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EFFECTS OF MARKETED PREPARATION OF *ALLIUM SATIVUM* ON STZ INDUCE HYPERGLYCEMIA IN WISTER ALBINO RATS

Nawin Kumar*, Sanjay Kumar, Narendra Patel

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, (M.P.)

*Corresponding Author's E mail: nawinkumar1968@gmail.com

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ABSTRACT

Management of type 2 diabetes with an agent having no side effects is still a challenge for the researchers, however if the side effects are lessened & there may be a chance for reduced adverse reactions or severe side effects due to drug interaction. These interactions may be due to either any concomitant drug therapy or any dietary supplements taken together with the drugs. *Allium sativum* (Garlic) is used as an important medicinal & dietary supplement in Greece, India, china & Egypt from ancient time. This study revealed that a drug interaction also provides some beneficial effects. As in this study; use of vildagliptin with garlic treatment not only resulted in glycaemic control in diabetic rats but also provided beneficial hypolipidemic effects, closely related to diabetes progression. The present study has demonstrated significant hypoglycemic & hypolipidemic effects of garlic when added with standard anti-diabetic agent. Comprehensive clinical studies are desirable to verify the effectiveness of garlic either alone or in combination with other antidiabetic agent in the treatment & prevention of diabetes risk factors.

K**eywords:** *Allium sativum*, vildagliptin, hyperglycemia, hypolipidemic

INTRODUCTION

Diabetes mellitus (DM) is one of the most significant chronic metabolic disorders characterized by hyperglycemia. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response [1]. The overall prevalence of diabetes mellitus in the global population is approximately 6%, of which 90% is type 2 diabetes. India had 32 million diabetics in 2000, and this number is expected to increase to 80 million by 2030 [2, 3]. Characteristic of diabetes is associated with disturbances in the metabolism of carbohydrates, lipids and proteins due to defects in insulin secretion, insulin action or both [4]. Diabetic complications are nephropathy, retinopathy, neuropathy, atherosclerosis and fatty liver. In all these cases continual hyperglycemia plays a significant role in the induction of oxidative stress by increasing glucose autooxidation, nonenzymatic protein glycation and activation of polyol pathway [5]. Also hyperglycemia induced stress sensitive signaling pathways including nuclear factor (NF)-kB. Activation of NF-kB increased cytokine concentrations such

as tumor necrosis factor-a (TNF-α). The renal cells are capable to synthesis TNF- α moreover the sensitive to changes of serum's TNF- α level. This process suggests a causal role for hyperglycemia in the immune activation of diabetes [6]. Since ancient times, consumption of medicinal herbs has considered in treatment of several diseases [7]. In recent years this kind of treatment has received growing attention because it is natural and has a few side effects [8]. Many medicinal plants extracts such as Bougainvillea spectabilis, Moringa oleifera, Curcuma longa, Cynodon dactylon and Trichosanthes dioica were used for treatment of diabetes mellitus due to having hypoglycaemic effects [9-13]. One of the most common medicinal plants is *Allium sativum* (garlic) from Liliaceae family that it is used for the treatment of various diseases such as heart disease, liver dysfunction, cancer, infection and diabetes mellitus [14]. Therefore, the aim of the present study was to evaluate the drug interaction of marketed garlic preparation on Streptozotocin (STZ) +nicotinamide-induced diabetes in rats.

Material and Methods

Marketed garlic preparation

Marketed Garlic Preparation was purchased from the Himalaya Herbal Healthcare, India. Concentrations of the Marketed Garlic Preparation was prepared and used for the dose of 400 mg/kg body weight of the rats.

Drugs and chemicals

Streptdzotocin (STZ) was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, SA), and Nicotinamide was obtained from Merck, Mumbai The working standard of Vildagliptin was provided as gift sample from Spectrum Labs, Hyderabad, India. All other chemicals and solvents used in this study were of analytical grade and purchased from commercial sources. The assay kits used for biochemical analysis were products of Span diagnostics Ltd, Surat, India

Animals

Mail Wister Albino rats, weighing between 150-220 gm were selected for the study. The animals were obtained from committee for the purpose of control and supervision of experiments on animals (CPCSEA) approved animal house of the Department of Pharmacology, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.), India. Animals were housed in spacious cages and allowed one week to adapt to their new environment. The animals were maintained in an environment of room temperature (25 ± 2^0C) under a 12-h light-dark cycle and standard rodent chow and were provided

throughout the experimental period. All animal procedures used were in strict accordance with the CPCSEA and GLP all experimental Protocols were approved by the institutional animal ethics committee guidelines.

Qualitative analysis of phytochemicals

The marketed formulation extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in various extracts of medicinal plants. The above phytoconstituents were tested as per the standard method [15].

Acute oral toxicity

Acute toxicity study of the prepared leaves extracts was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [16] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 5, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method [17].

Selection of Dose

Dose was Selected on the basis of maximum tolerable those (NOAEL), as there was no Lethality observed upto 2000mg/kg. Thus Dose was selected as 1/5th of 2000mg/kg i.e. 400mg/kg for further investigation.

Induction of Diabetes in Rat

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single inter- peritoneal injection of 45mg/kg streptozotocin, 15 min after the i.e. administration of 110mg/kg of nicotinamide [18]. Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 75 hrs. Only rats confirmed to have permanent NIDDM were for the antidiabetic study. The animal with blood glucose concentration more than 250mg/dl has been used for the study.

Experimental

Five groups of rats were employed in the present study and each group contains six animals, as follows:[19]

Group 1 (Normal control) Rats were given only vehicle (water). No drug was given in this group (diabetes free rats).

Group 2 (Diabetic control) Streptozotocin and Nicotinamide induced diabetic rats. No drug was given to diabetic rats.

Group 3 (Standard) Vildagliptin (3 mg/kg body weight) was administered to Streptozotocin and Nicotinamide induced diabetic rats.

Group 4 (Marketed Garlic Preparation) Marketed Garlic Preparation (400 mg/kg) to Streptozotocin + Nicotinamide induced type-2 diabetic rats.

Group 5 (Vildagliptin + Marketed Garlic Preparation) Vildagliptin (3 mg/kg) + Marketed Garlic Preparation (400mg/kg) to Streptozotocin + Nicotinamide induced type-2 diabetic rats.

After induction of diabetes, drugs (Vildagliptin and Marketed Garlic Preparation) were administered as intra gastric gavage daily for 28 days.

Collection of blood samples

Blood samples were collected from the retro-orbital plexus of rats under anaesthesia at 0, 7, 14, 21 and 28 days intervals. Blood was collected in heparinized tubes (eppendorf) and used for the estimation of blood glucose level and lipid profile.

Estimation of biochemical parameters

Blood glucose was estimated by the SGOT, SGPT, ALP, Total bilirubin method by spectrophotometrically using a commercially available kit (Span diagnostics ltd, Surat, India). Serum cholesterol and high density lipoprotein (HDL) levels were estimated by the method spectro-photometrically using a commercially available kit (Span diagnostics ltd, Surat, India). Blood triglyceride levels were estimated by the method spectro-photometrically using a commercially available kit (Span diagnostics ltd, Surat, India).

Statistical Analysis

All the values of blood sugar, lipid profile and biochemical estimations were expressed as Mean ± S.E.M. (Standard Error of Mean) for six rats in each group and analyzed with one way analysis of variance (ANOVA) followed by Bonferroni t-test. Differences between groups were considered significant at $P < 0.050$ & P 0.001 levels.

RESULT AND DISCUSSION

Table 1: Result of phytochemical screening of marketed formulation

S. No	Tests	Marketed Garlic
1	Carbohydrate	+ve
2	Proteins & Amino acids	+ve
3	Fats & Oils	+ve
4	Flavonoids	+ve
5	Saponin Glycosides	+ve
6	Tannin & Phenolic	+ve
7	Test for Vitamins C	+ve
8	Test for Calcium	+ve
9	Test for Potassium	+ve
10	Test for Sulphate	+ve
11	Test for Phosphate	+ve

Table 2: Acute oral toxicity Studies

S.No.	Dose	Lethality
1	5 mg/kg	0/3
2	5 mg/kg	0/3
3	50 mg/kg	0/3
4	50 mg/kg	0/3
5	300 mg/kg	0/3
6	300 mg/kg	0/3
7	2000 mg /kg	0/3
8	2000 mg/kg	0/3

Table 3 Effects of marketed garlic preparation and anti-diabetic drug on blood glucose level in Diabetes

Treatment (mean ± SEM)	Blood Glucose level (mg/dl)				
	0 Day	7 Day	14 Day	21 Day	28 Day
Control	92.5±1.381	94.7±1.456	95.5±1.433	96.2±1.757	96.4±1264
Diabetic Control	291.3±3.596	297.5± 0.007	302.3±4.818	305.1±4.988	307.1±4.935
Vildagliptin (3mg/kg)	292.1±2.1892	213.8±3.869	156.4±5.488	146.6±5.603	136.9±4.512
MGP (400 mg/kg)	288.6±2.831	245.9±3.526	206.1±2.679	196.9±3.789	185.8±7.359
MGP (400 mg/kg)+ Vildagliptin (3mg/kg)	191.1±2.01	202.9±3.897	144.1±4.151	129.9±5.899	106.9±3.344

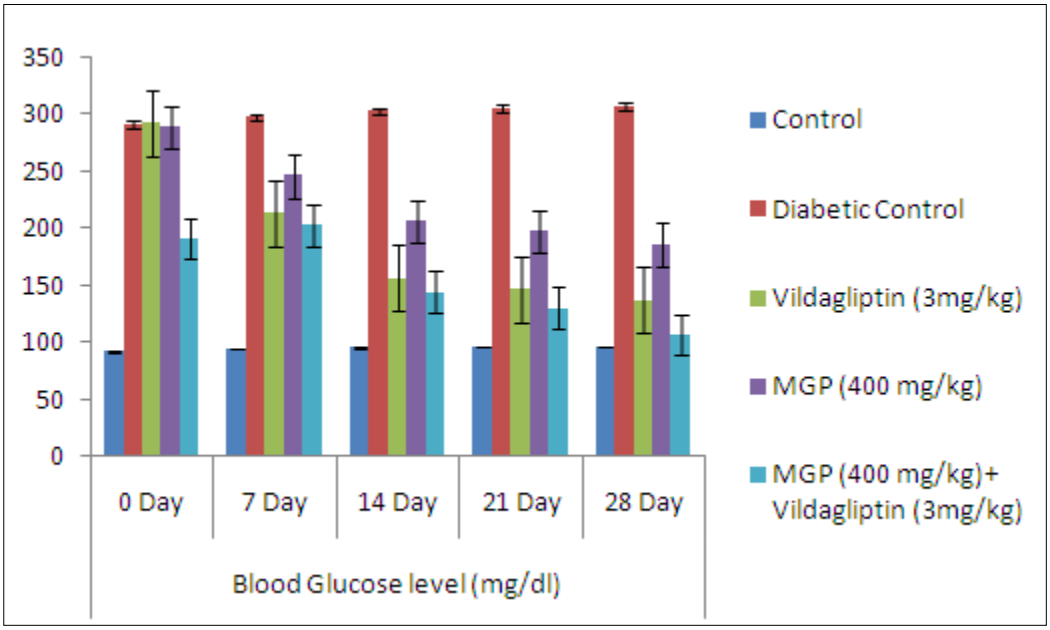

Fig 1. Effects of marketed garlic preparation & anti-diabetic drug on blood glucose level in diabetes

Table 4 Effects of marketed garlic preparation & anti-diabetic drug on lipid profile in diabetes

Treatment (mean ± SEM)	Lipid Profile (mg/dl)			
	TC	TG	LDL	HDL
Control	93.3±3.11	53.7±1.589	45.1±2.69	35.9±2.589
Diabetic Control	132.4±3.581	113.6±3.99	93.6±3.891	19.8±2.989
Vildagliptin (3mg/kg)	108.0±4.249	65.2±2.389	53.6±2.292	31.8±1.941
MGP(400 mg/kg)	119.1±4.798	18.1±4.151	62.5±3.989	25.5±3.445
MGP(400 mg/kg)+ Vildagliptin (3mg/kg)	99.8±4.125	58.4±2.474	48.4±2.690	35.9±1.793

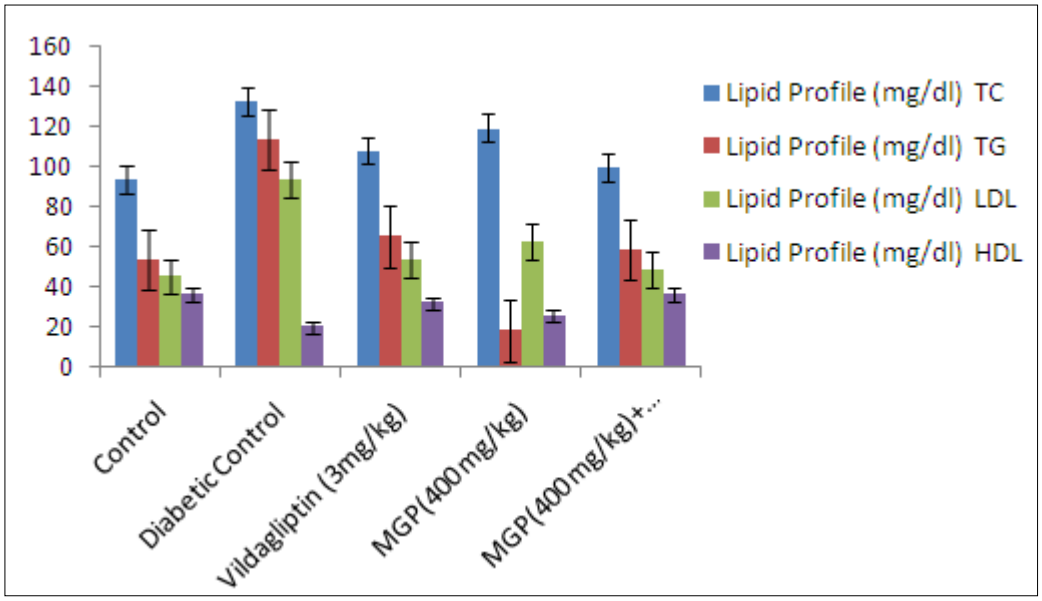

Fig. 2 Effects of marketed garlic preparation & anti-diabetic drug on lipid profile in diabetes

Table 5 Effects of marketed garlic preparation & anti-diabetic drug on liver function tests in diabetes

Treatment	Liver Function Test(mg/dl)			
	SGOT	SGPT	ALP	Bilirubin
Control	56.98±3.498	63.97±2.861	104.11±5.143	0.57±0.065
Diabetic Control	97.17±2.178	106.52±3.646	199.77±5.084	1.04±0.038
Vildagliptin (3mg/kg)	62.53±2.469	66.41±2.762	121.42±4.452	0.62±0.058
MGP(400 mg/kg)	69.58±6.656	72.41±2.890	133.72±10.547	0.78±0.048
MGP(400mg/kg)+ Vildagliptin (3mg/kg)	59.87±0.0482	65.22±1.695	112.45±3.879	0.58±0. .513

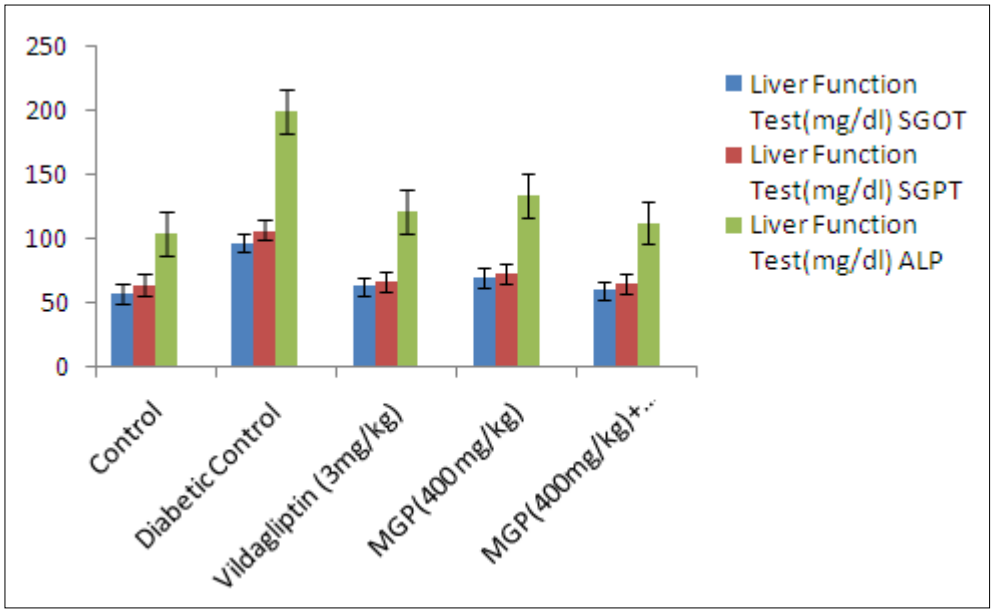

Fig. 3 Effects of marketed garlic preparation & anti-diabetic drug on liver function test in diabetes.

The qualitative phytochemical analysis showed (Table 1) the presence of Carbohydrate, Proteins & Amino acids, Fats & Oils, Flavonoids, Saponin Glycosides and Tannin & Phenolic etc. The effects of the marketed garlic preparation on blood glucose level, lipid profile, liver function tests (LFT), acute oral toxicity were investigated in the normal control group, diabetic control group, vildagliptin group, marketed garlic preparation group and marketed garlic preparation + vildagliptin group by STZ-induced diabetic rats using vildagliptin as standard anti-diabetic drug (Table 2-5). The significance of anti-diabetic & hypolipidaemic properties of garlic has been proven in animal studies. This study discovered that there are significant pharmacodynamic as well as pharmacokinetic drug dietary interactions, because the co-administration of proven hypoglycaemic properties of garlic as well as vildagliptin when compared to vildagliptin alone. Earlier studies on garlic suggested that sulfur containing amino acid S-allyl cysteine sulfoxide (alliin) in garlic has a potential to control diabetic condition in rats. This study also revealed that there is dose dependent hypoglycaemia seen in diabetic rats when given together with vildagliptin, as there is one more possible reason for additive hypoglycaemic effects of marketed garlic preparation with vildagliptin. Figure 2 showed the effects of vildagliptin alone & vildagliptin with marketed garlic preparation on, cholesterol & triglyceride levels of diabetic wistar rats which were found to be increased in diabetic control on the 28th day. The cholesterol & triglyceride values among all the treated groups are significantly reduced in dose dependent manner on the 28th day when compared with

diabetic control. The marketed garlic preparation showed reduction of triglyceride because of impairment of triglyceride synthesis by inhibiting fatty acid production. The cholesterol & triglyceride lowering effects of vildagliptin were seen during the study but those were not found statistically significant when compared with vildagliptin 0 day result. The effect of marketed garlic preparation with vildagliptin on HDL was not found statistically significant as some other studies suggested this too however the levels of HDL on 28^{th} day were increased when compared with 0 day readings. This study is proposed that, SCOT & SGPT enzymes are responsible for production of ketone bodies from amino acids & subsequently produce high concentration of glucose level. The higher levels of SGOT & SGPT, may give rise to a high concentration of glucose. In other words, the gluconeogenic action of SGOT & SGPT plays the role of providing new supplies of glucose from other sources such as amino acids. In the present study there was significant reduction in the level of TC, TG, LDL, SGOT & SGPT. But the level of HDL was increased.

CONCLUSION

It can be suggested that, administration of garlic to diabetic patients can decrease the blood glucose level. Garlic has been used as food additive & can be recommended as a dietary supplement for long term use without toxic effects. We certainly believe that garlic overall is a magical medicinal herb & if consume as much as possible has got the prophylactic effects in all people.

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