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Research Article

Anti-Anemic Effect of *Annona muricata* Ethanolic Leaf Extract in Dimethyl Nitrosamine (DMN)-Induced Hepatic Fibrosis in Rats

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Abstract

Background: This study was carried out to evaluate the anti-anemic effect of *Annona muricata* leaves on hematological parameters in dimethyl nitrosamine (DMN)-induced fibrotic rats.

Methods: Healthy adult male wistar rats were divided into four groups. Group I rats received normal saline and served as control, Group II rats were administered 200 mg/kg *Annona muricata*, Group III received 200 mg/kg *Annona muricata* orally simultaneously with 10 mg/kg DMN, Group IV received 10 mg/kg DMN without extract. DMN administered intraperitoneally was on first three days of each week for two weeks while *Annona muricata* was administered for 14 consecutive days. 24 hours after last administration, all animals were sacrificed and blood collected in EDTA containers for hematological analysis. Data were expressed as mean value ± standard deviation while differences between groups were determined by One-way ANOVA using SPSS. A probability level of less than 5% ($p < 0.05$) was considered significant.

Results: DMN-induced anemia were evidenced by significant decrease in the levels of Hemoglobin (8.53 ± 0.45 g/dl), Red blood cells (3.07 ± 0.23 x106/ųL), Platelets (30.00 ± 5.57x103/ųL), packed cell volume (24.45 ± 1.77%), Lymphocytes (26.58 ± 4.66%) and a significant increase in monocytes (16.40 ± 3.12%) and granulocytes (58.17 ± 10.12%). Simultaneous treatment with *Annona muricata* leaf extract however significantly ($p < 0.05$) reversed alterations in the above indices towards normal.

Conclusion: *Annona muricata* leaves exhibit anti-anemic and anti-fibrotic effect against DMN-induced hepatic fibrosis suggesting that it may help in amelioration and treatment of anemia.

Keywords: *Annona muricata; Anti-anemic; Dimethyl nitrosamine; Fibrosis; Hematology*

Introduction

Dimethyl nitrosamine (DMN), a member of the family of extremely potent carcinogens, the N-nitrosamines, is generated from the in situ reaction of dimethylamine (DMA) with monochloroamine in the disinfection process or the nitrosation of DMA by nitrite [1]. DMN exerts its carcinogenic effects, causes fibrosis, cirrhosis and induces hepatic necrosis in experimental animals through metabolic activation by CYP2E1 [2-3]. The occurrence of DMN in drinks, foods, tobacco products, cosmetics, gloves, balloons, toys, baby bottle teats, soothers, condoms, rubber or rocket fuel factories and leather tanneries is well established [4-8]. The pathogenesis of hepatic fibrosis is mediated through oxidative stress and hepatocyte injury and is always accompanied by impaired hepatic metabolism and deposition of connective components especially collagen and hyaluronic acid in the liver [9-11].

Annona muricata, a member of the family, Annonaceae, is traditionally used for treatment of headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an anti-inflammatory, antispasmodic and anti-dysenteric [12-13]. Research on *Annona muricata* have shown that bioactive compounds including a novel set of phytochemicals (Annonaceous acetogenins) found in the leaves, seeds and stem in addition to antioxidant properties, are cytotoxic against various cancer cells [14-15]. This study was carried out to evaluate the anti-anemic effect of *Annona muricata* leaves on hematological parameters in dimethyl nitrosamine (DMN)-induced fibrotic male rats.

Materials and Methods

Plant collection, Identification, Preparation and extraction

Fresh leaves of *Annona muricata* were collected from the tree in Upper Sakponba in Benin City, Edo state, Nigeria. The leaves were identified by a Botanist and ethanol extract of dry powdered *Annona muricata* leaves was prepared by soaking 100g each of the dry powdered plant materials in 1000 ml of absolute ethanol at room temperature for 48 hrs. At the end of the 48 hours, the extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The *Annona muricata* ethanolic extract was concentrated using a rotary evaporator with the water bath set at 40°C to one-tenth its original volume and then finally freeze dried. The dry residue (crude extract) was then stored at 4°C until used for animal study.

Animal study

Male wistar albino rats divided into four (4) groups of five (5) rats each, weighing between 160-200g were obtained from the Animal Unit facility of the University of Ibadan, Oyo state, Nigeria and housed in wooden cages in the animal house of the Department of Biochemistry, University of Benin. The rats were maintained under controlled environmental conditions (temperature- 24 ± 2ºC; relative humidity-50-70%; 12h light/dark cycle), housed for one week after their arrival to the animal house for acclimatization. The rats had free access to drinking water and normal pellet diet (NPD) ad libitum until they were assigned to individual groups.

The "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the appropriate ethics committee".

DMN used in this work was synthesized in a fume chamber at the Department of Biochemistry, University of Ibadan according to the method of Vogel [16].

Group I rats received normal saline and served as control, Group II rats were administered 200 mg/kg *Annona muricata*, Group III received 200 mg/kg *Annona muricata* orally simultaneously with 10 mg/kg DMN, Group IV received 10 mg/kg DMN without extract. DMN administered intraperitoneally (dissolved in 0.15M NaCl) was on first three days of each week for two weeks while *Annona muricata* was administered for 14 consecutive days. DMN administration (10 mg/kg) for first three days of each week for two (2) weeks was used as the model for induction of hepatic fibrosis [9,10]. 24 hours after last administration, all animals were sacrificed and blood collected in EDTA containers for hematological analysis using full automated blood cell counter PCE -210N.

Statistical analysis

Data were expressed as mean value ± standard deviation while differences between groups were determined by One-way ANOVA using SPSS. A probability level of less than 5% ($p < 0.05$) was considered significant.

Results and Discussion

DMN-induced anemia as shown in table 1 and 2 were evidenced by significant decreases in the levels of Hemoglobin (Hb), Red blood cells (RBC), Platelets, packed cell volume (PCV), lymphocytes and a significant increase ($p < 0.05$) in white blood cells, monocytes and

granulocytes. Simultaneous treatment with *Annona muricata* leaf extract however significantly ($p < 0.05$) reversed alterations in the hematological parameters towards normal [17].

Treatment Groups	Hb (g/dl)	RBC (x10^6/µL)	PCV (%)	PLT (x10^3/µL)
Control (normal saline)	13.73 ± 0.64^a	6.61 ± 0.21$^{a, b}$	40.10 ± 1.20^a	347.00 ± 61.36^a
AME alone (200 mg/kg)	12.83 ± 1.18^a	7.23 ± 0.77^a	41.33 ± 3.37^a	233.00 ± 33.63^b
AME (200 mg/kg) + DMN (10 mg/kg)	10.07 ± 0.09^b	5.28 ± 0.65^b	33.27 ± 3.35^b	82.50 ± 8.50^c
DMN alone (10 mg/kg)	8.53 ± 0.45^z	3.07 ± 0.23^c	24.45 ± 1.77^z	30.00 ± 5.57^d

Table 1: *Effect of ethanolic leaf extracts of Annona muricata on Hb, RBC, PCV and PLT in DMN-induced hepatic fibrotic rats.*

Values are expressed as Mean ± SD, (n = 5), AME = Annona muricata, DMN = Dimethyl nitrosamine, Hb = Hemoglobin, RBC = Red blood cell, PCV = Packed cell volume, PLT = Platelet.

Mean values in each column having different superscript (a, b, c, z) are significantly different ($p < 0.05$).

Treatment Groups	LYM (%)	MONO (%)	GRA (%)	WBC (x10^3/ųL)
Control (normal saline)	92.36 ± 2.20^a	2.30 ± 0.87^a	5.33 ± 2.11^a	6.40 ± 1.31^a
AME alone (200 mg/kg)	84.53 ± 3.92^a	3.63 ± 1.35^a	11.83 ± 2.15^a	5.26 ± 0.75^a
AME(200 mg/kg) + DMN (10 mg/kg)	63.90 ± 3.50^c	13.90 ± 3.20^b	22.20 ± 0.30^b	10.75 ± 3.58^b
DMN alone (10 mg/kg)	26.58 ± 8.66^z	16.40 ± 3.12^b	58.17 ± 10.12^z	17.60 ± 2.26^c

Table 2: *Effect of ethanolic leaf extracts of Annona muricata on WBC, LYM, MONO and GRA Hematological parameters in DMN-induced hepatic fibrotic rats.*

Values are expressed as Mean ± SD, (n = 5), AME = Annona muricata, DMN = Dimethyl nitrosamine, WBC = White blood cell, LYM = Lymphocyte, MONO = Monocyte, GRA = Granulocyte.

Mean values in each column having different superscript (a, b, c, z) are significantly different ($p < 0.05$).

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds on the blood constituents of an animal [18]. The significant reductions in Hb, PCV and RBC contents of the blood of DMN-fibrotic rats in this study is an indication that the oxygen transport blood would be reduced and thus will result in decrease of physical activity. The reductions in RBC, Hb and PCV may be attributed to increase in lipid peroxidation of the erythrocyte cell membrane [10,19]. The reduction in RBC counts observed with DMN treatment could be attributed to RBC haemolysis due to haemorrhage and reduced erythropoiesis. The significant decrease in the Hb concentration may be due to either impaired biosynthesis of haem in bone marrow, an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis. The marked reduction observed in Hb, PCV and RBC is thus an indication of anemia in DMN-alone rats.

However, the significantly increased RBC count of extract-DMN treated rats compared to DMN alone might be due to the lowered lipid peroxide level in RBCs membrane leading to a decrease susceptibility of RBCs to hemolysis. The significant reversal of the hematological indices in DMN-extract treated groups suggests that *Annona muricata* have some stimulatory effect on the production of RBC (erythropoiesis) and platelets.

Conclusion

Annona muricata leaves thus exhibit anti-anemic and anti-fibrotic effect against DMN-induced hepatic fibrosis suggesting that it may

help in amelioration and treatment of anemia. The ameliorative effect may have arisen from their antioxidant properties and mineral components.

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