GLOBAL JOURNAL OF ENVIRONMENTAL SCIENCES VOL. 2 NO. 1 2003:11-16
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TOXICOLOGICAL EFFECTS OF GARLIC *(ALLIUM SATIVUM)* ON SOME HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RAT

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(Received 9 April 2002; Revision accepted 28 August 2002).

ABSTRACT

The toxicological effect of garlic (*Allium sativum*) on some haematological and biochemical parameters in albino rats was studied. High doses of garlic infusion were found to cause a significant decrease ($p<0.05$) in the concentration of haemoglobin, packed cell volume, lymphocyte count, aspartate aminotransferase and alanine aminotransferase. The total white blood cell count and differential leucocyte counts (especially neutrophils and eosinophils) showed a significant increase ($p< 0.05$) consistent with high doses of garlic infusion. Red cell morphology was also appreciably affected.

Keywords: Haemoglobin (Hb), packed cell volume (PCV), *lymphocyte, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).*

INTRODUCTION

Since the origin of man on planet earth edible plants have been known to be of rich medicinal importance to man. For hundreds of years, there have been certain myths associated with garlic *(Allium sativum)* (Schauenberg and Ferdinand, 1977). Medicinally, garlic has been shown to have a wide range of actions. It is an all purpose herb that has been used in the treatment of hypertension, stroke, Diabetes mellitus, worm infection, cough, wounds, asthma, digestive disorders, epilepsy and lots more (Kafaru, 1994, The Guardian, 1977, Soffarand and Mokhar, 1991, Perez *et al*, 1994.). Presently, there is paucity of scientific information on the toxicological effects of garlic despite its enormous and indiscriminate use. This study was thus designed to investigate the effects of garlic infusion on some haematological and biochemical parameters in Swiss albino rats.

MATERIALS AND METHODS

Male Swiss albino rats with an average weight of 88-190g were housed in large cages with free access to food and water. The rats were randomly divided into five cages of four (4) rats per cage and allowed to acclimatize for 4 days. One group served as control while the other groups were labeled 1-4. Garlic bulbs were purchased from the Mile 1 market at Diobu in Port Harcourt, Nigeria. 40g of peeled fresh garlic was homogenized in a porcelain mortar. 100ml of boiling water was added and the mixture was allowed to stand for two hours or until cold. The infusion was filtered through a muslin cloth and the extract collected into a stoppered glass jar. The extract was later sterilized using a Portable Electrocaloric type autoclave at 121 °C for

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15min. The extract was used fresh and any left over discarded. Fresh preparations were made on every injection day.

Administration of Garlic Infusion.

The garlic infusion was administered intraperitoneally to the rats. On days 1 and 2 of the treatment, the rats received 1ml (0.4g), 2ml (0.8g), 3ml (1.2g) and 4ml (1.6g) each per groups 1, 2, 3, and 4. respectively. Control animals received 1ml of saline injections. After day 1, the treatment was withheld for the animals in-group 4 because of their weakened and sedative states. By day 3, because of the weakness of most of the animals in all the groups, the doses were halved and given 48 hourly for the rest of the treatment period. The entire treatment period lasted for 12 days. On the completion of garlic administration, there was a two-day drug free period.

Sample Collection

On every day of the injection, the rats were weighed per cage. Blood samples were

TABLE 1: Average weight of rats administered garlic infusion

Treatment Period (days)	Average weight in grams (test animals)				
	Control	1	2	3	4
0	88	124	142	152	172
1	97	120	139	152	170
2	103	114	145	140	159
4	111	116	153	140	157
6	116	124	154	137	155
8	128	141	160	134	150
10	131	142	160	129	147
12	134	143	161	123	143

TABLE 2 : Signs and symptoms following garlic treatment

Signs/symptoms	Groups showing signs /symptoms	Approximate time of appearance of sign and symptoms after garlic injection
Spontaneous defecation/ micturition	All	</min
Lacrimation	All	5-10mins
Sedation	4	30-45mins
	3	2hours
	2	2hours
	1	3hours
Respiratory distress	3,4	6 days
Coughing	3.4	6days
Severe hair and epidermal loss	4	8 days

collected from the animals for analysis on the 14th day of the treatment period. The rats were immobilized by gripping the skin and muscles around the cervical region while gently pulling the tail in the opposite direction. They were bled by cardiac puncture using 23G needles and 2ml syringes and the blood for haematological parameters was placed in K_2 EDTA bottles and properly mixed while that for enzyme studies was allowed to clot in the syringe. The serum was separated from the cells using the MSE centrifuge at 5,000 rpm for 5min. The samples were analyzed within one hour of separation.

Determination of Haematological Parameters

The haemoglobin (Hb) content of the samples was determined using the cyanmethaemoglobin method of Baker and Silverton (1985), while the packed cell volume (PCV) was determined by the microhaematocrit method. Total white blood cell (WBC) count which involved the use of Turk's solution and white cell differential count were done after preparation of thin blood films stained with freshly prepared leishmann stain. Observation was by the battlement method of Baker and Silverton (1985), using the oil immersion objective (100 x) on an HM-LUX Leitz Wetzlar binocular microscope.

Determination of biochemical parameters.

Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the method of Reitman and Frankel (1957).

Statistical analyses of mean, standard deviation, the student t-test were used for analysis of the results.

RESULTS

Chronic administration of garlic infusion at high doses resulted in general loss of weight in animals that received highest doses of the infusion. However, the animals that received lower doses (groups 1 and 2) gained weight. Control animals showed consistent weight gain (Table 1). During injection of the garlic infusion, the rats exhibited various signs and symptoms (Table 2). There was severe hair loss, which was accompanied later by loss of epidermis (skin) at the ventral side and pelvic region. The hind limbs became numb (paralyzed) and lower abdomen was slightly distended. The respiratory distress symptoms manifested in the form of frequent yawning and hiccups. The symptoms became severe at night resulting in coughing with wheezing sound accompanied with gasping for air. These events were more pronounced in animals in-groups 3 and 4 which also developed loss of appetite and thus could not eat properly.

The haemoglobin level, packed cell volume, lymphocyte count, AST and ALT levels showed appreciable decrease as the doses of garlic infusion administered increased. The haemoglobin concentration and the packed cell volume for groups 3 and 4 were significantly different from those of control at $p<0.05$. While the neutrophil count increased with increase in concentration of garlic infusion, there was a corresponding significant increase ($P<.0.05$) in the lymphocyte count when compared with control (Table 3). The effect of garlic infusion on red blood cell morphology resulted mainly in cell crenation, fragmentation and polychromasia (marked at high doses) (Table 4).

DISCUSSION

The rats that were not treated with the garlic infusion (control) gained weight progressively throughout the duration of the experiment. (Table 1). On the other hand, those administered with aqueous garlic infusion showed decrease in weight. It has been widely reported (Kafaru, 1977) that garlic could cause

TABLE 3:Hematological and biochemical parameters of rats administered garlic infusion

	Group	Hb	WBC	Differential count			Biochemical	
	PCV		(x10 ^{9}/L)	Neutrophil	Eosinophil	Lymphocytes	AST (I.U/L)	ALT
	g/dll	%						(I.U/L)
Control	12.70	39.30	6.15	29.00	0.50	70.50	19	19
1	11.00	33.10	6.73	36.70	-	63.30	13	12
2	10.90	32.70	10.03	41.50	1.30	51.30	15	8
3	8.70	27.00	11.50	54.70	3.30	42.00	10	8
4	7.30	22.00	11.50	56.00	2.00	38.00	6	6

Table 4 : Red cell morphology of garlic treated rats

Group	Nature of erythrocytes
Control	Normal red cells.
1	Crenation, polychromasia, normal red cells
2	Crenation (++), fragmented cells, polychromasia (++),
3	Crenation (++), polychromasia (++), marked fragmented cells, slight dimorphism, marked schistocytosis, ghost cells (+).
4	Marked crenation, marked fragmented cells (+++)

weight loss in humans especially at very high doses. The present finding demonstrates that weight loss could also occur in lower animals. The present finding also demonstrated a wider extrapolative use of garlic particularly in cases where weight reduction is necessary especially in the obese subjects

Extracts of the leaves of *Euphorbia hirta* have been shown to induce spontaneous micturition and defaecation, pallor and prostration, respiratory distress, sedation, coma and death in mice upon acute toxicity (Johnson *et al,* 1992). Garlic extract in the present study was shown to also cause spontaneous micturition and defaecation, lacrimation, sedation, respiratory distress and cough in rats. The severity of these signs and symptoms, however, relates more to the dose of garlic extract administered.

Saini *et al* (1996) observed a statistically significant decrease in hemoglobin level, packed cell volume and red cell counts in rats given propylene glycol for 2 days, which returned to basal values on the 8th day. Garlic

infusion in the present study also caused a significant decrease in hemoglobin level and packed cell volume ($p<0.05$) at high doses. The red cells also exhibited several morphological alterations such as crenation, fragmentation, polychromasia and schistocytosis whose severity increased with high doses of garlic administered. Cell crenation is often an artifact *in vitro* but it can also occur *in vivo* in a variety of disorders including gross electrolyte imbalance (Hall and Malia, 1984). Garlic administration at high doses might have increased the tonicity of the extra cellular fluid, which caused cell crenation. As crenation increased it might have gotten to the extent where the tension in the red cell membrane caused the membrane to rupture resulting in fragmented cells and cell membrane loss and possibly loss of the cell constituents. The resultant decrease in hemoglobin level and packed cell volume might have arisen from these factors. These conditions could cause anemia resulting in body weakness of the rats. As the circulating red cells are continually being reduced by the influence of garlic extract the bone marrow must have been stimulated to produce more red cells to meet the needs of the peripheral blood. Consequently, immature red cells (reticulocytes) were pumped into the circulation as revealed by the presence of polychromatic cells, which increased in number with increase in garlic dosage. The dimorphism observed in the study might have also resulted from the presence of immature reticulocytes and initial population of mature red cells. The control rats showed higher lymphocyte count than neutrophil but this trend was reversed upon the administration of garlic. It is important to recognize that elevated neutrophil count due to mobilization of the marginal neutophil pool can readily be produced by physiogical factors such as stress,. emotion, physical activity and are due to vasodilatation and increased blood flow through the capillaries which also occur in the presence of fever from whatever source (Hall and Malia, 1984). This phenomenon might explain the increase in the total white blood cell and neutrophil counts recorded in this study.

Administration of gossypol reduced the levels of AST and ALT in the peripheral blood of protein-malnourished and normal protein fed rats (Nwoha, 1995)., In this study administration of garlic to normal protein fed rats also reduced the levels of AST and ALT in the peripheral blood. These plasma enzymes are present in high concentrations in the heart, liver, skeletal muscle, kidney and erythrocytes and damage to any of these tissues may increase plasma levels of these enzymes (Zilva *et al*, 1988). The result of this study indicate that garlic even at high concentration (doses) in rats does not seem to cause release of these enzymes from tissues, thus, extrapolatirely, it may explain the claim that garlic has palliative function in diseases that affect these organs. The authors are of the opinion that the full potential of garlic in the prevention and treatment of diseases and its inherent effect are not fully known. Thus further investigation of the pharmacological and toxicological application of garlic in pharmacotherapy is worthwhile.

ACKNOWLEDGEMENTS

This research was performed at the University of Port Harcourt Teaching Hospital and the Diagnostic Research Laboratory, Rivers State University of Science and Technology, Port Harcourt, Nigeria.

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