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Effects of Water and Ethanol Extracts of *Allium Sativum* on Selected Haematological and Biochemical Parameters in Wister Rats

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Abstract

Allium sativum (garlic) is known to have anti-hypertensive, anti-rheumatic, etc, properties and therefore used in the treatment or alleviation of various ailments, such as asthma, diabetes, paralysis, forgetfulness, tumor, colichy pain and chronic fever. In this study, water and ethanol extracts of its cloves were tested for their effects on selected haematological and biochemical effects on Wister albino rats. Each of the extract was administered at 100 and 200 mg/kg body weight (mg/kg bw) to the appropriate groups. The water extract at 100 mg/kg bw significantly ($P < 0.05$) elevated the levels of RBC, WBC and lymphocytes but not PVC and Hb, all relative to control (Group 1, GP 1). At 200 mg/kg bw but however, the same extract caused non-significant ($P > 0.05$) elevations of all the parameters, except WBC which was lowered but not significantly ($P > 0.05$). Relative to GP 1, the ethanol extract at 100 mg/kg bw significantly ($P < 0.05$) elevated the levels of most haematological parameters studied. At 200 mg/kg bw it significantly ($P < 0.05$) elevated the RBC and lymphocyte level while the other parameters displayed irregular changes relative to other groups. The water extract at 100 mg/kg bw significantly ($P < 0.05$) elevated the serum levels of alkaline phosphatase and total bilirubin but not acid phosphatase, which was insignificantly ($P > 0.05$) lowered. At 200 mg/kg bw, alkaline phosphatase displayed significant ($P < 0.05$) elevations relative to the GP 1, while acid phosphatase was lowered insignificantly ($P > 0.05$). The ethanol extract significantly ($P < 0.05$) elevated the serum levels of alkaline phosphatase and total bilirubin at 100 mg/kg bw and lowered acid phosphatase. The levels of alkaline phosphatase and total bilirubin were significantly elevated at 200 mg/kg bw when compared with GP 1 but lowered acid phosphatase significantly ($P < 0.05$). Both the water and ethanol extracts of *A. sativum* at 100 mg/kg bw had the most profound effects on all the parameters studied but these was a glaring dose-dependent inconsistency in the effects observed.

Key words: *Allium, sativum,* Haematology, Biochemical

Introduction

Allium sativum, commonly known as garlic, is a species in the onion family Liliaceae and genus *Allium* (Krishnaraju *et al*., 2006). It is cultivated in some parts of Nigeria and used as meat tenderizer and spice in many delicacies (Morakinyo *et al.*, 2008). This plant has many local Nigerian names: "*ayo"* in Igbo, "*ayuu"* in Yoruba and "*tafarnuwa"* in Hausa (Gill, 1992). Its close

relatives include the onion, shallot, leek and chive. *A. sativum* has been used throughout history for both culinary and medicinal purposes. It is used as a spice and medicinal herb, with most recent research using it in raw, boiled, cooked and dried form as various therapeutic agents (Gorinstein *et al*., 2006). Commercially available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for therapeutic purposes, including lowering blood pressure and improving lipid profile (Elkayam *et al*., 2003).

With the exception of the single-clove species, the bulb is the most frequently used part of the plant (Gorinstein *et al.*, 2006). The bulb is divided into numerous fleshy sections called cloves. The cloves are used for cloning, consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavour that mellows and sweetens considerably with cooking.

The composition of the bulb is water (84.09%), organic matter (13.38%), inorganic matter (1.53%), while approximate composition of the leaves are water (84.14%), Organic matter (11.27%) and inorganic matter (1.59%) (Macpherson *et al.*, 2006).

When is *A. sativum* crushed, it yields allicin, a powerful antibiotic, antifungal and anthelminthic compound. It has been reported to be a home remedy that aids recovery from sore throat or other minor ailments because of its antibiotic properties. It also contains the sulfur-containing compounds: alliin, ajoene, diallylsulfide, dithiin, S-allycysteine, as well as vitamin B, proteins, minerals, saponins and flavonoids (Yamasaki and Milner, 1991). It also contains phytoalexin called allixin, which has an anti-tumor effect *in vivo*, especially inhabiting skin tumor formation initiated in mice (Yamasaki and Milner, 1991). Garlic and its extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (Koscienlny *et al.,* 1992; Rahman, 2001). Garlic modulates lipid metabolism (Rassoul *et al.,* 1999; Richter *et al.,* 1992; Gebhardt, 1993; Yeh and Yeh, 1994). Several studies have also shown that garlic contains active hypocholesterolemic and hypoglycemic components known as diallyl disulfide and dipropyl disulfide respectively (Bordia and Bansel, 1973; Bordia *et al.*, 1975; Jain, 1977).

This study is intended to evaluate the effect of ethanol and water extracts of *A. sativum* on selected haematological and biochemical parameters, using animal model.

Materials and Methods

A total of thirty (30) male Wister rats weighing between 125 and 200 g were obtained from Animal Genetics and Breeding Laboratory, Madonna University, Elele, Nigeria. These rats were treated in accordance with the internationally accepted principles for laboratory animal use and care (Nahed *et al.*, 2009). They were also allowed free access to water and standard rodent food pellets and housed in well-aerated cages in the

laboratory at a mean ambient conditions of temperature (23 ± 1 °C) humidity (55 ± 5 %) and 12 h/12 h light/dark cycles for 14 days to acclimatize.

Allium sativum cloves were sorted, peeled and washed with distilled water and there after shade-dried. They were subsequently reduced to a fine powder by grinding and then passed through mesh sieves. However, 500g of *A. sativum* powder was macerated in water for 24 hours and ethanol 70 % for 72 h in a container, the extract was decanted and then filtered through Whatman No. 1 filter paper to obtain a clear extract as described by (Carlos, 2015). The ethanol extraction was further concentrated at 50 ^{0}C using a rotary evaporator to obtain the crude extract which was stored in a refrigerator maintained at 4 ^{0}C until used. Extracts were later reconstituted in distilled water to give the required doses of 100 and 200 mg/kg body weight used in this study.

The Wister rats were placed in 5 groups of 6 each. The first group that received water and rodent feed pellets ad–lib was the control, while the second and third groups were each given 100 and 200 mg/kg body weight of the *A. sativum* water extract respectively. The fourth and fifth group were given 100 and 200 mg/ kg of *A. sativum* ethanol extract respectively.

Administration of the extracts was performed once every two days for a period of 20 days by gastric intubation using sterilized syringe and needles. At the end of the 20 days of treatment, each Wister rat in every group was bled through the orbital sinus into heparinised bottles for haematological studies and another set of blood samples also collected in clean non-heparinised bottles which were allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for the determination of alkaline phosphatase, acid phosphatase and bilirubin. Packed cell volume (PCV) and haemoglobin concentration were determined by the microhematocrit and cyanmethaemoglobin methods, respectively while erythrocyte count was determined by the haematocytometry (Jain, 1986). Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from giemsa stained slides viewed from each of the 30 fields of oil immersion objective of a microscope (Coles, 1989). Lymphocytes count was determined by using an automatic blood analyzer (Swelab Alfa, Biozen, Sweden)

Statistical analysis

Statistical analysis was carried out using SPSS version 21 and values obtained were expressed as mean ± S.D. Level of statistical significance across various groups for each of the parameters measured was determine using one-way analysis of variance (ANOVA) and probability less than 5 % was considered statistically significant.

RESULTS

Table 1: Effect of *Allium sativum* water and ethanol extracts on selected haematological parameters

Group	Treatment	Dose (mg/kg)	RBC ($x10^6$/mm	WBC($x10^6$/mm)	Lymphocyte (%)	PCV (%)	Hb(g/L)
Group 1	Dis. H_2O	0	3.01±1.00	4.43±0.86	69.3±1.5	41.60±3.00	5.8±0.11
Group 2	H_2O. Extract	100	4.04±2.08*	4.30±0.49[a]	59.8±1.36*[a]	41.93±9.17	6.0±0.09
Group 3	H_2O. Extract	200	5.27±2.31*	3.20±0.92	65.3±0.32	42.00±3.00	6.4±0.13
Group 4	Et. Extract	100	7.09±1.73*	4.40±1.00[b]	69.8±1.6*[b]	43.67±3.51*	7.8±0.11*[b]
Group 5	Et. Extract	200	6.75±2.89*	3.30±0.74	49.2±0.7*	42.03±4.27	5.8±0.10

Tabulated values are mean ± S.D of six determinations
Key: Et. = ethanol,* *(P<0.05), significant when compared with to control (Group);* [a] *(P<0.05), significant when compared to group 3;* [b] *(P<0.05), significant when compared to group 5*

In Table 1, irregular dose response effects are observable. There was a general inverse relationship between the effects of the water and ethanol extracts on the haematological parameters studied. *A. sativum* water extract administered at 100 mg/kg bw caused a significant ($p<0.05$) elevation in the RBC, WBC and lymphocytes when compared to the control, but treatment with 200 mg/kg bw brought about down regulations in the WBC and lymphocyte levels. The lowering of the levels of two parameters with increase in dosage is suggestive of immune compromised since they are cells of combat immune response. The effect of the ethanol extract displayed a regular pattern of dose-dependent down-regulation of all the afore-listed parameters in addition to Hb i.e. at a dose of 200 mg/kg bw, they all had lower values than those caused by the extracts at 100 mg/kg bw. However, relative to the observed values in rates fed adlib. with only water and pellets, there were significant ($P < 0.05$) increase.

Table 2: Effect of *Allium sativum* water and ethanol extracts on selected biochemical parameters

Group	Treatment	Dose (mg/kg)	Alkaline Phosphatase (mg/kg)	Acid Phosphatase (mg/kg	Total Bilirubin (mg/kg)
Group 1	Dis. H_2O	0	64.33±1.50	2.50±0.12	1.40.3±1.5
Group 2	H_2O. Extract	100	69.27±1.40*[a]	2.28±0.18	2.72±0.58*
Group 3	H_2O. Extract	200	66.73±2.47*	2.20±0.17	1.48±0.43
Group 4	Et. Extract	100	85.47±1.56*[b]	1.51±0.12*	2.93±0.25*
Group 5	Et. Extract	200	66.7±1.13*	1.27±0.25*	4.03±0.27*[a]

Tabulated values are mean ± S.D of six determinations
Key: Et. = ethanol, ** (P < 0.05), significant when compared with to control (Group); [a] (P < 0.05), significant when compared to group 3; [b] (P < 0.05), significant when compared to group 5*

As shown on Table 2, significant ($P < 0.05$) elevation was caused by the water extract in the respective serum activity and level of alkaline phosphatase and bilirubin but the converse was the case for acid phosphatase (Group 2 and 3) when compared with GP1. The water extract at 200 mg/kg bw caused a significant ($P < 0.05$) elevation of alkaline phosphatase and slight elevation of bilirubin level but decreased the serum level of acid phosphatase and bilirubin when compared with GP 1. The ethanol extract caused significant ($P < 0.05$) decrease in the serum level of acid phosphatases, while that of alkaline phosphatase and total bilirubin was significantly ($P < 0.05$) elevated.

Discussion

The present study investigated the effect of *A. sativum* water and ethanol extract on selected on haematological and biochemical parameters. No mortality was recorded in any of the groups irrespective of the administered doses of *A. sativum* water and ethanol extracts.General assessment revealed no negative behavioural changes.

Elevations in RBC, PCV and Hb with treatment of *A. sativum* extracts were observable when compared with control andcould be interpreted as increase synthesis of erythropoietin by the kidney with subsequent enhancement of synthesis in the bone marrow by mobilizing the appropriate precursor. Bendich (1993) and Oluwole (2001) in a similar study, discovered that Wister rats administered with garlic extract have preponderance of RBC. Increase in RBC, Hb and PCV implies that there was a change in the oxygen carrying capacity of the blood by the *A. sativum* extract and in the transferring of respiratory gases (Degruchy, 1976). Nwinuka and Monanu (2008) stated that elevated values of RBC are suggestive of polycythaemia. Increased RBC, PCV and Hb observed in the present study suggest that *A. sativum* extracts may be pursued for their clinical relevance in the management of anaemia disorders.

Lowering of WBC and lymphocyte - immune combat cells against infectious organisms, in the *A. sativum* extracts treated groups when compared to the control is an indicative of compromised immune machinery. Sumiyoshi (1997) and Idown *et al.* (2009) in a similar research had contrary reports.

This study also showed that the used dosages of ethanol and water extracts of *A. sativum* brought about an increase in alkaline phosphatase which can be an indication of disproportionate intracellular fat deposit which is released into the bloodstream. Nagmoti *et al.* (2010) also reported that increased alkaline phosphatase level indicates considerable hepatocellular damage. Elevated values of bilirubin in *A. sativum* treated groups in the present

study are indicative of anti-inflammatory effects of the extracts. Frei *et al.* (1988) discovered that increased bilirubin levels had anti-inflammatory effects as well as acts as scavengers of reactive oxygen species. Bilirubin is the end product of haeme catabolism and has been reported to have strong cytoprotective effects (Hansen, 2002).

In conclusion the results of the present study suggest that *Allium sativum* extracts may have beneficial effects as it demonstrated high tendency to elevated red blood cell level but administered dosage should be properly monitored as there were indications of low WBC with increase in doses.

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