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EVALUATION OF THE ANTIDIABETIC ACTIVITY OF THE ETHANOLIC EXTRACT OF *ALLIUM SATIVUM* LEAVES IN WISTAR ALBINO RATS SUBJECTED TO STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA

Sandila Chandana*[1], Dr. Neelam I[2], Dr. Y.Sridhar[3], G Vijaya Kumar[4]

[1]Department Of Pharmacology, Kgr Institute Of Technology And Management, Rampally, Secunderabad, Telangana 501302.

[2]M Pharm Phd, Associate Prof,Kgr Institute Of Technology And Management, Rampally, Secunderabad, Telangana 501302.

[3]M Pharm,Ph.D, Prof, Kgr Institute Of Technology And Management, Rampally, Secunderabad, Telangana 501302.

[4]M. Tech Phd Prof And Principal Of Kgr Institute Of Technology And Management, Rampally, Secunderabad, Telangana 501302.

Abstract:

*In India, the number of people suffering from diabetes is believed to be rising steadily and the current antidiabetic therapies are frequently reported to have adverse side effects. Ethno medicinal plant use has shown promise for the development of cheaper, cost-effective antidiabetic agents with fewer side effects. Te aim of this study was to investigate the antidiabetic activity and mechanism of action of Ethanolic extract prepared from **Allium sativum**. Since this claim has not been investigated scientifically, the aim of this study was to evaluate the antidiabetic effect and phytochemical screening of Streptozotocin -induced diabetic Albino Rats.*

*The leaves of **Allium sativum** have been used in traditional health systems to treat diabetes mellitus. However, the antidiabetic activity of this medicinal plant is not scientifically validated and authenticated. The present study aimed to investigate the in vitro and in vivo anti-diabetic activity of flower crude extract and solvent fractions of **Allium sativum**.*

*The in vitro α–amylase inhibition of the crude extract and solvent fractions of **Allium sativum**. Blood glucose lowering activity of 80% Ethanolic crude extract and solvent fraction was studied in animal models: Hypoglycemic Albino rats model, oral glucose loaded rat model, dose-treated Streptozotocin -induced diabetic Rat model. The effect of the crude extract on diabetic lipid profile was studied.*

*The acute toxicity study of **Allium sativum** leaves extract did not show mortality in the animals at the limit dose during the observation period. The result of α–amylase enzyme inhibition activity was found in a dose-dependent manner, the strongest activity was shown by Crude extract fraction (89.60 % inhibition at 1000 µg/mL) compared to the standard acarbose having 97.19% inhibition at 1000 µg/mL. The crude extract of **Allium sativum** showed significant blood glucose-lowering effect on hypoglycemic Albino rats and oral glucose loaded Albino rats. In Streptozotocin -induced diabetic Albino rats' model, the crude extract fraction significantly decreased the fasting blood glucose level after 14 days of treatment.*

*The result demonstrated the beneficial biochemical effects of **Allium sativum** extract by inhibiting α–amylase improving serum lipid profile levels. The leaves crude extract are effective in lowering blood glucose levels in diabetic and hypoglycemic Albino rats. The claimed traditional use as antidiabetic has scientific ground.*

Key words: *Diabetes mellitus, Herbal medicine, Allium sativum, Streptozotocin, Anti diabetic activity.*

Corresponding author:

Sandila Chandana,
Department of Pharmacology,
KGR Institute Of Technology And Management,
Secunderabad, Telangana.
Mobile:
Email Id: *sandilachandana@gmail.com*

QR CODE

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INTRODUCTION:

Diabetes Mellitus (DM):

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment[1].

1.2-Classification of DM

Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as

Type 1, or insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and

Type 2, or non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Type 2 diabetes commonly occurs in adults associated with obesity. There are many underlying factors that contribute to the high blood glucose levels in these individuals. An important factor is the resistance to insulin in the body essentially ignoring its insulin secretions. A second factor is the decreased production of insulin by the cells of the pancreas. Therefore, an individual with Type 2 diabetes may have a combination of deficient secretion and deficient action of insulin. In contrast to Type 2 diabetes, Type 1 diabetes most commonly occurs in children and is a result of the body's immune system attacking and destroying the beta cells. The trigger for this autoimmune attack is not clear, but the result is the end of insulin production[2].

Multiple risk factors for the development of Type 2 diabetes mellitus[3]:

- Family history (parents with diabetes).
- Obesity (i.e., ≥20% over ideal body weight or body mass index ≥25kg/m^2).
- Habitual physical inactivity.
- Impaired glucose tolerance.
- Hypertension (≥140/90mm Hg in adults).
- High density lipoprotein (HDL) cholesterol ≤ 35mg/dl and/or triglyceride level ≥ 250mg/dl.

1.3-History

The term “Diabetes” was first used around 250 B.C. It is a Greek word meaning “to syphon”, reflecting how diabetes seemed to rapidly drain fluid from the affected individual. The Greek physician Aretaeus noted that affected individuals passed increasing amounts of urine as if there was “liquefaction of flesh and bones into urine”. The complete term “diabetes mellitus” was coined in 1674 by Thomas Willis. Mellitus is Latin for honey, which is how Willis described the urine of diabetics[4-5].

Historical accounts reveal that as early as 700-200 BC, diabetes mellitus was a well recognized disease in India and was even distinguished as two types, a genetically based disorder and other one resulting from dietary indiscretion. Ancient Hindu writings document how black ants and flies were attracted to the urine of diabetics. The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals, and for many centuries to come, the sweet taste of urine was a key to the diagnosis.

Physicians have observed the effects of diabetes for thousands of years. One of the effects of diabetes is the presence of glucose in the urine (glucosuria). For much of the time, little was known about this fatal disease that caused weight loss of body, extreme thirst, and frequent urination. It was in 1922 that the first patient was successfully treated with insulin. Till the mid-1800s, the treatments offered for diabetes varied tremendously. A breakthrough in the puzzle of diabetes came in 1889. German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs. The dogs immediately developed diabetes. Now that a link was established between the pancreas and diabetes, research focused on isolating the pancreatic extract that could treat diabetes. Dr. Frederick Banting succeeded in his experiments of isolating a pancreatic extract. The diabetic dog was kept alive for eight days by regular injections until supplies of the extract, at that time called "isletin", was exhausted. Experiments on dogs showed that extracts from the pancreas caused a drop in blood sugar, caused

glucose in the urine to disappear, and produced a marked improvement in clinical condition.

A young boy, Leonard Thompson, was the first patient to receive insulin treatment in the year 1922 and lived for thirteen years. Over the next 70 years, insulin was further refined and purified. A revolution came with the production of recombinant human DNA insulin in 1978. Instead of collecting insulin from animals, new human insulin could be synthesized. In 1923, Banting and Macloed were awarded the Nobel Prize for the discovery of insulin. In his Nobel Lecture, Banting concluded the following about their discovery: "Insulin is not a cure for diabetes; it is a treatment."

1.4-Epidemiology[6]

Present status projects that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization (WHO), there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in year 2001, about 441,004 deaths were registered and 49,855 of them provoked by diabetes, representing 11.2% of the total population. In United States, diabetes is the sixth leading cause of death. The prevalence of diabetes mellitus is rapidly increasing worldwide and India is estimated to have 31 million diabetics from the total population of the world. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people.

The driving force behind the high prevalence of diabetes is the rise of obesity, sedentary lifestyle, consumption of energy rich diet, etc. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups.

The prevalence of Type 2 diabetes is now at epidemic proportions. Type 2 diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Type 2 diabetes accounts for about 90-95 % of population while Type 1 diabetes accounts for about 5 -10% of the total population. In the past, Type 2 was rarely seen in the young, but recent reports describe Type 2 diabetes being diagnosed even in children and adolescent[7].

1.5-Sugar Regulation: Carbohydrate, protein and lipid metabolism[8]

Glucose is an essential fuel for the body and is the main source of energy for the tissue cell. The amount of glucose in the blood is controlled mainly by the hormones insulin and glucagon. The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Glucose is transported into the beta cell by Type 2 glucose transporters (GLUT2). As glucose metabolism proceeds, ATP is produced which closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell. The rise in positive charge inside the beta cell causes depolarization thereby opening the voltage-gated calcium channels and allowing calcium ions (Ca^{2+}) to flood into the cell. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

Insulin increases the uptake and use of glucose by tissues such as skeletal muscle and fat cells. Once inside the cell, some of the glucose is used immediately via glycolysis. Any glucose that is not used immediately is taken up by the liver and muscle where it can be converted into glycogen (glycogenesis). When glycogen stores are fully replenished, excess glucose is converted into fat in a process called lipogenesis by increasing the number of glucose transporters (GLUT4) expressed on the surface of the fat cell, causing a rapid uptake of glucose. Glucose is converted into fatty acids that are stored as triglycerides for storage[9].

The rise in glucose also inhibits the release of glucagon, inhibiting the production of glucose from other sources, e.g., glycogen break down. Glucose may also indirectly contribute to protein synthesis by synthesis of amino acids. Glucagon is the main hormone opposing the action of insulin and is released when food is scarce. Glucagon also helps the body to switch to using resources other than glucose, such as fat and protein during starvation[10].

6. MATERIALS AND METHODS:

Instruments

Following instruments were required for the study:

Table No 6.1: List of Instruments used for study

Name of the instrument	*Source*
Centrifuge	Dolphin
Digital weighing balance	Horizon
Glucometer	Horizon
Heating mantle	ASGI®
Refrigerator	Videocon
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI®, Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

Table No 6.2: List of Chemical used for study

Name of the Chemical	**Source**
Allium sativum	Tirumala hills, Tirupati, Andhra Pradesh. India
Glibenclamide	Sanofi
Streptozotocin	Loba Chemie
Ethanol	Honeywell

a) Plant collection

The leaves of *Allium sativum* were collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

The leaves of *Allium sativum* were collected and authenticated by Department of Botany. After shade-dried (Temp<40°C.), plant material was grounded into a moderately coarse powder. The extract was made by maceration and the ethanolic extract was made by using soxhlet apparatus. The extract was allowed to dry. Both the extracts were preserved in the refrigerator till further use.

***Invitro* antidiabetic activity of *Allium sativum* leaves extracts**

Alpha-amylase inhibition assay

The a-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.50 The crude and solvent fractions of *Allium sativum* were dissolved in buffer ((Na2HPO4/ NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of a-amylase solution (Molychem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30 C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 mL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The inhibition of a-amylase was expressed as percentage of inhibition and was calculated by the following equation: Inhibition (%) ¼ [(Ac -Acb) (As Asb) / (Ac -Acb)] × 100, where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and

Asb is the absorbance of sample blank. The % a-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph.

Preliminary phytochemical screening of Ethanolic leaves extract of *Allium sativum*

The Ethanolic leaves extract of *Allium sativum* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavanoids

Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.

Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins

Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoid

Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids

Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.

Hager's test: The extract was treated with few ml of Hager's reagen.

Wagner's test: The extract was treated with few ml of Wagner's reagent.

Tests for Glycosides

Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins

Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids

Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

g) Acute oral toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen. In most case these are desired and useful, but many effects are not advantageous. Acute, sub-acute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD50 (It is the lethal dose (causing death) to 50% of tested group animals).

LD50 (median lethal oral dose)

LD 50 (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of animal (mg/kg).

In this study acute oral toxicity study was carried out in Rats. The procedure was followed by using OECD 423(Acute toxic class method). The Rats are fasted overnight, prior to dosing. The three dose levels are administered by orally the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be withheld for a further 3-4 hours in Rats. The purpose of

sighting study is to allow selection of the appropriate starting dose for main study.

The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg). Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected.

***Invivo* antidiabetic activity of *Allium sativum* leaves extract in Streptozotocin induced diabetic Rats.** [54]

Prior to the experiment the rats were housed in a clean polypropylene cage (6 Rats / cages) for a period of 7 days under standard temperature (25 - 30ºC), relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organizational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

Hypoglycemic Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)
Group II served as Positive control – Glibenclamide (2mg /kg)
Group III served as ethanolic extract of *Allium sativum* – (200mg/kg)
Group IV served as ethanolic extract of *Allium sativum* – (400mg/kg).
Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)
Group II served as Positive control – Glibenclamide (2 mg /kg)
Group III served as Ethanolic extract of *Allium sativum* – (200mg/kg)
Group IV served as Ethanolic extract of *Allium sativum* – (400mg/kg).
All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of Rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of Streptozotocin dissolved in sodium citrate buffer was used for the induction of diabetes in Rats after overnight fasting. After 1 hr of Streptozotocin administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of Rats six in each groups received the following treatment schedule for 14 days.
GROUP I - Normal control (normal saline 10 ml /kg, P.O)
GROUP II - Streptozotocin treated control (100 mg/kg, I.P)
GROUP III - Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).
GROUP IV - Streptozotocin (100 mg/kg, i.p.) + EEAS (200 mg/kg, P.O)
GROUP V - Streptozotocin (100 mg/kg, i.p.) + EEAS (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control Rats. Group IV and Group V (which previously received Streptozotocin 100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Allium sativum* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EEAS-Ethanolic extract of *Allium sativum* Leaves).

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of Rats at weekly intervals till the end of the study 1, 7, and 14 day.

Estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0. All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one-way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

7. RESULTS:

a) Appearance and percentage yield of EEAS (Ethanolic Extract of *Allium sativum* Leaves)

Table 7.1: a-Amylase Inhibitory Activities of the Crude Extract and Solvent Fractions.

Percentage inhibition					
Concentration (mg/mL)	**Chloroform fraction**	**Ethyl acetate fraction**	**Aqueous fraction**	**Crude extract**	**Acarbose**
50	4.52 + 0.2	17.25 + 0.95	26.03 + 1.03	31.20 + 0.36	57.65 + 0.79
100	13.51 + 0.10	23.65 + 0.51	32.11 + 0.11	43.01 + 1.42	68.10 + 0.46
200	26.26 + 0.03	28.99 + 2.58	40.65 + 0.56	60.12 + 0.98	76.93 + 1.53
400	31.31 + 0.12	44.98 + 0.20	57.92 + 0.19	73.84 + 0.76	88.51 + 0.17
600	40.09 + 0.35	52.15 + 1.47	70.82 + 0.56	80.19 + 0.24	93.06 + 0.26
800	47.26 + 0.64	66.09 + 0.12	77.60 + 0.74	83.55 + 0.19	96.27 + 0.17
1000	55.39 + 0.33	72.69 + 0.41	84.21 + 0.22	88.98 + 0.74	97.19 + 0.92
IC50	33.25 + 0.19	23.56 + 0.82	15.36 + 0.69	9.69 + 0.91	3.34 + 0.14

Abbreviation: IC50, half maximal inhibitory concentration.

Each value of percentage inhibition of a-amylase is presented as means + standard error of the mean (SEM), n ¼ 3.

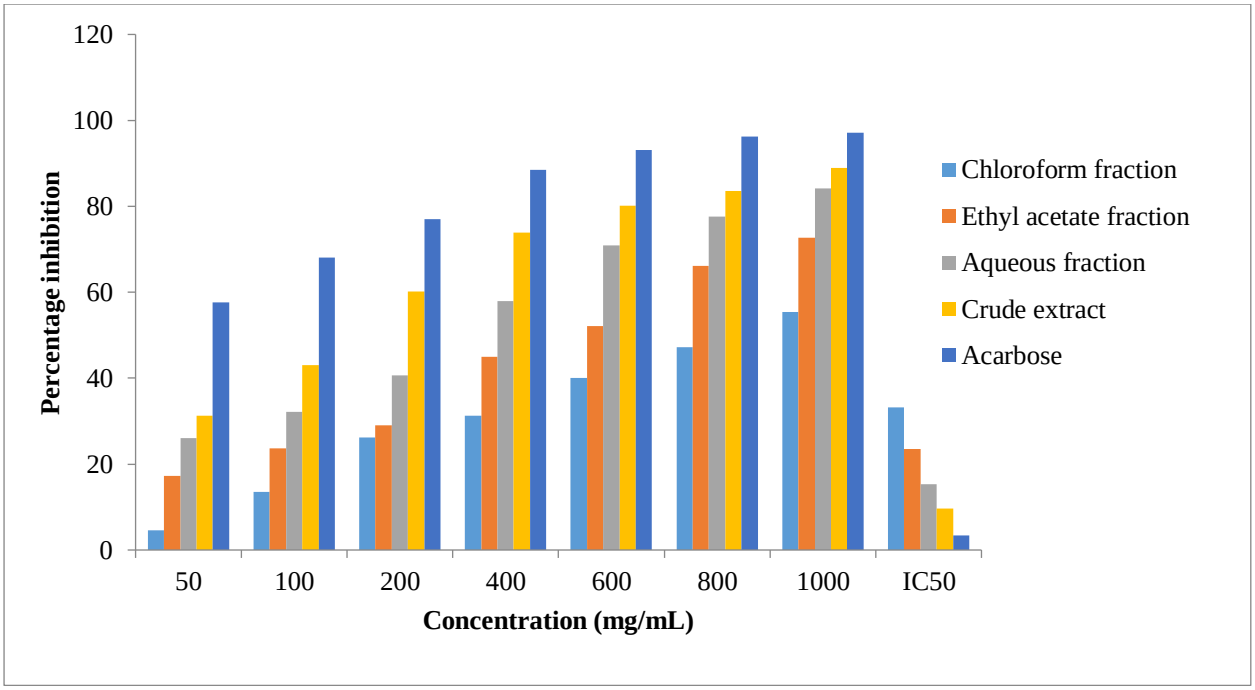


Figure 7.1: a-Amylase inhibitory activity of the ripe crude extract and solvent fractions of *Allium sativum*

In Vitro a-Amylase Inhibition Activity of Crude Extract and Solvent Fractions *In vitro* a-amylase inhibitory study evaluating the percent of a-amylase inhibition as a function of extract

concentrations and the IC50 values were calculated (Figure). Concentration dependent inhibitions were observed for various concentrations of the tested extracts and the standard. Among the extracts, the crude extract exhibited the lowest IC50 of 67.21 + 0.91 mg/mL and the IC50 values of water fraction, ethyl acetate fraction, and the chloroform fraction were 15.36 + 0.69, 23.56 + 0.82, and 33.25 + 0.19mg/mL, respectively. The standard positive control acarbose showed an IC50 of 3.34 + 0.14mg/mL (Table).

Minimum % Inhibition was found *Allium sativum* leaves which resemblance to %Inhibition of positive control, So Ethanolic extract of *Allium sativum* contain active constituents of antidiabetic.

Phytochemical studies

Table No 7.2: Results of Ethanolic extract of *Allium sativum* leave

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	-
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test Lead acetate test	+ +
Tannins	Braemer's test	-
Alkaloids	Wagner's Mayer's Draggendrof's test	+ + +
Glycosides	Legal's test Brontranger's test	+ +
Saponins	Foam test	+
Sterols	Salkowski's test	-
Amino acids	Ninhydrin test	-
Terpenoids	Lieberman Burchardt test	+

+Present in moderate amount
-Absence

The phytochemical studies results revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids Flocculent white precipitate also indicated the same. There is no dark blue or greenish grey coloration of the solution indicated the absence of tannins in the drug. No characteristic observation for steroids and dark pink or red coloration of the solution indicated the presence of Terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish-brown precipitation indicated the presence of alkaloids. Pink to red Colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. If the response to the test is indicated table-1high it can be noted or which indicates that the particular group is present as the major class. If the response is average then note it as indicates the presence in moderate quantity and note it as indicating the presence of only in traces. If no response is then negative.

TABLE 7.3: Hypoglycemic Test

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	30min	1hr
CONTROL Carboxymethyl Cellulose (CMC)	0.5%	72.15±1.125	70.29±1.241	71.85±2.369
Positive Control Glibenclamide	2	65.20±2.241	53.96±2.102**	45.36±1.296***
Ethanolic Extract of *Allium sativum*	200	66.39±1.396	56.25±1.209*	55.20±2.109*
Ethanolic Extract of *Allium sativum*	400	66.12±1.269	53.62±1.263**	40.1+±1.836***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.

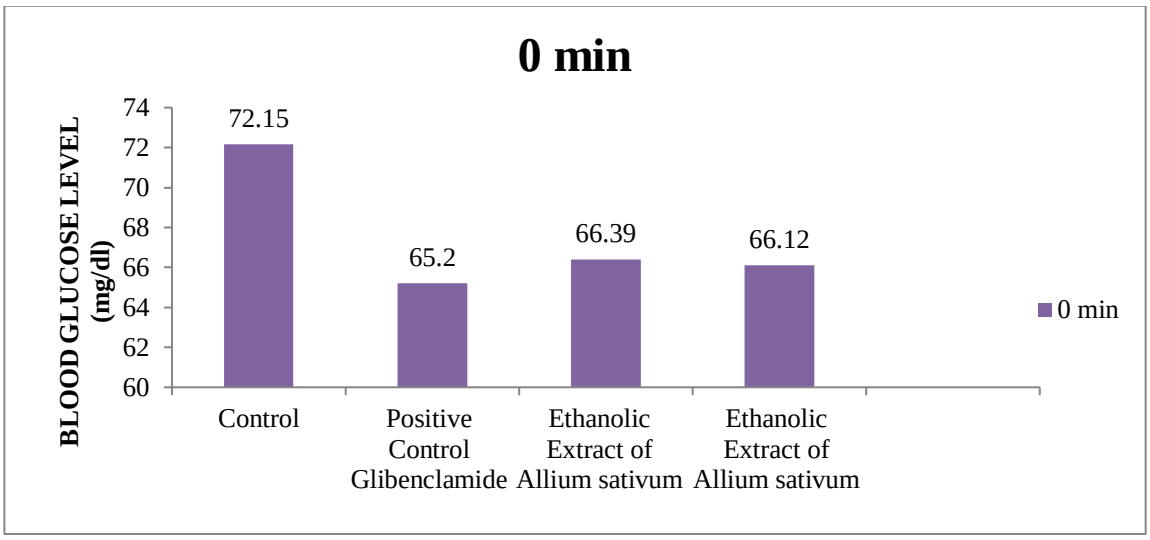


Fig 7.2: Blood glucose level 0min

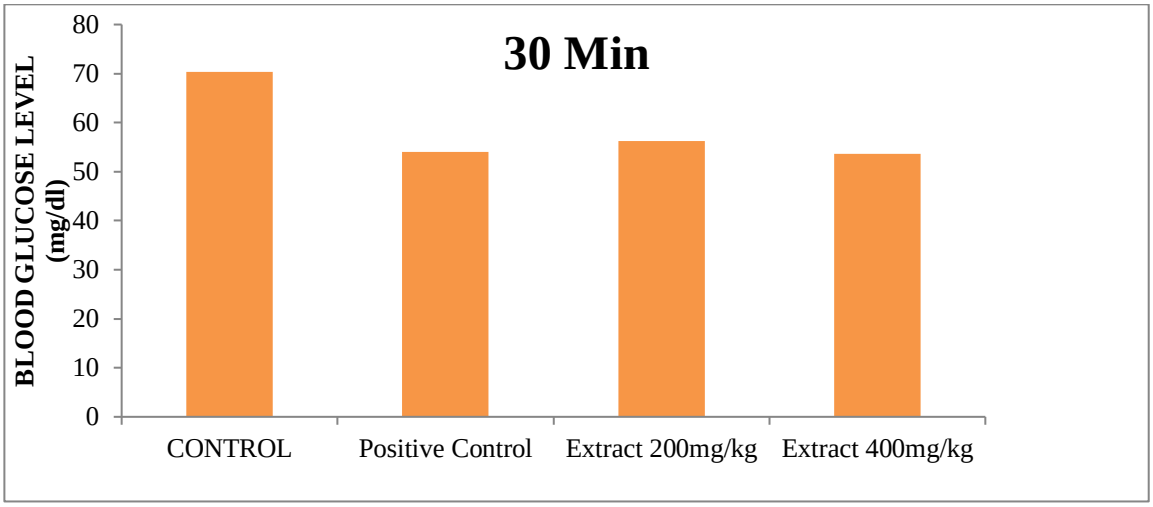


Fig 7.3: Blood glucose level 30min

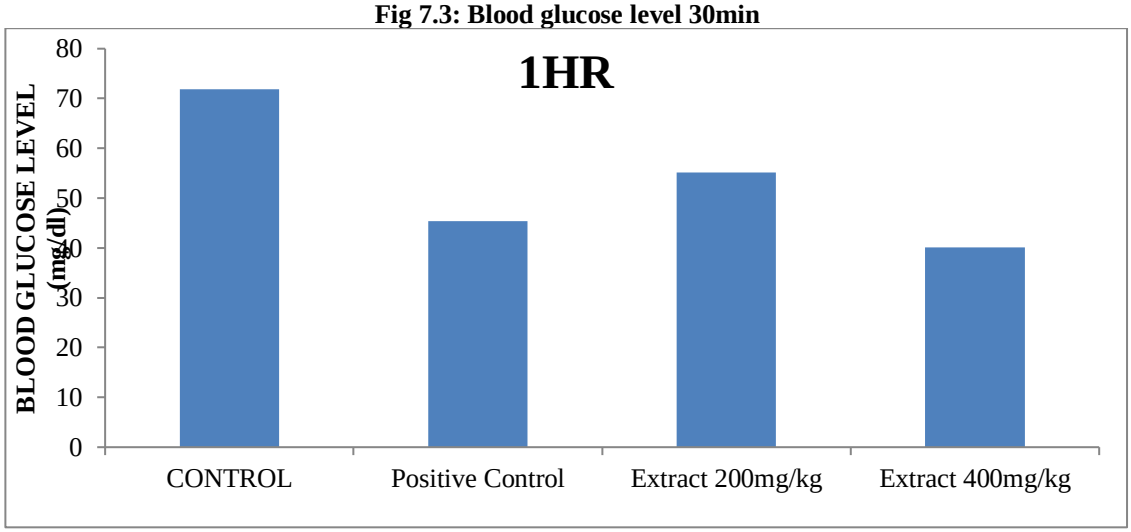


Fig 7.4: Blood glucose level 1hr

The hypoglycemic test results have shown Table No: which indicated ethanolic extract of *Allium sativum* treated animals 200 & 400, significantly decreased in blood glucose level when compared to control and positive control.

g) *Invivo* antidiabetic study

Table 7.4: Results of the effects of Ethanolic extract on blood Glucose levels

TREATMENT	BLOOD GLUCOSE LEVEL (mg/dl)		
	0 min	30min	1hr
Normal control 10 ml/kg P.O	77.25±2.132	74.0±1.232	73.2± 2.151
Negative control	260.1±1.20	258.2±1.060	262.2±2.1
Positive control (Glibenclamide 2mg/kg) P.O	251.36±1.125	130.12±1.5***	112±1.5***
EEAS 200 mg/kg P.O	250.01±1.50	243.2±1.151**	235.2±1.136**
EEAS 400 mg/kg P.O	260.30±1.91	169.0±2.72***	152.1±1.6***

(The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

The experimental results have indicated on Table the negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased Glibenclamide 2mg/kg treated group when compared with control group at 7th and 14th days. The Ethanolic leaves extract of *Allium sativum* treated groups 200 & 400 mg/kg were dose dependent manner decreased when compared with control group but positive control have more anti diabetic activity at 7th day.

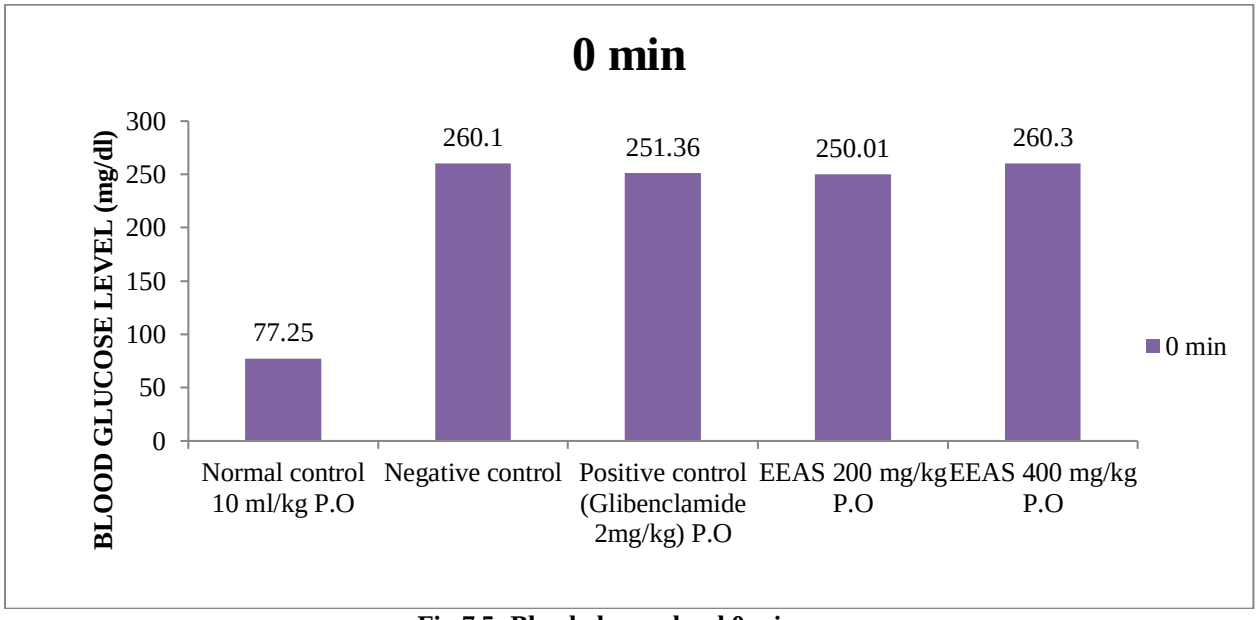


Fig 7.5: Blood glucose level 0 min

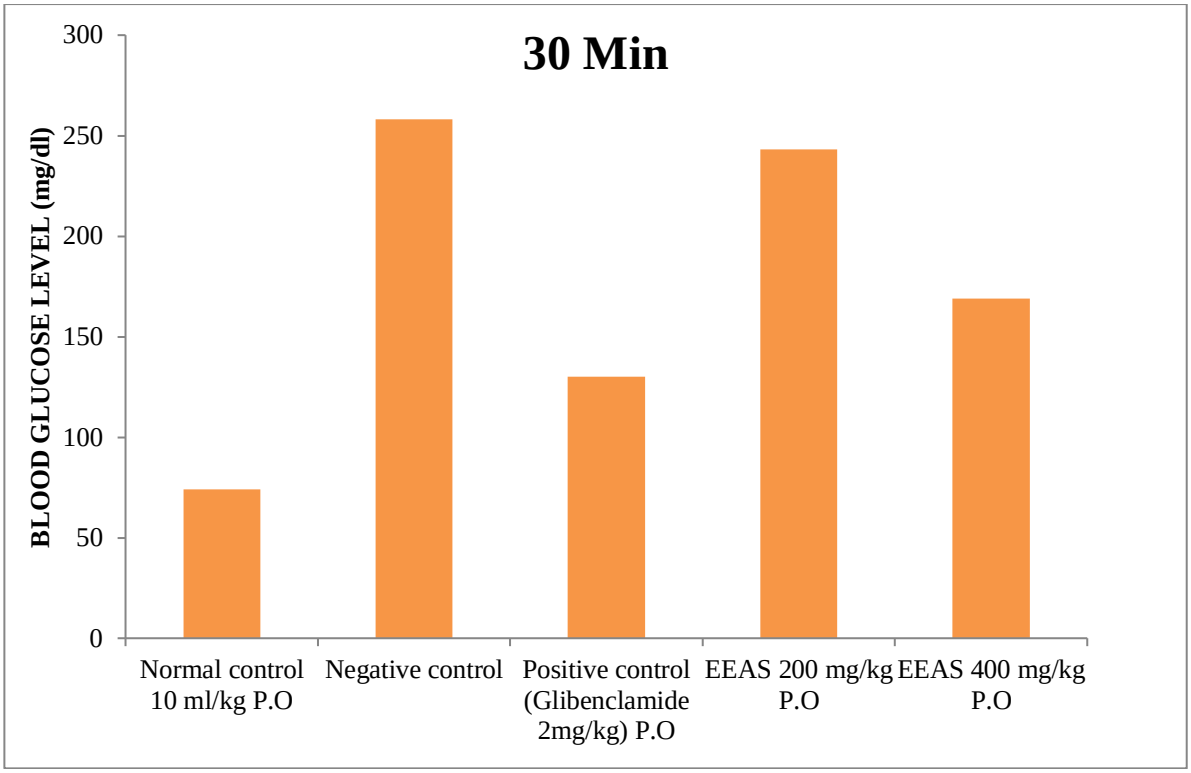


Fig 7.6: Blood glucose level 30 min

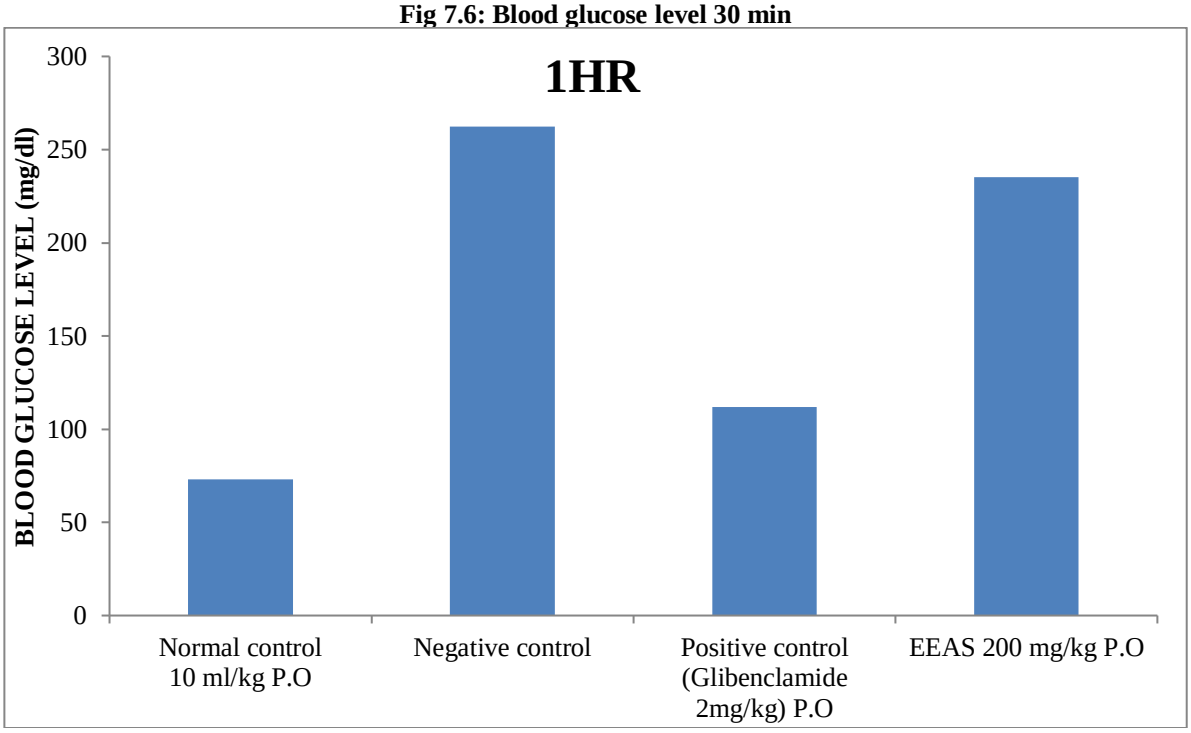


Fig 7.7: Blood glucose level 1 hr

The Ethanolic leaves extract of *Allium sativum* at the dose level 400mg/kg have equipotent activity when compared with positive control at 7th day. The Ethanolic leaves extract of *Allium sativum* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action when compared to control and positive control. On day 14th, Ethanolic leaves extract of *Allium sativum* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level when compared to control and positive control.

Table 7.5: Oral Glucose Tolerance Test

Treatment	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1hr	1.5hr	2hr	2.5hr	3hr
Control (CMC)	0.5%	68.05±1.141	143.2±1.325	184.3±1.120	173.2±10.42	153.2±4.121	150.0±3.142	128.3±9.36
Positive Control Glibenclamide	2	70.24±0.21	104.1±3.154***	112.1±3.24***	93.10±1.121***	80.14±3.011**	74.03±1.201***	70.50±3.512***
EEAS	200	65.10±1.109	123.5±1.001	142.3±1.121*	131.5±0.162*	121.3±0.101*	110.12±0.20**	103.0±2.106**
EEAS	400	67.01±2.141	111.2±0.156**	120±4.116**	100.0±2.211***a	90.12±3.251*** a	83.01±1.02***a	79.03±201***a

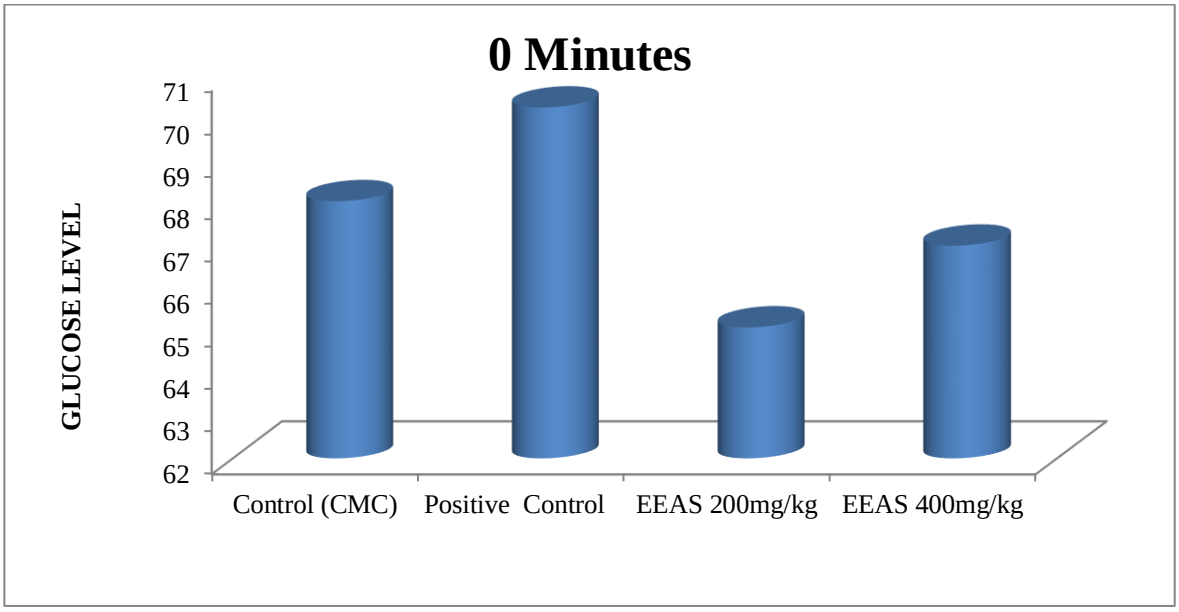


Fig 7.8: Blood Glucose Level (mg/dl) 0 minutes

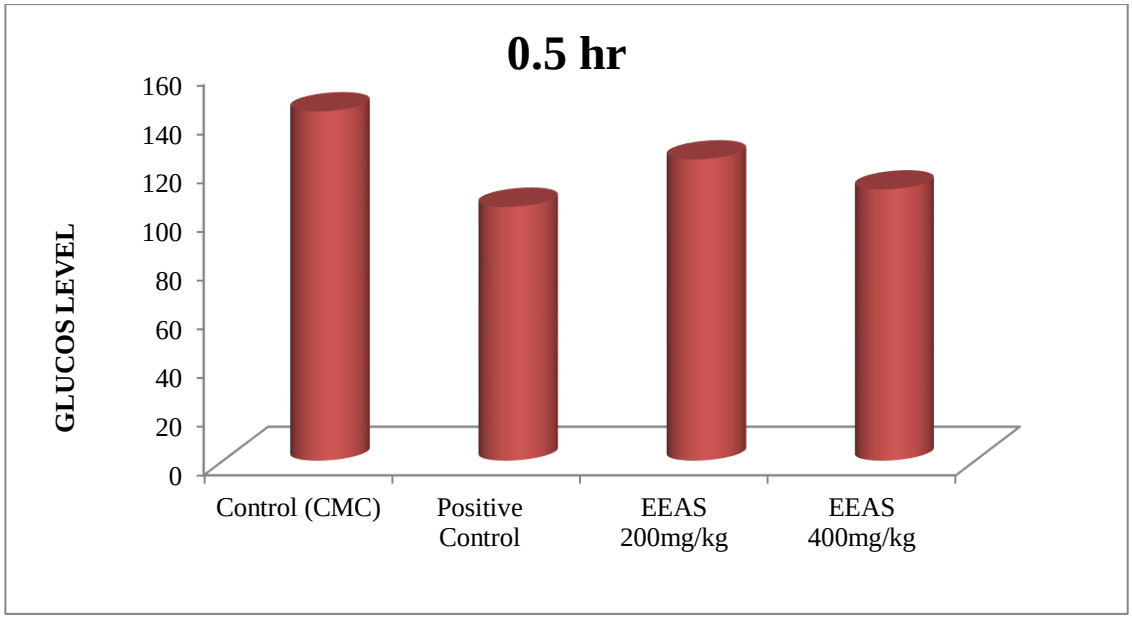


Fig 7.9: Blood Glucose Level (mg/dl) 0.5hr

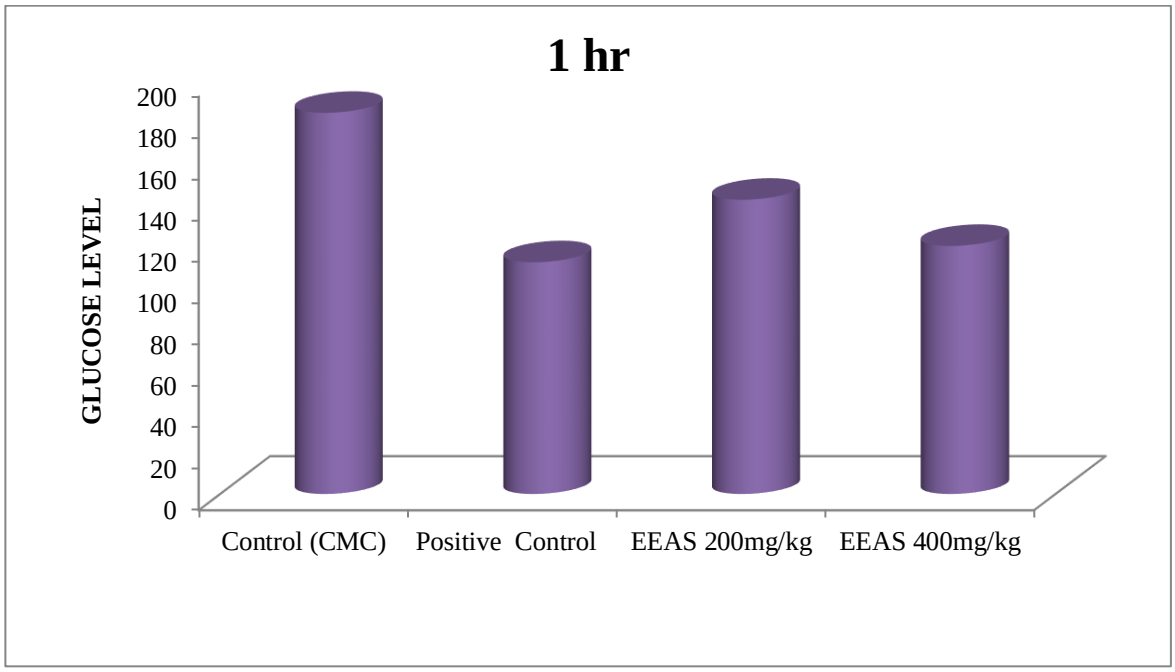


Fig 7.10: Blood Glucose Level (mg/dl) 1hr

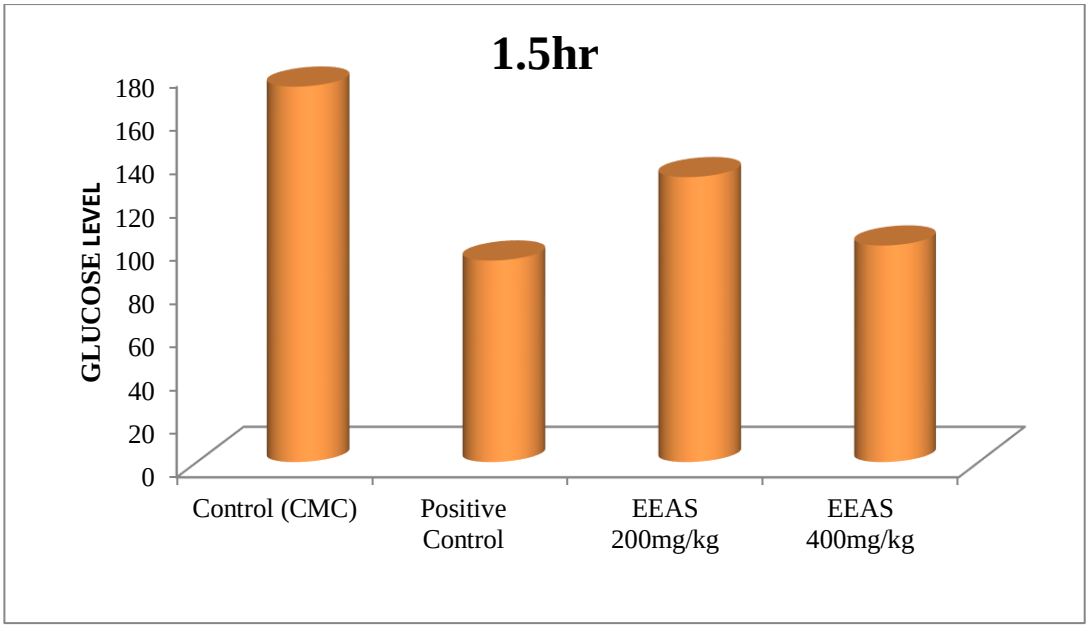


Fig 7.11: Blood Glucose Level (mg/dl) 1.5hr

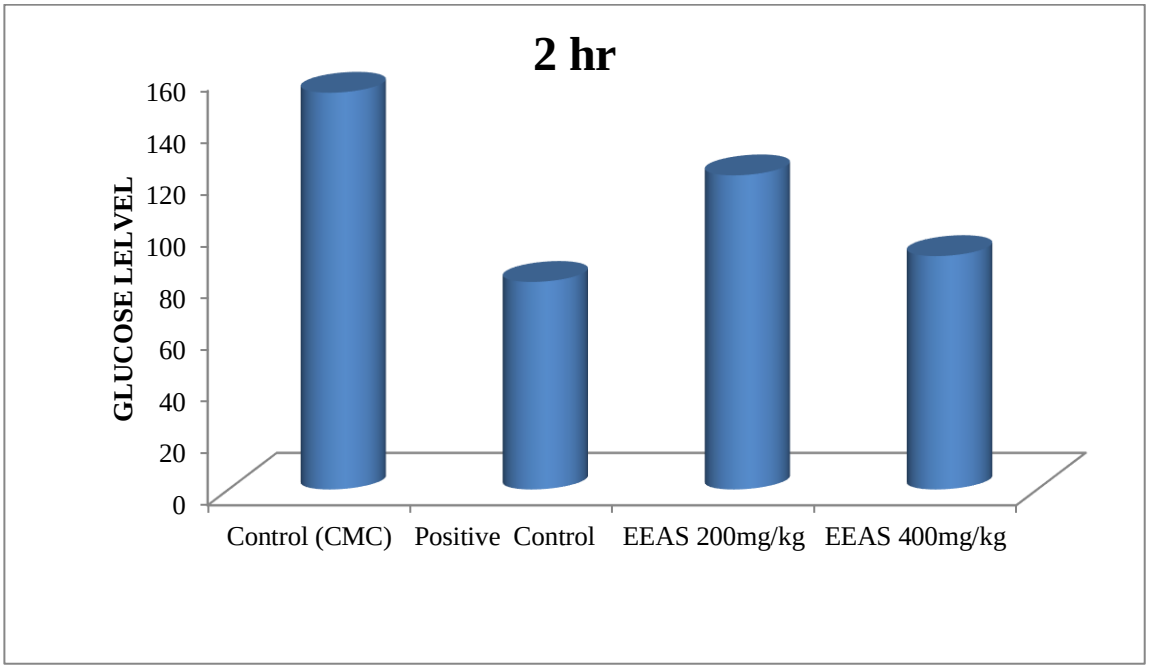


Fig 7.12: Blood Glucose Level (mg/dl) 2 hr

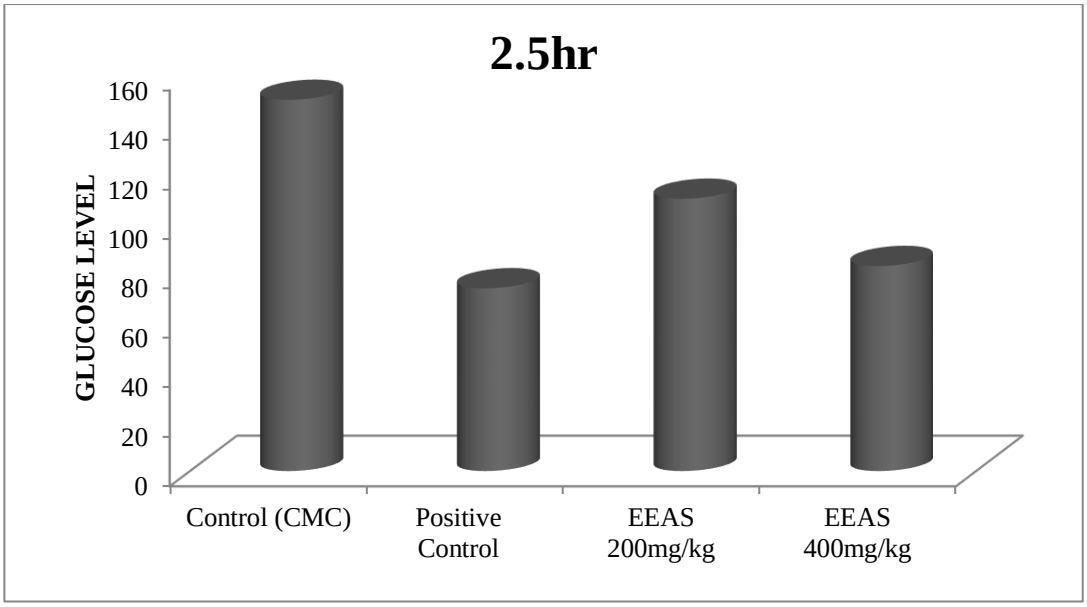


Fig 7.13: Blood Glucose Level (mg/dl) 2.5hr

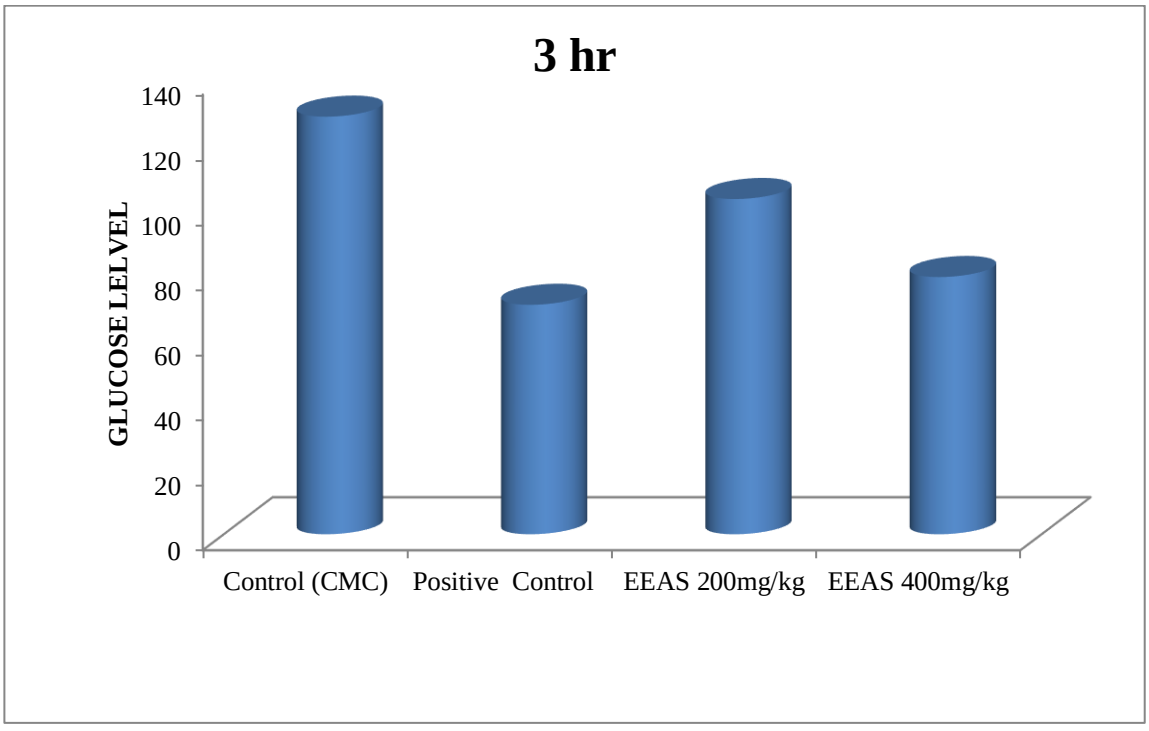


Fig 7.14: Blood Glucose Level (mg/dl) 3hr

Oral Glucose Tolerance Test (OGTT) results have been expressed on Table. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased for, Ethanolic extract of *Allium sativum* 200 & 400 mg/kg when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels (200 mg/kg were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the equipotent activity.

DISCUSSION:

Invitro study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic αamylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order. Each extract was tested for α-amylase inhibition to get the extraction with minimum IC50 value. As per the above mechanism all the extract has concentration dependent affinity

towards the inhibition of α-amylase. Finally, acarbose extract was observed as more active extract.

In this present study acute toxicity study was carried out in Albino Rats. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Average two to three steps may be necessary. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). Observe for signs for toxicity and were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. The principle involved in the Streptozotocin induced diabetes mellitus in Albino Rats, Streptozotocin, a cytotoxic, diabetes induced chemical but wide variety of animal species by damaging the insulin secreting cells of the pancreas.

Literature sources indicate that the Streptozotocin induced Albino Rats are hyperglycaemic. The treatment of lower doses of Streptozotocin (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals become permanently diabetic.

Thus, these animals have surviving β cells and regeneration is possible. It is well known that the sulfonylurea's act by directly stimulating the β-cells of the islets of Langerhans to release more insulin and these compounds are active in mild Streptozotocin induced diabetes. *Invivo* anti diabetic screening was performed for the confirmation of above mechanism of action was undergone the Ethanolic extract of *Allium sativum* biological system (Which was already resulted for α-amylase inhibitory activity.

At the end of the Ethanolic extract of *Allium sativum* (200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. So, the Ethanolic extract of *Allium sativum* showed antidiabetic activity. This work will be useful for further diabetes mellitus and its related diseases research worker to develop new entity for the treatment of diabetes mellitus.

CONCLUSION:

This study revealed that the crude extract and solvent fractions of *Allium sativum* have showed significant lowering of blood glucose level on diabetic, Hypoglycemic and oral glucose loaded Albino Rats and not permitted bodyweight loss of diabetic. The results also verified that inhibition of intestinal α-amylase by the extracts may contribute to the antihyperglycemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. *Allium sativum* would be promising for further clinical studies in the management of DM. Further studies to find out the mechanism of this plant for its antidiabetogenic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for the antidiabetic activity.

The present study suggested that the isolation of active constituents from Ethanolic extract of *Allium sativum* leaf and characterize the compounds by using preliminary phytochemical studies.

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