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The Beneficial Effects of Methanol Extract of Annona Muricata Seeds on Acute Hemolytic Anemia in Albino Wistar Rats

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Abstract
The effect of methanol extract of annona muricata seed on packed cell volume, hemoglobin concentration, red cell count, alanine and aspartate aminotransferases, and alkaline phosphatases was investigated using albino wistar rats. Acute hemolytic anemia was induced with phenylhydrazine. The rats packed cell volume was used to determine anemia. Only rats that exhibited anemia were used. They were treated with annona muricata extract for four weeks and these hematological and liver enzymes were investigated. The results showed that extract caused a significant increase in packed cell volume, hemoglobin concentration, and red cell count ($P < 0.05$), but caused a fall in alanine and aspartate aminotransferases, and alkaline phosphatases ($P < 0.05$) back to normal. The histopathological studies showed absence of and/or mild liver congestion and inflammation after treatment with extract.The results suggest that annona muricata seed extract is beneficial in the treatment of hemolytic anemia induced liver injury.
Keywords: annona muricata. phenylhydrazine. packed cell volume. hemoglobin concentration. red blood cell. alanine aminotransferase. aspartate aminotransferase. alkaline phosphatase

INTRODUCTION

Annona muricata (annonaceae) leaf, bark, roots, stem and seed extracts are not edible but have been shown as antibacterial in vitro against numerous pathogens.[1-3] The phytochemicals (*annonaceous acetogenins*) that are found in the leaves, seeds and stems have been reported to be cytotoxic against various cancer cells.[4-6] Our recent studies also showed that *annona muricata* seeds reduced the levels of liver function enzymes and restored hepatocytes in alloxan induced diabetic mice.[7] The plant seeds have also been reported to increase some reproductive hormones, sperm parameters and restored testicular cells in alloxan induced diabetic rats.[8]

Neonatal jaundice contributes to over 5 million deaths occurring annually in children during their first few months of life.[9] Hemolytic anemia is most widely induced with phenylhydrazine.[10] Phenylhydrazine causes a decrease in hemoglobin level, red blood cell concentration and packed cell volume. It also impairs erythrocyte deformability, free plasma hemoglobin and extramedullary hematopoiesis in the liver and spleen. [11-13] It had been established that hemolysis can induce liver damage either by direct effect[25] or via phenylhydrazine.[26] This present study investigated the effect of methanol extract of *annona muricata* seed on some hematological parameters and liver enzymes in phenylhydrazine treated rats.

MATERIALS AND METHOD

Twenty adult albino wistar rats (200 - 300 g) were used in this study. They were divided into five groups of four rats each. The animals were housed in the departmental animal room with a temperature of between 27℃ - 30℃, and lighting rhythm of 12 hours light and 12 hours of darkness. All animals were housed four to a cage. They had free access to food (Vital feeds, Nigeria) and also tap water *ad libitum*. Animal care and treatment were conducted in conformity with the guidelines of Animal Ethical Committee of our institution.

Fresh seeds from *annona muricata* fruits were collected, sundried and ground into coarse form. About 200 mg/kg of the coarse form was soaked in 600 ml of methanol and placed in a mechanical shaker for 48 hours to make a mixture. The mixture was sieved using white clean handkerchief. The filtrate was then concentrated using rotator evaporator (Buchi), and was further concentrated to dryness at 40℃ in an electric oven (Gallenkamp). The extract was later kept in the refrigerator 4℃ until ready for use. The extract was administered orally at dose concentration of 100, 300 and 500 mg/kg (table 1).

The rats were weighed and blood samples collected from the tail vein to measure PCV (basal). The volume of phenylhydrazine injected (intra peritoneal) was 0.4 ml depending on the body weight of the rat. PCV was used to assess level of anemia as described by Idowu et al.[14] after 48 hours before treatment as showed below.

At the end of 28 days of experiment, the animals were sacrificed under chloroform anesthesia. 5 ml of blood was collected via cardiac puncture into EDTA container for biochemical analysis. The liver was excised quickly and subjected to histological studies. PCV was estimated using microhaematocrit method and Hb content of the blood using Sahli's hemoglobinometer methods, RBC count was done using the methods by Dalcie and Lewis.[15]

Table 1: Experimental Grouping and Injection Protocols for Experimental and Control Cases

Groups (n = 5)	Phenylhydrazine (ml)	*Annona muricata* extract (mg/kg)
Group I (Control)	-	-
Group II	0.4 ml	-
Group III	0.4 ml	100 mg/kg
Group IV	0.4 ml	300 mg/kg
Group V	0.4 ml	500 mg/kg

Blood was diluted to 1: 200 with Hayem's fluid which preserved the corpuscles and then counted with Neubauer counting chamber under a light microscope. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed based on Randox diagnostic kit on the principles of Reitman and Frankel.[16] Alkaline phosphatase (ALP) was determined by the method of Bessey et al.[17]

Statistics

Data obtained was analyzed using SPSS version 18.0 for windows. Analysis of variance (ANOVA) was used to compare mean ± SEM. Data was considered significant at P = 0.05 or less. Post hoc multiple comparisons was done using least significant difference (LSD).

RESULTS AND DISCUSSION

The PCV levels of experimental Groups II, III, IV and V significantly decreased ($P < 0.05$) after induction of 0.4 ml phenylhydrazine compared to Group I. After four weeks of treatment, the level

of PCV significantly increased ($P > 0.05$) in Groups III and IV compared with Group I. There was statistically significant difference ($P < 0.05$) between Group V compared to Group I.

Phenylhydrazine hydrochloride has a reducing effect on PCV.[18-19] Results showed that PCV levels was reduced at third week although statistically significant at the fourth week after treatment with *annona muricata* extract especially in Group III and IV respectively. Phenylhydrazine administration has been shown to cause hematotoxicity which leads to hemolytic anemia by altering iron metabolism, activating immune response which triggers phagocytosis in the spleen and liver and interferes with the binding of erythropoietin on its receptors.[20] Antioxidants have been implicated in the reduction of blood loss to lipid peroxidation[21-22] whereas *annona muricata* aqueous extract has been shown to possess antioxidant properties and protective action on pancreatic beta cells.[23] Therefore, the antioxidant effect of *annona muricata* extract may be responsible for the increase in the PCV after phenylhydrazine anemia.

Erythropoietin increases the number of erythropoietin sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors and subsequently to mature erythrocytes and hemoglobin synthesis.[24] Based on the fact that phenylhydrazine interfered with binding erythropoietin on erythropoietin receptors,[25] it is wise to suggest that *annona muricata* extract disinhibited the binding thus stimulated erythropoiesis and increased hemoglobinization of red cells as showed (table 3). These effects on PCV levels, Hb concentration and RBC count seemed to be dependent on time.

Experimental studies have shown that intravascular hemolysis in any condition may damage liver and other vascular organs.[25] Apart from hemolysis induced liver injury, phenylhydrazine has been reported to cause oxidative damage to liver, kidney and spleen;[26] therefore, we investigated the effect of *annona muricata* extract on some liver enzymes AST, ALT and ALP. Results (table 4) showed that liver enzymes were restored to normal. Our findings on liver enzymes are in accord with earlier reports by Fareed et al.[27] The effect of phenylhydrazine may be direct and/or indirect on the liver. The mechanism of action of *annona muricata* seed may solely depend on the restoration of these hematological parameters thereby preventing damage to the liver and/or restoration of injured hepatocytes.

We also studied histopathological analysis of sections of the liver in order to determine the effect of the injury on hepatocytes. Photomicrographs (figures 3-5) of liver of the rats treated with *annona muricata* seed extract restored the architecture of the liver almost to normalcy characterized with absent of portal inflammation, focal dilation of central veins and congestion, although these restoration also seemed to be dependent on time. Most of these changes especially congestion is associated with oxidative

stress and inflammation.[28] Phenylhydrazine causes alteration of iron metabolism by causing overload in the liver,[20] releasing heme which catalyzes free radical reaction thereby promoting oxidative damage.[29-30] ALT is a specific marker for hepatic injury[31] because it is present mainly in the cytosol of the liver and in low concentrations elsewhere and could explain the degree of oxidative stress on the hepatocytes. The photomicrographs (figure 3-5) confirmed the biochemical findings in this present study indicating that *annona muricata* seed extract is hepatoprotective against hemolytic anemia and/or phenylhydrazine induced hepatotoxicity in rats. Our findings agreed with previous reports in streptozotocin-treated diabetic rats[32] and in carbon tetrachloride and acetaminophen-induced liver damage.[27]

CONCLUSION

The fact that *annona muricata* seed extract increased PCV, Hb and RBC levels and caused a reduction in AST, ALT and ALT with restoration of hepatocytes after phenylhydrazine induced hemolytic anemia suggest that the extract may be beneficial in the treatment of hemolytic anemia and/or hemolytic anemia induced liver injury.

Table 2: PCV Levels Before and After Induction of Anemia with 0.4 ml Phenylhydrazine

Groups	Group I	Group II	Group III	Group IV	Group V
Before induction	45.25± 2.49	44.50 ± 2.47	42.50 ± 1.55	47.00 ± 1.22	46.00 ± 1.29
After induction	45.25 ± 2.49	26.25 ± 2.18[a]	28.25 ± 2.78[ab]	30.00 ± 0.41[ab]	32.50 ± 1.32[ab]
First week of treatment	45.25 ± 2.49	22.25 ± 1.97[a]	31.75 ± 0.85[ab]	29.00 ± 2.35[ab]	33.00 ± 1.08[ab]
Second week of treatment	42.50 ± 1.55	20.50 ± 1.44[a]	35.25 ± 1.93[ab]	32.25 ± 1.03[ab]	34.25 ± 1.44[ab]
Third week of treatment	47.00 ± 1.22	24.75 ± 2.75[a]	43.00 ± 1.41[ab]	44.50 ± 1.19[ab]	43.25 ± 1.75[ab]
Fourth week of treatment	46.00 ± 1.29	24.25 ± 1.84[ab]	45.25 ± 1.65[b]	45.50 ± 0.96[b]	42.50 ± 1.76[ab]

Table 3: RBC Count, Hb Content and Hematocrit after Four Weeks Treatment

Groups	RBC count (10^{12}/l)	Hb concentration (mmol/l)	Hematocrit (%)
Group I	7.36 ± 0.42	10.88 ± 0.30	40.63 ± 1.94
Group II	2.61 ± 0.60[a]	5.40 ± 0.63[a]	20.10 ± 0.35[a]
Group III	7.62 ± 0.74[b]	10.78 ± 0.74[b]	40.70 ± 3.54[b]
Group IV	6.68 ± 0.54[ab]	10.58 ± 0.69[b]	40.78 ± 2.50[b]
Group V	8.49 ± 0.37[b]	10.75 ± 0.73[b]	39.33 ± 3.69[ab]

Table 4: Liver Enzymes: ALT, AST and ALP after Four Weeks Treatment

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Group I	21.50 ± 1.20	47.75 ± 9.80	7.18 ± 1.90
Group II	38.50 ± 0.87[a]	171.80 ± 19.23[a]	21.10 ± 2.18[a]
Group III	19.00 ± 2.10[b]	31.75 ± 0.74[b]	5.93 ± 1.10[b]
Group IV	21.75 ± 2.60[b]	39.25 ± 3.40[b]	5.75 ± 0.60[b]
Group V	18.70 ± 2.30[b]	38.00 ± 4.50[b]	6.30 ± 1.50[b]

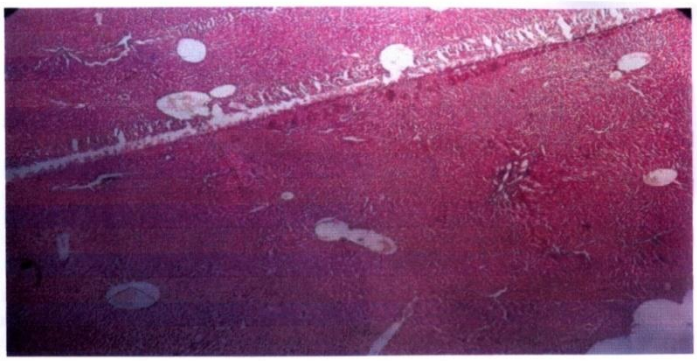

Figure 1: Photomicrograph of the liver of normal/Group I rats after the fourth week of the study. Stained preparation, H & E x 40

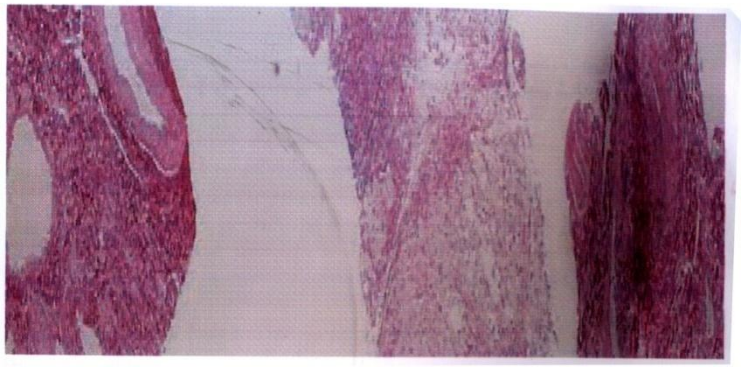

Figure 2: Photomicrograph of the liver of Group II rats (phenylhydrazine only) after the fourth week of the study. Stained preparation, H & E x 40

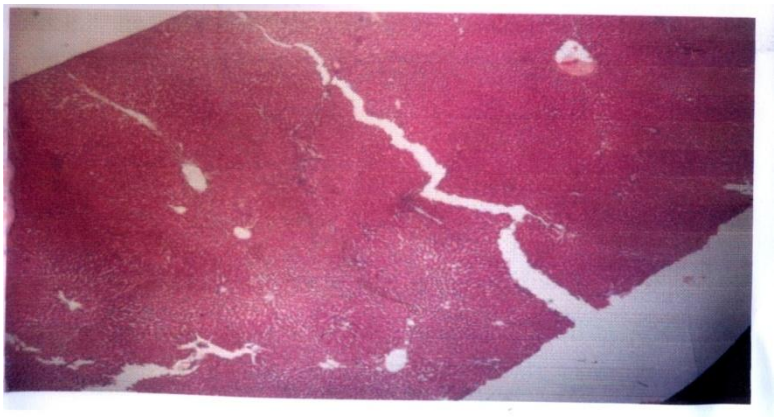

Figure 3: Photomicrograph of the liver of group III rats (300 mg/kg of *annona muricata* seed extract) after fourth week of the study. Stained preparation, H & E x 40

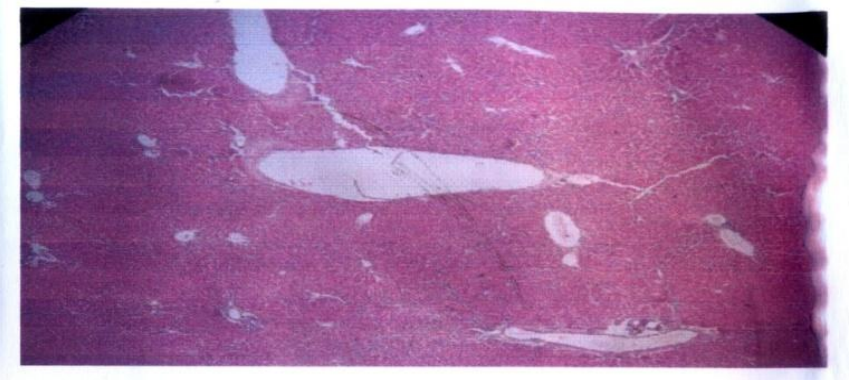

Figure 4: Photomicrograph of the liver of Group IV rats (300 mg/kg of *annona muricata* seed extract) after the fourth week of the study. Stained preparation, H & E x 40

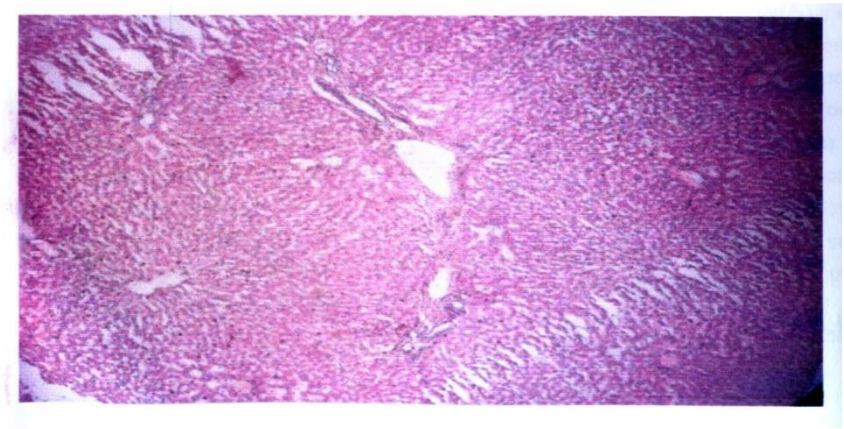

Figure 5: Photomicrograph of the liver of Group V rats (500 mg/kg of *annona muricata* seed extract) after the fourth week of the study. Stained preparation, H & E x 40

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