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ANTIDIABETIC ACTIVITY OF ETHONOLIC EXTRACT OF *Allium Cepa* SEEDS AGAINST ALLOXAN INDUCED DIABETIC RATS

Ayyanna Chakali ^{*1}, Sekar Munuswamy ², Niranjan kumar Raghupathi ³, Ravi kumar Kota ⁴^{1*}Department of Pharmacology, CES college of pharmacy, Kurnool-518001, Andhra Pradesh, India.²Department of Pharmaceutical chemistry, Santhiram College of Pharmacy, Nandyal-518501, A.P, India.³Department of Pharmacology, Santhiram College of Pharmacy, Nandyal-518501, A.P, India.⁴Department of Pharmaceutics, Santhiram College of Pharmacy, Nandyal-518501, A.P, India.

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*Corresponding author:

Ayyanna Chakali

Email:

ayyannac5@gmail.com

Tel.: +91-9885020087.

ABSTRACT:

Background: Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both. Alloxan was used to induce diabetes mellitus in experimental rats and protective activity of ethanolic extract of *Allium cepa* seeds was studied.

Methods: The present study was carried out on Wister albino rats, where Diabetes mellitus was induced by alloxan. Rats were randomly separated into five groups and normal group animals received 2% acacia, control group animals received alloxan dose of 150 mg/kg, standard group animals received glibenclamide dose of 0.5 mg/kg and test group animals received ethanolic extract of *Allium cepa* seeds dose of 200 and 400 mg/kg for 21 days. Different biochemical parameters were used to determine the blood glucose and lipid profile was studied.

Results: Ethanolic extract of *Allium cepa* seeds (EEACS) showed significant dose dependent decrease in blood glucose, cholesterol, triglycerides, LDL, VLDL levels and significant dose dependent increase in HDL levels.

Conclusion: EEACS possesses significant and dose dependent antidiabetic effects which was more pronounced at the dose of 400 mg/kg against alloxan induced Diabetes mellitus.

Keywords: Diabetes mellitus, alloxan, Glibenclamide, *Allium cepa* seeds.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism due to deficient action of insulin on target tissues resulting, from defects in insulin secretion, insulin action, or both [1, 2]. Diabetes mellitus may present with characteristics symptoms such as polyphagia, polydipsia, polyuria, blurring of vision and weight loss.

Treatment of type-2 is complicated by several factors inherent to the disease process, typically, insulin resistance, hyperinsulinemia, impaired insulin secretion and reduced insulin-mediated glucose uptake and utilization [3, 4]. The most frequently used i.v dose of

this drug to induce diabetes in rats is 65 mg/kg b.w. [5, 6]. When alloxan is given i.p or sub-cutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg between may be insufficient for inducing diabetes in the rat [7, 8]. Fasted animals are more susceptible to alloxan [7], [9]. Diabetogenic action of alloxan is mediated by reactive oxygen species. The action of alloxan in the pancreas is preceded by its rapid uptake by the β -cells [10, 11].

MATERIALS & METHODS

Collection of seeds

Allium cepa seeds were collected from local market, Kurnool and were identified pharmacognostically by a botanist. Then powdered, weighed and stored in a

clean, dry and air tight container. The powder was subjected to successive extraction with solvents n-hexane (n-hexane used to remove fatty substances) and ethanol.

Ethanol extraction procedure

The dried powder was defatted by using n-hexane with maceration technique. The defatted powder is dried at room temperature. After that the dried defatted powder was extracted with ethanol at 70°C by soxhlet apparatus. The solvent present in the extract removed by distillation and dried to get a solid mass.

Experimental animals:

Either sex Wister rats weighing about 150 to 180 g were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee of Santhiram College of Pharmacy (1519/PO/a/11/CPCSEA). Animals were obtained from Sainath enterprises, Hyderabad. Rats were housed in polyacrylic cages (38x23x10 cm). They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12 h light/ 12 h dark) and maintained humidity 60±5% and an ambient temperature of 25±2%. All experiments were performed between 9:00 am to 4:00 pm. The animals were free access to standard diet and water *ad libitum* and allowed to acclimatize for one week before the experiments.

Drugs and chemicals:

N- Hexane -Moly chem. India pvt. Ltd, Mumbai,
Ethanol -Santhiram College of pharmacy
Alloxan -NP CHEM-BOMBAY,
Kits-(Glucose, LDL, TG, HDL.) Excel diagnostics Pvt. Ltd. Hyderabad.

Acute toxicity study

The acute toxicity study was carried out for ethanolic extract of *Allium cepa* seeds (EEACS) using fixed dose method according to OECD guideline no.423. Healthy adult Wister rats weighing between 150 to 170 g were used for study. Animals were divided into five groups of six animals each and kept fasted overnight. The different doses like 500, 1000, 2000, 3000 and 4000 mg/Kg b.w. were administered to the group I, II, III, IV, V respectively. After administering the ethanolic extract of *Allium cepa* seeds to different groups the mortality and behavioural changes like body temperature, CNS activity, micturition and defecation were observed for 24 h and also observed for 14 days without giving drug.

Experimental Induction of Diabetes:

In the present study, diabetes was induced by single intraperitoneal injection of alloxan (150mg/kg) [7].

The alloxan was freshly prepared by dissolving 150 mg of alloxan in 1ml of normal saline solution. The animals are fasted over night and allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia then alloxan was given by intraperitoneal route in dose of 150 mg/kg to all rats except group-1 animals. 72 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of > 200 mg/dl were included in groups II-V.

The rats were divided into five groups consisting of six rats in each group, the animals treated for 21 days.

Experimental design:

Animals were randomized and divided into five experimental groups (n=6) as follows.

Group 1: Normal was received vehicle (2% acacia, 10ml/kg body weight, p.o.),

Group 2: Control was received alloxan (150mg/kg, i.p)

Group 3: Standard was received alloxan (150mg/kg, i.p) and Glibenclamide (0.5 mg/kg/P.O)

Group 4: Low dose was received alloxan (150mg/kg, i.p) and low dose of EEACS (200 mg/kg, p.o.),

Group 5: High dose was received alloxan (150mg/kg, i.p) and high dose of EEACS (400 mg/kg, p.o.).

The doses were given for 21 days as multiple dose studies. Animals were provided with food and water as usual before experiment.

Biochemical Estimations:

The blood samples were drawn on 7th, 14th and 21st day from the retroorbital venous plexus of rats under ether anaesthesia using a glass capillary tube and the blood was centrifuged (2,500rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, and VLDL- cholesterol.

Statistical Analysis:

All the data are expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (graph pad prism 5.03). $P < 0.05$ was considered statistically significant.

RESULTS

Pharmacological studies:

Acute toxicity studies:

The EEACS was found to be safe at the maximum single dose of 4000mg/kg when administered orally the animals did not show any mortality and gross behavior changes. Hence according to OECD guide lines 423 the dose can be reduced up to 1/10 of the LD₅₀ that is 400mg/kg were used as high dose and 1/20 of the LD₅₀

that is 200mg/kg were used as low dose in the subsequent study respectively.

Antidiabetic activity:

Administration of alloxan (150 mg/kg, i.p.) lead to 1.5 fold elevation of fasting blood glucose levels in group-2 animals compare to group-1 animals, which was maintained over a period of 3 weeks. Daily treatment of ethanolic extract of *Allium cepa* seeds leads to a significant and dose dependent ($P < 0.001$) reduce in blood glucose levels in group 4 and 5 when compare to group-2 animals and the results showed in table no 1 and figure no 1.

Alloxan administered animals will increase the serum enzymes levels such as cholesterol, triglycerides, LDL, VLDL and decrease the HDL level, but Glibenclamide (0.5 mg/kg/P.O) and ethanolic extract of *Allium cepa* seeds reversed the above alloxan induce changes. There was a significant and dose dependent decrease of cholesterol, triglycerides, LDL, VLDL and significant and dose dependent increase in HDL levels after 21 days in group 4 and 5 animals when compare to group-2 animals ($p < 0.001$) and the results showed in table no 2-6 and figure no 2-6.

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary & energetic states via glucose concentration in the blood & in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas. Alloxan, a beta cytotoxin, destroys beta cells of islets of langerhans of pancreas resulting in a decrease endogenous insulin secretion paves the ways for the decreased utilization of glucose by the tissue. It results in elevation of blood glucose level. Expression of elevated fasting blood glucose level confirmed induction of diabetes in Alloxan induced experimental rats, there by inducing hyper glycaemia. Insulin deficiency leads to various metabolic alterations in the animals increased blood glucose, increased cholesterol, increased levels of alkaline phosphate & transaminases. From the outcome of the current study, it appears that insulin producing cells are still performance and the stimulation of insulin release could be responsible for most of the metabolic effects.

Fig no: 1 Effect of EEACS on blood glucose levels

Fig no: 2 Effect of EEACS on serum total cholesterol levels

Fig no: 3 Effect of EEACS on serum triglycerides level

Fig no: 4 Effect of EEACS on serum HDL levels

Fig no: 5 Effect of EEACS on serum LDL levels

Fig no: 6 Effect of EEACS on serum VLDL levels

Table no: 1 Effect of EEACS on blood glucose levels

S.No	Groups	Treatment	Serum glucose (mmol/L) (Mean $\pm$ SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	95.32 $\pm$ 6.32	101.65 $\pm$ 15.32	89.14 $\pm$ 5.47	94.87 $\pm$ 4.68
2	Group-2	alloxan (150mg/kg, i.p)	114.25 $\pm$ 5.14	235.54 $\pm$ 11.24 ^{###}	221.85 $\pm$ 11.52 ^{###}	242.84 $\pm$ 17.25 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	84.57 $\pm$ 4.78	158.64 $\pm$ 14.21 [*]	135.28 $\pm$ 8.25 ^{**}	102.67 $\pm$ 7.36 ^{**}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	102.84 $\pm$ 9.24	195.35 $\pm$ 9.14	174.45 $\pm$ 5.12 [*]	139.72 $\pm$ 8.08 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	79.15 $\pm$ 4.84	175.32 $\pm$ 9.47 [*]	155.94 $\pm$ 8.89 ^{**}	121.84 $\pm$ 9.45 ^{***}

All values were expressed as Mean $\pm$ S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

Table no: 2 Effect of EEACS on serum total cholesterol levels

S.No	Groups	Treatment	Serum total cholesterol (mmol/L) (Mean $\pm$ SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	75.14 $\pm$ 4.03	72.36 $\pm$ 1.51	79.35 $\pm$ 4.21	66.84 $\pm$ 2.06
2	Group-2	alloxan (150mg/kg, i.p)	81.14 $\pm$ 2.14	145.26 $\pm$ 2.62 ^{###}	185.62 $\pm$ 9.87 ^{###}	176.37 $\pm$ 9.97 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	56.85 $\pm$ 0.98	125.14 $\pm$ 7.36 [*]	85.73 $\pm$ 2.84 ^{***}	71.48 $\pm$ 9.04 ^{***}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	65.41 $\pm$ 2.47	138.62 $\pm$ 11.25	124.36 $\pm$ 9.47 [*]	104.58 $\pm$ 10.57 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	67.62 $\pm$ 1.62	126.74 $\pm$ 9.97 [*]	107.91 $\pm$ 4.98 ^{**}	84.79 $\pm$ 7.08 ^{***}

All values were expressed as Mean $\pm$ S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

Table no: 3 Effect of EEACS on serum triglycerides level

S.No	Groups	Treatment	Serum triglycerides (mmol/L) (Mean ± SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	123.25±5.65	112.54±4.98	124.92±7.38	109.74±7.25
2	Group-2	alloxan (150mg/kg, i.p)	101.65±7.36	152.32±8.89 ^{###}	165.84±11.02 ^{###}	149.87±9.09 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	95.84±6.67	138.36±7.06 [*]	124.69±6.81 ^{**}	107.62±4.37 ^{***}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	121.78±9.74	162.54±11.58	132.68±7.83 [*]	121.78±6.47 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	97.84±7.91	142.84±9.07	124.84±8.04 ^{**}	114.97±5.83 ^{***}

All values were expressed as Mean±S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

Table no: 4 Effect of EEACS on serum HDL levels

S.No	Groups	Treatment	Serum HDL (mmol/L) (Mean ± SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	35.48±3.65	34.84±2.65	29.74±0.36	41.84±2.64
2	Group-2	alloxan (150mg/kg, i.p)	36.87±3.41	12.47±0.64 ^{###}	15.36±0.78 ^{###}	14.87±0.49 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	41.84±1.75	14.87±2.06	21.36±0.97 ^{**}	32.84±1.84 ^{***}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	38.47±1.64	13.84±0.74	18.36±1.97 [*]	22.36±1.72 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	32.84±1.11	12.97±0.78	23.97±0.91 ^{**}	29.14±0.49 ^{***}

All values were expressed as Mean±S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

Table no: 5 Effect of EEACS on serum LDL levels

S.No	Groups	Treatment	Serum LDL (mmol/L) (Mean ± SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	26.03±1.37	38.64±3.65	41.87±2.85	36.47±3.02
2	Group-2	alloxan (150mg/kg, i.p)	31.24±2.84	75.48±2.86 ^{###}	95.48±4.94 ^{###}	89.75±3.97 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	39.47±1.94	62.87±1.26 [*]	55.48±0.32 ^{**}	43.67±2.78 ^{***}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	33.65±2.74	69.14±2.84	63.94±1.81 [*]	55.14±1.09 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	37.97±3.47	61.78±2.22	59.47±1.36 ^{**}	42.79±2.67 ^{***}

All values were expressed as Mean±S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

Table no: 6 Effect of EEACS on serum VLDL levels

S.No	Groups	Treatment	Serum VLDL (mmol/L) (Mean ± SEM) on			
			Initial day	7 th day	14 th day	21 st day
1	Group-1	2% acacia, 10ml/kg b.w	21.36±1.14	24.78±1.54	26.47±0.99	18.62±1.01
2	Group-2	alloxan (150mg/kg, i.p)	31.58±1.84	65.47±2.36 ^{###}	59.47±2.87 ^{###}	62.84±1.84 ^{###}
3	Group-3	alloxan (150mg/kg, i.p) & glibenclamide (0.5 mg/kg, P.O)	25.94±1.34	45.84±0.92 [*]	39.72±0.98 ^{**}	29.73±1.81 ^{***}
4	Group-4	alloxan (150mg/kg, i.p) & EEACS (200 mg/kg, p.o.),	19.84±0.97	51.97±2.73	48.15±0.65 ^{**}	44.97±0.76 ^{**}
5	Group-5	alloxan (150mg/kg, i.p) & EEACS (400 mg/kg, p.o.)	24.73±0.49	48.15±0.77 [*]	42.36±2.31 ^{**}	33.97±0.92 ^{***}

All values were expressed as Mean±S.E.M and n=6

indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;

* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to alloxan induced group (One-way ANOVA followed by Tukey's test)

The medicinal plant compounds may have mechanisms acting as insulin like effect, improving insulin sensitivity, augmenting glucose-dependent insulin secretion, and stimulating the regeneration of islets of langerhans in pancreas of alloxan-induced diabetic rats.

Presence of phenolic compounds such as alkaloids, flavonoids, and tannins in *Allium cepa* was noticed in preliminary phytochemical analysis. We hypothesized that *Allium cepa* can be attributed to their insulin-trophic effect that enables the reduction of blood glucose levels due to their antidiabetic effect. The possible mechanism of action of *Allium cepa* extract treated groups could be potentiating the pancreatic secretion of insulin from β -cells of pancreatic islets, as was evident by significantly lowering the level of glucose [12].

CONCLUSION

In conclusion, the present study suggests a potential role of fresh ethanolic extract of *Allium cepa* seeds against alloxan induced diabetes mellitus model. Further studies are required for understand the basic mechanism and characterization of active constituents responsible for antidiabetic effect.

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