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Evaluation of Anti-Diabetic Activity of Isolated Fractions of *Allium Sativum*

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Abstract

The present study focuses the determination of the anti-diabetic activity of the extracted fractions of Allium sativum in rats with diabetes induced through STZ. The 20 mg/kg and 40 mg/kg doses of Allium sativum were given to the rats for 28 days. Using the accu-chek active test meter, blood glucose levels were measured to assess the anti-diabetic effects of the isolated fractions. Additionally, a comparison was made with the standard anti-diabetic medication, Pioglitazone, was given to another group of rats at a normal dose of 2.7 mg/kg. The results revealed that Allium sativum had significant anti-diabetic activity. Also, the Allium sativum remained safe till 300 mg/kg in acute toxic and 1000 mg/kg in sub-acute toxic studies and had photochemically. From these findings it can be inferred that Allium sativum has lowered FBG in experimentally induced diabetic rats.

Keywords: *Anti-diabetic activity, Allium sativum, STZ, FBG-fasting blood glucose.*

1. Introduction

Diabetes mellitus is a complex metabolic condition characterized by chronic hyperglycemia and impaired insulin secretion, disrupting carbohydrate, fat, and protein metabolism, as well as insulin action. While type II diabetes primarily affects middle-aged individuals, it's concerning that 55% of diabetes-related deaths occur in women [1]. The global prevalence of diabetes was estimated to be 2.8% in 2000 and is projected to increase to 4.4% by 2030[2]. Current therapies for diabetes can have side effects, prompting the search for effective, safe, and affordable alternatives, such as medicinal plants, which have been used since ancient times for disease prevention and treatment, including diabetes [3]. Advancements in molecular biology and information technology have deepened our understanding of the mechanisms of action of herbal drugs and phytomedicines, which differ in various aspects from synthetic drugs or single chemical entities [4].

Garlic (*Allium sativum* L.) is considered one of the twenty most important vegetables, with various uses throughout the world, either as raw vegetable for culinary purposes, as also an ingredient in traditional and modern medicine.[5] The importance of garlic is due to its use not only for culinary but also for therapeutic and medicinal purposes in both traditional and modern medicine. It is consumed either as raw vegetable (fresh leaves or dried cloves), or after processing in the form of garlic oil, garlic extracts and garlic powder with differences in chemical composition and bioactive compounds content between the various forms.[6] The main responsible compounds for that flavor are sulfur-containing non-volatile amino acids (thiosulfinates), among which alliin or S-allyl-cysteine sulfoxide (ACSO)comprises the most predominant garlic flavor precursors.

Apart from their flavor attributes, these sulfur compounds are also responsible for the renowned medicinal properties of garlic and additionally may improve the biosynthesis of glutathione, from which important antioxidant functions are known. Several sulfur-containing compounds, such as allicin, 1,2-vinyldithiin, allixin and S-allyl-cysteine and sulfides, such as diallyl-, methylallyl-, and dipropyl mono-, di-, tri- and tetra-sulfides), which are formed after the decomposition of thiosulfinates

Quality of garlic, as expressed by chemical composition and bioactive compounds content is highly dependent of both pre- and post-harvest conditions of particular concern must be the objective of

achieving a maximum quality through cultivation practices, genotype selection and growing conditions.[7,8,9]

2. Materials And Methods

Collection of plant material

Fresh garlic bulbs (*A. sativum* L.) were purchased from the local market (T.S., India). The bulbs were planted and after 2 months fresh plants are harvested and chopped into small pieces and kept for drying till there was no moisture left.

Soxhlet extraction method

500g of the whole plant of *Alliuma sativum* was mashed into smaller pieces and placed inside a thimble made from thick filter paper, which was then loaded into the main chamber of the Soxhlet extractor. The extraction solvent used was ethanol. The solvent was heated to reflux at temperature above 100°C for 5 and 10 hours. After the extraction, the products were collected and purified using rotary evaporator at fixed temperature 50°C. After rotovap, the samples were left under fume hood for one hour to make sure all the ethanol left in the oil crude was completely vaporized to the environment.

Test for active compound using HPLC

The active compound in *Alliuma sativum*, e-ajoene was tested using High Performance Liquid Chromatography (HPLC). The HPLC was run using a reversed-phase C18 column. The mobile phase comprising a mixture of n-hexane/10% iso-propyl alcohol in ethyl acetate (67/33) 1 ml/min, detection was done at 254 nm. Time used for the process was 20 min with temperature 38°C. The volume injection for each sample was 50µl, water used was dehydrogenized. The mobile phase was as per previous literature and showed successful, result when comparing the e-ajoene standard. 10µg of sample were diluted in 10ml methanol used for HPLC.[10] The standard used was 95% pure procured from Sigma Aldrich.

Thin layer chromatography

Garlic powder was analysed for the presence of chemical constituents using thin layer chromatography. Test solution was prepared using 1 g of garlic powder dissolved in 5 ml of meth- anol and spotted on TLC plates. Reference solution was pre- pared using 5 mg of alanine in 20 ml of methanol and diluted with distilled water. A mixture of glacial acetic acid: propanol: water: ethanol (20:20:20:20) was used as a mobile phase and ninhydrin reagent was used as spraying reagent.

Animals

Swiss Albino male mice weighing 25 – 30 g for acute and sub chronic toxicity studies and adult Wistar rats of either sex weighing 180-220 g were used for antidiabetic study. The inbred animals were procured from the animal house of Mahaveer Enterprises, Hyderabad. They were housed five per cage under standard lab conditions with a room temperature at 22 ± 2 0C with 12 hr light/dark cycle. The animals were adjusted to lab conditions one week and given standard pellets chow and water ad libitum. Ethical committee clearance was obtained from IAEC of Trinity College of Pharmaceutical sciences, Peddapalli, IAEC/0018/07/2020 CPCSEA.

Toxicity study [11]

The procedure was followed as per OECD guidelines - 423, three male albino mice weighing between 20-25 gm were taken into the study. 300 mg/kg body weight p. o. is taken as the starting dose level of the isolated fractions. Dose was administered accordingly to the overnight fasted mice with water *ad libitum*, food was not given till 3-4 hours post drug administration and seen for the evidence of toxicity.

Body weights of the mice were taken at the start and end of the treatment, monitored for any alterations in eyes, skin, fur and mucous membranes and any systems like circulatory, respiratory, central, autonomic nervous systems, behavior pattern and locomotor activity and signs like convulsions, tremors, salivation, lethargy, diarrhea, sleep and coma were took a note. Both the onset and signs of toxicities if any were observed for 14 days.

Sub-chronic toxicity study [12]

The below experimental procedure was used to determine the sub-chronic toxicity of *Allium sativum* in mice. Group I: Control animals received 10% tween 20, 2 ml/kg/p. o. for 28 days. Group II: Isolated fractions of at a dose level of 1000 mg/kg/p. o. suspended in 10% tween 20; 2 ml/kg/p. o. for 28 days. Food-water intakes and body weight were noted twice per day with subsequent review for any toxic modulation and mortality. All animals were immolated by the end of 28 day treatment period, under

anesthesia using over dose ether. Blood was taken from the jugular vein in anticoagulant pretreated tubes and shaken gently and was used for estimation of hemogram and leukogram using fully automatic hematology analyzer. Liver, spleen, brain, heart, kidney, lung, testis and ovaries were separated and preserved for histopathologic study using 10% formalin.

Induction of diabetes

Streptozotocin 90 mg/kg (Acetate buffer 0.1M freshly made, having pH 4.5) was given intraperitonially to the neonatal rats of 10-12 g weight on day five, postnatally.[13] Freshly prepared buffer serves as control was also given in the same way to the neonatal rats. After four weeks, all these rats were segregated from their mothers, provided with standard pellet feed (Rayan's Biotech, Hyderabad) along with water ad libitum.

Experimental design

Sl.no	Grouping of animals
1	**Grouping of animals**
	Group I - Normal Rats (vehicle control)
	Group II - Rats serve as negative control
2	**Pretreated set**
	Group III - Rats given *Allium sativum* 20 mg/kg
	Group IV - Rats given *Allium sativum* 40 mg/kg
	Group V - Rats given Pioglitazone 2.7 mg/kg
3	**Post treated set**
	Group VI- Rats given *Allium sativum* 20 mg/kg
	Group VII- Rats given *Allium sativum* 40 mg/kg
	Group VIII - Rats given Pioglitazone 2.7 mg/kg

Rats were categorized into two sets, one is pre-treatment and other is post-treatment (i.e. after taking streptozotocin, they remain untreated for 12 weeks), both have five groups (n = 10) each, of the pre-treatment groups, administration of drugs starts from 4th week of STZ administration till 21st day after 12 weeks whereas in the case of post treated groups, fractions are given after 12th week of taking streptozotocin for 21 days. Group I is to serve as control, group II as negative control, takes only vehicle. Pre-treated set has five groups from group III to group VII, which were treated in the way as explained above. *Allium sativum* and pioglitazone were given as suspension in 10% tween 20 (vehicle) p. o. Dilutions were made as such to give 0.2ml/100g intra-gastrically. Negative control group received vehicle alone. Post treated set also has five groups, but they remain untreated till 12th week after streptozotocin is given. All treatments were given intra-gastrically.

Oral glucose tolerance test (OGTT)

OGTT was done in both the pre-treated and post treated groups on 7^{th} and 12^{th} week after the streptozotocin treatment. An extra four groups of normal rats with similar age were used to study the effects of these treatments on OGTT in normal rats. The effect of the fractions on glucose overloaded hyperglycemia was learned in all the groups. Normal rats kept under fasting overnight nearly 12h, were taken into 6 groups (n = 6) of which group I being a control, group II, III, IV, V and VI were given *Allium sativum* 20, 40 mg/kg p. o. respectively, group VII rats were given 2.7 mg/kg of pioglitazone intra-gastrically. Group III to VII are pre-treated set whereas post-treated set remain untreated. Zero-hour sample was measured for blood glucose levels by tail vein puncture. Animals were given oral glucose (4g/kg BW) after half an hour past drug administration and the blood glucose levels were measured at 0.5, 1, 2 and 3 h past glucose administration [14]. Blood glucose levels were read through a glucometer.

Hypoglycemic effect in n5-STZ rats after chronic administration

After OGTT, was done on 12th week after taking streptozotocin, both pre and post treatment rats were used to find the effect on the levels of blood glucose. Rats having more than 150 mg/dL blood glucose concentrations were regarded diabetic and taken into the study [15]. All the rats were given isolated fractions and pioglitazone as stated before. Blood glucose levels were measured through glucometer (Accu-chek ActiveTM Test meter) by tail vein puncture on days 1, 7, 14 and 21, 30 min past drug administration.

Effect on diabetes

Induction of diabetes mellitus in experimental animals

Diabetes was produced in male wistar albino rats of 2–3 months age (180–200 g body weight) by giving streptozotocin (single dose of 55 mg/kg B.W.) intraperitoneally, made by dissolving in freshly prepared 0.01 M citrate buffer with pH 4.5. After taking STZ, the animals were given food and water ad libitum and 5% glucose given with drinking water for the initial 24 hours to balance any hypoglycemia. The generation of diabetes was established past 72 hours of STZ injection, under light anesthesia the blood was drawn by cutting the tip of tail of each rat and the blood glucose concentration was measured. Animals with > 200 mg/dl blood glucose were regarded diabetic and divided into groups accordingly.

Sl.no	**Grouping of animals**
1	**Grouping of animals**
	Group I - Normal Rats (vehicle control).
	Group II - Rats served as negative control
2	**Pretreated set**
	Group III - Rats given *Allium sativum* 20 mg/kg
	Group IV - Rats given *Allium sativum* 40 mg/kg
	Group V - Rats given Pioglitazone 2.7 mg/kg

Experimental design

The animals were sorted into seven groups each having six rats. Group I were normal rats, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats. Group III and group IV were given *Allium sativum*. group V rats were given pioglitaozone (PIO) 2.7 mg/kg for 28 days. Blood glucose concentrations under fasted state were noted during the pre-administration of fractions on 1st, 7th, 14th, 21st and 28th days of treatment period. Blood was collected by making an incision on the rat tail. Blood glucose concentrations were measured through a glucometer (Accu-chek ActiveTM Test meter). Effect on liver glycogen and glucose-6-phosphatase was measured accordingly glycogen was analyzed in fresh isolated livers of anesthetic state rats (sodium thiopental, 50 mg/kg). Parts of nearly 2 g were done homogenization and extraction with 8 ml of 6% HClO4. The floating liquid in the upper part was under neutralization with 5 N K_2CO_3 and taken into the enzymatic glycogen assay [16]

Statistical analysis

All the values were expressed as mean ± standard error (SEM). One way analysis of variance followed by Dunnet's test comparing with p less than 0.05 were noted significant among the groups.

3. Results and Discussion

Table 1: Phytochemical screening of *Allium sativum*

Sr.No.	Phytochemical constituents	Test	Methanol extract	Water extract
1	Alkaloids	Maverstest	+	+
		Dragendroffstest	+	+
		Wagnerstest	+	+
		Hagerstest	+	+
2	Glycosides	Borntraoerstest	+	+
		Legalstest	+	+
3	PhenolicsandTannins	Ferricchloridetest	+	+
		Gelatinetest	+	+
		Leadacetatetest	+	+
4	Flavonoids	Shinodatest	++	+
5	Proteins	NinhydrinandMillonstest	+	+
6	Coumarin	Sodiumhydroxidetest	++	++
7	Saponins	Frothtest	+	+

+=Presence; ++=More abunclantly present

Isolated fractions

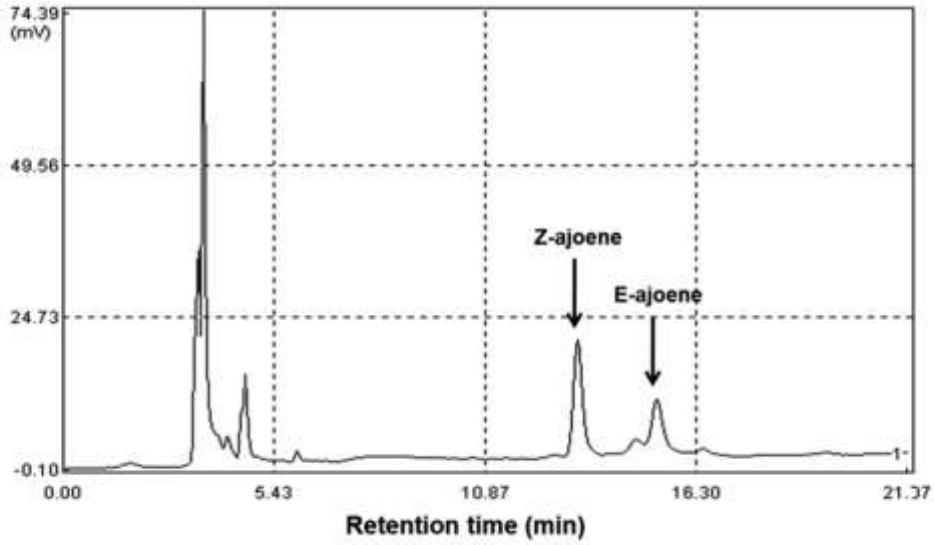


Figure 1. Chromatogram of ajoene extract in high performance liquid chromatography (HPLC).

HPLC conditions are as follows, column: Spherisorb silica, 5 µm, 4.6×250 mm, eluent: n-hexane/10% iso-propyl alcohol in ethyl acetate (67/33) 1 ml/min, detection 254 nm.

Figure 2. TLC OF *Allium sativum* SAMPLE

The test sample of *Allium sativum* run through mobile phases and indicated Rf 0.21 and 0.22 when compare with the standard samples.

Acute oral toxicity study

The acute oral toxicity study was conducted in accordance with the OECD guidelines 423 (Acute toxic class procedure). A beginning dose of 300 mg/kg *Allium sativum* fractions were given to three male mice and monitored for three days. No significant change is there in body weight in group with and without taking treatment and no toxicity signals were seen even with the repeated experiments at same dosing level, the mice were monitored for 14 days, no alterations were there in the first experimental set. LD_{50} cut off mg/kg B.W was seen as above 300mg/kg bw and globally harmonized system (GHS) classes also come in category 4, as the dose studies are limited to 300 mg/kg bw.

Table 2: Acute oral toxicity studies

S.No	Drug treatment	Dose	Weight of animal Group		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration in days
			Before treatment (1st day)	After treatment (14th day)				
1.	*Allium sativum*	300 mg/kg	22	26	No	-	-	14
2.	*Allium sativum*	300 mg/kg	27	33	No	-	-	14
3.	*Allium sativum*	300 mg/kg	22	25	No	-	-	14

Sub chronic toxicity

Allium sativum fractions at the dose of 1000 mg/kg p.o were administered for 28 days. The changes in body weight, food and water intake were observed during the study. No prominent changes were seen. Drug treated mice does not exhibit any hematological alterations like Hb, red blood cells (RBC), white blood cells (WBC) or differential leukocytes such as neutrophils, monocytes, eosinophils, basophils and lymphocyte values compared to normal control animals.

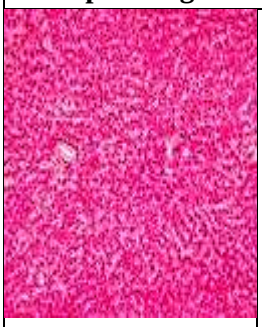
Table 3: Hematological parameters of mice after sub chronic toxicity studies

HEMATOLOGICAL PARAMETERS	Control	*Allium sativum* 1000 mg/kg p.o.	*Allium sativum* 1000 mg/kg p.o.
Erythrocytes ($x10^{12}$/l)	5.95 ± 0.31	5.75 ± 0.42^{ns}	5.2 ± 0.1^{ns}
Leukocytes ($x10^{9}$/l)	3.32 ± 0.15	3.6 ± 0.14^{ns}	3.34 ± 0.4^{ns}
Hematocrit (%)	0.42 ± 0.02	0.4 ± 0.01^{ns}	0.61 ± 0.02^{ns}
Hemoglobin (g%)	13.3 ± 1.42	**13.34 ± 2.15^{ns}**	13.14 ± 1.23^{ns}
DIFFERENTIAL COUNT per/cmm			
Neutrophils ($x10^{9}$/l)	2.35± 0.32	2.48 ± 0.24^{ns}	2.45 ± 0.3^{ns}
Eosinophils ($x10^{9}$/l)	0.08 ± 0.003	0.08 ± 0.004^{ns}	0.08 ± 0.002^{ns}
Lymphocytes ($x10^{9}$/l)	3.13 ± 0.18	3.43 ± 0.85^{ns}	3.14 ± 0.68^{ns}
Monocytes ($x10^{9}$/l)	0.14 ± 0.02	0.17 ± 0.05^{ns}	0.15 ± 0.03^{ns}
Basophils ($x10^{9}$/l)	0.02 ± 0.0014	0.03 ± 0.0015^{ns}	0.02 ± 0.002^{ns}

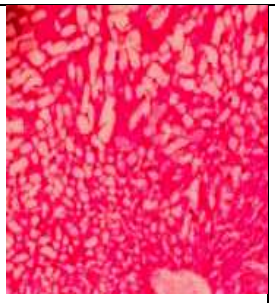
Histopathological Effects

Histopathological examination of internal organs like kidney, liver, heart, spleen, lungs, testis, brain and ovary did not exhibit changes in their normal architecture suggesting no damage caused by both the fractions.

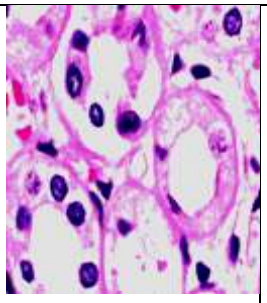
Histopathological slides of control and Allium sativum (CZ) treated mice tissues



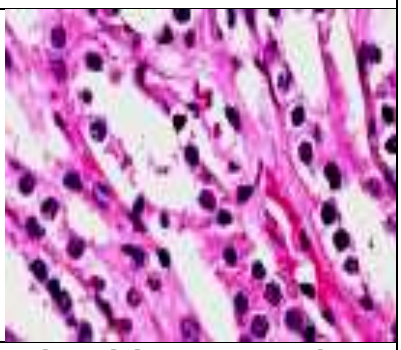
Liver slide- control



Liver slide-cz 1000mg/kg



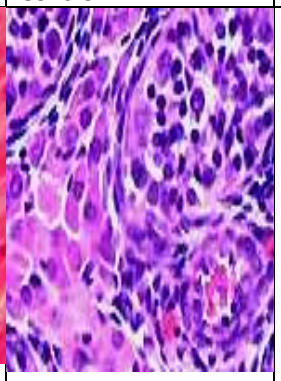
Kidney slide- control



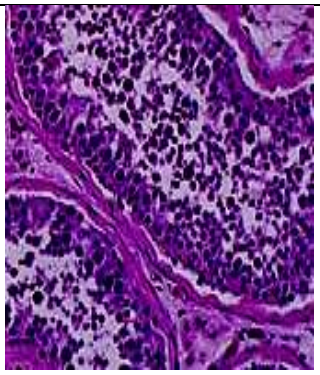
Kidney slide - cz 1000mg/kg

Heart slide – control

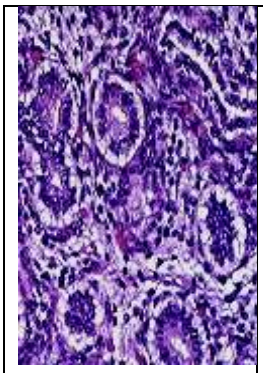
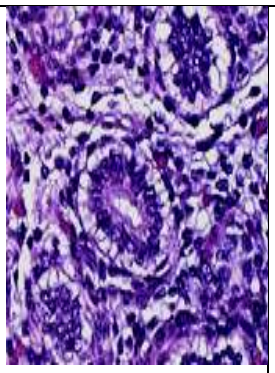
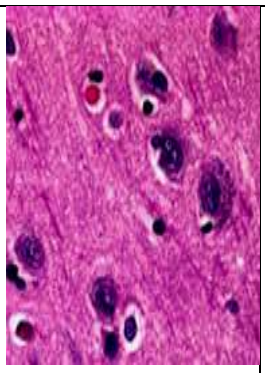
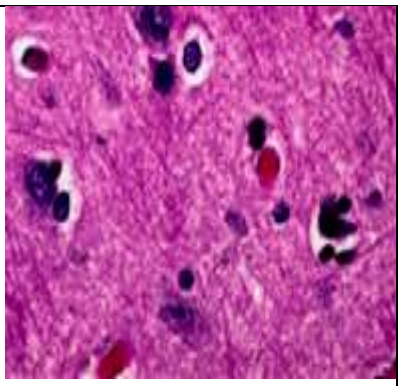
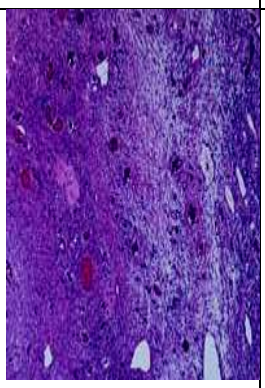
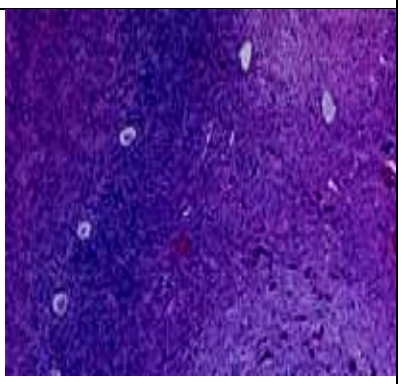
Heart slide cz - 1000mg/kg



Slide - testis-control



Slide -testis – cz 1000mg/kg

			
Slide -lung-control	Slide -lung- cz 1000mg/kg	Slide – brain-control	Slide- brain- cz 1000mg/kg
			
Slide- pancreas-control	Slide- pancreas- cz 1000mg/kg	Slide - ovary-control	Slide - ovary- cz 1000mg/kg

Liver slides control- slide showing that normal hepatocytes with central vein with the hepatocyticcords. *Allium sativum* 1000mg/kg- slide showing that normal liver cells with central vein with the hepatocytic cords. Kidney slides control- slide showing that normally nephron glomeruli capsule and the renal tubules *Allium sativum* 1000mg/kg- slide showing that normal glomeruli capsule in kidney and kidney tubules. Heart slides control – slide showing that the normal car- showing diac myofiber *Allium sativum* 1000mg/kg- slide showing that the normally cardiac myofober. Testis slides control – slide showing that the normal testicular tubules with spermatogenesis normally. *Allium sativum* 1000mg/kg- slide showing that normal tubules with normal spermatogenesis in the testis.

Lungs slides control – slide shows normal lung tissues with bronchi & alveoli cells. *Allium sativum* 1000mg/kg- slide shows normal lung's tissue with bronchi and cells of alveoli. Brain slides control – slide shows normal tissues. *Allium sativum* 1000mg/kg- slide shows normal tissue. Pancreas slides control – slide shows normal pancreatic β-islets. *Allium sativum* 1000mg/kg- slide shows normal pancreatic cells. Overy slides control – slide 47- showing that normal ovary with maturing follicles cells. *Allium sativum* 1000mg/kg- slide 48- showing that normal ovary with maturing follicles.

Effect of Glucose Administration

Effect of blood glucose levels after oral glucose tolerance test in normal rats treated with drugs, glucose administration had shown a marked enhancement in the blood glucose levels of control rats from 0.5 hr and remained significant for 1st and 2nd hours respectively.

Table 4: Effect of isolated fractions on glucose overloaded hyperglycemia in normal rats

Treatment	Blood glucose levels (mg/dL) at various intervals (days)				
	0	0.5	1	2	3
Control	79 ± 2.5	148.3 ± 5.8 $^{b\#}$	163.4± 5.3 $^{b\#}$	134.4 ± 5.8 $^{b\#}$	88.6 ± 3.8^{bns}
Allium sativum 20 mg/kg	72 ± 3.3^{ans}	135.4 ± 4.9$^{ans\ b\#}$	143.8 ± 5.9$^{ans\ b\#}$	121.8 ± 5.3$^{ans\ b**}$	82.8 ± 1.6^{ansbns}

Allium sativum 40 mg/kg	66 ± 3.4^{ans}	123.5 ± 3.4$^{ans\ b\#}$	129.4 ± 3.5 $^{a*\ b**}$	102.5 ± 2.8 $^{a**\ b*}$	74.5 ± 1.3^{ansbns}
Pioglitazone 2.7mg/kg	67 ± 1.6^{ans}	135.6 ± 4.4$^{ans\ b\#}$	114.8 ± 4.4 $^{a**\ b**}$	94.4 ± 2.8 $^{a**\ bns}$	74.6 ± 3.4^{ansbns}

Data represents mean ± SEM of blood glucose levels. a = represents comparison of blood glucose levels of all the groups (n=6) with that of control, b = blood glucose levels at various time intervals compared with 0 hr blood glucose levels using one way ANOVA followed by Dunnett's test. *p<0.05; **p<0.01; #p<0.001, ns-non significant.

The various blood glucose values are indicative of the intricate balance between carbohydrates absorbed from the gut, hepatic glucose output/uptake, and peripheral glucose uptake. Hepatic glucose yield was reviewed by the blood glucose values in fasting and resting state and is the sum of hepatic glucose output at the two-hour test value and glucose load. The fasting and two-hour blood glucose values related to the inception of particular micro vascular diabetic complications (nephropathy, retinopathy and neuropathy) and macrovascular issues (atherosclerotic vascular disorder) were seen and the values were regarded as diagnostic for the absence or presence of diabetes or pre-diabetes.

Effect of OGTT on 7th week pretreated rats

The effect on OGTT on 7th week in rats treated with drugs, a marked increase p<0.05 was seen in the blood glucose concentrations of control at 0 hour compared to other groups. Experimental values were clearly. *Allium sativum* 20 mg/kg showed a marked reduction in the blood glucose levels at 1, 2 and 3 hours after glucose over load at p<0.05, p<0.05 and p<0.01 respectively, compared to negative control, *Allium sativum* 40 mg/kg exhibited a marked reduction p<0.05 in the blood glucose levels at all the time points after glucose overload.

Table 5: Effect of isolated fractions on OGTT on 7th week in pretreated rats

Effect of OGTT on 7th week in post treated rats

Treatment	**Blood glucose levels (mg/dL) at various intervals (days)**				
	0	0.5	1	2	3
Control	73 ± 5 ^{a*}	158 ± 13.5$^{ans\ b\#}$	144 ± 8.4$^{ans\ b*}$	122 ± 5.9 $^{a*\ b*}$	96 ± 4.3 $^{a**\ b*}$
Negative control	117 ± 11.4	185 ± 14.4	173 ± 6.4	164 ± 12.4	148 ± 8.4
Allium sativum 20 mg/kg	97 ± 4.4^{ans}	138 ± 3.8$^{ans\ b*}$	123 ± 4.4 $^{a*\ b*}$	105 ± 3.8 $^{a*\ bns}$	97 ± 8.4 $^{a**\ bns}$
Allium sativum 40 mg/kg	112 ± 3.3^{ans}	133 ± 9.2 $^{a*\ bns}$	117 ± 8.6 $^{a*\ bns}$	109 ± 5.5 $^{a*\ bns}$	104 ± 9.4 $^{a*\ bns}$
PIO 2.7mg/kg	97 ± 5.8^{ans}	139 ± 6.5$^{ans\ b*}$	115 ± 5.9 $^{a**\ b*}$	95 ± 3.4 $^{a\#\ bns}$	80 ± 2.8 $^{a**\ bns}$

In the effect on OGTT on 7th week in rats which did not receive prior treatment (post treated rats), a marked increase p<0.001 in blood glucose was observed in all the groups compared to control rats at 0-hour, treatment with *Allium sativum* 20 mg/kg.

Table 6. Effect of isolated fractions on OGTT on 7th week in post treated rats.

Treatment	**Blood glucose levels (mg/dL) at various intervals (days)**				
	0	0.5	1	2	3
Control	67 ± 1.7 $^{a\#}$	154 ± 6.4$^{ans\ b\#}$	136 ± 2.3 $^{a*\ b\#}$	106 ± 2.6 $^{a**\ b*}$	94 ± 1.6 $^{a**\ bns}$
Negative control	122 ± 4.3	186 ± 8.3	173 ± 4.3	159 ± 4.3	138 ± 3.4
Allium sativum 20 mg/kg	113 ± 3.8^{ans}	165 ± 3.3$^{ans\ b*}$	145 ± 6.4^{ansbns}	135 ± 3.6^{ansbns}	117 ± 4.8^{ansbns}
Allium sativum 40 mg/kg	120 ± 5.4^{ans}	158 ± 3.8$^{ans\ b*}$	134 ± 4.6 $^{a*\ bns}$	125 ± 4.4 $^{a*\ bns}$	118 ± 3.2 $^{a*\ bns}$
PIO 2.7mg/kg	123 ± 2.3^{ans}	159 ± 2.8^{ansbns}	128 ± 3.8 $^{a*\ bns}$	130 ± 2.3^{ansbns}	114 ± 3.8^{ansbns}

Effect of OGTT on 12th week in post treated rats

The effect on OGTT on 12th week in rats which did not receive prior treatment (post treated rats), a marked increase p<0.001 in blood glucose was observed in all the groups compared to control rats at 0 hour, treatment with *Allium sativum* 20 and 40 mg/kg didn't show any marked changes in the blood glucose concentrations compared to negative control, a marked rise p<0.05 in the blood glucose concentrations were observed 30 minutes after glucose overload with *Allium sativum* 20 mg/kg and a significant increase was observed after 30 minutes and 1 hour of glucose overload in *Allium sativum* 40 mg/kg treated rats.

Table 7: Effect of isolated fractions on OGTT on 12th week in post treated rats.

Treatment	Blood glucose levels (mg/dL) at various intervals (days)				
	0	0.5	1	2	3
Control	73 ± 2.2 a#	165 ± 3.4ansb#	133 ± 4.6a**b**	106 ± 2.7a#b*	84 ± 3.9a#bns
Negative control	195 ± 9.6	267 ± 12.5 b**	254 ± 11.8b*	236 ± 10.8b*	225 ± 15.8b*
Allium sativum 20 mg/kg	203 ± 10.4ans	275 ± 14.2ans b*	254 ± 9.4ansbns	224 ± 6.4ansbns	218 ± 12.6ansbns
Allium sativum 40 mg/kg	188 ± 8.4ans	264 ± 11.5ans b*	248 ± 10.4ansb*	234 ± 11.6ansbns	233 ± 13.5ansbns
PIO 2.7mg/kg	203 ± 9.5ans	285 ± 11.8ans b**	265 ± 8.3ansb*	250 ± 13.6ansbns	243 ± 11.5ansbns

In this study the hypoglycemic activity of the *Allium sativum* fractions were evaluated in streptozotocin induced diabetic rats. Both the fractions significantly reduced the blood glucose amounts compared to the standard drug. Acute and sub-acute toxicities of the fractions were tested and LD50 cut off mg/kg B.W was seen as above 300mg/kg b.w. and globally harmonized system (GHS) classes also come in category 4, as the dose studies are limited to 300 mg/kg b.w. Drug treated mice does not exhibit any haematological alterations compared to normal control animals. Histopathological examination of internal organs did not exhibit changes in their normal architecture suggesting no damage caused by both the fractions.

Pretreatment with *Allium sativum* 20 mg/kg shows a marked decrease in blood glucose concentrations on first ($p<0.05$), 7th ($p<0.01$), 14th ($p<0.001$) and 21st day ($p<0.001$) compared to the blood glucose concentrations of negative control group, while the decrease in blood glucose concentrations were prominent on 14th ($p<0.05$) and 21st ($p<0.01$) days only, compared to blood glucose concentrations of day one. *Allium sativum* 40 mg/kg exhibited a marked decrease in blood glucose concentrations on 1ST ($p<0.01$), 7th ($p<0.001$), 14th ($p<0.001$) and 21st days ($p<0.001$) compared to blood glucose concentrations of negative control group, while the decrease in blood glucose concentrations were prominent on 14th ($p<0.05$) and 21st ($p<0.01$) days only, compared to blood glucose concentrations of day one.

Post treated *Allium sativum* 20 mg/kg did not exhibit a marked decrease in the blood glucose concentrations on 14th and 21st days ($p<0.05$) and $p<0.001$ respectively compared to negative control group and 14th ($p<0.05$) and 21st ($p<0.001$) days compared to basal blood glucose concentrations on day one and treatment with *Allium sativum* 40 mg/kg did not show a marked reduction on 7th ($p<0.05$), 14th ($p<0.01$) and 21st day ($p<0.001$), compared to negative control group and 7th ($p<0.05$), 14th ($p<0.01$) and 21st days ($p<0.001$), compared to basal blood glucose concentrations on day one. The results are comparable with that of standard treated groups.

In the post treatment rats, the basal blood glucose amounts were more than those seen in rats which take isolated fractions and pioglitazone from 4th week, whose protective action on pre-treated was described through the certainty that there causes the pancreatic β-cells disruption, there will be insulin sensitivity, evidencing the less basal blood glucose amounts in them. In conclusion, utilizing these drugs as prophylactic in basal hyperglycemic stage persons could decrease the risk of progressing into T2DM and also have therapeutic importance in the treating T2DM.

In the study on the effect of stz induced diabetes, diabetic rats with blood glucose levels above 175mg/dl were taken, treatment with the isolated fractions of *Allium sativum* 20 mg/kg exhibited a marked decrease in the blood glucose amounts on 14th ($p<0.05$), 21st ($p<0.05$) and 28th day ($p<0.01$) compared to blood glucose amounts on day one.

Longer term treatment (28 days) with active fraction of *Allium sativum* produced mild advancement in plasma insulin amounts. This proposes that *Allium sativum* like Pioglitazone initiates insulin secretion from the residual beta cells of islets of Langerhans or the drug might imitate one or more activities of insulin at the receptor level or/and it might impact one or more post receptor events.

4. Conclusion

Acute and sub-acute toxicities of the fractions were tested and LD^{50} cut off mg/kg B.W was seen as above 300mg/kg b.w. and globally harmonized system (GHS) classes also come in category 4, as the dose studies are limited to 300 mg/kg b.w. Drug treated mice does not exhibit any haematological alterations compared to normal control animals. Histopathological examination of internal organs did not exhibit changes in their normal architecture suggesting no damage caused by both the fractions. The isolated *Allium sativum* fractions shown marked hypoglycemic activity on STZ induced diabetes.

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