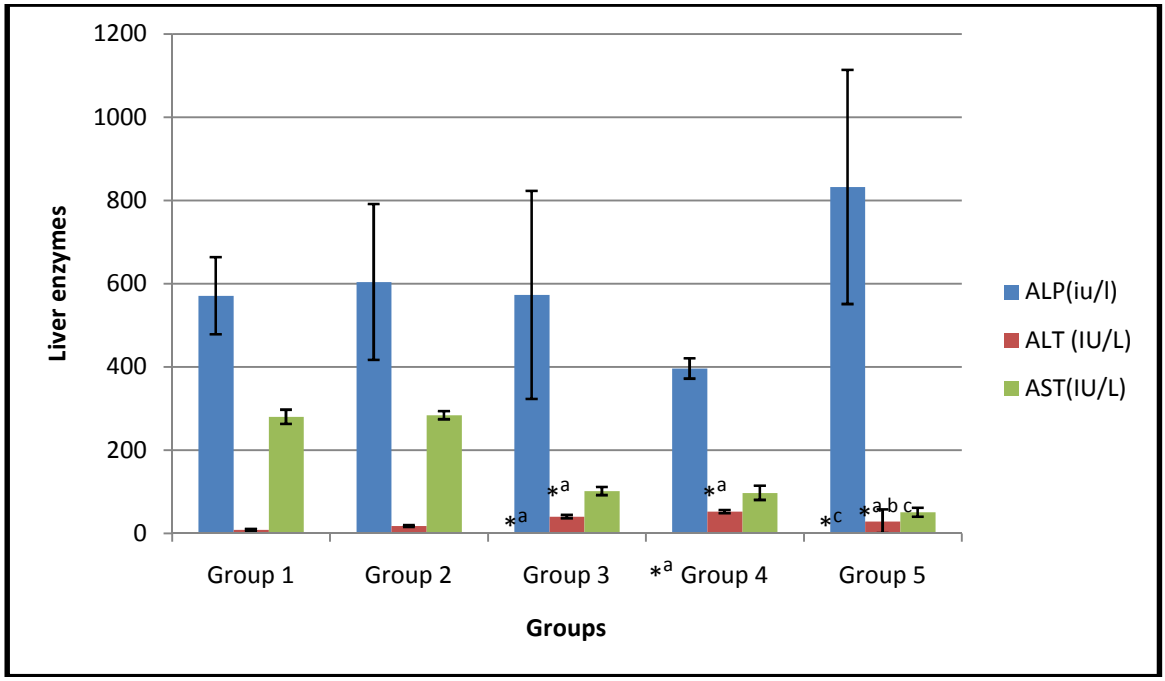

Figure I: Bar chart showing the serum liver enzymes among the various groups
Values are mean±SEM, n=5.
**p<0.05 vs control; a=p<0.05 vs grp 2; b=p<0.05 vs grp 3; c=p<0.05 vs grp 4*

Group 1=normal rats fed normal rat chow(negative control).
Group 2=anaemic rats fed normal rat chow(positive control).
Group 3=anaemic rats fed normal rat chow with combined diet of garlic, garden egg and groundnut.
Group 4=anaemic rats fed normal rat chow with garden egg.
Group 5=anaemic rats fed normal rat chow with groundnut.

DISCUSSION

The liver, the key organ involved in numerous metabolic functions and plays a central role in the detoxification process and faces the threat of maximum exposure to xenobiotics and their metabolic by-products [23]. The histopathological results of this study demonstrated that exposure of rats to phenylhydrazine resulted in degenerative changes in hepatocytes, distorted sinusoids and dilated central vein with lysed blood cells. The observed cytoarchitecture of liver in response to phenylhydrazine could be due to its toxic effect primarily by the generation of reactive oxygen species causing damage to various membrane components of the cell [24]. The oxidative damage

might ultimately enhance apoptosis unless the deleterious effects of the oxidative stress are counteracted by the endogenous cellular defence mechanisms that include enzymatic and non-enymatic free radical scavenger [25]. It has been previously reported that during liver damage, there was an observed decrease in antioxidant defences in the liver [26].

The ability of combined diet of garlic, garden egg and groundnut to improve liver dysfunction may be due to its antioxidant properties. Studies have shown that garlic, garden egg and groundnut have antioxidant properties due to certain phytochemical components such as allicin and other organosulphur compounds in garlic [27, 28, 29, 30]; phenols and flavonoids present in garden egg [31, 32] and stilbenoids present in groundnut [33]. This present study suggest that the combined diet of garlic, garden egg and groundnut, or diet of garden egg and/or groundnut ameliorating effects on the liver to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity.

The biochemical indices monitored in the liver in this study are useful markers for assessing the functional capacities of the liver. Biochemical indices of organ if altered will impair the normal functioning of the organs [34]. These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury [35, 36]

In this study, the administration of Phenylhydrazine showed increased in serum ALP, AST and ALT activities in animals in the positive control when compared to animals in negative control. This suggests hepatic damage in the anaemic rats and the extent of damage was observed in the histological appearance of the liver of rats in the positive control, showing altered cytoarchitecture of the liver. The present data suggests that Phenylhydrazine exerts possible hepatotoxic effect. This is similar to works carried out by [37] and [38] that Phenylhydrazine has a hepatotoxic effect. ATP depletion may underline the hepatoxicity [39] or oxidative damage [40].

Anaemic rats fed with diet of garlic, garden egg and groundnut showed decrease ALP levels when compared with rats in positive control (anaemic). The decrease was significant ($p<0.05$) in AST levels as compared to the positive control. There was rather a significant ($p<0.05$) elevation of ALT activity in group 3 rats compared with both negative and positive controls rats (Table I). The reduced serum ALP and AST activities may generally be attributed to decreased production of the enzymes from the liver [41], hence denoting the reversing effect of garlic, garden egg and groundnut on Phenylhydrazine toxicity in rats. Liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity [35]. The cause for the isolated increase in serum ALT levels observed in group 3 animals was not ascertained in this study.

In this study, there was increase in ALP and significant increase ($p<0.05$) in ASP activity in rats fed with normal rat chow and garden egg (Table I). There was a significant ($p<0.05$) increase in ALT levels as compared to both the positive and negative groups. The decrease serum levels of ALP and ASP in group 4 animals indicates the possible hepatoprotective function garden egg. The significant ($p<0.05$) in ALT maintains some concern about hepatic toxicity. Considering this, the findings on normal liver histology in garden egg fed rats are reassuring. This finding is similar to work done by [42] in which the histology of the liver was normal although there was an isolated increase in serum ALP level.

In rats fed with groundnut and normal chow (Group 5), AST level was significantly ($p<0.05$) decreased compared to both the negative and positive controls. There was rather an increase in ALP levels when compared to both control groups and significant increase ($p<0.05$) in ALT levels when compared with the negative (normal) control. The increase in ALT was not significant when compared to positive control animals. Groundnut if infested can contain aflatoxin. Aflatoxin tends to increase ALP [43]. The cause for elevated ALT level is not ascertained in this study. From the histology of the liver, the diet of groundnut did not show alteration to liver cytoarchitecture.

One of the possible mechanisms of injury to the liver is by oxidative stress [44]. Oxidative stress is produced by free radicals which attacks the cells of the body. Antioxidants neutralize these highly unstable and extremely reactive free radicals [45]. Garlic is considered one of the best disease-preventing foods based on its potent and varied effects [3]. Organosulphide compounds and saponins present in garlic are known to exhibit antioxidant activity [46]. Flavonoid such as nasunin and delphinidine isolated in garden egg has been shown to have antioxidant properties [10, 32]. Thermal treatment of garden egg before consumption can increase the content and biological activity of antioxidant components. Numerous polyphenols have been identified in groundnut [47]. It is well known that phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour and also in providing health-beneficial effects. They also serve in defence mechanisms to counteracts reactive oxygen species (ROS) and prevents molecular damage [48]. Phenols are antioxidant. Groundnut fed rats in group 5 showed relatively normal levels of liver enzymes and this is supported by the normal liver structure observed histologically. The increase in ALP level is similar to work done by [43] that aflatoxin present in peanut

increased ALP levels. Stilbenoids present in peanut inhibited intracellular generation of reactive oxygen species, exhibiting a strong antioxidant effect [33].

CONCLUSION

Overall, our results confirm that administration of phenylhydrazine in rats caused some level of liver or hepatic damage in the animals and post-lead treatment with *A. sativum* (garlic), S. melongena (garden egg) and A. hypogaea (groundnut) have some hepatoprotective effects on these rats.

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