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EFFECTS OF *ALLIUM SATIVUM* ON BODY WEIGHT AND BIOCHEMICAL PARAMETERS IN HEALTHY RATS

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ABSTRACT: This case-control study had undertaken to evaluate effect of *Allium sativum* in normal rats. In this study ethanol extract of *Allium sativum* had macerated with aqueous gum acacia (2%, w/v) suspension and fed orally (500 mg/kg b w p. o.) to male adult normal rats of charles foster strain for 15 days. Results of this study showing that alcoholic extracts caused no any significant reduction in blood glucose, total cholesterol, triglyceride, phospholipids, free fatty acid, lipid peroxide and no significant increased in post heparin lipolytic activity, but on the other hand as per pre-existing data and my published studies in diabetic patients and diabetic experimental animals showed that extracts exert all above effects significantly. That's why it is very clear here if a healthy person will take natural products, it never causes hypoglycemia, hypolipidemia, and under the weight. Natural products also not cause any significant change in hepatospecific parameters. Thus, from this study, we conclude that natural products are safe, non-toxic, and free from side effects, in comparison to synthetic drugs.

INTRODUCTION: *Allium sativum* (Lat.), (Eng: Garlic, Urdu: 'Lahsan') is widely distributed in all parts of the world and used not only as a spice but also as a popular remedy for prevention and treatment of a variety of diseases like rheumatism, dermatitis, abdominal disorders, and diabetes mellitus. Effect of garlic in cardiovascular diseases was more encouraging in experimental studies, which prompted several clinical trials. Dietary factors play a key role in the development of various human diseases, including cardiovascular disease.

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Garlic has attracted particular attention of modern medicine because of its widespread health use around the world and the cherished belief that it helps in maintaining good health warding off illnesses and providing more vigor. To date, many favorable experimental and clinical effects of garlic preparations, including garlic extract, have been reported. These biological responses have been largely attributed to reduction of risk factors for cardiovascular diseases, cancer and stimulation of immune functions, enhanced detoxification of foreign compound, hepatoprotective, antimicrobial effect and antioxidant effect [1].

Garlic is reported to prevent cardiovascular disease by multiple effects, one of which is the decrease total cholesterol and triglycerides [2], LDLC, VLDLC, while increase HDLC [3] and suppression of the cholesterol biosynthesis [4]. Studies prior to 1995 consistently concluded hypolipidemic action

of garlic [5, 6]. However, studies after 1995 using enteric-coated preparation of raw garlic did not manifest any hypolipidemic effect [7, 9]. These paradoxical observations warrant a systemic study to resolve the controversy.

It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, anti-thrombotic, hypolipidemic, antiarthritic, hypo-glycemic and antitumor activity. Additionally, garlic has known hypoglycemic properties, which have been demonstrated in alloxan induced diabetic rats and rabbits. The extract of garlic and its component, S-allylcysteine sulfoxide, significantly decreased blood glucose concentration. Its activity appears to be in part due to the stimulation of insulin secretion from β-cell in the pancreas [10].

Cardiovascular diseases are leading cause of death in both industrialized and developing nations [11]. Disorders of lipid metabolism following oxidative stress are the prime risk factors for the initiation and progression of heart diseases [12]. The current therapies used for controlling hyperlipidemia, fibrates, stains, and bile acid sequestrations are almost inefficient to regulate lipid metabolism. Furthermore, these drugs also cause a number of serious adverse effects in patients. Currently available treatment for hyperlipidemia in modern medicine, fibrates, statins, or bile acids sequestrants and their combinations do not regulate lipid metabolism up to an appreciable mark, also have several adverse effects in patients [13]. Therefore, there is a need to develop safe and effective treatment modalities for hyperlipidemia. Further-more, medicinal plants play an important role in the treatment of lipid disorders, especially due to their lesser toxicity, side effects, and cost-effectiveness. Therefore, the research and development of hypoglycemic and lipid-lowering drugs from natural products are the best option and also are in great demand. In view of the above considerations, the present study was designed to investigate the adverse effects of *A. sativum* in normal rats.

MATERIALS AND METHODS:

Preparation of *Allium sativum* Extract: *Allium sativum* were collected from the local area of Lucknow and identified taxonomically by the Department of Pharmacology, Era's Lucknow Medical College Lucknow. A voucher specimen (AS-005/10) was also submitted. The bulbs of garlic (*A. sativum*) were cut into small pieces and extracted with alcohol. The alcohol content was evaporated to dryness. The final yield of 20.0 gms of crude extract (concentrate) was added with 50 ml of triple distilled water and was used for *in-vivo* studies. A dose of 400 mg/Kg was administered to rats orally, daily for 15 days [14].

Animals: *In-vivo* experiments were conducted as per guidelines provided by the Animal Ethics Committee of Central Drug Research Institute, Lucknow, India. Male adult rats of Charles Foster strain (200-225 g) bred in the animal house of the Institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26 °C, relative humidity 50-60%, and 12/12 h light/dark cycle (light from 8:00 an m to 8:00 pm) and provided with standard rat pellet diet and water *ad libitum*.

Preparation of Doses: A quantity of 50 mg *Allium sativum* extract was suspended /ml tripled distilled water (TDW) containing 2% (w/v) gum acacia. The suspension was given in a volume of 1 ml/100 g animal b. w. (500 mg drug / kg b. w.) by oral intubation.

Chemicals: Blood glucose (BLG), Total cholesterol (TC), triglycerides (TG), Phospholipid (PL) were analyzed using standard kits from Erba Diagnostic (Mannheim Gmb H, Germany) by an auto-analyzer (Erba Mannheim, EM 360, Germany). Intralipid from victrum AB, In the Kabivitrum Group, Stockholm, Sweden [15].

Experimental Animals: Healthy male adult rats of Charles Foster strain (200-250 g) bred in the animal house of the Central Drug Research Institute, Lucknow, were used. The animals were kept in controlled conditions; temperature 25-26 °C, relative humidity 60-70%, and 12/12 h light / dark cycle (Light from 08:00 AM to 08:00 PM), provided with standard pellet diet (Lipton India Ltd.) and water *ad libitum* [15].

Experimental Design: The rats were divided in five groups having six animals in each as follows: group 1; normal control rats (on normal saline); group 2; *Allium sativum* treated normal rats [15].

Assessment of Biochemical Parameters: The blood was centrifuged, and plasma was separated. The fasting blood sugar (FBS) was analyzed in plasma; superoxide dismutase (SOD), catalase (CAT), hepatic triglyceride lipase (TGL), and lipoprotein lipase (LPL) were estimated in liver homogenate.

Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein total cholesterol (HDL-TC), serum bilirubin, SGPT, SGOT, and alkaline phosphatase were assayed by standard spectro-photometric kit methods. Low-density lipoprotein total cholesterol (LDL-TC) and very-low-density lipoprotein total cholesterol (VLDL-TC) were calculated by Friedewald's equation. Serum was also used for the assay of lecithin cholesterol acyltransferase activity (LCAT), lipid peroxide (LPO), and reduced glutathione (GSH).

A portion of serum was fractionated into very-low-density lipoprotein (VLDL), low-density lipo-protein (LDL), and high-density lipoprotein (HDL) by polyanionic precipitation methods. Lipoproteins were measured for their total cholesterol (TC), phospholipids (PL), triglyceride (TG), and apo-protein by standard spectrophotometric methods in the liver homogenate [15-30].

Statistical Analysis: One-way analysis of variance (ANOVA-Newman's Student t-test) was performed by comparison of values for a normal treated group with normal healthy control. All hypothesis testing was two-tailed. $P < 0.05$ was considered statistically significant, and the results were expressed as Mean ± SD. The graph pad in State 3.0 software was used to carry out the statistical analysis [31].

RESULTS: Effect of drug treatment on body weight of drug-treated normal rats after 1 month with respect to normal control without drug-treated. Weight of all experimental rats (Normal control without drug-treated and drug-treated) was recorded in the beginning of the study and after completion of the study. Percent change in weight of rats after 15 days, at the end of the study, as compared with respect to the value of the weight of rats on study day 0 (Beginning). No significant change in weight of drug-treated normal rats was found with respect to normal control without drug-treated, after 15 days, in the end of the study, **Table 1**.

TABLE 1: EFFECT OF DRUG TREATMENT ON BODY WEIGHT OF NORMAL RATS AFTER 15 DAYS

Group	Body Weight (gm)		Percent change with respect to the value of weight of rats on study day 0 (Beginning)	'p' value
Normal control + 2 % aqueous gum acasia	Study day 0 (Beginning)	247.50 ± 33.90	-	-
	study day 15 (end of study)	315 ± 23.56	+ 22	NS
Normal control + *Allium sativum* treated (500 mg/kg body weight)	Study day 0	258.16 ± 18.32	-	NS
	Study day 15	329 ± 20.70	22	NS

Values expressed as gram (gm) are mean ± SD of six rats, drug-treated groups were compared with normal control. Percent change with respect to the value of the weight of rats on study day 0 (beginning). NS = Not significant

Effect of *Allium sativum* on Blood Glucose, Serum Lipid, and Lipoprotein Profile in Healthy Control Rats: In normal rats, administration of *Allium sativum* at the dose of 500 mg/kg b. w. orally once daily for 15 days lowered the levels of blood glucose (2 %), TC (2%), PL (2%), TG (1%), and increase in total serum protein (1%) **Table 2**. Furthermore, treatment with natural test products, the lipid, and protein components of serum lipoproteins remains almost the same as that of control **Table 2**.

TABLE 2: EFFECTS OF *ALLIUM SATIVUM* ON BLOOD GLUCOSE AND SERUM LIPIDS IN NORMAL RATS

Experimental schedule	Blood glucose (mg/dl)	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	Protein (g/dl)
Normal control	89.76 ± 10.30	87.50 ± 11.16	75.96 ± 7.36	82.21 ± 5.80	7.00 ± 0.53
Allium sativum Treated (500 mg/kg body weight)	88.20 ± 1060 (-2%)	85.65 ± 11.69 (-2%)	74.62 ± 7.53 (-2%)	81.61 ± 5.76 (-1%)	7.09 ± 0.56 (+1%)

Values expressed are mean ± SD of six rats. Values with parenthesis are percentage change drug-treated groups are compared with control (Change is non-significant

It was seen that *Allium sativum* caused a decrease in the level of ± lipoprotein TC (3-5%), PL (4-8%), TG (0.82-3.0%), and apolipoproteins (0.3-1.5%) respectively. However, herbal preparation increases lipid and protein contents of ± lipoprotein HDL by (0.3-7%) in **Table 3**.

TABLE 3: EFFECT OF *ALLIUM SATIVUM* ON LIPOPROTEIN PROFILE IN NORMAL RATS

Experimental schedule	Very low-density lipoprotein (VLDL)				Low-density lipoprotein (LDL)				High-density lipoprotein (HDL)			
	TC	PL	TG	Apo-protein	TC	PL	TG	Apo-protein	TC	PL	TG	Apo-protein
Normal control	8.10	16.80	40.10	7.00	17.90	11.98	20.61	16.87	51.64	41.18	14.90	173.80
	±0.66	±2.06	±3.29	±0.99	±1.73	±1.43	±1.76	±2.37	±5.59	±3.36	±1.51	±11.63
Allium sativum	7.70	16.06	39.77	7.01	17.40	11.07	19.90	16.92	54.78	41.95	15.07	174.08
treated (500 mg/kg	±0.54	±2.12	±3.43	±0.73	±1.66	±1.25	±1.42	±2.42	±8.27	±3.32	±1.49	±11.59
body weight)	(-4.9%)	(-3.8%)	(1.00%)	(-0.20%)	(-3%)	(-8%)	(-3%)	(+0.6%)	(+6%)	(+2%)	(+1%)	(+0.14%)

Values expressed are mean ± SD of six rats. Values with parenthesis are percentage change. Drug treated groups are compared with control (Changes were no significant)

Effects of *Allium sativum* on Serum Free Fatty Acids, Hepatic Triglyceride Lipase and Total Hepatic Lipoprotein Lipase Activity Status in Normal Rats: In normal rats administration of *Allium sativum* for 15 days lowered the levels of free fatty acid (1%) hepatic triglyceride lipase (4%) and total hepatic lipoprotein lipase activity (4%) respectively **Table 4**.

TABLE 4: EFFECT OF *ALLIUM SATIVUM* ON SERUM FREE FATTY ACID TOTAL HEPATIC LIPOPROTEIN LIPASE AND HEPATIC TRIGLYCERIDE LIPASE ACTIVITY IN NORMAL RATS

Experimental schedule	Serum-free fatty acid[a]	Hepatic lipoprotein lipase activity[b]	Total hepatic lipoprotein lipase activity[b]
Normal control	1.69 ± 0.20	71.78 ± 7.60	85.01 ± 0.06
Allium sativum treated (500 mg/kg body weight)	1.65 ± 0.12(-1.0%)	74.65 ± 7.48 (+4%)	88.53 ± 4.58 (+4.0%)

Values are expressed as mean ± SD of six rats drug-treated groups are compared with control. (The changes are non significant); a = μ mol FFA/L, b = μ mol FFA released h / mg protein

Effect of *Allium sativum* on Serum Lipid Peroxide, Hepatic Sod and Hepatic Catalase Activity in Normal Rats: In normal rats administration of *A. indicus*, *Hibiscus rosasinensis* *C. tora* and *T. cordifolia* for 15 days lowered the level of serum lipid peroxide (8%) and increase in the levels of hepatic SOD (4%), catalase (1.4%) respectively **Table 5**.

TABLE 5: EFFECT OF *ALLIUM SATIVUM* ON SERUM MDA AND HEPATIC SOD, HEPATIC CATALASE ACTIVITY IN NORMAL RATS

Experimental schedule	Serum lipid peroxide[a]	Hepatic SOD[b]	Hepatic CAT[b]
Normal control	2.90 ± 0.40	2.78 ± 0.20	3853 ± 251.40
Allium sativum treated (500 mg/kg body weight)	2.67 ± 0.40 (-7.8.0%)	2.88 ± 0.20 (+4.00%)	3910 ± 267.08 (+1.46%)

Values are expressed as mean ± SD of six rats. Drug treated groups are compared with control. (The changes are non-significant); a = μ mol MDA / ml, b = units / min /mg protein

Effect of *Allium sativum* on Hepatospecific Marker in Normal Control Rats: In normal rats administration of above mentioned herbal preparations for 15 days lowered the levels of Bilirubin, SGPT, SGOT, ALP in serum by (4.83%), (6.99%), (0.99%) and (1.64%) respectively **Table 6**.

TABLE 6: EFFECT OF *ALLIUM SATIVUM* ON THE HEPATO SPECIFIC MARKERS IN THE SERUM OF NORMAL RATS

	Serum bilirubin[a]	SGPT[b]	SGOT[b]	ALP[c]
Normal control	0.60 ± 0.00	22.20 ± 2.70	57.22 ± 7.30	17.63 ± 1.00
Allium sativum (500 mg/kg body weight)	0.50 ± 0.10 (-4.83)	20.73 ± 2.91(-7.00)	56.65 ± 6.90(-0.99)	17.34 ± 1.09(-1.60)

Values are expressed as mean ± SD of six rats, values in the parenthesis are percent change (changes are non significant). Units a = mg/dl, b = units/l, c = units/dl

DISCUSSION: In normal rats treatment with *Allium sativum* at the doses of 500 mg/kg body weight orally once in a day for 15 days did not alter significantly, their body weight **Table 1** or blood biochemical parameters namely the levels of glucose, serum protein-lipid profile **Table 2**, lipoprotein profile **Table 3**, a free fatty acid with lipolytic enzyme activities **Table 4**, lipid peroxide and antioxidant enzymes **Table 5**. Also, there was no significant change in the hepatospecific parameters like serum bilirubin (S. bil), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) **Table 6**. This indicated that the herbal preparations, as such, did not exert any adverse and toxic side effects during treatment in rats. The results of the present study demonstrated that natural products did not cause a significant decrease in biochemical parameters in healthy normal rats [32-34]. That's why it very clear here if a healthy person will take natural products, it never causes hypoglycemia, hypolipidemia and under the weight. Natural products also not cause any significant change in hepatospecific parameters. Thus from this study, we conclude that natural products are safe, non-toxic, and free from side effects in comparison to synthetic drugs [35-38].

CONCLUSION: It should be pointed out here that plant-derived natural compounds have established a proven platform for developing new drug synthesis with fewer side effects or free from side effects.

Ethical Approval: This article does not contain any studies with human participants performed by any of the authors. The study was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute and was carried out in accordance with the current guidelines set by Organization for Economic Co-operation and Development (OECD), received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for the Care of Laboratory Animals:

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