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Haematological Effects of Aqueous Extract of *Allium sativum* Bulb on Adult Wistar Rats

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Abstract: Garlic (*Allium sativum)* which is a member of the Alliaceae family, has been widely recognized as a valuable spice and used as remedy for various ailments and physiological disorders. It is principally used to lower blood pressure, cholesterol, blood sugar and fight infections. The purpose of this study is to evaluate the effects of oral administration of aqueous extract of *Allium sativum* on the haematological parameters in adult Wistar rats. Twenty adult Wistar rats weighing between 200-270 were procured and acclimatized for two before the commencement of the experiment. They were grouped into four (groups- A, B, C and D). Aqueous extract of *Allium sativum* was administered orally at doses of 150, 300 and 600mg/kg body weight respectively to groups B, C and D for a period of five weeks. Rats in group A served as control and were given distill water and animals feed daily for period of five weeks. At the end of the experiment, the animals were sacrificed and blood samples were collected for laboratory investigations. The result of the investigation showed that white blood cell (WBC) count and its differentials viz agranulocytes (lymphocytes and monocytes) as well as the level of RBC and its related indices like Hb, MCV and MCHC especially in the groups treated with dose of 300 and 600 mg/kg body weight respectively were significantly increased when compared to control group. In conclusion: The result showed that *Allium sativum* extract enhanced the immune cells by activating the production of leukocytes, lymphocytes and increased hemoglobin.

Keywords: Haematology, Photomicrographs, Agranulocytes, Hemoglobin, Microscope.

Introduction

Allium sativum, commonly known as garlic is a member of the Alliaceae family, has been widely recognized as a valuable spice and used as remedy for various ailments and physiological disorders. *Allium sativum's* principal medicinal uses are to lower blood pressure, cholesterol, blood sugar and fight infections (McMahon and Vargas,1993; Jeyaraj *et al.,* 2006; Eidi *et al.,* 2006). *In vitro* studies on animal data suggest that *Allium sativum* may help to prevent some solid tumors and therefore it is also effective in cancer prevention. Other therapeutic effects of *Allium sativum* includes hepatoprotection (Mirunalini *et al*., 2010), antihelmentics (Iqbal *et al*., 2001), anti inflammatory (Tsai *et al*., 2005), antioxidant (Yin and Cheng, 2003), antifungal (Ledezma and Apitz-Castro, 2006), antiviral (Josling, 2001), anticoagulant (Song, 1963) and wound healing (Jalali *et al*., 2009). Extracts of garlic has also been shown to improve the activation of natural killer cells as well as the level of interleukin-2 (Clement *et al*., 2010). Allicin, diallyl disulfide-oxide, an active ingredient released from garlic is a systemic vasodilator (Sang *et al*., 1995).

Research has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters. These alterations could either be positive or negative (Ofuya and Ebong, 1996; Onifade and Suleiman, 1999). Anaemia is one of the negative consequences of oral ingestion of medicinal compounds or drugs and occurs as result of sequestration of red blood cell in the spleen, impaired red blood cell production or primary bone marrow dysfunction (Watt and Breyer-Brandwijk, 1962; Cheeke 1998).

Materials and Methods

Collection and identification of Allium sativum: *Allium sativum* bulbs were purchased at Ikpoba hill market, in Benin metropolis and the bulb were identified and authenticated in the Pharmacognosy Department of the University of Benin by Mr. Sunny Nweke.

Extract preparation: The bulbs were peeled, blended using a blender and soaked in distilled water for 12 hrs. The aqueous extract was filtered using a Buchner funnel and Whatman No.1 filter paper. Dried aqueous extracts were obtained after removing the solvent by evaporation under reduced pressure using Rotary evaporator. The extract was stored in an air-tight container and kept in the refrigerator at 4°C until use.

Test animals: Twenty (20) Wistar rats of both sexes, weighing between 200- 270 g were used for this study. The animals were rats obtained from the animal house of the Anatomy Department of the University of Benin, Benin City. Food and water were provided *ad libitum.* Animals were exposed to controlled environmental temperature of (28± 2°C), relative humidity (50± 5%) and 12 h light or dark. The handling procedures were conducted in accordance with the Faculty of Pharmacy, University of Benin Ethical Committee on Experimental Animals. The animals were also allowed two weeks under these conditions to acclimatize before the commencement of the experiments. The animals were weighed before, during, and at the end of the experiment.

Experimental design and treatment regime: The rats were divided into four groups, each group consisting of five animals. All administrations were done orally using an orogastric tube daily for five (5) weeks. The control group also received 1ml of distilled water via orogastric tube daily.

Group A: (Control) received only 1ml of distilled water for 5 weeks.
Group B: Received 150mg/kg of body weight of *Allium sativum* extract for 5 weeks
Group C: Received 300mg/kg of body weight of *Allium sativum* extract for 5 weeks
Group D: Received 600mg/kg of body weight of *Allium sativum* extract for 5 weeks

At the end of five weeks of the experiment, the animals were sacrificed under chloroform anesthesia and blood was collected directly from the abdominal aorta of the Wistar rat into lithium heparin and EDTA bottles for hematological studies. Finally, photomicrographs of processed blood slides were taken using the Light Microscope.

Biochemical analysis: Red Blood Cell (RBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell (WBC), Granulocytes (basophils, neutrophils, and eosinophils,) and Agranulocytes (lymphocytes and monocytes) were estimated using automated hematology analyser.

Statistical analysis: Data were expressed as (Mean ± SEM) of six replicates and were subjected to one-way analysis of variance (ANOVA) using SPSS version 10.0. Individual comparisons were obtained by the Duncan multiple range test (DMRT). A value of $P<0.05$ was considered to indicate a significant difference between groups (Duncan 1957).

Results

Agranulocytes (lymphocytes and monocytes) in the test groups were observed to be significantly ($p<0.05$) increased when compared to the control as show in Table 1. The study also revealed decrease in granulocytes in the test groups treated at dose 300 mg/kg bwt when compared to the control (Table 1). Also there were significant increases ($p<0.05$) level of platelet in the test group treated with *Allium sativum* extract at the dose of 300 mg/kg. Administration of *Allium sativum* extract led to significant ($p<0.05$) increase level of RBC and its related indices like Hb, MCV and MCHC especially in the group treated with *Allium sativum* extract at dose of 300 and 600 mg/kg bwt when compared to control group (Table 2). There were decreases in the level of MCH for all the doses tested.

Microscopic findings: At low dose, the blood film showed an increase in two polymorphonuclear leukocytes, and production of one lymphocyte amidst sheets of erythrocytes per high power field. At moderate dose, there were three polymorphonuclear leukocytes and two lymphocytes amidst sheets of erythrocytes per high power field. At high dose, changes were the same with that observed in the moderate dose.

Table 1: Effect of aqueous extract of *Allium sativum* on white blood cell count and its differentials

Parameter	Group A	Group B	Group C	Group D
WBC($\times10^3$\µl)	7.98±2.00	9.48±2.00	12.74±2	12.94±2.00[a]
Lymphocytes (%)	57.68±6.00[a]	59.72±9.00 [a]	57.00±1.00	61.16±7.00
Monocytes (%)	13.72±2.00[a]	15.62±4.00[a]	16.96±0.90[a]	13.60±1.00
Granulocytes (%)	28.60±4.00	24.60±6.00[a]	26.54±2.00	25.18±7.00
Platelets ($\times10^3$/µl)	305.40±80.00	300.00±75.00	326.40±32.00[a]	299.20±51.00

Data is expressed as Mean ±SEM [a]$p<0.05$ is considered significant

Table 2: Effect of aqueous extract of *Allium sativum* on red blood cell count and its differentials

Parameter	Group A	Group B	Group C	Group D
RBC($\times10^6$\µl)	5.99±0.50	6.05±0.90	6.32±0.40[a]	6.94±0.50[a]
Hb (g/dl)	12.40±1.00	12.26±2.00	13.84±0.60[a]	13.76±0.90[a]
MCV(fl)	52.22±5.00	51.90±1.00	57.78±2.00[a]	55.84±5.00[a]
MCH (pg)	27.58±6.00	20.06±0.60[a]	22.06±1.00[a]	19.90±0.80[a]
MCHC(g/dl)	37.54±0.60	38.70±0.90	38.14±2.00	36.46±2.00

Data is expressed as Mean ±SEM [a]$p<0.05$ is considered significant

Discussion

Administration of *Allium sativum* extract at doses of 150, 300 and 600 mg/kg body weight led to significant ($p<0.05$) increase in the level of RBC and its related indices like Hb, MCV and MCHC especially at dose of 300 and 600 mg/kg body weight when compared to control group. This gives an indication that the plant extract may contain some phytochemicals that can induce erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006). The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCV and MCHC especially at doses of 300 and 600 mg/kg body weight for MCV and MCHC respectively reported by (Abu-Zaiton, 2010). There was decrease in the level of MCH for all the doses tested. Haematological parameters like MCV and MCHC mathematically define the concentration of haemoglobin and its increase suggested the improvement of oxygen carrying capacity of the blood. Therefore the extract did not alter the morphology and osmotic fragility of RBCs. A decrease in the level of MCH as revealed from the study may imply that cells do not have enough hemoglobin and is suggestive of deficiency in haemoglobin production due to sequestration of iron by some photochemicals which might be present in the extract.

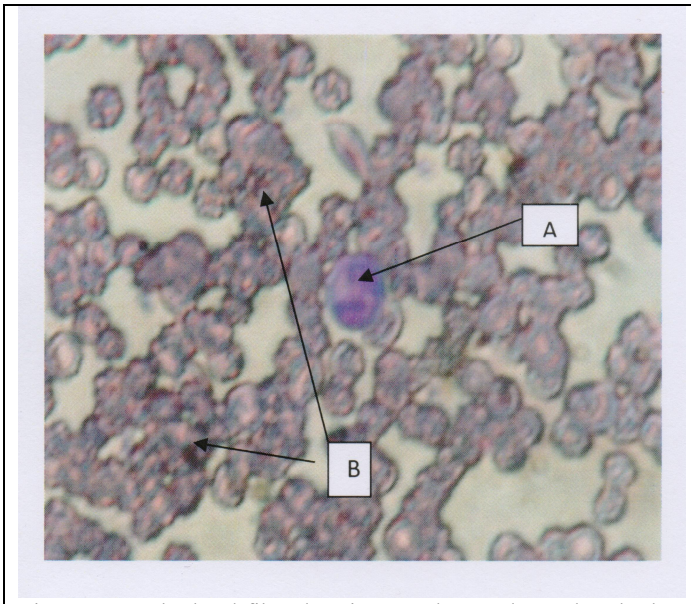

Fig 1: Control Blood film showing a polymorphonuclear leukocyte (A) with sheets of red blood cells (B) per high power field (Giemsa x 400)

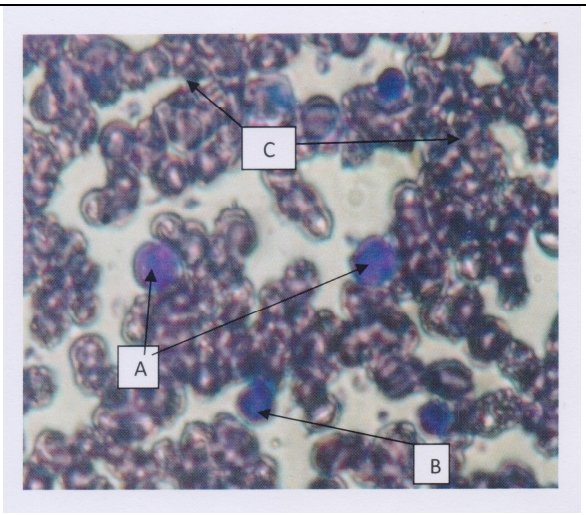

Fig 2: Blood film of rat given moderate dose garlic extract showing polymorphonuclear leukocyte (A) lymphocytes (B) amidst sheets of erythrocytes (C) per high power field (Giemsa x 400)

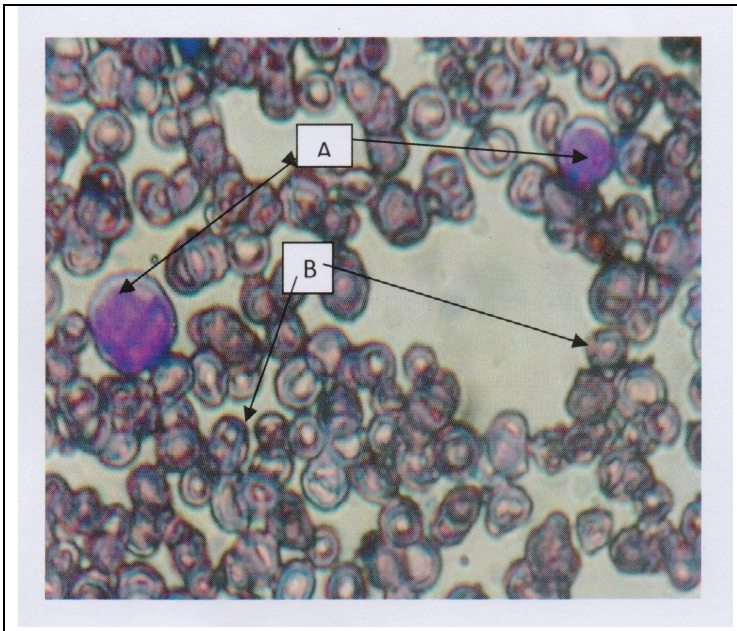

Fig 3: Blood film of rat given high dose extract showing two lymphocytes (A) amidst sheets of erythrocytes (B) per high power field (Giemsa x 400)

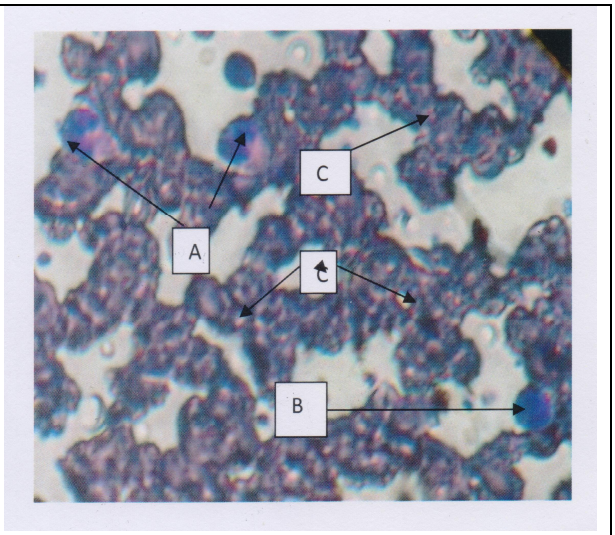

Fig 4: Blood film of rat given low dose Garlic extract showing two polymorphonuclear leukocytes (A), a lymphocyte (B) amidst sheets of erythrocytes (B) per high power field (Giemsa x 400)

In most clinical situations, when a total WBC count is requested, it is usually performed with its differentials. The agranulocytes are free of visible grains under the microscope and include lymphocytes and monocytes. In this study, WBC count and its differential agranulocytes (lymphocytes and monocytes) in the test groups were observed

to be significantly ($p<0.05$) increased when compared to the control. The extract may have induced leucopoiesis, lymphocytosis, and monocytosis respectively (Afolayan *et al.,* 2009). The lymphocytes were the most proliferated cells of all the white blood cell population counted. The increased total WBCs, lymphocytes and monocytes (Agranulocytes) counts may account for the boosting effects of *Allium sativum* on the immune system and phagocytic activity of the blood cells of the animals. These findings are consistent with previous works (Afolayan *et al.,* 2009). The study also revealed decrease in granulocytes in the test groups treated with *Allium sativum* extract at dose 300mg/kg bwt when compared to the control. Platelets is a meshwork of fibrin fibre which adhere to any vascular opening and thus mediate blood clotting. It plays a crucial role in reducing blood loss and repairing of vascular injury (Oyedemi *et al.,* 2010). In this study, there was significant increase ($p<0.05$) in the level of platelet in the test group treated with *Allium sativum* extract at the dose of 300 mg/kg .This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors (Adebayo *et al.,* 2005) due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during haemorrhage (Dahlback, 2008). Continued administration of the extract at high doses did not show anticoagulation benefit.

The leucopoiesis, lymphocytosis, monocytosis and erythropoiesis effect of the *Allium sativum is* further supported by microscopic findings as revealed from the blood film, at low dose, it showed an increase to two polymorphonuclear leukocytes, and production of one lymphocyte amidst sheets of erythrocytes per high power field. At moderate dose, there were three polymorphonuclear leukocytes and two lymphocytes amidst sheets of erythrocytes per high power field. At high dose, changes were the same with that observed in the moderate dose.

In conclusion, our investigation shows that aqueous extract of *Allium sativum* enhanced the immune cells by activating the production of leukocytes, lymphocytes and increased hemoglobin concentration when orally administered as shown in the values of haematological parameters and blood film.

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