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Research Article

Dietary supplementation of garlic (*Allium sativum*) extract alters the glycogen deposition in liver and protein metabolism in gonads of male albino rats

Srivastava S*, Pathak PH

Department of Zoology, D. D. U. Gorakhpur University, Gorakhpur – 273 009, U. P. India

ABSTRACT

The prophylactic efficacy of garlic (*Allium sativum* Linn.) extract was studied on glycogen deposition and protein metabolism in male albino rats. The garlic extract was tested in three different doses 1ml, 2ml and 4ml/ kg body weight daily for a period of 7, 14, 21 and 28 days. The significant ($P<0.01$ & $P<0.05$) increase in Glycogen and Protein 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 significant ($P<0.01$) decrease in Glycogen level and a not significant increase in Protein level was observed.

Keywords: Garlic Extract; Glycogen Deposition; Protein Metabolis; Albino Rats

INTRODUCTION

Epidemiological studies, during the last decade, have revealed an inverse relationship between garlic, (*Allium sativum* Linn.) (Alliaceae), consumption and the incidence of certain forms of diseases, including stomach, colon and laryngeal cancers (Yeh and Lijuan, 2001). The importance of garlic has already been recognized in early Egyptian, Chinese and Indian civilizations, centuries ago as a herbal or traditional medicine. Today, in many parts of the world garlic is being used both as prophylaxis and for the cure of variety of diseases including acute and chronic infections like gastritis, dysentery, typhoid fever, cholera, tuberculosis, pneumonia, diabetes mellitus, heart disease and hypertension (Khataibeh et al., 2006). Previously it was reported that allyl-containing sulfides in garlic increase the uncoupling protein (UCP) content in brown adipose tissue, and noradrenaline and adrenaline secretion in rats (Oi et al., 1999). It also reported that administration of diallyldisulfide, a major volatile sulfur-containing compound in garlic, enhanced triglyceride catabolism and growth of interscapular brown adipose tissue (IBAT)2 by increasing noradrenaline secretion in rats (Oi et al., 1995; 1998). We speculated that garlic may affect whole-body protein metabolism by the stimulation of hormone secretion and that dietary supplementation of garlic may enhance hormone-regulated protein anabolism.

Biochemical investigations of the effect of garlic in rats are limited to lipid metabolic studies only. The purpose of this study was to investigate the effect of dietary supplementation of garlic extract on the level of Glycogen in liver and Protein in gonads of male albino rats.

* Corresponding Author
Email: sashank_sri@rediffmail.com
Contact: +91-9935875372
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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 (Sonepati et al., 2001).

Experimental Animal

Healthy adult male albino rats weighing approximately 150 – 200 gm were selected for the experiment. All animals were acclimatized for a week in the laboratory before use (Parthasarathy and Prasanth, 2009). 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 (Nagaraja et al., 2006). 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 (Thomson et al., 2006; Cikler et al., 2005).

Glycogen Estimation

Glycogen was measured by the Anthrone method of Van der Vies (1954). Glycogen, present in submicroscopic from in tissue, yield an amorphous product on reacting with KOH. This produces a green – brown colour with anthrone reagent. 50 mg tissue of liver was homogenized with 5 ml of 5% TCA and filtered. To one ml of filtrate, one ml of 10N KOH was added and the mixture was boiled for exactly 60 minutes. Excess alkali was neutralized with 0.5 ml glacial acetic acid and distilled water is added to make a final volume of 10 ml. One ml of the above mixture was added to two ml of freshly prepared anthron reagent (2 mg anthrone/ml of 36N sulphuric acid), shaken laterally and heated in a boiling water bath for 10 minutes. The green-brown colour develops which was measured calorimetrically at 650nm against blank, prepared simultaneously by using one ml of 5% TCA instead of one ml of tissue filtrate. The optical density was compared with a set of glucose standard of varying concentration (0.01 mg/ml to 0.04 mg/ml). Results have been expressed as µg/mg tissue.

Protein Estimation

Quantitative assessment of protein was made according to the procedure of Lowry et al., (1951). Animal were dissected, their gonads was removed, weighed and homogenized (1mg/ml, w/v) in 10% TCA using an electrical homogenizer for 5min. The homogenate thus obtained was centrifuged (6000g × 20min) and the precipitate was collected. The precipitate was washed twice with 5.0ml of 5% TCA and centrifuged again at the same speed for another 20 minutes. The precipitate was dissolved in 4.0ml of 1N NaOH. In 1.0 ml of diluted supernatant 5 ml of freshly prepared reagent C was added. The reagent C was prepared by addition of 50 ml of reagent A (2% sodium carbonate in 0.1N NaOH) and 1.0 ml of reagent B (2% sodium potassium tartarate, 1% copper sulphate, mixed in 1:1 ratio at the time of experiment). The reaction mixture was left standing for 10 minutes at room temperature following which 0.5 ml of Folic – ciocatu's reagent diluted 1:2 ratio with distilled water at the time of experiment was added and mixed thoroughly. After 10 minutes, the blue colour develops that was measured at 600 nm. Standard curves were prepared with different concentration of bovine serum albumin. Values have been expressed as µg protein/mg tissue.

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 (Paterson, 1939).

RESULTS

Effect of extract on Glycogen deposition

There was a significant increase/decrease in the mean values of Glycogen level in male albino rats. In group B and C, the level increased significantly ($P<0.01$) to the extent of 5.09% and 7.88% respectively whereas in group D there was a significant decrease ($P<0.01$) of 3.46% in glycogen level was observed (Table 1, Figure 1).

Effect of extract on Protein metabolism

There was a significant increase in the mean values of Protein level in male albino rats. In group B and C, the level increased significantly ($P<0.01$) to the extent of 10.53% and 12.61% respectively, whereas a not significant increase of 3.41% in protein level was observed in group D (Table 1, Figure 1).

DISCUSSION

Carbohydrates fulfill both structural and metabolic roles. Carbohydrates are major constituents of animal food and tissues. The glucose is the most important Carbohydrate in the animal biochemistry because nearly all carbohydrates in food are converted to glucose for further metabolism. Glucose is a major fuel of the tissues of animals. It is converted into other carbohydrates having highly specific function, viz., glycogen for storage, in certain complex lipids and in combinations with proteins in glycoproteins. Glycogen is a storage polymer of glucose in animals. The level of glycogen was significantly higher in the B, C and D groups of rats than in the A group at low and medium dose, but at higher dose its level decreases. It is reported that garlic acts as a hypoglycemic agent (Ahmed and Sharma, 1997; Khataibeh et al., 2006). Therefore, it is assumed that administration of garlic increases response of insulin and also promotes the conversion of the inactive form of glycogen synthetase to the active form and enhances conversion of blood glucose into glycogen. These findings get support from Villar-Palasi and Lamer (1961), Jain et al., (1973), Jain and Vyas (1974; 1975) and seemed to support the theory that garlic acted as a hypoglycemic agent. The hypoglycemic effect might be due to an increase in the insulin response during feeding, probably due to enhanced transport of blood glucose to the peripheral tissues. The abolition of this effect due to ingestion of garlic may inhibit some steps in the formation of glucose or in the deposition of this glucose as liver glycogen (Todd et al., 1964).

On this basis, it is possible that the low levels of liver glycogen initially may also be related to inhibition of a part of the process of deposition. Reduction of hepatic glycogen may be explained as due to pathophysiological changes in liver (increased relative liver weight). The present study is in accordance to Nigam (1987), Igbedioh and Akinyele (1992), who reported reduced hepatic glycogen with pathological changes in the liver of common house sparrow due to furadon SP_{50} exposure.

Table 1: Percent change in Glycogen and Protein level after following the programmed feeding of Allium sativum (garlic) extract daily for 7, 14, 21 and 28 days respectively in male albino rats

REGIMENS	TREATMENTS	DAYS			
		07	14	21	28
Glycogen Level (µg/mg)	CONTROL (0)	568.950 ± 1.152	569.678 ± 1.479	567.676 ± 1.062	568.404 ± 1.208
		(100%)	(100%)	(100%)	(100%)
	1ml/kg (bd.wt)	575.140 ± 1.062^{NS}	584.972 ± 1.268**	593.165 ± 1.362**	588.249 ± 1.451**
		(1.09%) ↑	(2.68%) ↑	(4.49%) ↑	(5.09%) ↑
	2ml/kg (bd.wt)	581.249 ± 1.100**	591.862 ± 1.208**	605.454 ± 1.332**	616.765 ± 1.718**
		(2.16%) ↑	(3.89%) ↑	(6.67%) ↑	(7.88%) ↑
	4ml/kg (bd.wt)	566.765 ± 1.187^{NS}	561.458 ± 1.130*	555.268 ± 1.221**	528.714 ± 1.268**
		(0.38%) ↓	(1.44%) ↓	(2.19%) ↓	(3.46%) ↓
Protein level (µg/mg)	CONTROL (0)	101.894 ± 1.226	105.614 ± 1.242	107.554 ± 1.144	109.010 ± 1.564
		(100%)	(100%)	(100%)	(100%)
	1ml/kg (bd.wt)	105.775 ± 1.314^{NS}	110.789 ± 1.696^{NS}	115.641 ± 1.329*	120.493 ± 1.279**
		(3.81%) ↑	(4.91%) ↑	(7.52%) ↑	(10.53%) ↑
	2ml/kg (bd.wt)	107.878 ± 1.216*	112.568 ± 1.237*	116.935 ± 1.103**	122.758 ± 1.605**
		(5.87%) ↑	(6.58%) ↑	(8.72%) ↑	(12.61%) ↑
	4ml/kg (bd.wt)	102.702 ± 1.279^{NS}	106.907 ± 1.433^{NS}	109.172 ± 1.226^{NS}	112.730 ± 1.368^{NS}
		(0.79%) ↑	(1.22%) ↑	(1.50%) ↑	(3.41%) ↑

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.

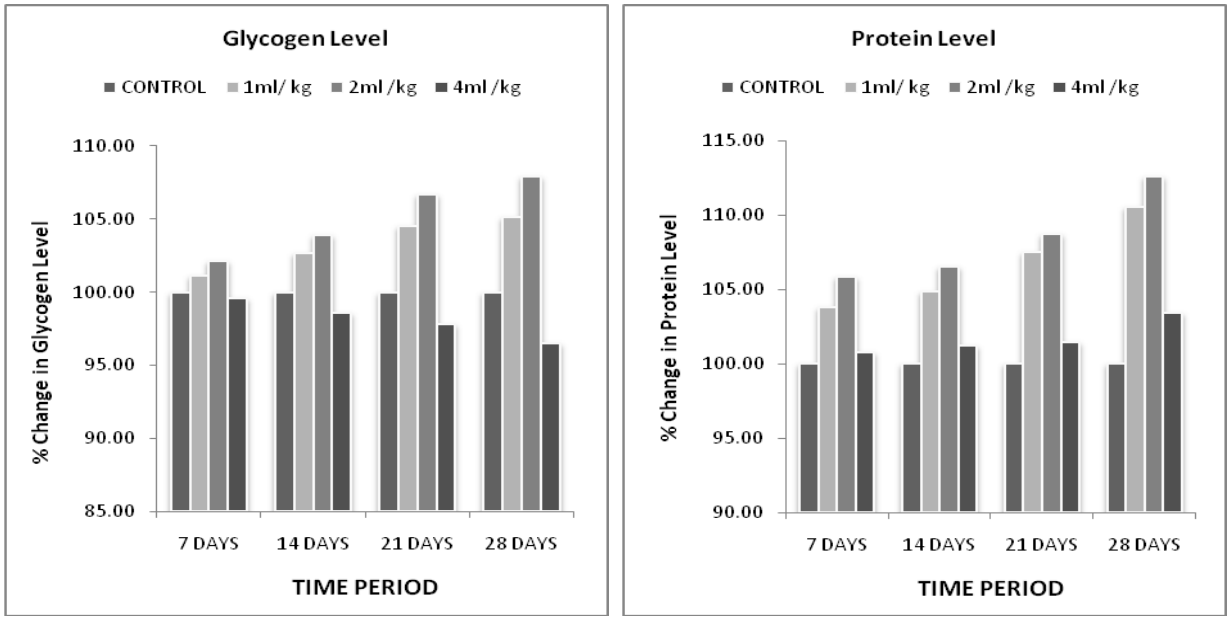

Figure 1: Change in percent level of Glycogen and Protein in male albino rats after fed with different volumes of raw garlic extract for 7, 14, 21 and 28 days daily

The effects of garlic supplementation on protein metabolism have not been fully clarified. It is believed that garlic involved in hormonal secretion, may affect whole-body protein metabolism due to hormonal regulation by stimulating hormone secretion (noradrenaline and adrenaline), or it may affect protein metabolism by enhancing protein anabolism. These results get support from OI, et al., (1995; 1998) who reported that supplementation of garlic powder at 0.8 g/100 g with a high fat diet and the administration of diallyldisulfide, a major volatile sulfur-containing compound in garlic, enhanced triglyceride catabolism and growth of interscapular brown adipose tissue (IBAT) by increasing noradrenaline secretion in rats. Further in (1999), they

reported that allyl-containing sulfides in garlic increase the uncoupling protein (UCP) content in brown adipose tissue, and noradrenaline and adrenaline secretion in rats. They further reported that the allyl-containing polysulfides in garlic are responsible for the enhancement of noradrenaline and adrenaline secretion and increased in thermogenesis, indicated by the increased UCP content in IBAT. Thus, garlic administration may affect whole-body protein metabolism due to hormonal regulation by stimulating hormone secretion. On the other hand decrease in protein level, observed in present investigation may be due to their degradation and possible utilization for metabolic purposes. Decreased protein content might also be attributed to the destruction or necrosis of cells and their consequent impairment in protein synthesis machinery. The quantity of protein depends on the rate of protein synthesis or on the rate of its degradation.

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