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RESEARCH ARTICLE

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Immunomodulatory activity of Garlic (*Allium sativum*) in Wistar Rats

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ABSTRACT

The consumption of Garlic as spice for food and medicinal purposes is very common among Northern Nigerians. It is believed that Garlic helps fight infections thereby keeping the body healthy. This study was designed to investigate the effect of aqueous extract of Garlic (Alllium sativum) on in vivo leukocytes mobilization in Albino strain Wistar rats. Twenty (20) Albino Wistar rats, divided into four groups of five animals each were used for this study. The group 1 (control group) received 1ml of distilled water, groups 2 and 3 received graded doses of 50 and 100 mg/kg aqueous extract of Garlic, respectively, while the fourth group received Diclofenac (50mg/kg). After one hour, the animals all received 1ml of 3% (w/v) agar suspension in normal saline intra-peritoneally. The animals were killed after four hours (4hrs). The total and differential leukocytes counts were then carried out on the peritoneal fluid obtained from them. The total leukocytes count in the experimental groups were found to be higher than the control group, but the increase was statistically insignificant ($p>0.05$). The differential leukocyte counts in the control groups also showed a non significant increase in lymphocyte and neutrophils counts when compared with the control groups. The findings in this study showed that aqueous extract of Garlic has no significant effect on leukocytes mobilization in albino strain Wistar rats.

INTRODUCTION

Leukocytes are the principal components of the immune system that function mainly by destroying foreign pathogenic substances, such as bacteria. The real value of leukocytes is that most of them are specifically transported to areas of infection and inflammation thereby providing a rapid and potent defense against infectious agents [1]. Inflammation (which may be internal or external) provides a multifaceted defense against tissue damage/invasion by pathogens. Blood cells participating in the inflammatory response release a variety of inflammatory mediators such as neutrophils) that perpetuate the response [2]. The inflammatory response is initiated by circulating proteins and blood cells when they contact invaders in a tissue. A key event in inflammatory response is the localized recruitment of various leukocytes subsets [3]. Garlic has been known for ages to be a very effective medicinal plant which was used for treatments of various disease conditions including leprosy, psoriasis, digestive disorders, infectious diseases, cough, cardiovascular diseases and a host of others. Garlic has been employed as an anti-viral, anti-bacterial and immunostimulant. However, too much of it may sometimes induce migraines [4]. Health promoting properties of garlic includes anti-thrombotic, lipid lowering [5] anti-tumoring and antioxidant effects [6]. The anti-inflammatory properties of garlic acts by modulating cytokines [7] resulting in inhibition of tumor necrosis factor in the surrounding tissues. Also, study has shown that supplementation of diet with aged garlic

extract may enhance immune cell function [8]. The present study was designed to evaluate the immunomodulatory activity of aqueous extract of garlic *(Allium sativum)* in Wistar rats.

MATERIALS AND METHODS

Materials

Agar, Normal saline (0.9% NaCl), Leishman's stain, Distilled water (PH 6.8), Turks solution (White blood cell fluid), Phosphate buffered saline (PH 7.4, containing 8g/L NaCl; 0.2g/L KCl; 1.44g/L $NaHPO_4.2H_2O$; 0.24g/L KH_2PO_4), EDTA Powder, Pasteur pipettes, White blood cell counting chamber (Improved Neubauer chamber), Glass slides/Cover slips, Light microscope, Immersion oil, Syringes, Dissecting kit.

Animals

20 adult Wistar strain albino rats (120-250g) of both sexes were purchased from the Animal house, Department of Human physiology, Faculty of Medicine, Ahmadu Bello University, Zaria for the study. The animals were housed in a wired mesh cages with saw dust as beddings. They were fed with Vital feed (growers) pellet form and had unrestricted access to clean drinking water.

Preparation of plant extract

Fresh cloves of the plant (garlic) were purchased from Samaru local market, Zaria, Nigeria. The cloves were identified and authenticated at the herbarium of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria, and the voucher (324) was deposited. Plant extraction was done in the Department of Pharmacognosy and Drug Administration, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The fresh garlic cloves were crushed using a pestle and a mortar after peeling the garlic. The paste was then soaked in a conical flask containing about 25ml of distilled water for 24hrs. After this period, the mixture was filtered and the filtrate was then poured into an evaporating dish for concentration in a water bath at a temperature of 45°C.

Experimental design

Twenty (20) adult wistar strain albino rats were divided into four groups of five animals in each group. Group 1 received 1ml of distilled water; Groups 2 and 3 received 50 and 100 mg/kg body weight of aqueous extract of *Allium sativum* respectively, while, Group 4 received 50 mg/kg body weight of Diclofenac potassium. All administrations were through oral route. One hour after this administration, all the groups (Group 1-4) were administered with 1ml of 3 % (w/v) agar suspension in normal saline intraperitoneally. After four hours (4hrs), the animals were killed and the peritoneal fluid collected.

Collection of peritoneal fluid

Each animal was anaesthetized using chloroform. The animal was then placed on the dissecting board (in a supine position) with its paws held down to the board with pins and the abdomen cut open using a pair of scissors. The abdominal cavity was washed with 5ml of 5% solution of EDTA in phosphate buffered saline. An appreciable volume of the peritoneal fluid was withdrawn using a syringe, after which the total and differential leukocytes counts of the peritoneal fluid were carried out as described by Dacie and Lewis [9].

Statistical analysis

Values obtained were expressed as mean ±SEM using Statistical Package for Social Scientists (SPSS). Level of Statistical significance was determined by one way analysis of variance (ANOVA) using Post Hoc test, with values $p<0.05$ considered significant.

RESULTS

Table 1: Effect of Aqueous Extract of Garlic *(Alllium sativum)* on *In vivo* Leukocytes Mobilization in Wistar Rats

Dose	Total Leucocytes Count ($\times 10^9$/L)	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocytes (%)	Basophils (%)
Control	9.18 ± 3.23[a]	12.8 ± 3.52 [a]	86.6 ± 3.36 [a]	0.20 ± 0.20 [a]	0.20 ± 0.20	0.20 ± 0.44[a]
Garlic 50mg/kg b w	16.02 ± 6.06[b]	13.2 ± 3.78 [a]	86.8 ± 3.68 [a]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Garlic 100mg/kg b w	19.20 ± 3.60[b]	10.8 ± 3.28 [a]	88.4 ± 4.03 [a]	0.40 ± 0.40 [a]	0.40 ± 0.40	0.60 ± 0.89[b]
Diclofenac 50mg/kg b w	16.86 ± 8.77[b]	13.20±1.46[b]	96.4 ± 1.63 [a]	0.20 ± 0.20 [a]	0.00 ± 0.00	0.20 ± 0.47[a]

Values with different superscript letters (a, b) are significantly ($P < 0.05$) different when compared to control group (Normal + Distilled water)

RESULTS AND DISCUSSION

Table 1 shows the mean values of control and experimental groups administered with graded doses of garlic extract. The results obtained showed that there was a significantly ($P < 0.05$) increased total leukocytes count in the groups that received all doses of garlic extract when compared to control group. The differential leukocyte counts showed no statistical significant difference ($P > 0.05$) in the groups that received graded doses of aqueous garlic extract, when compared to control group. This study disagree with the report of Oluwole [10] and Sumiyoshi [11] that showed that aqueous Garlic extract significantly increased total leukocyte count, neutrophil and lymphocyte counts. However, the basophil, eosinophil and monocyte counts showed effects similar to that reported by Iranloye [12] as no significant effect was observed in the present study.

CONCLUSION

In conclusion, the present study showed that the various doses of aqueous garlic extract produced a significant increase in the total leukocytes count, with no significant effect observed in the differential leukocyte counts when compared to control group.

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