International Journal of Bio-Science and Bio-Technology
Vol.8, No.1 (2016), pp.51-64
http://dx.doi.org/10.14257/ijbsbt.2016.8.1.05

Evaluation of Methanolic Extract of Cressa Cretica Linn on Alloxan Induced Hyperglycemic Wistar Rats

[1]Lalitha Kumari, B.

[1]*Asst. Prof., Dept.of S&H, VFSTRU, Vadlamudi, Guntur (Dt.), A.P. India*
Mail id: lalithakumaribes@gmail.com

Abstract

The anti-hyperglycemic effect of methanolic soluble fractions of Cressa cretica (Convolvulaceae) made up of the whole plant (flower, root, leaf, stem) was investigated in alloxan (150 mg/kg) induced hyperglycemic Wistar rats. It's effect was compared with that of negative control, glibenclamide (0.9 mg/kg) as standard reference drug and Vit-E (100 IU/kg) as anti-oxidant standard. The physico-metabolic parameters measured were: Body weight, glycogen content in liver and muscle, serum glucose, speroxide desmutase (SOD) and histopathological examination of pancreas were also observed. Oral administration of MECC (200 mg/kg, 400 mg/kg) for 28 days exhibited a significant reduction in blood glucose. An improvement of glycogen content in liver and muscle, body weight, hepatic enzyme like SOD was observed. The results of this work suggest that MECC may possess anti-hyperglycemic and anti-oxidant property.

Keywords*: Cressa cretica Linn, Acute-toxicity, in-vivo antioxidant activity, alloxan diabetes*

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to absolute deficiency of circulating insulin levels (Venkatesh, *et al.,* 2003). Diabetes mellitus could also mean a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both (Edwin, *et al.,* 2007). The disease is a major ailment in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Vivek, *et al.,* 2010).

Generally it is known that there are two main basic types of diabetes; Type 1 or insulin dependent diabetes mellitus (IDDM) characterized by a deficiency of insulin due to loss of the insulin producing β-cells of the islets of langerhans in the pancreas and Type 2 diabetes or non insulin dependent diabetes mellitus (NDDM) which is due to insulin resistance or reduced insulin sensitivity combined with relatively reduced insulin secretion which in some cases become absolute (Wild, *et al.,* 2004). The defective responsiveness of body tissues to insulin is believed to involve the insulin receptors of the cell membranes. Globally type 2 diabetes mellitus is by far the commonest form of the disease and developing countries are the worst hit as far as this epidemic concerned (Sharma, *et al.,* 2007).

Currently available therapy for diabetes include insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone,etc. (Kameswararao, *et al.,* 2003). In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents (sulfonyl ureas, *etc.*,) as noted by (Pepato, *et al.,* 2005). These drugs are used as mono-therapy or in combination to achieve better glycemic control. They have their limitations and are known to produce serious side effects, therefore, the search so safer, specific and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from

ISSN: 2233-7849 IJBSBT

readily available traditional medicinal plants offering potentials for discover of new antidiabetic drugs (Klein, *et al.,* 2007). Cressa cretica Linn is one of such medicinal plants used in folkoric medicine for management of diabetes.

Cressa cretica L (convolvulaceae), commonly known as rudravanthi is an erect, dwarf shrub, salt tolerated plant, usally grows in sandy or muddy saline habitates. This is distributed throughout india. Traditionally, the plant is used in diabetes, asthma and it is used as an expectorants, stomachic, antibilious, anthelminthic, dyspepsia, flatulence, anorexia, tonic and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, urinary discharges (Vidyaratnam, *et al.,* 1997). Dry leaves of Cressa cretica crushed with sugar are used as emetic in sudan (Sangeeta, *et al.,* 2011).

It has been reported to possess anti-fungal (Pizada, *et al.,* 2009), anti-bacterial (Sunita, *et al.,* 2009), anti-tussive (Suganthi, *et al.,* 2008), testicular function (Guptha, *et al.,* 2006), anti-inflammatory and in-vivo antioxidant activities (Sunita, *et al.,* 2011). The methanolic extract of whole plant has been reported to contains flavonoids, ursolic acid, stigmasterol, β-amyrin, triacontoic acid (Hussain, *et al.,* 2005). By virtue of antioxidant property of the plant could be useful in restoring disturbed antioxidant defence system. The present study is therefore planned to evaluate the effect of Cressa cretica L methanolic extract on alloxan induced hyperglycemic wistar rat.

Material and Methods:

Collection and Identification of Plant Material

The whole plant of Cressa cretica L was collected from A. V. Rao (LAAM), Guntur district, AP, identified by Prof. Harsha Hegde, scientist 'B', Indian Council of Medical Research, Belgaum, with the reference no. RMRC-566.

Preparation of Plant Extract

The whole plant material of Cressa cretica was dried under the shade, powdered and passed through the sieve (coarse 10/40). The powder was subjected to extraction in soxhlet extractor, was deffated with petroleum ether (40-60) and later extracted with methanol. The extraction was continued for 12 cycles or until the solvent in the thimble was clears. With the use of rotator evaporator solvent was evaporated, extract obtained was stored under refrigerating condition. The extract was suspended in 1% tween-80 and used for the oral administration.

$$\% \text{ yield } = \frac{\text{weight of the extract}}{\text{weight of the plant material}} \text{X } 100$$

Experimental Animals

The complete course of experiment was carried out using healthy male wistar rats weighing between 150-200 g, were procured from sri venkateswara enterprises Bangalore. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle. The animals were provided with standard pelleted diet obtained commercially from the manufacturer (Amrut Laboratories, sangli) and water ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC Reg. No: 221/CPCSEA) KLEU's College Of Pharmacy, Belgaum, before conducting the experiment.

Acute Toxicity Study

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) Guideline 423-Acute toxicity class method. Female rats weighing 150-200 g (8-12 weeks) were used for toxicity study.

Induction of Diabetes

Diabetes was induced in overnight fasted male wistar rats (160-250 mg/kg) by i.p injection of 150 mg/kg body weight of alloxan. Alloxan monohydrate 150 mg/kg b.w, was dissolved in normal saline and injected i.p after 18 hours fasting. The rats were then given 4% w/v glucose solution in feeding bottles for the next 24 h in their cages to prevent hypoglycemia. After 48 hrs, alloxanization in blood samples collected by tail tipping method using glucometer, rats with marked hyperglycemic fasting blood glucose more than 200mg/dl were selected and used for the study. All the animals were allowed free access to water, pellet diet and maintained at room temperature in polyethylene cages.

Experimental Study Design:

The rats were divided into six groups consisting of six rats each.
Group I : Euglycemic or Normal animals (normal saline 5ml/kg, once daily)
Group II : Hyperglycemic rats (Alloxan 150 mg/kg, i.p, once)
Group III : Hyperglycemic rats + Cressa cretica L (low dose 200 mg/kg, p.o, once daily)
Group IV : Hyperglycemic rats + Cressa cretica L (high dose 400 mg/kg, p.o, once daily)
Group V : Hyperglycemic rats + Glibenclamide (0.9 mg/kg, p.o, once daily)
Group VI : Hyperglycemic rats + Vitamin E (100 mg/kg, p.o, once daily)

The body weight, blood glucose of all the rats were recorded at weekly intervals (1st day,7th day, 14th day,21st day,28th day) during the experimental period (28 days). At the end of experimental period the animals were overnight fasted and blood was taken from retro-orbital plexus under mild anesthesia, for estimation of glucose and serum parameters, liver for glycogen content and anti-oxidant enzymes estimation, muscle for estimation of glycogen content, pancreas for histopathological examination.

Statistical Analysis

Values were expressed as Mean ± SEM. The data were analyzed by one-way ANOVA followed by Dunnet's Multiple Comparison Test. $P<0.05$ was considered as significant.

Results and Discussion

Plant Extraction

The yield of MECC whole plant extract was 8% (w/w) dry matter and was greenish in colour

Preliminary Phytochemical Screening

By preliminary phytochemical analysis of MECC showed the presence of flavonoids and terpnoids.

Acute Toxicity Study

The acute toxicity study in rats produced no death or signs of toxicity even at high dose (5000mg/kg) of the extract

Anti-Diabetic Study

Effect of Various Treatments on Body Weight and Blood Glucose

The body weight of alloxan (150 mg/kg) treated group was gradually reduced from 7th day to 28th day compared to that of normal rats from 7th day to 28th day. The hyperglycemic rats treated with MCC Low dose of extract showed increase in the body weight gradually from 14th day to 28th day as compared with Alloxan treated rats, but hyperglycemic rats treated with MCC high dose of extract and Vit E (100 IU/kg), Glibenclamide (0.9 mg/kg) showed gradual increase in body weights from 7th day to 28th day respectively compared to that of Low dose of extract and Alloxan (150 mg/kg) treated group (Table 1, Figure 1).

Table 1. Effect of Various Treatments on Body Weight (gm)

GROUPS	1st day	7thday	14th day	21st day	28th day
GROUP I Normal (normal saline)	161.50±2.75	169.0±2.78	176.7±23.17	182.3±11.09	192.00±1.30
GROUP II Alloxan (150 mg/kg)	200.50±2.89	172.00±2.59	158.00±2.51	145.00±1.72	133.7±1.542
GROUP III Alloxan(150 mg/kg) + Glibenclamide (0.9 mg/kg)	173.50±2.84	181.00±2.64	186.50±2.02	194.00±1.90	203.00±1.90
GROUP IV Normal + Low dose (200 mg/kg)	162.9±6.81	167±8.594	178.7±20.91	183.3±15.07	190.6±26.28
GROUP V Alloxan + Low dose (200 mg/kg)	176.8±6.169	165.3±6.614	167.3±2.91	170.12±2.78	171.2±2.51
GROUP VI Normal + High dose (400 mg/kg)	165.9±27.87	169.3±12.89	175.5±17.77	179.9±4.703	189.8±19.12
GROUP VII Alloxan (150 mg/kg) + High dose (400mg/kg)	166.50±5.46	168.0±6.812	172.2±9.82	175.7±26.28	180.9±2.51
GROUP VIII Alloxan + Vit E (100 IU/kg)	163.50±6.78	166.8±15.07	168.3±2.64	179.00±2.51	182.7±1.54

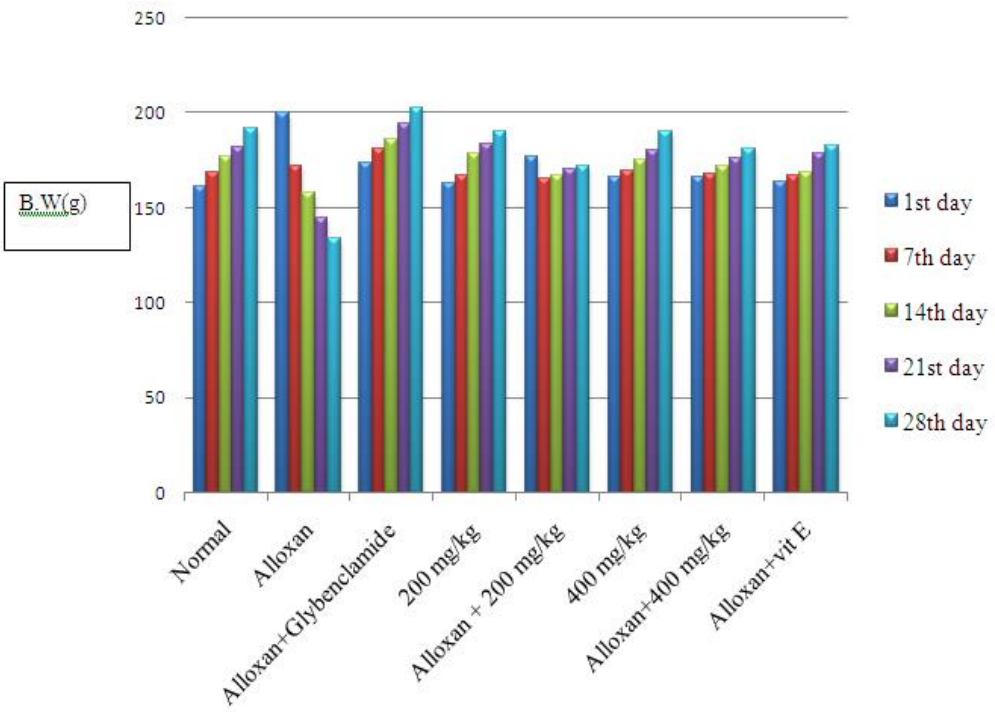


Figure 1. Effect of Various Treatments on Body Weight (gm)

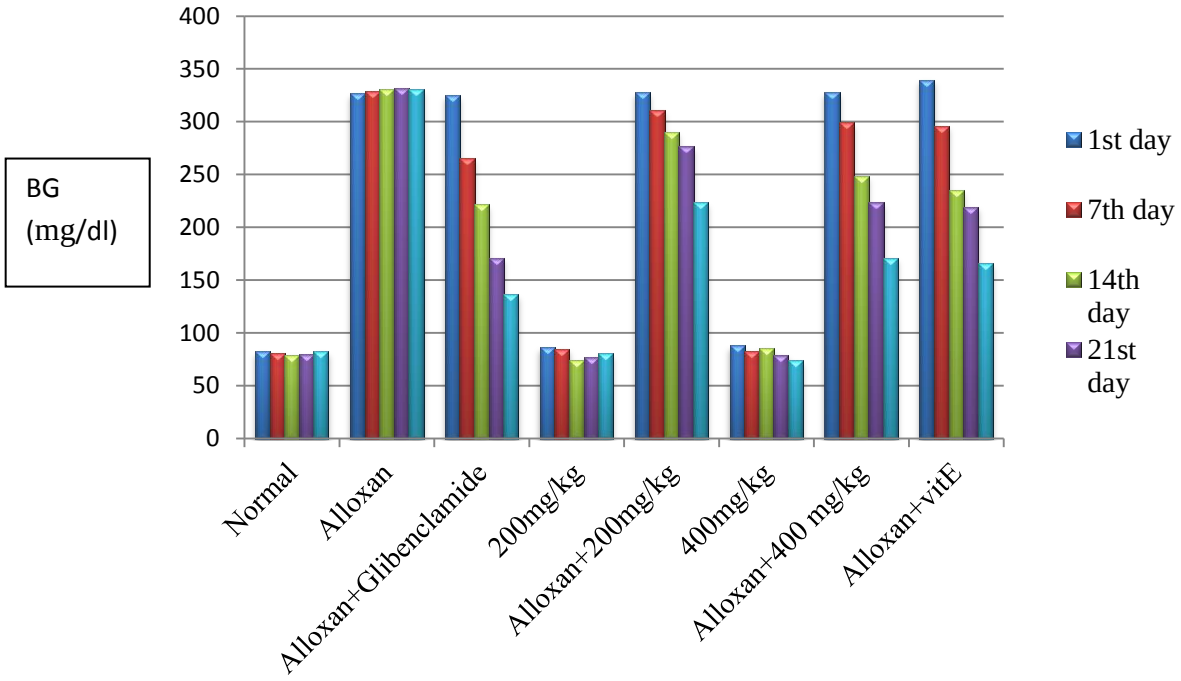
The blood glucose of Alloxan (150 mg/kg) treated group was increased from 7th day to 28th day compared to that of normal group from 7th day to 28th day . The hyperglycemic rats treated with low dose of MECC showed gradually decrease in blood glucose from 7th day to 28th day as compared with Allloxan (150 mg/kg) treated group, but the hyperglycemic rat treated with high dose of MECC, Vit-E (100 IU/kg) and glibenclamide (0.9 mg/kg) showed gradually decrease in the blood glucose from 7th day to 28th day when compared to that of Alloxan (150 mg/kg) and Low dose treated group (Table 2, Fig 2) .

Table 2. Effect of Various Treatments on Blood Glucose (mg/dl)

GROUPS	1st day	7th day	14th day	21st day	28th day
GROUP I Normal (normal saline)	82.09±1.078	80.62±1.134	78.93±1.006	79.35±0.960	82.36±0.602
GROUP II Alloxan (150 mg/kg)	326.6±1.744	328.5±1.449	330.1±1.245	331.7±1.638	330.7±0.872
GROUP III Alloxan (150 mg/kg) + Glibenclamide (0.9 mg/kg)	324.8±1.692	265.5±1.785	221.8±1.621	171.1±2.418	136.3±1.276
GROUP IV Normal +Low dose (200 mg/kg)	85.83±2.600	84.67±1.563	73.67±3.190	76.67±3.190	80.33±0.802

GROUP V Alloxan (150 mg/kg) + Low dose (200 mg/kg)	327.8±7.857	311.3±2.720	290.3±8.459	276.8±12.14	223.5±4.031
GROUP VI Normal +High dose (400 mg/kg)	88.00±9.092	82.67±1.606	85.67±3.449	78.93±1.006	73.67±3.890
GROUP VII Alloxan (150 mg/kg)+High dose (400mg/kg)	327.8±5.344	298.9±3.150	248.8±4.031	223.5±4.031	171.0±2.000
GROUP VIII Alloxan(150 mg/kg) +Vit E (100 IU/kg)	329.5±29.25	276.0±1.245	235.0±6.00	219.4±3.021	166.3±19.08

Figure 2. EFFECT OF VARIOUS TREATMENTS ON BLOOD GLUCOSE (mg/dl)



Effect of Various Treatments on Glycogen Content in Liver and Muscle

There was a significant decrease in the glycogen levels in liver and muscle in alloxan treated group ($P<0.001$) compared with that of normal group. Treatment with low dose (200 mg/kg) of extract showed significant ($P<0.05$) effect compared to that of Alloxan (150 mg/kg) treated group. But, the groups treated with high dose (400 mg/kg) of extract, vit E (100 IU/kg) and Glibenclamide (0.9 mg/kg) standard showed significant ($P<0.001$) increase in levels of glycogen compared with Alloxan (150 mg/kg) induced group. The group treated with high dose (400 mg/kg) of extract showed no significant ($P<0.001$) increase in the glycogen levels compared with Glibenclamide (0.9mg/kg) treated group (Table 3, Figure 3).

Table 3. Effect of Various Treatments ON Glycogen Content in Liver and Muscle

GROUPS	LIVER (mg/gm)	MUSCLE (mg/gm)
GROUP I Normal (normal saline)	19.38±1.713	9.62±0.102
GROUP II Alloxan (150 mg/kg)	9.56±0.680 $^{###}$	1.482±24.25 $^{###}$
GROUP III Alloxan (150 mg/kg) + Glibenclamide (0.9 mg/kg)	17.14±1.645 ***	7.504±0.078 ***
GROUP IV Normal + Low dose (200 mg/kg)	20.05±1.060	9.95±0.798
GROUP V Alloxan (150 mg/kg) + Low dose (200 mg/kg)	11.66±1.382 *	2.638±0.410 *
GROUP VI Normal+ High dose (400 mg/kg)	19.20±0.9236	9.18±0.214
GROUP VII Alloxan (150 mg/kg) + High dose(400 mg/kg)	13.36±2.777 ^{***,no}	4.054±0.430 ^{***,no}
GROUP VIII Alloxan (150 mg/kg) + Vit E (100 IU/kg)	13.85±0.9304 ***	4.976±0.375 ***

Compared with Normal control: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$.
Compared with Disease control: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, ns = no significance
Compared with standard : $^{a}P<0.05$, $^{aa}P<0.01$, $^{aaa}P<0.001$, no = no significance

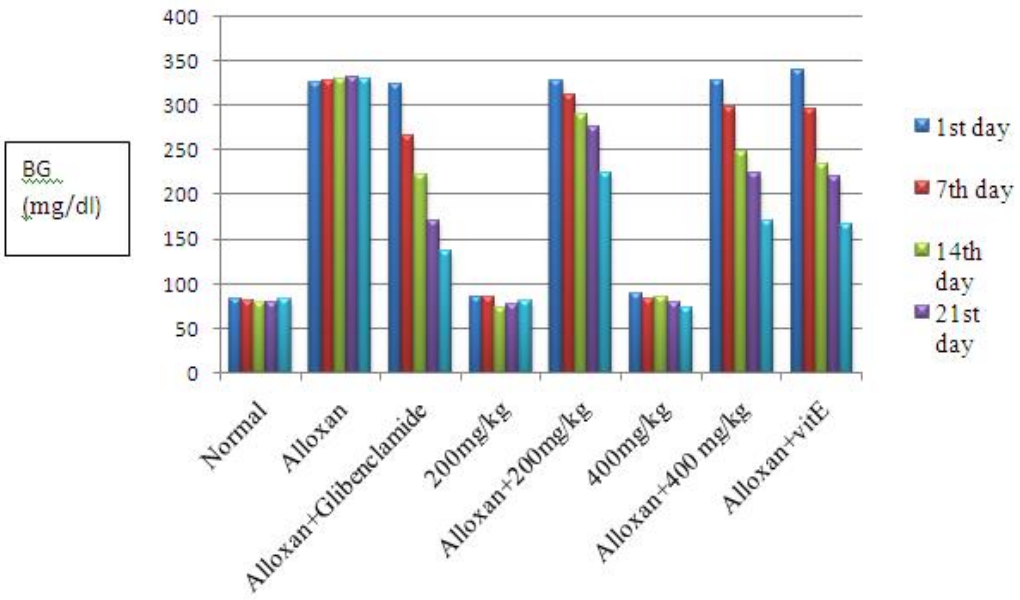


Figure 3. Effect of Various Treatments on Glycogen Content in Liver and Muscle

Pancreas of normal control and various treated diabetic rats were kept in 40% formalin, submitted to Jeevan Regional Diagnostics for histopathology. The specimens were processed by slandered procedure and embedded in paraffin wax. The blocks were sectioned from the ventricular portion and 5-micron thick sections were stained according to the hematoxylin and eosin (H & E) method given by Smith and Burton the sections were examined by light microscopy.

Photomicrograph showed normal acini and normal cellular population in islets of Langerhans and absence of both damage to islets and hyperplasia in normal contorl rats. In diabetic control rats chronic Islet damage and reduced Islet size was seen. In GLB treated rats showed normal acini and normal cellular population with little congestion in islets of Langerhans. In low dose of MECC treated group showed mild to moderate destruction of islets of Langerhans. In high dose of MECC and vit-E (100 IU/kg) treated groups showed mild destruction with near to normal cellular population size (Figures: 4-9).

EFFECT OF VARIOUS TREATMENTS ON HISTOPATHOLOGY OF PANCREAS

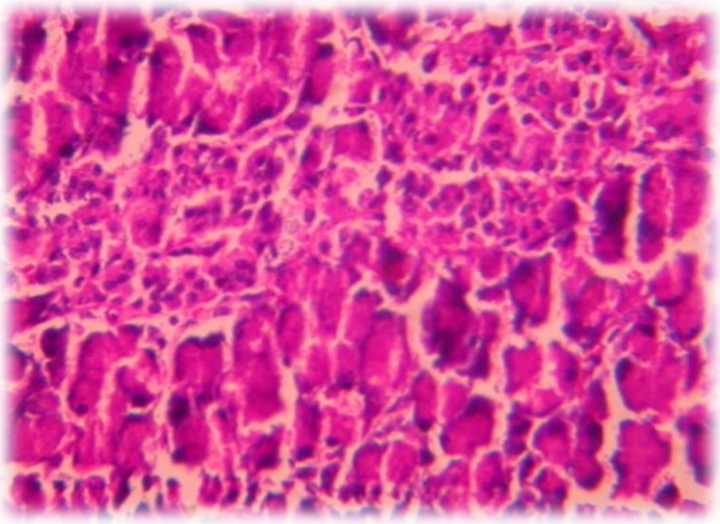


Figure 4. Normal Acini and Normal Cellular Population in Islets of Langerhans and Absence of Both Damage to Islets and Hyperplasia in Normal Control Rats

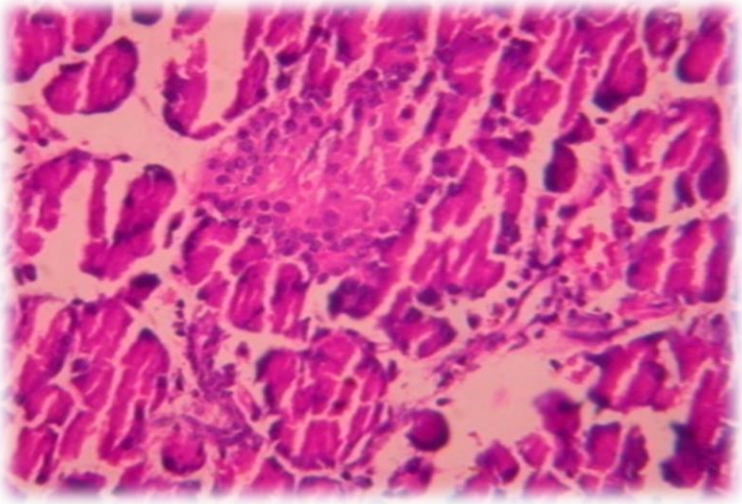


Figure 5. Alloxan (150 mg/kg) Treated Rats Showed Sever Congestion and Focal Necrosis of Exocrine Pancreas. There is Marked Reduction in Number, Size and Beta Cellularity of Islets of Langerhans

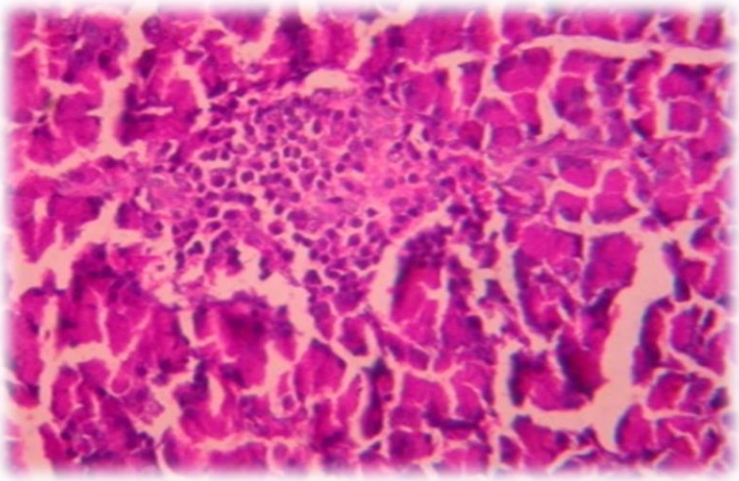


Figure 6. Alloxan + Glib (0.9mg/kg) Treated Rats Showed Mild Congestion with Near Normal Number of Islets of Langerhans

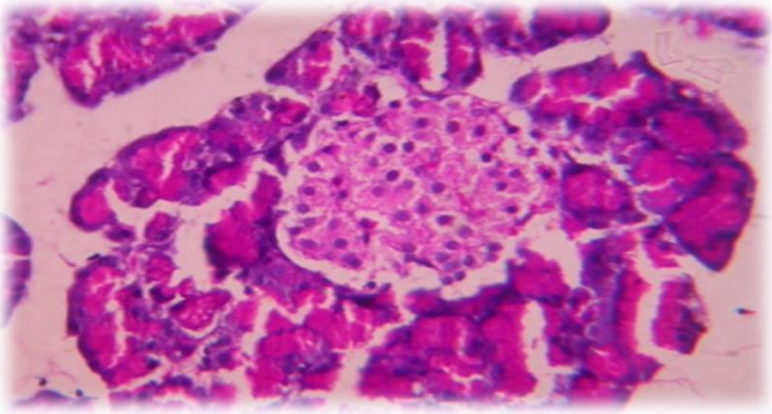


Figure 7. Alloxan + Low Dose (200 mg/kg) Treated Rats Showed Mild Congestion of Exocrine Pancreas. There is Mild Reduction in Number and Size in Islets of Langerhans

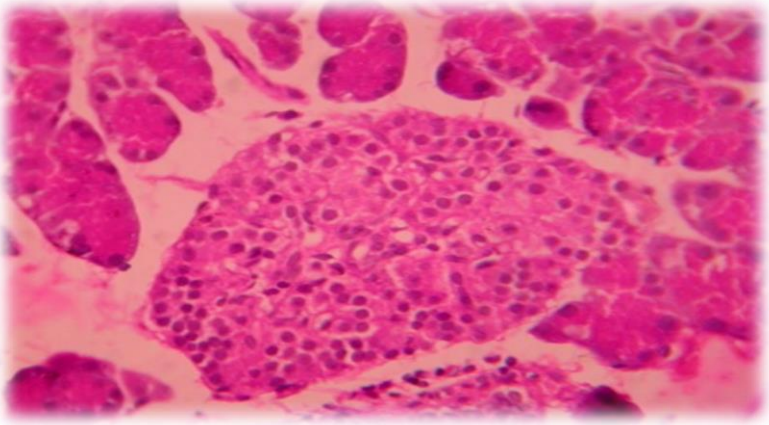


Figure 8. Alloxan + High Dose (400mg/kg) Treated Rats Showed Mild Congestion of Exocrine Pancreas. There is Mild to Moderate Reduction in Number and Size of Islets of Langerhans

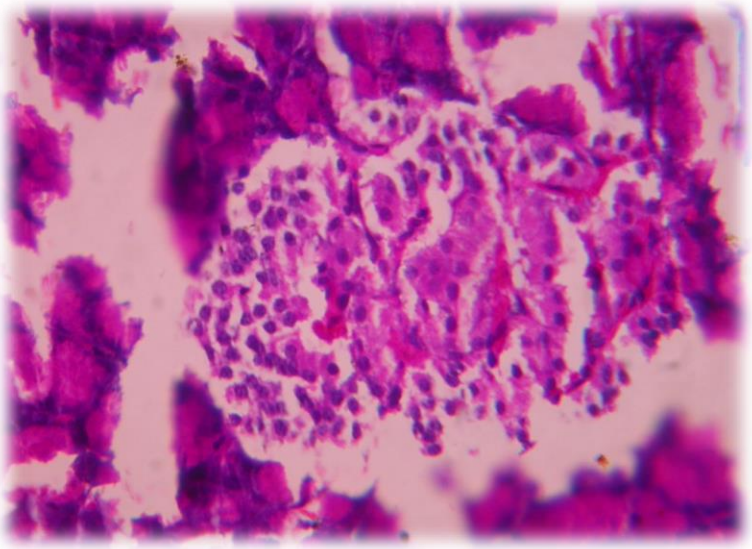


Figure 9. Alloxan + VitE (100 IU/kg) Treated Rats Showed Mild Congestion of Exocrine Pancreas and Mild Reduction in Number, Size of Islets of Langerhans

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins and an increased complications from vascular disease. Because of a lack of, or ineffective use of, the hormone insulin. In DM Oxidative stress and oxidative damage to tissues are common causes of chronic diseases such as atherosclerosis, diabetes and rheumatoid arthritis. The oxidative stress and oxidative damage in DM was thought to be a result of free radical generation during autoxidation of glucose (Inoguchi, *et al.*, 2000).

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their origin and less side effects. The present study is carried out on methanolic extract of *Cressa cretica* Linn in Alloxan induced hyperglycemic rats.

Cressa cretica Linn was adminstered at two different doses (low dose 200 mg/kg, high dose 400 mg/kg) considered as safe. The previous studies on *Cressa critica* Linn provides a clear evidence that presence of β-amyrin, umbelliferone (anti-oxidant), quercetin (lowers LDL- cholesterol level), rutin, olaenolic acid, stigmasterol (hypoglycemic) in the methanolic extract have potent antioxidant and anti-peroxidant activity (Hussain et at., 2005). *Cressa cretica* L offers significant hypoglycemic agent in terms of preservation of endogenous antioxidants, scavenging of superoxide and hydroxyl radicals and inhibition of lipid peroxidation.

The action of alloxan in the pancreas is preceded by rapid uptake by insulin-secreting cells (β-cells) and would allow generation of reactive oxygen species (ROS), sufficient to damage the cells is preceded by alloxan reduction. Has been proposed to be one of the important features determining alloxan cytotoxicity (Szkudelski, *et al.*, 2001).

ADA criteria include symptoms of DM, *e.g.*, polyurea, polydipsia, and unexplained weight loss, increased plasma glucose concentration. Alloxan treated group showed decrease in body weight and increase in blood glucose, which indicates loss or ineffective use of glucose by the peripheral tissues due to the destruction of β-cells of pancreas (Szkudelsi, *et al.*, 2001). However, all of the above changes were found to be inhibited in the *Cressa cretica* Linn high dose of extract, vit-E and glibenclamide treated groups as compared with alloxan and low dose of extract treated groups.

Pharmacological augmentation of endogenous liver antioxidants has been identified as a promising therapeutic approach in disease associated with increased oxidative stress

(Bever et al., 1979). The antioxidative enzymes SOD greatly attenuates the toxicity of both alloxan and dialuric acid to insulin-producing cells in the presence of GSH. The reason for this cytoprotective effective is the ability of SOD to scavenge superoxide radicals, which are generated in the O_2^- dependent chain reaction between dialuronic acid and alloxan. Through this mechanism counteracts the toxicity to insulin-producing cells. Similarly, increased ROS may lead to cell death or to acceleration in aging and age-related diseases. Traditionally, the impairment caused by increased ROS is thought to result from random damage to proteins, lipids and DNA (Elsner, *et al.*, 2007) (Munday, *et al.,* 1989).

Alloxanized rats treated with low dose *Cressa cretica Linn.*extract showed significant ($P<0.05$) increase in liver and muscle glycogen levels, but rats treated with high dose of extract, vit E and Glibenclamide showed significant ($P<0.001$) increase in liver and muscle glycogen levels. But, as compared with standard drug high dose showed no significant increase in glycogen content in liver and muscle.

Conclusion

The results obtained from the present study demonstrated that the orally administered high dose (400 mg/kg) of MECC significantly decreases the elevated blood sugar levels, and significantly increased insulin, glycogen content in liver and muscle, possesses antioxidant property by virtue of its normalizing the impaired oxidative stress defence mechanism in the hyperglycemic rats.

Cressa cretica has been already reported to have anti-inflammatory and anti-fungal activity by virtue of its anti-oxidant constituents, so the present study on anti hyperglycemic activity seems to have these active principles. Further work includes effect of MECC treatments on serum lipid profile; serum biomarker enzymes; serum insulin; and Anti oxidant enzymes in LTH.

References

[1] S. Venkatesh, R. G. Dayanand, R. B. Madhava, M. Ramesh and A. V. N. Apparao, "Anti-hyperglycemic activity of Caralluma attenuate", Fititerapia, vol. 74, **(2003)**, pp. 274-79.
[2] E. Edwin, E. Sheeja, S. P. Dhanabal and B. Suresh, "Anti-hyperglycemic activity of Passiflora mollissima Bailey", IJPS, vol. 64, **(2007)**, pp. 570-71.
[3] K. S. Vivek, K. Suresh, J. P. Hitesh and H. Schivakumar, "Hypoglycemic activity of Ficus glomerate in alloxan induced diabetic rats", IJPS, vol. 1, **(2010)**, pp. 18-22.
[4] S. Wild, G. Roglic, A. Green and H. Sicree-King, "Global prevalence of diabetes: estimates for 2000 and projections for 2030", Diabetes Care, vol. 5, **(2004)**, pp. 1047-53.
[5] S. Sharma, M. Chaturved, E. Edwin, S. Shukla and H. Sagrawat, "Evaluation of the phytochemicals and antidiabetic activity of Ficus bengalensis", International Journal of Diabetes in Developing countries, vol. 27, **(2007)**, pp. 57-59.
[6] M. T. Pepato, D. M. Mori, A. M. Baviera, J. B. Harami, R. C. Vendramini and I. L. Brunetti, "Fruit of Jambolan tree and experimental diabetes", Journal of Ethnopharmacology, vol. 96, **(2005)**, pp. 43-48.
[7] G. Klein, J. Kim, K. Himmeldirk, Y. Cao and X. Chen, "Antidiabetes and anti-obesity activity of Lagerstroemia speciosa", Evidence-Based Complementary and Alternative Medicine, vol. 4, **(2007)**, pp. 401-07.
[8] P. S. Vaidyaratnam, "Indian Medicinal Plants", Orient Logman Ltd, vol. II, **(1997)**, pp. 206.
[9] R. Sangeeta, S. Chaudhary, P. Singh, G. Mishra, K. K. Jha, R. L. Khosa and C. C. Linn, "An important medicinal plant- a review on its traditional uses, Phytochemical and pharmacological properties", Scholar Research Library, vol. 1, no. 1, **(2011)**, pp. 91-100.
[10] A. J. Pirzada, W. Shaikh, K. U. Ghani and Laghari, "Study of Anti fungal activity and basic elements of medicinal plant Cressa cretica Linn against fungi causing skin diseases", SURJ, vol. 42, no. 2, **(2009)**, pp. 15-20.
[11] P. Sunita, S. Jha and S. P. Pattanayak, "In-vivo anti tussive activity of Cressa cretica Linn using cough model in rodents", Pharmacognosy Research, vol. 1, no. 3, **(2009)**, pp. 157-61.
[12] G. Suganthi, K. Shudhshini, K. Sripathy and K. Manian, "HPTLC and Antibacterial analysis of extracts of Cressa cretica Linn", Ancient Science of Life, vol. 27, no. 3, **(2008)**, pp. 1-14.

[13] R. S. Guptha, J. B. S. Kachhawa, V. Khushalani, K. Thanwar and Y. C. Joshi, "Effect of Cressa cretica Linn methanolic extract on testicular function of albino rats", Pharmaceutical Biology, vol. 44, no. 5, **(2006)**, pp. 382-88.
[14] P. Sunita, S. Jha and S. P. Pattnayak, "Antiinflammatory and In-vivo Antioxidant Activity of Cressa cretica Linn a halophytic Plant", Middle-East Journal of Scientific Research, vol. 8, no. 1, **(2011)**, pp. 129-40.
[15] S. Hussain, E. Ahmed, A. Malik, A. Jabbar and M. Arshad, "Phytochemical studies on Cressa cretica Linn", Journal of the Chemical Society of Pakistan, vol. 27, no. 3, **(2005)**, pp. 296-98.
[16] "Revised document (OECD 423) guidance document on acute toxicity", Guidelines for testing of animals, pp. 1-14.
[17] T. Inoguchi, P. Li, F. Umeda, H. Y. Yu, M. Kakimoto, M. Imamura, *et al.*, "High glucose level and free fatty acid stimulate reactive oxygen species production through proteins C-dependent activation of NAD(P)H oxidase in culture vascular cells", Diabetes, vol. 49, **(2000)**, pp. 1939-1945.
[18] T. Szkudelski, "The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas", Physiol Res., vol. 50, **(2001)**, pp. 536-46.
[19] O. B. Bever and G. R. Zahnd, "Plants with oral hypoglycemic activity", Pharmaceutical Biology, vol. 17, no. 3-4, **(1979)**, pp. 139-196.
[20] M. Elsner, E. G. Convey and S. Lenzen, "Relative importance of cellular uptake and reactive oxygen species for the toxicity of alloxan and dialuric acid to insulin-producing cells", Free Radic Bio Med., vol. 41, **(2007)**, pp. 825-834.
[21] R. Munday and C. C. Winterbroun, "Reduced glutathione in combination with superoxide dismutase as an important biological antioxidant defence mechanism", Biochem Pharmacol, vol. 30, **(1989)**, pp. 4349-52.