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GARLIC (*ALLIUM SATIVUM*) EXTRACT AFFECTS THE FREE AMINO ACID LEVEL IN THE GONADS OF FEMALE ALBINO RATS

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ABSTRACT
Effect of *Allium sativum* Linn. (Alliaceae) (Raw Garlic Extract (RGE)) was studied on free amino acids (FAA) level in female albino rats. The garlic extract was tested in three different doses 1ml, 2ml and 4ml/ kg body weight daily as low, medium and high dose respectively for a period of 7, 14, 21 and 28 days. The significant ($P<0.01$) increase in Free amino acid (FAA) level was observed when rats were fed with low and medium dose but when rats were fed with 4ml/kg body weight of garlic extract there was a not significant increase in Free Amino Acid (FAA) level was observed.
Key Words: Garlic Extract, Free Amino Acid, Albino Rats, Biochemical Estimation.

INTRODUCTION

Epidemiological studies in the past 10 years have revealed an inverse relationship between *Allium sativum* (garlic) consumption and the incidence of certain diseases, including stomach, colon and laryngeal cancers[1]. The importance of garlic was recognized many centuries ago, in early Egyptian, Chinese and Indian civilizations as a herbal or traditional medicine. Today, in many parts of the world, garlic is being used for prophylaxis and for the treatment of acute and chronic gastritis, dysentery, typhoid fever, cholera, tuberculosis, pneumonia, diabetes mellitus and heart disease[2]. Allicin, diallyldisulfide-oxide, alliin, active ingredients from garlic (alliin) are systemic vasodilator[3]. Ether extracts and partially purified distilled extracts of garlic have been reported to inhibit human platelet aggregation *in-vitro*[4]. Treatment with garlic extracts was found to improve the activation of natural killer cells, the function of T-lymphocytes[5]. In *in-vitro* and *in-vivo* studies, aged garlic extracts stimulated immune functions. Kamin and Handler[6] reported that metabolism of the amino acids follows their accumulation in various tissues. By-products of such metabolism are urea and ammonia which appear in the urine. Bose et al[7] reported that glycine labeled with carbon-14 is incorporated into liver cholesterol and fatty acids by rats. Caspi et al[8] also reported that rats incorporated the S-methyl group of methionine into cholesterol. Medicinal uses of *Allium sativum* (garlic) have existed for centuries, but its therapeutic properties need more investigation. The present study investigates the effect of garlic extract on amino acid level in female albino rats.

MATERIALS AND METHODS

The Extract

Six months old (after harvest) garlic bulbs were collected from the local market. Garlic bulbs were separated, peeled and washed with distilled water. After drying in shed, about 500 gm of clean garlic bulbs were crushed with the help of electronic grinder. The extract was strained through muslin cloth after squeezing the crushed materials[9].

Experimental Animal

Healthy adult female albino rats weighing approximately 150 – 200 gm were selected for the experiment. All animals were acclimatized for a week in the laboratory before use[10]. The animals were housed five per cage under controlled conditions of a 12 h light/dark cycle, 50% of humidity and 26°C ± 2°C, with minimum noise levels[11]. Animals had free access to tap water *ad libitum* and normal diet.

Experimental Design

The animals were divided into four groups. Group A animals, which served as healthy control, were given normal feed and tap water *ad libitum* throughout the experimental tannure. Rats of group B, C and D were fed with 1ml, 2ml and 4ml/ kg body weight garlic extract daily for 7, 14, 21 and 28 days daily. In all the groups, the extract was forced fed by using ball – tipped needle every day between 11.00 a.m. to 12.00 pm[12, 13].

Free Amino Acids Estimation

Estimation of total free amino acids in gonads of Albino rats was made according to the method of Spies[14]. The tissue homogenate (1% w/v) was prepared in 96% ethanol in an electrical homogenizer for 5 minutes and centrifuged at 8000g for 20 minutes. In 0.1 ml of supernatant, 0.1 ml of distilled water and 2.0 ml of ninhydrin reagent was added and mixed thoroughly. Ninhydrin reagent was prepared by mixing one gram of ninhydrin in 250 ml of absolute ethanol and 0.04 gm of stannous chloride in 25.0 ml of citrate buffer (p^H 5.0). The reaction mixture was kept in boiling water bath for 15 minutes. Two ml of 50% ethanol was added to the above after cooling. The violet colour developed which was measured at 575 nm. Glycine was used to determine standard. Free amino acids have been expressed as µg /mg tissue.

Ethical Clearance: Letter No. DDU/N.F.Sc./7607

Statistical Analysis

All the experiments were replicated five times and subjected to statistical analysis by two way analysis of variance (ANOVA), followed by student's t-test, wherever required[15].

RESULTS AND DISCUSSION

There was a significant increase in the mean values of Free Amino Acid level in female albino rats. In group B and C, the level increased significantly (P<0.01) to the extent of 8.84% and 11.44% respectively whereas in group D there was a not significant increase of 2.86% in FAA level was observed (Table 1, Figure 1).

Table 1: Percent change in Free Amino Acid (FAA) level after following the programmed feeding of *Allium sativum* (garlic) extract daily for 7, 14, 21 and 28 days respectively in female albino rats

REGIMENS	TREATMENTS	DAYS			
		07	14	21	28
Free Amino Acid level (µg/mg)	CONTROL (0)	257.704 ± 1.088 (100%)	260.336 ± 1.783 (100%)	264.095 ± 1.880 (100%)	267.854 ± 1.758 (100%)
	1ml/kg (bd.wt)	262.779 ± 1.773^{NS} (1.97%) ↑	269.358 ± 1.349* (3.47%) ↑	280.636 ± 1.561** (6.26%) ↑	291.538 ± 1.665** (8.84%) ↑
	2ml/kg (bd.wt)	268.606 ± 1.498** (4.23%) ↑	275.185 ± 1.743** (5.70%) ↑	287.779 ± 1.468** (8.97%) ↑	298.493 ± 1.748** (11.44%) ↑
	4ml/kg (bd.wt)	260.336 ± 1.030^{NS} (1.02%) ↑	265.599 ± 1.136^{NS} (2.02%) ↑	270.862 ± 1.556^{NS} (2.56%) ↑	275.193 ± 1.486^{NS} (2.86%) ↑

Values are expressed as Mean ± SE of five replicates, Values in parentheses are percent change with control taken as 100 percent.
Data were analyzed through Two Way Analysis of Variance (ANOVA) followed by Student's t-test.
'NS' not significant, '*' significant (P<0.05) and '**' significant (P<0.01), when treated groups were compared with controls.
↓ Decrease in % change and ↑ Increase in % change.

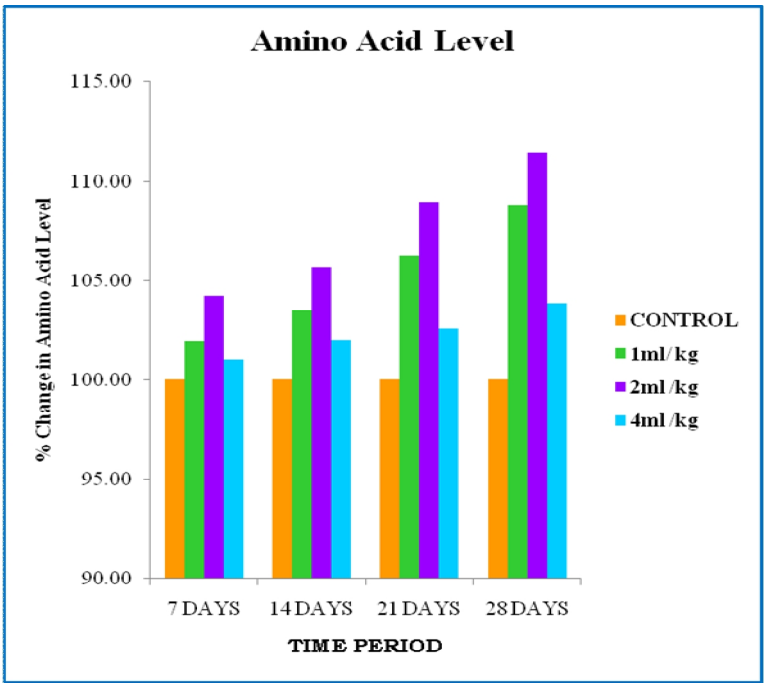

Figure 1: Change in percent level of Free Amino Acid (FAA) in female albino rats after fed with different volumes of raw garlic extract for 7, 14, 21 and 28 days daily.

Amino acids can be metabolized to produce energy. Some amino acids act as neurotransmitters, and some act as starting materials for the biosynthesis of neurotransmitters, hormones, and other important biochemical compounds. Amino acids are the primary building blocks for proteins. This description fits a very large number of compounds, most of which are non-physiological. In addition, many physiologically important amino acids are not used in proteins. However, in biochemistry, the term "amino acid" generally refers to one of the 20 types of monomeric units most commonly used to construct proteins. We have no adequate explanation for the changes in amino acid concentrations. A number of possibilities have been considered to explain the mechanism at work but we have as yet no actual proof to substantiate them. This is especially important during fasting, when the breakdown of muscle protein is a major energy source. The increased Amino Acid level suggests possible tissue damage probably due to increased proteolytic activity under stress. However, the elevated

levels of amino acid may be (a) utilized for energy production by feeding them into the TCA cycle through amino transferase reaction, (b) attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis, (c) due to transamination and animation of keto acids, and (d) attributed to use of amino acids and their involvements in the maintenance of an acid – base balance.

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