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# Effects of hydroalcoholic extract of *Allium sativum* on STZ induce hyperglycemia

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**Abstract**
Management of T2DM 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 anti-diabetic agent in the treatment & prevention of diabetes risk factors.

**Keywords:** *Allium sativum,* vildagliptin, hyperglycemia, hypolipidemic

## Introduction
Diabetes is an autoimmune disease in which your body is unable to adequately use make glucose, according to Mayoclinic.com. Whether you have Type 1 or Type 2 diabetes, this condition indicates an excess amount of glucose in your blood. Garlic has a long-standing medicine use in folk medicine for the treatment of diabetes [1]. Herb, it is still unclear as to its exact effect on diabetes and you should therefore always consult your physician before beginning an herbal regimen [2].

## Lower Blood Sugar
Garlic can lower blood sugar levels in diabetics as it bound up chemical receptors that would otherwise deactivate insulin, the hormone that control sugar usage, according to Phyllis Balch in her book." prescription for Herbal. Balch also says that garlic help to stimulate the pancreas to secrete insulin without inducing weight gain, which is a common side effect and concern of those suffering from this condition. Balch says using just 600mg of garlic per day can benefit a diabetic in gaining control over the disease [3].

## Lower Cholesterol
High cholesterol and triglyceride level are regular complication for diabetics. This is due to a usual high-sugar, high-fat diet that incites the development of Type 2 diabetes. For Type 1 diabetics, poor eating habits merely exacerbate the disease and cause further complication such as increased risk of heart attack, according to Julian Whitaker in the book "Reversing Diabetes [4]." Diabetics who have high cholesterol and triglyceride levels are at an increased risk of heart and coronary diseases due to plaque build-up within the arteries.
The lipid-lowering effects of some oil-soluble sulfur compounds in hepatocytes coincide with cytotoxicity, as revealed by increased lactate dehydrogenase release from cells [5]. Water-soluble sulfur compounds, on the other hands, although effective at reducing cholesterol, were not cytotoxic an array of water-soluble constituents, including S-allylcysteine (SAC), may account for the reduced toxicity of the hydroalcoholic extracts of garlic compared with raw preparation [6]
The reduction in cholesterol seen from oil-soluble sulfur compounds (e.g., diallyl sulfide (DAS), diallyl disulfide (DADS) or diallyl trisulfide (DATS) appears to be due to toxicity as revealed by increased lactate dehydrogenase from exposed hepatocytes [54] and release of acetone in the breath after oral consumption by humans [7]. Water-soluble sulfur compounds, although effective at reducing cholesterol, were not cytotoxic. Therefore, extraction by aqueous ethanol solution (aging process) reduces damage to the liver [8].

Several clinical reports, including meta- analyses, have revealed a cholesterol-lowering effect of garlic in humans. These report have promoted public awareness about the cholesterol lowering effect of garlic. However, recent publication suggested that not all preparation may be hypocholesterolemic [9].

## Material and Method

### Collection and extraction

Garlic extract were purchased from the Himalaya Herbal Healthcare, India. It was identified by (No. NISCAIR/RHMD/Consult/-2015-10/026/12) India. Concentration of the extract were prepared and used for the dose of 400 mg/kg body weight of the animals [10].

### Phytochemical Screening

### Phytochemical screening

Identification of the chemical constituents were carried out on the powdered Garlic and the extract was concentrated and subjected to various chemical tests to detect the presence of different Phytoconstituent [11].

### 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, PBRI, Bhopal, India. Animals were housed in spacious cages and allowed one week to adapt to their new environment [12]. The animals were maintained in an environment of room temperature (25±2$^{O}$ C) 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 PBRI institutional animal ethics committee guidelines [13].

### Drug and Chemical

Streptozotocin (STZ) was obtained from sigma Aldrich, Germany and used in this study to induce diabetes in rats. Nicotinamide was obtained from Merck, Mumbai. Vildagliptin was used as a standard drug in STZ-induced moderate diabetic model to evaluate the interaction potential of Allium sativum. All other chemical and solvent used in this study were analytical grade and purchased from commercial sources [14, 15].

### 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 [16]. 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 anti – diabetic study.

The animal with blood glucose concentration more than 250mg/dl has been used for the study.

### Experimental Design [17]

Five group of were employed in the present study and each group contains six animals, as follows:

- Group I (Normal control): Rats were give only vehicle (water). No drug was given in this group II (diabetes free rats).
- Group III (Diabetic control): Streptozotocin and Nicotinamide induced diabetic rats. No drug was given to diabetic rats.
- Group IV (Standard): Vildagliptin (3mg.kg body weight) was administered to streptozotocin and Nicotinamide induced diabetic rats.
- Group V (Garlic extract): Garlic extract (400mg/kg) to Streptozotocin+ Nicotinamide induced type-2 diabetic rats.
- Group (Vildagliptin+ Garlic extract): Vildagliptin (3mg/kg) + Garlic extract (400mg/kg) to Streptozotocin+Nicotinamide induced types-2 diabetic rats.

After induction of diabetes, drug (Vildagliptin and Garlic extracts) were administered as intra gastric gavages daily for 28 days.

### Collection of Blood Samples

Blood sample were collected from the reto- orbital Plexus of rats under anesthesia at 0, 7, 14, 21 and 28 days intervals [18]. Blood was collected in heparin zed tued (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) level were estimated by the method spectro-photometrically using a commercially available kit (Span diagnostics ltd, Andheri (East), Mumbai). Blood triglyceride levels were estimated by the method spectro- photometrically using a commercially available kit (span diagnostics ltd Andheri (East), Mumbai).

### Statistical Analysis

All the values of blood sugar, lipid profile and biochemical estimation Were expressed as Mean±SEM. (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 group were considered significant at $p<0.050$ & $P<0.001$ levels [19-21].

### Experimentation

### Induction of Type 2 diabetes by streptozotocin

- **Step-1:** Streptozotocin was dissolved in citrate buffer ($p^{H}$ 4.5) and nicotinamide was dissolved in normal physiological saline.
- **Step-2:** Non-insulin dependent diabetes mellitus was included in overnight fasted rats by single inter-peritoneal injection of 45 mg/kg of nicotinamide.
- **Step-3:** Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 75 hrs. Only rats confirmed to have permanent NIDDM were used for the anti-diabetic study.

The animal with blood glucose concentration more than 250mg/dl has been used for the study [22].

### Experimental Design for Anti-Hyperglycemic Activity

The rats were divided into five group of six (n=6) each randomly [23],

- GRP I-Vehicle
- GRP II-Vehicle + (STZ+Nicotinamide)
- GRP III-Vildagliptin (3mg/kg) + (STZ+Nicotinamide)
- GRP IV-Ext(400mg/kg) + (STZ+Nicotinamide)
- GRP V-Ext(400mg/kg) + (STZ+Nicotinamide

## Result and Discussion

**Table 1:** Comparatives study of qualitative phytochemical tests

| S. No | Tests | Comparative study | |
|---|---|---|---|
| | | Hydro-alcoholic garlic extract | Marketed Garlic |
| 1 | Carbohydrate | +ve | +ve |
| 2 | Proteins & Amino acids | +ve | +ve |
| 3 | Fats & oils | +ve | +ve |
| 4 | Flavonoids | +ve | +ve |
| 5 | Saponin Glycosides | +ve | +ve |
| 6 | Tannin & phenolic compounds | +ve | +ve |
| 7 | Test for Vitamins C | +ve | +ve |
| 8 | Test for Calcium | +ve | +ve |
| 9 | Test for Potassium | +ve | +ve |
| 10 | Test for sulphate | +ve | +ve |
| 11 | Test for phosphate | +ve | +ve |

**Table 2:** Effects of garlic Extract and anti-diabetic Drug on blood Glucose Level in Diabetes

| S. No. | Treatment | Blood Glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 Days | 7 Days | 14 Days | 21 Days | 28 Days |
| 1. | Control | 92.4±1.381 | 94.6±1.447 | 95.6±1.436 | 96.1±1.754 | 96.4±1.265 |
| 2. | Diabetic Control | 292.6±3.598 | 297.7±5.009 | 302.24.817 | 305.1±4.989 | 306.5±4.934 |
| 3. | Vildagliptin (3mg/kg) | 292.0±2.192 | 213.8±3.965 | 156.45.499 | 146.5±5.581 | 137.3±4.474 |
| 4. | Ext. (400mg/kg) | 288.6±2.825 | 246.1±3.576 | 206.1±2.681 | 197.2±3.792 | 186.1±7.360 |
| 5. | Ext. (400mg/kg) + Vildagliptin (3mg/kg) | 190.9±2.211 | 203.0±3.999 | 143.9±4.147 | 130.0±5.916 | 107.8±3.333 |

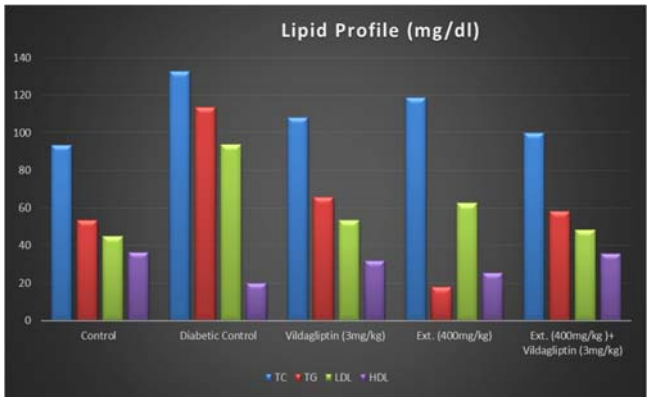


**Fig 1:** Effect of Garlic extract & Anti- diabetic drug on blood glucose level in diabetes. The result were expressed as mean±SEM.

**Table 3:** Effect of Garlic Extract and anti-diabetic drug on lipid profile in diabetes

| S. No. | Treatment | Lipid Profile (mg/dl) | | | |
|---|---|---|---|---|---|
| | | TC | TG | LDL | HDL |
| 1 | Control | 93.4±3.010 | 53.6±1.595 | 44.9±2.704 | 36.2±2.594 |
| 2 | Diabetic Control | 132.6±3.578 | 113.4±4.096 | 93.7±3.961 | 19.9±2.985 |
| 3 | Vildagliptin (3mg/kg) | 107.9±4.258 | 65.4±2.394 | 53.4±2.291 | 31.8±1.938 |
| 4 | Ext. (400mg/kg) | 118.7±4.797 | 18.0±4.152 | 62.6±3.987 | 25.4±3.444 |
| 5 | Ext. (400mg/kg )+ Vildagliptin (3mg/kg) | 99.7±4.124 | 58.4±2.475 | 48.3±2.689 | 35.8±1.797 |

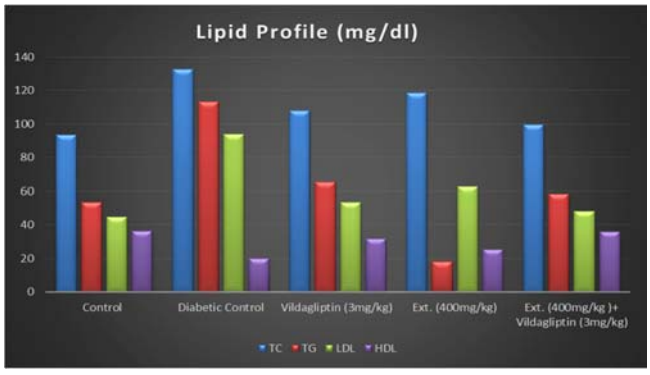


**Fig 2:** Effect of Garlic extract & Anti – diabetic drug on lipid profile in diabetes. The result was expressed as mean± SEM.

**Table 4:** Effects of Garlic Extract and anti-diabetic drug on liver Function Test in diabetes

| S. No. | Treatment | LFT (mg/dl) | | | |
|---|---|---|---|---|---|
| | | SGOT | SGPT | ALP | Bilirubin |
| 1 | Control | 57.11±3.511 | 63.98±2.856 | 103.86±5.043 | 0.56±0.064 |
| 2 | Diabetic Control | 97.18±2.177 | 106.53±3.647 | 199.78±5.085 | 1.04±0.038 |
| 3 | Vildagliptin (3mg/kg) | 62.53±2.470 | 66.41±2.761 | 121.43±4.451 | 0.61±0.059 |
| 4 | Ext. (400mg/kg) | 69.58±6.657 | 72.40±2.889 | 133.71±10.546 | 0.77±0.047 |
| 5 | Ext. (400mg/kg)+ Vildagliptin (3mg/kg) | 59.88±2.512 | 65.21±1.694 | 112.46±3.878 | 0.58±0.049 |

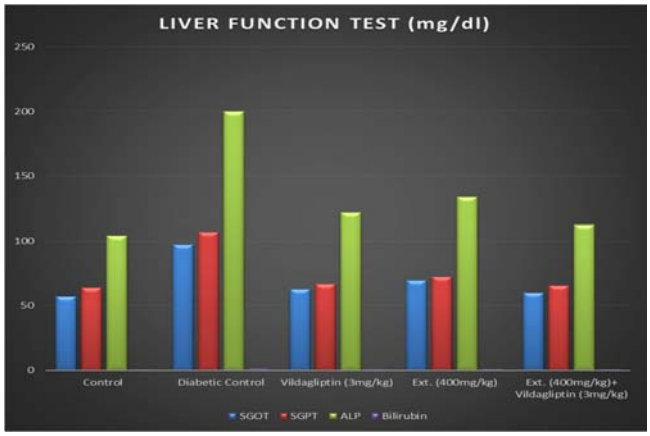


**Fig 3:** Effect of extract & Anti- diabetic drug on liver function test in diabetes. The result were expressed as mean±SEM.

**Table 5:** Acute oral toxicity Studies

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

**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 1/5$^{th}$ of 2000mg/kg i.e. 400mg/kg for further investigation.

**Discussion**

The effect of the garlic extract on blood glucose level, lipid profile, liver function test (LFT), acute oral toxicity was investigated in the normal control group, diabetic control group, vildagliptin group, garlic extract group and garlic extract+ vildagliptin group by STZ-induced diabetic rats using vildagliptin as standard anti-diabetic drug [24, 25]. The significance of anti-diabetic & hypolipidemic properties of garlic has been proven in animal studies. This study discovered that there is significant pharmacodynamic as well as pharmacokinetic drug dietary interactions, because the co-administration of garlic with vildagliptin causes tight glycaemic control due to the proven hypoglycemic 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 [26].

This study also revealed that there is dose dependent hypoglycemia seen in diabetic rats when given together with vildagliptin, as there is one more possible reason for additive hypoglycemic effects of garlic with vildagliptin. Fig.2 showed the effects of vildagliptin alone & vildagliptin with garlic extract on cholesterol & triglyceride levels of diabetic wistar rats which was found to be increased in diabetic control on the 28$^{th}$ day. The cholesterol & triglyceride values among all the treated groups are significantly reduced in dose dependent manner on the 28$^{th}$ day when compared with diabetic control. The garlic extract showed reduction of triglyceride synthesis by inhibiting fatty acid production [27]. The cholesterol & triglyceride lowering effects of vildagliptin were seen during the study but those were not found statistically when compared with vildagliptin's 0-day result. The effect of garlic 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, SGOT & SGPT enzymes are responsible for production of ketone bodies from amino acid & subsequently produce high concentration of glucose level. The higher level of SGOT &SGPT, may give rise to a high concentration of glucose. In other words, gluconeogenic action of SGOT & SGPT plays the role of providing new supplies of glucose from other sources such as amino acids. Following i.p. administration of different plant fraction, SGOT & SGPT levels were significantly reduced. 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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