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Antidiabetic activity of *Echinochloa crusgalli* (L.)P.Beauv grains extract in alloxan induced diabetic rats.

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ABSTRACT

The plant used ethnically in India for controlling blood sugar, this promotes us to undertake a study to examine the possible anti-diabetic activity of the grains 70% hydroalcoholic (HAEC) extract in normal and ALX induced diabetic rats. A single dose study was studied in the normal rats for 12 hrs. Oral glucose tolerance test (OGTT) was performed in normal rats after receiving glucose orally (2g/kg). Diabetes was induced by ALX (120mg/kg, i.p.) three different doses of HAEC (200, 400 and 600mg/kg, p.o.) and HAEC was administered orally to experimental diabetic induced rats as treatment for 21 days. Glibenclamide (5mg/kg p.o.) was used as standard reference. Fasting blood glucose levels, changes in body weight and organ weight, serum albumin, urea, total protein, creatinine, total lipid profile, haemoglobin, GSH, SOD and TBARS were evaluated. Finally histopathological examination of pancreas was performed. Oral glucose tolerance test clearly indicate that 400mg/kg and 200mg/kg p.o HAEC shown a significant reduce in the blood glucose levels whereas, 600mg/kg p.o shown a little effect. Single dose study of HAEC on normal rats showed a significant decrease in the fasting blood glucose levels when compared with the normal control rats. In diabetic rats, treatment with the 400, and 200mg/kg, p.o. showing significant reduction in the fasting blood glucose levels, serum cholesterol, serum triglycerides, LDL-C and VLDL-C levels. A significant escalation is seen in the levels of HDL-C, haemoglobin, body weight and liver weight whereas, the anti-oxidant levels TBARS, GSH and SOD levels improved than the untreated diabetic rats. HAEC has shown significant, *In-vivo* antioxidant property and antidiabetic activity.

Keywords: Antidiabetic activity; Antioxidant Activity; Alloxan; *Echinochloa crusgalli (L)P.Beauv*; Oral Glucose Tolerance Test.

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by chronic hyperglycaemia (an abnormally high amount of glucose levels in blood) with disturbances of carbohydrate, fat and protein metabolism resulting from inadequate production or use of insulin. Insulin is an endocrine hormone produced in specialized cells (beta cells in the Islets of Langerhans) in the pancreas that allows the body to use and store glucose. Hyperglycemia in diabetes affects multiple organ systems in the body especially kidneys, eyes, nerves, and blood vessels. It is an endocrine disorder of pancreas. Diabetes mellitus, a chronic metabolic disorder has profound effect on quality of life in terms of social, psychological well-being as well as physical ill health [1]. Clinical manifestation of the disease includes thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death [2,3]. It is considered as one of the five leading causes of death in the world. It is characterized hyperglycemia (an increased blood glucose levels) and also a defect in insulin secretion and insulin action or both [4]. World Health Organization (WHO) predicts that developing countries will bear the brunt of this epidemic in the 21st century. Currently, more than 70% of people with Diabetes live in low and middle income countries. Diabetes is one of the major causes of premature illness and death worldwide. Non-communicable diseases including Diabetes account for 60% of all deaths worldwide. According to the International Diabetes Federation, it is revealed that the country with the largest numbers of people with Diabetes is India (32 million), followed by China (26 million), United States (18 million) and in Canada, 2.4 million people live with Diabetes. About 90% of Diabetic people die of Diabetic Cardio vascular diseases. Russia (9.6 million) and Germany (7.4 million) by the year 2025, there will be as many as seven million new Diabetic cases in the world [5]. The WHO estimates more than 32 million Diabetics in India. India is the “Diabetic capital” of the world, responsible for 19% of the worldwide incidence in Diabetes in 2005 and this trend will be continuing into the future. It has been estimated that in 1995, 19.4 million individuals were affected by Diabetes mellitus in India and these numbers are expected to rise to 79 million by 2030 unless urgent preventive steps are taken [6,7].

Echinochloa crusgalli (*L.)P. Beauv* is a grass belonging to the family Poaceae. Cockspur (or Cockspur Grass), Common Barnyard Grass are the common names. It is distributed in India, China, Europe, Indonesia, Cambodia, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam. It is annual growing to 1.2 m (4ft) by 0.2m. It is in flower from July to September, and the seeds ripen from August to October. The flowers are hermaphrodite (have both male and female organs) and are pollinated by wind. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils and can grow in very alkaline soils. It cannot grow in the shade. It requires moist soil. The plant can tolerate strong winds but not maritime exposure [8].

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MATERIALS AND METHODS

Plant material

The grains of *Echinochloa crusgalli* is widely found in the India. The grains were collected from the Bangalore, Karnataka, identified and authenticated by Dr.M.V.C Gowda Project Co-ordinater, AICRP on Small Millets, ICAR, UAS, GKVK, Bangalore, Karnataka, India.

Preparation of Extract [9,10]

The fresh grains were collected, cleaned and shade dried at room temperature. The dried grains were coarse powdered by using grinder. The coarse powder was packed in Soxhlet column and then extracted with 70% hydro-alcohol (75-80^{o}C). Thereafter, the extract was concentrated using rotary flash evaporator (50^{o} C).

Determination of Acute Toxicity (LD_{50})

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs [11].

Phytochemical screening [12]

The preliminary phytochemical analysis was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, phytosterols/terpenes, proteins and saponins were qualitatively analysed.

Experimental animals

Albino wistar rats weighing 150-220g were procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at 27^{o} ± 2^{o} C and 12 hr light-dark cycles for one week. They were housed in polypropylene cages and containing paddy husk as bedding. They had a free access to standard pellets and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-IAEC/05/05/2011) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

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Preparation of test sample

The grains extract [200,400 and 600mg/kg, b.w. (bodyweight)] were suspended in tween 80 prior to oral administration to animals. The standard hypoglycemic drugs glibenclamide (5mg/kg, Darwin Formulations®) and alloxan monohydrate (120mg/kg, Loba Chemie ®) dissolved on 0.9% sodium chloride solution (normal saline) is used in this study.

Experimental design

Effect of *Echinochloa crusgalli* extract on blood glucose level of normal rats [13].

Albino wistar rats weighing 150-200 mg/kg were divided into five groups of six in each group. Animals were fasted overnight for 16 hrs prior to the experiment (Jimam NS et al 2010). The blood glucose levels were measured just prior to and 1^{st}, 2^{nd}, 4^{th}, 8^{th} and 12^{th} hrs after drug administration. The blood glucose levels were measured from the tail vein by using Sugarcheck glucometer manufactured by Wockhardt.

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and served as standard
Group-III: Animals received a dose of 200 mg/kg of HAEC p.o.
Group-IV: Animals received a dose of 400 mg/kg of HAEC p.o.
Group-V: Animals received a dose of 600 mg/kg of HAEC p.o.

Effect of *Echinochloa crusgalli* extract on Oral Glucose Tolerance Test in Normal Rats (OGTT) [14].

The oral glucose tolerance test was performed in rats weighing 150-200g. The animals were fasted for 16 hr before the experiment but allowed free access to water. These Rats were divided into five groups, six in each group. Rats of all groups were loaded with glucose 2g/kg p.o 30 min after drug administration. Blood samples were collected from the tail vein prior to drug administration and at 30, 60, 90 and 120 min of glucose administration.

Group-I: Animals received distilled water and after 30min a glucose load of 2g/kg is administered p.o. which was served as control.
Group-II: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and after 30 min a glucose load of 2g/kg is administered p.o which was served as standard.
Group-III: Animals received a dose of 200 mg/kg of HAEC p.o. and after 30 min a glucose load of 2g/kg is administered p.o.
Group-IV: Animals received a dose of 400 mg/kg of HAEC p.o. and after 30 min a glucose load of 2g/kg is administered p.o.
Group-V: Animals received a dose of 600 mg/kg of HAEC p.o. and after 30 min a glucose load of 2g/kg is administered p.o.

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Effect of *Echinochloa crusgalli* Grains extract on ALX induced diabetic rats. [15-17].

Experimentally Induced Diabetes Mellitus

Female Wistar rats weighing 150-220g were used for this study. The animals were overnight fasted for 16h before the induction of diabetes. Diabetes was induced by a single dose of 120 mg/kg body weight of alloxan by intraperitoneal route. After a period of 2 days blood glucose levels were checked by snipping the tail of alloxan treated fasted rats. Rats showing the blood glucose levels more than 300 mg/dl is taken into the study.

Experimental Procedure

Diabetes was induced in fasted female Albino wistar rats (150-220g) by intraperitoneal injection of 120mg/kg body weight of alloxan except Group I. After 72hrs, animals with fasting blood glucose levels higher than 300 mg/dl were selected and used.

Group-I: Animals received distilled water only and served as normal control.
Group-II: Animals received distilled water only and served as diabetic control
Group-III: Animals received a dose of 5 mg/kg of Glibenclamide p.o. and served as standard
Group-IV: Animals received a dose of 200 mg/kg of HAEC p.o.
Group-V: Animals received a dose of 400 mg/kg of HAEC p.o.
Group-VI: Animals received a dose of 600 mg/kg of HAEC p.o.

The study was carried out for 21 days. Fasting blood glucose levels were measured before the administration of HAEC. It was recorded as 0 day. The doses of the HAEC (200, 400 and 600mg/kg p.o.) along with the standard (Glibenclamide) were given daily to the animals for 21 days. The blood glucose levels were checked on 0, 7^{th}, 14^{th}, and 21^{st} day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured by using the glucometer Sugarcheck.

Determination of Body Weight:

Body weight of the entire animal in each group was noted on the 0, 7^{th}, 14^{th} and 21^{st} day of the experiment period. The weight difference was calculated.

Determination of Weights of Pancreas, Liver, Heart, Kidneys, Spleen:

Animals were sacrificed and Pancreas, liver, heart, kidneys and spleen were isolated, washed with saline and weighed by using an electronic balance.

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Estimation of Biochemical Parameters

The following parameters are estimated by using standard procedures of Excel, Beacon, Erba diagnostics estimating kits. Total Protein, Serum Albumin, Serum Urea, Serum Creatinine, Hemoglobin (Hb) and Lipid Profile (HDL, LDL, VLDL, TG and Total Cholesterol) [18].

Estimation of Antioxidant Activity [19-21].

Livers of the animals were homogenized with ice-chilled 10% Phosphate buffer and centrifuge at 2000 rpm to 10 minutes. The supernatant liquid is used for the estimation of following parameters. Superoxide Dismutase, Thiobarbituric Acid Reactive Substances (TBARS) and Glutathione.

Statistical analysis

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Extraction of grains of *Echinochloa crusgalli* was carried out by using the soxhlet apparatus with hydroalcoholic solvent (70 % v/v ethyl alcohol) the percentage yield of extract was found to be 4%.

Preliminary Qualitative Phytochemical Studies

Preliminary qualitative phytochemical studies of 70% (v/v) hydro-alcoholic extract of *Echinochloa crusgalli* grains revealed that the presence of alkaloids, glycosides, carbohydrates, flavonoids, phytosterols/terpenes, proteins, and saponins.

Effect of *Echinochloa crusgalli grains* extract on blood glucose level of normal rats.

Hypoglycaemic activity of HAEC was studied on normal rats and the results were tabulated in Table No. 1.

Low dose (200 mg/kg, p.o.) of HAEC shows a significant reduction action in blood glucose levels at 8 and 12 hours ($P<0.001$), but less significant reduction action in blood glucose levels was shown at 1^{st},2^{nd},4^{th} hours ($P<0.01$). Medium dose of HAEC (400 mg/kg, p.o.) shows a significant action in reducing the blood glucose levels at 4,8,12 hours ($P<0.001$) onset of action is starts from 1 hour after the treatment. But, less significant reduction in blood glucose levels was shown at 1^{st} and 2^{nd} hours ($P<0.01$) compared to the normal untreated group. High dose of HAEC (600 mg/kg, p.o) shows a very less significant action in reducing the blood glucose levels

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8th and 12th hours (P<0.05) after administration of the extract. But, it did not show significant reduction in blood glucose levels at 1st, 2nd and 4th hours. Glibenclamide showed its effect from 1 hour after treatment. The onset of Glibenclamide starts from 1 hour after the treatment. It reduces maximum blood glucose levels at 12 hours (P<0.001). Glibenclamide significantly reduced the blood glucose levels after treatment in normal rats. All the blood glucose levels of treated group were compared with the normal control group animals.

Table No.1: Effect of *Echinochloa crusgalli* Grains Extract on Blood Glucose Levels of Normal Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)					
		0	1	2	4	8	12
Group-I	Saline	90.33 ± 3.34	86.67 ± 5.65	92.50 ± 11.19	84.17 ± 5.44	81.83 ± 4.99	95.67 ± 4.49
Group-I I	Glibenclamide (5mg/kg)	78.00 ± 1.23	58.50 ± 3.68***	43.83 ± 4.61***	49.33 ± 2.87***	47.50 ± 1.60***	53.33 ± 3.68***
Group-III	HAEC (200mg/kg)	87.00 ± 6.36	70.83 ± 2.31**	65.83 ± 2.13**	65.67 ± 3.84**	61.17 ± 3.17***	69.00 ± 3.01***
Group-IV	HAEC (400mg/kg)	91.00 ± 5.12	65.50± 2.24**	59.17± 2.10**	60.83 ± 4.24***	54.33 ± 1.64***	62.33 ± 4.58***
Group-V	HAEC (600mg/kg)	95.33 ± 5.45	74.64 ± 3.21ns	74.00 ± 1.86ns	76.17 ± 0.91ns	70.00± 1.63*	78.00 ± 3.41*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. HAEC- Hydro-alcoholic (70%v/v) grains extract of *Echinochloa crusgalli.*

Effect of *Echinochloa crusgalli* grains extract on Oral glucose tolerance test normal rats

The effect of HAEC on oral glucose tolerance test was tabulated in the Table No-2.

Low dose (200 mg/kg, p.o.) of HAEC did not show significant reduction in blood glucose levels at 30, min and it shows less significant reduction effect at 60, 120 min (P<0.01) and significant reduction was observed at 90 min (P<0.001). In Medium dose of HAEC (400 mg/kg, p.o.) a significant reduction was observed at 30, 60 and 120 min (P<0.001) and les significant reduction was observed at 90 min (P<0.01). Whereas, high dose of HAEC (600 mg/kg) p.o also show a significant decrease in blood glucose levels, when administered 30 min before glucose loading. It showed a significant activity at the time intervals of 30, 60, 90 and 120 min (P< 0.001) and less significant reduction was observed at 30, 60 and 120 min (P< 0.01). Medium dose of HAEC showed a significant effect (P<0.001) when compared with the other doses of HAEC but low dose of this extract also showed significant effect at 90 min (P< 0.001) compared with the high dose of HAEC.Glibenclamide showed its potent antidiabetic activity in normal rats it bring backs the elevated blood glucose levels to normal levels compared to normal control group at 120 min (P<0.001). Overall the grains extract of *Echinochloa crusgalli* has showed a significant decrease in the blood glucose levels when compared with the normal control group rats at time intervals 30, 60, 90 and 120 min.

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. **Table No. 2: Effect of *Echinochloa crusgalli* Grains Extract on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats**

Groups	Treatment	Blood Glucose Levels (mg/dl) and Time in min				
		0	30	60	90	120
Group-I	Saline+ Glucose(2g/kg)	94.50 ± 3.65	124.8 ± 6.03	105.7± 2.37	97.50 ± 3.96	91.33 ± 3.38
Group-II	Glibenclamide(5mg/kg) + Glucose (2gm/kg)	92.67 ± 6.14	83.83± 4.74***	70.67± 4.35***	60.33 ± 3.01***	54.67±3.50***
Group-III	HAEC (200mg/kg) + Glucose (2gm/kg)	89.33 ± 4.19	108.2± 5.64^{ns}	86.83± 2.85**	77.83± 4.43***	67.33± 7.85**
Group-IV	HAEC (400mg/kg) + Glucose (2gm/kg)	86.00 ± 6.77	90.00± 5.90***	79.17± 4.75***	71.17± 3.90**	63.33± 2.30***
Group-V	HAEC (600mg/kg) + Glucose (2gm/kg)	90.17 ± 4.26	100.80± 6.32*	90.00± 4.30*	85.00± 4.24***	75.00± 1.84*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dennett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. HAEC- Hydro-alcoholic (70%v/v) grains extract of *Echinochloa crusgalli.*

Effect of Extract in Alloxan Induced Diabetic Rats

A chronic study of 21 days was done in ALX induced diabetic rats with gains extract of *Echinochloa crusgalli* and the results of blood glucose levels are tabulated in the Table No. 3. Blood glucose levels on zero day showed no significant intra group variation. Administration of ALX (120mg/kg, i.p.) showed a significant increase in fasting blood glucose levels (415.00 ± 20.16mg/dl). After 21 days, diabetic control rats exhibited significantly higher blood glucose levels (324.20 ± 19.17 mg/dl) as compared to the normal control rats (83.83 ± 8.526). A daily treatment of HAEC (200 mg/kg, p.o.) for a period of 21 days lowers the blood glucose levels in diabetic treated rats. Blood glucose levels on 21 days lowered the blood glucose levels from 371.50 ± 17.11 to 161.30 ± 7.99 (P<0.001). Similarly diabetic rats treated with the medium dose of HAEC (400mg/kg, p.o.) showed a significant activity when compared with the high dose 600mg/kg p.o treated group at 7th day to 21st day,(319.70 ± 20.16 to236.60 ± 10.69 mg/dl) (P<0.001). Overall the HAEC 400mg/kg p.o showed a significant decrease in the blood glucose levels when compared with the normal control and 200 and 600 mg/kg groups at day intervals day 7th, 14th, 21st (P<0.001). Glibenclamide showed its potent antidiabetic activity and reduced the blood glucose levels of diabetic rats to the level significantly (324.20 ± 19.17 to 104.70 ± 4.91mg/dl).

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Table No. 3: Effect of *Echinochloa crusgalli* Grains Extract on Blood Glucose Levels in ALX Induced Diabetic Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0 day	7th day	14th day	21st day
Group-I	Saline	90.50 ± 3.29	78.00 ± 3.15***	77.17 ± 4.11**	83.83 ± 8.52***
Group-II	ALX(120mg/kg) + Saline	415.00 ± 20.16	411.00 ± 16.78	362.80 ± 17.02	324.20 ± 19.17
Group-III	ALX(120mg/kg) + Glibenclamide (5mg/kg)	324.20 ± 19.17	290.30 ± 15.60***	199.20 ± 11.65***	104.70 ± 4.91***
Group-IV	ALX(120mg/kg) + HAEC(200mg/kg)	371.50 ± 17.11	332.50 ± 13.40**	252.70 ± 12.00***	161.30 ± 7.99***
Group-V	ALX(120mg/kg) + HAEC(400mg/kg)	410.00 ± 27.79	319.70 ± 20.16***	215.00 ± 15.89***	132.20 ± 14.82***
Group-VI	ALX(120mg/kg) + HAEC(600mg/kg)	433.70 ± 21.33	357.50 ± 6.19*	312.20 ± 11.31*	236.60 ± 10.69***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group. HAEC- Hydro-alcoholic (70%v/v) grains extract of *Echinochloa crusgalli*

Effect of *Echinochloa crusgalli* Grains extract on body weight in ALX induced diabetic rats

There is a significant change seen in the body weight of animals after the treatment inducing diabetes with ALX. The decreased body weight of the animals were significantly regains when compare with the diabetic control animals after treatment for 21 days with the extract. And also the body weight of normal control group was significantly increased compared to initial body weight. The changes in body weight of the animals during 0, 7th, 14th and 21st days were tabulated in the Table No. 4.

Table No. 4 Effect of *Echinochloa crusgalli* Grains Extract on Body Weight in ALX Induced Diabetic Rats

Groups	Treatment	Body Weight (gms)			
		0 Day	7th Day	14th Day	21st Day
Group-I	Saline	203.80 ± 5.10	210.2 ± 4.49***	213.00 ±5.39***	217.80 ± 4.82***
Group-II	ALX(120mg/kg) + Saline	189.20 ± 5.45	152.50 ± 4.24	151.50 ± 3.25	146.70 ± 1.28
Group-III	ALX(120mg/kg) + Glibenclamide(5mg/kg)	184.00 ± 4.09	182.00 ± 4.13***	187.7 ± 4.93***	191.70 ± 4.93***
Group-IV	ALX(120mg/kg) + HAEC(200mg/kg)	180.30 ± 2.23	168.50 ± 1.94*	176.30 ±1.83***	184.20 ± 2.60***
Group-V	ALX(120mg/kg) + HAEC(400mg/kg)	181.30 ± 2.71	171.70 ± 3.09**	179.50 ±2.86***	187.70 ±3.48***
Group-VI	ALX(120mg/kg) + HAEC(600mg/kg)	185.00 ± 3.53	164.80 ± 2.16ns	169.20 ±3.21*	178.00 ±3.21***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with diabetic control. All the values are compared with the diabetic control group. HAEC- Hydro-alcoholic (70%v/v) grains extract of *Echinochloa crusgalli*

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Effect of *Echinochloa crusgalli* grains Extract on Pancreas, Liver, Heart, Kidney and Spleen Weights in ALX Induced Diabetic Rats

Weights of different organs like Pancreas, Liver, Heart, Kidneys and Spleen were observed in ALX induced diabetic rats. The weights of these organs were increased slightly in ALX control group compared to normal control group, and group of 200mg/kg, p.o HAEC did not show any effect where as groups of 400mg/kg and 600mg/kg, p.o HAEF showed slight increased in organ weights were significantly compared to ALX control group. The values of these weights were tabulated in Table No.5.

Table No. 5: Effect of *Echinochloa crusgalli* Grains Extract on Pancreas, Liver, Heart, Kidney and Spleen Weights in ALX Induced Diabetic Rats

Groups	Treatment	Organ Weights (gms)				
		Pancreas	Liver	Heart	Kidney	Spleen
Group-I	Saline	0.86 ± 0.07***	6.37 ± 0.19***	0.74 ± 0.02***	1.61 ± 0.06***	0.87 ± 0.04***
Group-II	ALX(120mg/kg) + Saline	0.42 ± 0.02	4.29 ± 0.16	0.49 ± 0.02	1.09 ± 0.04	0.36 ± 0.03
Group-III	ALX(120mg/kg) + Glibenclamide(5mg/kg)	0.83 ± 0.06***	6.15 ± 0.22***	0.70 ± 0.03***	1.52 ± 0.04***	0.85 ± 0.03***
Group-IV	ALX(120mg/kg) + HAEC(200mg/kg)	0.69 ± 0.01^{ns}	5.96 ± 0.33^{ns}	0.64 ± 0.01*	1.42 ± 0.10**	0.75 ± 0.06*
Group-V	ALX(120mg/kg) + HAEC(400mg/kg)	0.71 ± 0.04**	6.09 ± 0.29**	0.71 ± 0.03**	1.44 ± 0.05**	0.753 ± 0.06**
Group-VI	ALX(120mg/kg) + HAEC(600mg/kg)	0.64 ± 0.05**	5.44 ± 0.02***	0.54 ± 0.02***	1.39 ± 0.03***	0.68 ± 0.11***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

Effect of *Echinochloa crusgalli* Grains Extract on Serum Albumin, Serum Urea, Serum Creatinine, Serum Total Protein, and Haemoglobin Levels in ALX Induced Diabetic Rats

Serum albumin levels were decreased in the diabetic animals, as compared with the normal control animals. Whereas albumin levels in the diabetic control group is 2.97 ± 0.10 mg/dl. But albumin levels after treatment with the *Echinochloa crusgalli* Grains extract shows an increased the serum albumin levels. The values of the albumin levels are mentioned in the Table No.6.

Diabetic rats showed an increased in the levels of serum urea. Treatment of these rats with the extract and glibenclamide showed a decrease in the urea levels when compared with the normal animals. The urea levels in the diabetic rats are 110.80 ± 14.99 mg/dl, whereas it is decreased to 53.89 ± 3.36 mg/dl in glibenclamide treated rats. Serum Urea levels of treated and Normal rats are expressed in the Table No.6.

Diabetic rats showed an increased in the levels of serum creatinine. Treatment of these rats with the extract and glibenclamide showed a decrease in the creatinine levels when

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compared with the normal animals. The creatinine levels in the diabetic rats are 1.06 ± 0.07 mg/dl, whereas it is decrease 0.65 ± 0.03 mg/dl in glibenclamide treated rats. Serum creatinine levels of treated and Normal rats are expressed in the Table No.6.

Serum protein levels are decreased (4.74 ± 0.22 mg/dl) in the untreated diabetic rats compares to the normal control rats (9.16 ± 0.45 mg/dl). After treatment with the Glibenclamide, 200mg/kg, 400mg/kg p.o dose of HAEC shown a significant increase in the serum protein levels compared with the diabetic control animals. High dose of HAEC showed a less significant activity in increasing the protein levels. The values of serum total protein levels are shown in the Table No.6.

A daily dose of the HAEC for a period of 21 days showed an increased in the haemoglobin level of diabetic rats. But, the high dose of HAEC showed a less significant activity when compared with the diabetic control rats. Glibenclamide restores the haemoglobin levels to normal levels after treatment. The values were tabulated in the Table No.6

Table No. 6: Effect of *Echinochloa crusgalli* Grains Extract on Serum Albumin, Serum Urea, Serum Creatinine, Serum Total Protein, and Haemoglobin Levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Albumin Levels (g/dl)	Serum Urea Levels (mg/dl)	Serum Creatinine Levels (mg/dl)	Serum Total Protein Levels (mg/dl)	Haemoglobin (mg/dl)
Group-I	Saline	5.14 ± 0.20***	45.18 ± 1.73***	0.64 ± 0.02***	9.16 ± 0.45***	12.48 ± 0.24***
Group-II	Saline + ALX(120mg/kg	2.97 ± 0.10	110.80 ± 14.99	1.06 ± 0.07	4.74 ± 0.22	7.78 ± 0.87
Group-III	Glibenclamide (5mg/kg) + ALX (120mg/kg)	5.04 ± 0.19***	53.89 ± 3.36***	0.65 ± 0.03***	8.99 ± 0.37***	12.13 ± 0.46***
Group-IV	HAEC(200mg/kg) + ALX(120mg/kg)	4.19 ± 0.22***	66.30 ± 4.16***	0.76 ± 0.03***	8.05 ± 0.75**	10.27 ± 0.33**
Group-V	HAEC(400mg/kg) + ALX(120mg/kg)	4.81 ± 0.14***	64.75 ± 4.40***	0.68 ± 0.05***	8.39 ± 0.62***	11.38 ± 0.37***
Group-VI	HAEC(600mg/kg) + ALX(120mg/kg)	3.74 ± 0.21*	74.47 ± 7.47**	0.83 ± 0.04**	7.29 ± 0.82*	9.65 ± 0.31*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

Effect of *Echinochloa crusgalli* Grains Extract on Serum Lipid Profile of ALX Induced Diabetic Rats

The lipid profile was evaluated by estimating triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (HDL-C),VLDL-Cholesterol (VLDL-C) in normal and diabetic animals. The ALX diabetic animals showed a significant increased in the TG, TC, LDL-C and VLDL-C levels and suppression of HDL-C levels compared to control group (Table No.7). But

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after treatment with the grains extract and glibenclamide diabetic animals Showed decrease in the TG, TC, LDL-C and VLDL-C levels and increase in the HDL-C levels compared to untreated diabetic rat.

Table No. 7: Effect of *Echinochloa crusgalli* Grains Extraction Serum Lipid Profile of ALX Induced Diabetic Rats

Groups	Treatment	Serum Lipid Profile mg/dl				
		TC	TG	HDL-C	LDL-C	VLDL-C
Group-I	Saline	86.03 ± 4.17***	71.33 ± 3.64***	26.53 ± 0.76***	45.21 ± 5.00***	14.25 ± 0.72***
Group-II	**Sali**ne + ALX (120mg/kg)	125.80 ± 3.56	127.90 ± 3.69	14.25 ± 0.94	45.21 ± 5.00***	25.58 ± 0.73
Group-III	Glibenclamide (5mg/kg) + ALX (120mg/kg)	94.53 ± 2.53***	80.57 ± 6.56***	24.74 ± 1.05***	85.96 ± 4.02	16.11 ± 1.31***
Group-IV	HAEC(200mg/kg) + ALX (120mg/kg)	97.60 ± 7.05***	87.89 ± 7.42***	22.76 ± 2.07***	53.69 ± 4.08***	17.58 ± 1.48***
Group-V	HAEC(400mg/kg) + ALX (120mg/kg)	95.27 ± 0.89***	85.43 ± 4.37***	24.08 ± 1.18***	57.18 ± 8.16**	0.8817.07 ± ***
Group-VI	HAEC(600mg/kg) + ALX (120mg/kg)	101.4 ± 3.88**	96.17 ± 3.32***	21.34 ± 1.95**	46.18 ± 6.54***	19.23 ± 0.66***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with diabetic control.

Effect of *Echinochloa crusgalli* Grains Extract on SOD, TBARS, GSH in ALX Induced Diabetic Rats

Diabetic rats exhibited significant lower SOD (7.02 ± 0.49) as compared to those of control rats (15.07 ± 0.63) treatment with the plant extract significantly elevated the reduced SOD levels. HAEC and Glibenclamide showed a marked increase in the SOD levels (P<0.001) compared to the diabetic control. These values are tabulated in the Table No.10. Rats treated with ALX had a TBARS level of 3.64 ± 0.14 nmoles of MDA/ 100 mg of tissue when measured on day 21. This was significantly higher when compared to levels in normal control rats of 1.07 ± 0.09 nmoles of MDA/ 100 mg of tissue. Diabetic rats treated with 200mg/kg, p.o. had a lipid peroxidation levels of12.19 ± 0.60 nmoles of MDA/ 100 mg of tissue and also rats treated with the 400mg/kg, o.p. and 600mg/kg, p.o. treated rats having a TBARS levels of 1.39 ± 0.11 and nmoles 2.54 ± 0.13 of MDA/ 100 mg of tissue respectively. Whereas in glibenclamide treated rats the levels are restored to normal levels of 1.278 ± 0.051 nmoles of MDA/ 100 mg of tissue. These values are expressed in Table No.8. Rats treated with ALX had a tissue GSH level of 26.38 ± 1.288 mM/ 100 mg of tissue when measured on day 21. Treatment with HAEC shows increase GSH levels in ALX treated rats. These values are having a significant higher (P<0.001) when compared to GSH levels in diabetic control rats. The values are tabulated in the Table No. 8.

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Table No. 8: Effect of *Echinochloa crusgalli* Grains Extract on SOD, TBARS, GSH in ALX Induced Diabetic Rats

Groups	Treatment	SOD U/mg Protein	TBARS (nmoles of MDA/ 100 mg of tissue)	GSH (mM/ 100 mg of tissue)
Group-I	Saline	15.07 ± 0.63***	1.07 ± 0.09 ***	42.63 ± 1.91 ***
Group-II	Saline + ALX (120mg/kg)	7.02 ± 0.49	3.64 ± 0.14	26.38 ± 1.28
Group-III	Glibenclamide (5mg/kg) + ALX (120mg/kg)	14.32 ± 0.75***	1.27 ± 0.05 ***	40.85 ± 1.15 ***
Group-IV	HAEC(200mg/kg) + ALX (120mg/kg)	12.19 ± 0.60***	1.87 ± 0.05 ***	39.75 ± 0.76 ****
Group-V	HAEC(400mg/kg) + ALX (120mg/kg)	13.86 ± 0.62***	1.39 ± 0.11 ***	40.07 ± 0.57 ***
Group-VI	HAEC(600mg/kg) + ALX (120mg/kg)	11.56 ± 0.88***	2.54 ± 0.13***	35.64 ± 0.63***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

Histopathological Study of Pancreas

Group –I (Normal Control + Saline)

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of aggregates of small Beta-cells (70%, Short-arrow), while the periphery comprises of large Alpha-cells (25%, Long-arrow). Intervening these cells are seen thin walled capillaries.

Group –II (Diabetic Control + Alloxan [120mg/kg)

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (30%, Long-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (65%, Short-arrow) having eosinophilic granules. Also seen are some degenerated beta cells and lymphocytic infiltration amidst these islet cells.

Group –III (Alloxan [120mg/kg] + Glibenclamide [5mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show areas of light-staining islets of langerhans. The center of islet cells consist of quantitative increase in Beta-cells [compared to Diabetes control] (65%, Long-arrow), while the periphery comprises of Alpha-cells (30%, Short-arrow). Also seen are few congested vascular spaces amidst these cells.

Group – IV (Alloxan [120mg/kg] + HAEC [200mg/kg])

Section studied shows pancreatic lobules separated by thin fibrovascular septa. The center of islet cells consist of quantitative increase in Beta-cells [compared to positive control] (65%, Short-arrow), while the periphery comprises of Alpha-cells (30%, Long-arrow). Also seen are few degenerated beta cells.

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Group – V (Alloxan [120mg/kg] + HAEC [400mg/kg])

Section studied shows pancreatic lobules separated by thin connective tissue septa. The center of islet cells consists of quantitative decrease in Beta-cells (50%, Long-arrow) having basophilic granules, while the periphery comprises of Alpha-cells (45%, Short-arrow)) having eosinophilic granules. There are seen degenerated beta cells amidst these islets.

Group – VI (Alloxan [120mg/kg] + HAEC [600mg/kg])

Section studied shows pancreatic lobules separated by thin connective tissue septa. The center of islet cells consists of quantitative decrease in Beta-cells (55%, Long-arrow), while the periphery comprises of Alpha-cells (40%, Short-arrow).

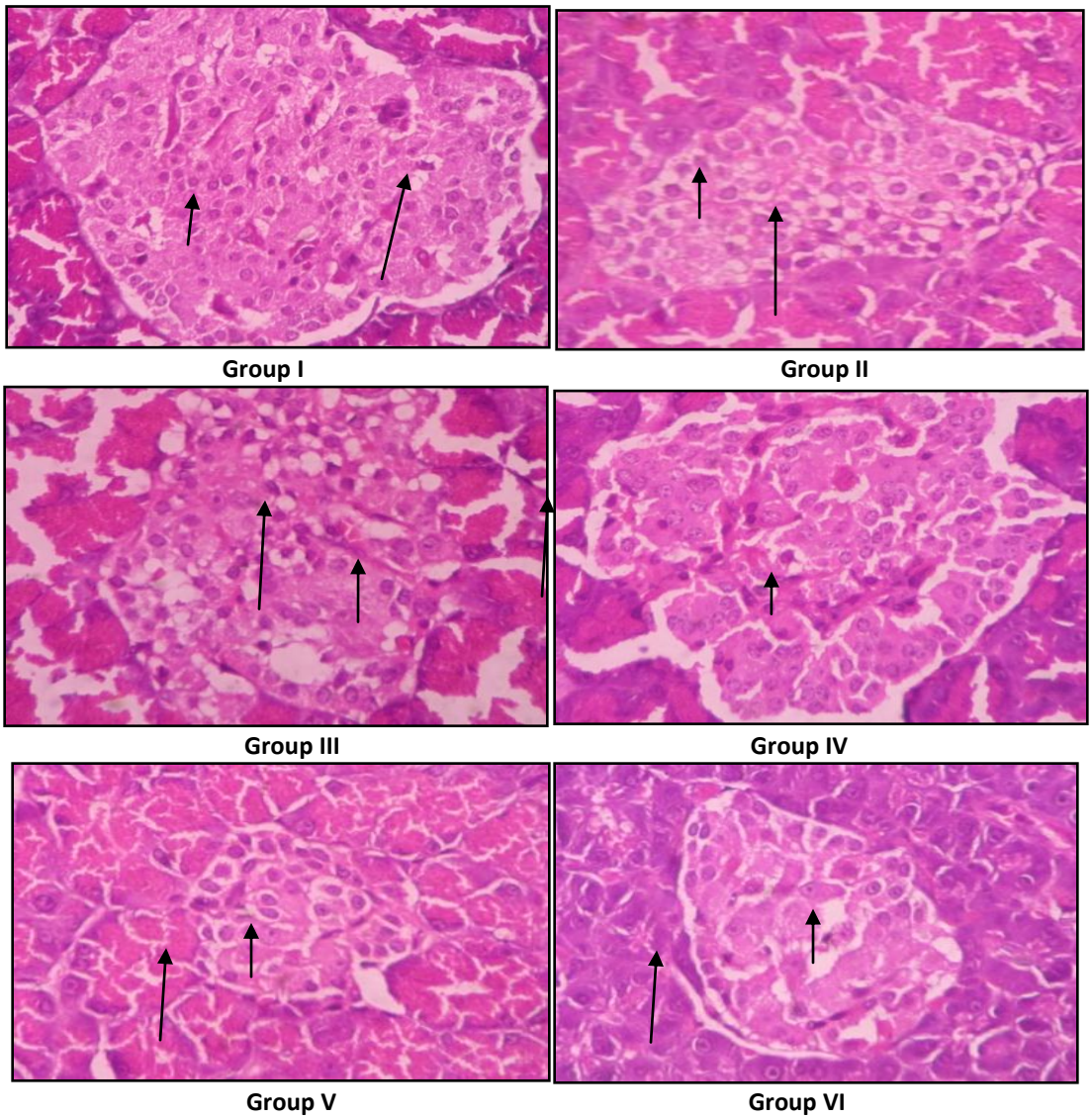

Figure No. 1: Histopathology of Pancreas

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DISCUSSION

Single dose study for 12 hours was carried out in normoglycemic rats. High dose of HAEC (600 mg/kg, p.o.) showed less significant decrease in blood glucose level at 8th hour and 12th hour compared to normal grouped. Medium dose of HAEC (400 mg/kg, p.o.) showed maximum decrease in blood glucose levels at 4th hour, 8th hour and 12th hour compared to normal group. Low dose of HAEC also showed a significant decrease from the 1st hour of the drug administration. Low dose of HAEC showed a significant decrease in blood glucose level at 8th hour and 12th hour compared to normal levels. It also showed its activity at 12th hour after the drug administration when compared with the normal control. High dose shown less significant compared with medium and low dose of HAEC. It may produce hypoglycemia in normal animals by stimulating the pancreatic beta-cells to produce more insulin and by increasing the glycogen deposition in the liver [22]. Glibenclamide (5mg/kg, p.o.) showed a maximum decrease of blood glucose levels in normoglycemic rats at 12th hr of our study.

Oral glucose tolerance test was studied on the normal rats. The lowering of glucose can be seen better in assay of glucose tolerance [23]. The fasting blood glucose levels decreases in glibenclamide, along with HAEC medium dose and high dose treated rats. Low dose shows reduced activity at 90th min. Such a phenomenon was already seen in the indigenous plants and reported. The lowering of glucose levels is may be due to the inhibition of intestinal absorption, or it may act by potentiating the secretion of insulin and by increase in the utilization of glucose levels in muscles [24].

As it was explained, that ALX damages β-cells in pancreas there is increased blood glucose levels in ALX treated rats. A prolonged treatment (21 days) of diabetic rats with the 200, 400 and 600mg/kg, p.o. of HAEC showed a significant reduction in the blood glucose levels than the control group rats. This hypoglycemic activity may be due to the stimulation of surviving β-cells to release more insulin. *Echinochloa crusgalli* may act by inhibiting hepatic gluconeogenesis or inhibiting α-glucosidase enzyme in the intestine, which is the enzyme helpful for breakdown of disaccharides to form glucose [25].

Induction of diabetes with ALX is associated with a characteristic decrease in body weight than the normal rats, this may be due to the wasting and loss of tissue protein. Whereas, diabetic rats treated with 200, 400 and 600mg/kg, p.o. of HAEC showed an improved result when compared with normal diabetic control. Which may be due to the protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement of glycemic control [26].

A decrease in the pancreas, liver, heart, kidneys, spleen weight was observed in diabetic animals. After 21 days of *Echinochloa crusgalli* in diabetic animals, an increased in the pancreas, liver, heart, kidneys, spleen weight is observed than the untreated rats. Whereas, high dose of HAEC did not show significant activity in organs weight.

A marked reduction in the levels of total protein and albumin levels was observed in the diabetic rats. The decrease in the albumin levels may be due to the increased protein catabolism. Present study showed that the treatment of diabetes 200mg/kg and 400mg/kg p.o dose of HAEC and glibenclamide showed increased albumin levels and protein levels significantly.

An enhanced increase in plasma urea levels were found in the diabetic rats when compared with the respective control group rats. While after the treatment with HAEC levels were significantly decreased. Similar observations are also reported in the D-400 herbal formulation.

In the present study, the groups of normal rats have shown gain in the body weight while fasting serum glucose was maintained in the normal range throughout the study period. The serum cholesterol and serum triglyceride levels of the normal rats were found to be increasing within the normal range during the four weeks of study period and the haemoglobin levels was also found to be maintained within the normal range throughout the study period.

The haemoglobin levels of the diabetic group of rats were found to be reduced significantly as against the normal haemoglobin levels of the normal group of rats this is due to an increased formation of its glycosylated form. During hyperglycemic condition protein synthesis is attenuated or reduced in all the tissues and thus the synthesis of haemoglobin also reduced [27].

Under normal condition, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes there by causing hypertriglyceridemia [28]. This altered lipid metabolism leads to diabetic complications. Practically it has been observed that there is an altered in levels of serum cholesterol and triglycerides levels in ALX treated rats, causes hypercholesterolemia and hyper-triglyceridemia [25]. Diabetic rats treated with the medium, high dose of HAEC and glibenclamide has shown a significant decrease in the levels of TG, TC, LDL-C and VLDL-C, where as it increases the levels of HDL-C when compared to the normal diabetic control rats. In low dose of HAEC treated rats HDL-C levels is less significant.

Diabetic rats treated with glibenclamide (5 mg/kg, p.o.) showed significant protection from the body weight loss and progressive reduction of 67.8% in fasting serum glucose levels after a daily dose for 21 days. The glibenclamide treatment also showed the reduced elevated serum cholesterol, albumin, total protein and urea levels produced significant reduction in elevated serum triglyceride and allowed significant recovery of reduced haemoglobin content during the period of study when compared with the diabetic group of rats. In agreement with the present results, several studies have shown protection in body weight loss [29], anti-diabetic activity, reduction in serum cholesterol and serum triglyceride [12] and recovery in haemoglobin content upon glibenclamide treatment [30].

Superoxide dismutase is an enzymatic antioxidant which reduces superoxide radical to hydrogen peroxide and oxygen. A decrease in the antioxidant activity in liver results in the accumulation of free radicals (hydroxyl radical) in diabetic rats. Administration of the medium dose of HAEC (400 mg/kg, p.o.) and glibenclamide increased the activity of SOD levels to a significant level of $P<0.001$, low dose of HAEC showed the significant level of $P<0.01$. While the SOD levels of untreated diabetic control rats having lowered levels. The HAEC may act by either directly scavenging the reactive oxygen metabolites or by increasing the anti-oxidant molecules. In diabetes, lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals react with the polyunsaturated fatty acids in cell membrane leading to lipid peroxidation. This in turn results in the development of free radicals. Low levels of lipoxygenase peroxides stimulate the release of insulin.

But, if the concentration of this peroxidase increases it results in uncontrolled release of lipid peroxidation. The most commonly used indicator of lipid peroxidation is TBARS. There was a significant elevation of TBARS in liver tissue in diabetic control animals when compared to the normal rats. Administration of HAEC and glibenclamide significantly reduced the TBARS levels. The effect shown is may be due to prevention of potential glycation of anti-oxidant enzymes.

Glutathione which is a tripeptide normally present at high concentrations intracellularly. Glutathione is helpful for reducing the toxic effects of lipid peroxidation.

Decreased level of GSH in liver during diabetes represents its increased utilization due to oxidative stress. Significant increased levels of GSH were shown in the diabetic rats treated with the HAEC and glibenclamide.

The histological evidence showed in the authenticated injury caused by ALX and the protection offered by HAEC and glibenclamide in pancreatic cells were shown. Microscopically examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in ALX induced rats. Histopathological study showed that *Echinochloa crusgalli* has the capacity to increase islet cell mass. However, the expansion was better with medium, high dose of HAEC dose.

CONCLUSION

In conclusion, the results of the present study indicate that hydro-alcoholic extract extract of grains of *Echinochloa crusgalli* at the doses 200, 400 and 600mg/kg, p.o. possess significant antidiabetic activity against ALX induced diabetic rats. 600mg/kg, p.o. showed very less effect than the 200 and 400mg/kg, p.o. both reduces the blood glucose levels in diabetic induced rats. The acute toxicity study indicated that the HAEC was devoid of major toxic effects. The effect of HAEC in normal rats and glucose loaded rats also indicated that the HAEC exhibited better glycemic control compared with the normal control animals, besides the drug treated (ALX induced, i.p.) diabetic rats showed a significant reduction in blood glucose levels and the other serum biomarker levels and also increases the haemoglobin levels. HAEC also

exhibited antioxidant activity in diabetic rats. The reports of histopathology study concluded protecting and regenerating the β-cells from the cytotoxic actions of ALX.

The results showed in dose 400mg/kg, p.o. is more similar to glibenclamide treated group which was used as reference standard. Overall observed significant activity may be due to presence of active constituents present in HAEC.

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