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# Hematopoietic and modulatory effects of leaf extract of *Annona muricata* on Male adults rabbits

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**Abstract**

*Annona muricata* L. (Magnoliales: Annonaceae) is a tropical plant species known for its edible fruit which has some medicinal merits, but also some toxicological effects. The present study was undertaken to evaluate the potential effects of *A. muricata* on hematopoietic and hematological parameters. Tenth rabbits were randomly divided into two equal groups (each group five rabbits). The first group was used as a control. The second group was used to study the effect of *A. muricata* L. (100 mg/kg body weight) for six weeks. Results indicated that treatment with *A. muricata* were not caused significant effect on packed cell volume (PCV), hemoglobin (Hb), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). *A. muricata* caused increased in the number of PLT compared with control group. In concluded, clinical studies are necessary to support the therapeutic potential of this plant.

**Keywords:** Hematopiotic, *A. muricata*, blood parameters

## Introduction

Food adulteration in India is getting deadlier day Haematological studies are important because the blood is the major transport system of the body, and evaluations of the haematological profile usually furnishes vital information on the body's response to injury of all forms, including toxic injury [1]. Haematological studies also represent a useful process in the diagnosis of many diseases as well as investigation of the extent of damage to the blood [2]. This is relevant since blood constituents' change in relation to the physiological conditions of animals. The blood transports or conveys nutrient and materials to different parts of the body. Therefore, whatever affects the blood; drugs, pathogenic organism or nutrition will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction [3]. The use of complementary traditional medicine which includes herbal medicines in the treatment of various diseases has expanded rapidly in both developed and developing countries, attributable to affordability, accessibility and efficacy [4]. Today many botanicals natural products are used in therapy of different diseases because they contain components of therapeutic value, such as graviola (*A. muricata* L). *A. muricata* (Linn.) commonly called soursop, Graviola or guanabana is an evergreen tree native to the tropical regions belonging to the Annonacea family [5]. *A. muricata* plays a crucial role in various traditional and alternative medicines.

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All parts of this tree are extensively used as traditional medicines. The bark, leaves, roots, fruit and fruit seeds have their own respective use [6-8]. A. muricata L is a member of the Annonaceae family comprising approximately 130 genera and 2300 species [5]. Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an anti-inflammatory, antispasmodic and antidysenteric [9]. Moreover, internal administration of the leaf's decoction is believed to exhibit anti-rheumatic and neuralgic effects, whereas the cooked leaves are topically used to treat abscesses and rheumatism [5]. The plant has been reported to possess acetogenins as major phytoconstitutents [10, 11], which are responsible for number of activities such as antitumor, immunomodulator, antispasmodic, antimalarial, pesticidal, antiparasitic, antibacterial, antifungal and antihelmintic activity [12].

Different studies reported the beneficial effects of *A. muricata* on the hematological status of rats, especially on the synthetic ability of some of the important plasma proteins, including albumin and platelets[13, 14]. The fruit, leaf, and root-bark extracts showed a dose-dependent increase in white blood cells and lymphocytes. The extracts of the various parts of the plant apart from the stem-bark recorded marked increase in platelet levels. The various extracts of the plant parts recorded striking increase in red blood cells, hemoglobin concentration, and packed cell volume. These observations could be linked to the remarkable quantity of alkaloids, flavonoids, and phenols present in the leaf and fruit fractions [13].

This plant is generally considered to be very safe and well tolerated in animals [15], whereas there have been few reports about the toxicity of *A. muricata* in human studies. In spite of the worldwide use of it, there has been concern about the lack of clinical toxicology information. This study aimed to examine whether the 6-weeks oral administration of extract of *A. muricata* exhibits any noticeable toxic effect in hematological parameters on male rabbits.

## Material and Methods

*A. muricata* was purchased from *A. muricata* leaf (powder) (maximum international company, Brasil) was purchased from local pharmacy. Each capsule contains 0.3125 g powder and the content of each capsule was dissolved in corn oil just before use.

**Experimental procedure:** ten mature male rabbits were randomly divided into couple groups (each five rabbits) as follows:-

- **Group I**: Rabbits were used as control and received an equivalent 1 ml of the vehicle (corn oil) alone by oral gavage twice per week for 6 successive weeks.
- **Group II**: Rabbits were treated with *A. muricata* L. which was given twice per week by gavage at a dose of 100 mg/kg B.W, [16-18] dissolved in corn oil for 6 successive weeks.

Blood samples were collected from the ear vein of all animals every week throughout the 6-weeks experimental period. Blood samples were obtained in the morning before accesses to feed and water. Values derived from complete blood count (CBC). All CBC tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc [19, 20]. CBCs were performed on EDTA as anti-coagulated samples. Differential cell counts were performed manually using Dif-Quik-stained blood smears. Data were recorded according to the following categories: white blood cell (WBCs); red blood cell (RBCs); hemoglobin (HB); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

**Preparation of blood smears**

Thin films, 3-5 cm in length, of the aspirated a drop of blood were made using a smooth-edged glass spreader of not more than 2 cm in width. The blood fragments are dragged behind the spreader and leave a trail of cells behind them. After drying, films were dried in the air, and then fixed by immersing in a jar of methanol for 15-20 min. Slides were transferred to a staining jar containing Giemsa's stain (1 volume of stain with two volumes of distilled water).

After staining for 15-30 min, the slides were transferred to a jar containing buffered water, pH 7, rapidly washed in three or four changes of water and finally allowed to standing undisturbed in water for a short time (usually 2-5 min) for differentiation to take place. The smears were examined under scanner, low power, high power and oil immersion objectives using Olympus CX31 light microscope with attached Olympus

camera. The erythrocytes and leucocytes were examined for variation in size and shape [19, 20]..

**Preparation of bone marrow smears**

Bone marrow cells were collected from rabbit's femora after cutting away the epiphyses and condyles. Using a 25-gauga needle inserted into the proximal end of the bone the marrow was flushed with ice-chilled buffered saline into slide. Make films, 3-5 cm in length, of the aspirated marrow using a smooth-edged glass spreader of not more than 2 cm in width. The marrow fragments are dragged behind the spreader and leave a trail of cells behind them. After drying, dry the films in the air, then fix by immersing in a jar of methanol for 15-20 min. Transfer to a staining jar containing Giemsa's stain (1 volume of stain with two volumes of distilled water). After staining for 15-30 min, transfer the slides to a jar containing buffered water, pH 7, rapidly wash in three or four changes of water and finally allow standing undisturbed in water for a short time (usually 2-5 min) for differentiation to take place. The smears were examined under scanner, low power, high power and oil immersion objectives using Olympus CX31 light microscope with attached Olympus camera. The preparation was considered satisfactory only when marrow particles and free marrow cells were observed in the stained films [19, 20].. .

**Statistical analysis:** Where applicable, statistical analysis was carried out in Minitab software (version17)/ statistical significance was assessed using two samples T- test analysis after detection normal distribution to the data and appropriate $P < 0.05$ consider significant [21].

## Result and Discussion

The relative organ weights (%) were calculated as g/100 g body weight. The relative weight of spleen was increased in *A. muricata* group without significant differences between two groups. Values derived from complete blood counts (CBC), including differential cell counts were recorded for each group and presented in Table 1. Results indicated that treatment with *A. muricata* were caused increased on number of red blood cells (RBCs), mean cell hemoglobin concentration (MCHC), percentage number of neutrophils and number of platelets (PLT). While, number of white blood cells (WBCs), levels of hemoglobin (Hb), packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell volume (MCV), and percentage number of lymphocytes (%) were decreased in *A. muricata* group compared to control group. MCV, MCHC and PLT were change with significant deference.

Results from blood smear were shown similar on morphological pattern in both groups as shown in Figure 1. Similar results were found in bone marrow smear (Figure 2).

**Table 1: Mean values of relative spleen weight, RBCs, HB, PCV, MCV, MCH, and MCHC in male rabbits control and treated with *A. muricata* at end of experiment**

| Parameters | Control Mean± SEM | *A.muricata* Mean± SEM |
|---|---|---|
| Spleen (%) | 0.041 ± 0.0038 [a] | 0.065 ± 0.016 [a] |
| **RBC $\times 10^6$ (µl)** | 5.533± 0.135[a] | 5.7400±0.07[a] |
| **Hb (g/dl)** | 13.208±0.294[a] | 12.892± 0.126[a] |
| **PCV$\times 10^3$(µl)** | 40.250± 0.880[a] | 39.042±0.411[a] |
| **MCV (fl)** | 72.91± 1.30[a] | 68.133± 0.728[b] |
| **MCH (pg)** | 24.304± 0.358[a] | 23.934± 0.420[a] |
| **MCHC (dl)** | 32.690± 0.086[b] | 33.467±0.262[a] |
| **WBCs $\times 10^3$(µl)** | 10.178 ±0.35[a] | 9.433 ± 0.78[a] |
| **Neutrophils (%)** | 34.33 ± 1.35[a] | 42.00 ± 0.58[a] |
| **Lymphocytes (%)** | 55.67 ± 1.35[a] | 46.0 ± 0.58[a] |
| **PLT $\times 10^3$(µl)** | 289.9 ± 21.97[b] | 337.9 ± 26.33[a] |

Values are expressed as means ± SEM; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different, $p<0.05$

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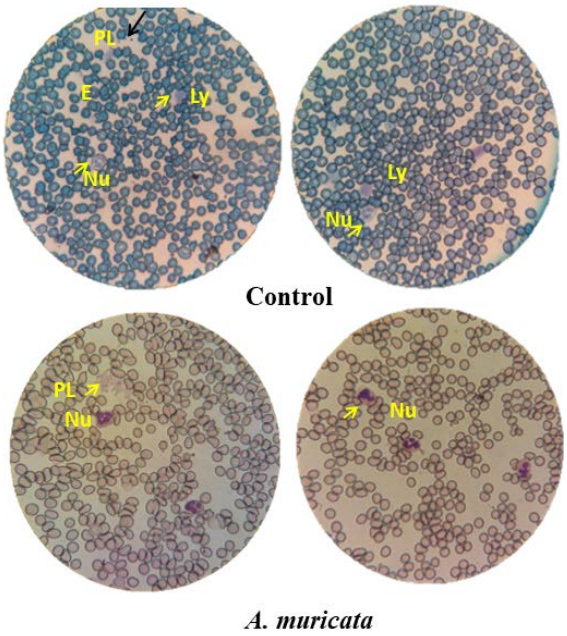


**Fig.1: Photomicrographs showing peripheral blood smears of rabbits in two groups: In both groups: arrows indicates blood platelets (PL), lymphocyte (Ly) and neutrophil (Nu). Rabbit treated with A. muricata note similarity to control Giemsa's stained preparation of dry smear from EDTA anticoagulated blood. Magnification: 100X**

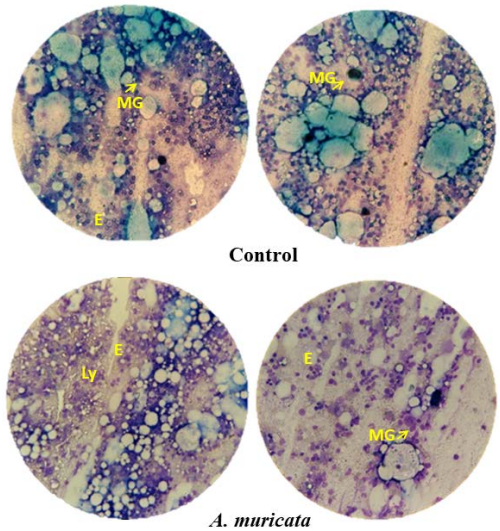


**Fig. 2: Different smears of bone marrow from two groups**

Three major cell lines are represented. These cell lines include myelomonocytic cells (LY) and erythroid cells (E). Megakaryocytes (MG) is apparent in these fields. (Magnification 40X).

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. It can also be used to explain blood relating functions of chemical compounds/plant extract [22]. The hematopoietic system is very sensitive to toxic substances [23]; this indicates the safety of the *A. muricata* leaves, as there were no hematological changes. The evaluation of pathological changes, by microscopy isn't indicators of toxicity the presence of altered red cells morphology is consistent with erythrocyte damage and is presumed to be related to direct oxidative injury to the red cells by the chemicals or to the pitting function of the spleen. The increasing on relative weight of spleen was consistent with increasing of CBC parameters. Only MCV was significantly decreased with *A. muricata* alone. The decrease in MCV indicates that older and healthier erythrocytes were destroyed [24], and this may due to the treatment induced cytotoxic stress erythrocyte membranes [25]. Recent study found that *A. muricata* play role as bio-functionalized in maintaining erythrocyte membrane integrity and,consequently, decreasing the degree of haemolysis in male Wister rats at dose 100mg/kg body weight of leave extract [25]. Alkaloids, flavonoids and tannins present in the plant extracts have been considered to be responsible for the bio-reduction process [25].

Platelet level increased in the groups treated with *A.muricata* suggesting enhanced cytoprotetive potential of *A. muricata* in which platelets can adhere to the walls of the blood vessels, release bioactive compounds, and aggregate to each other, resulting in increase in arterial thrombosis and atherogenesis [25, 26]. Agu, Okolie [27] hypothesize that this activity may involve stimulating increases in bone marrow platelet production, increased mobilization, as well as direct modulatory interactions with biomolecules synthesized, store, or released by the platelets. It is concluded that the administration of the

hydroalcoholic extract *A. muricata* leaves, does not present acute and subacute toxicity, as evidenced by clinical, hematological, parameters; thus, demonstrating the safety of the atomized extract at the tested doses. It is recommended to continue studies according to the standards of regulatory bodies, such as studies of chronic toxicity, reproductive toxicity, genotoxicity, endocrine changes, among others, in order to evaluate the total safety for use in humans.

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