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SCREENING OF EXTRACTS OF *PROSOPIS SPICIGERA* (LIN) FOR ANTIDIABETIC ACTIVITY IN EXPERIMENTAL MICE MODEL

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ABSTRACT:

The present study was aimed at evaluating the anti-diabetic potential of ethanolic extracts of leaves of *Prosopis spicigera* on streptozotocin induced mice models. Total RBCs & WBCs count and differential WBC count was studied along with serum protein, glucose and lipid content were estimated.ALP, AST,ALT & LDH were estimated to evaluate the two functions.STZ diabetes induced mice fed with *P. spicigera* extracts showed value of blood glucose 100 mg% comparable to those treated with insulin. Similarly all the parameters studied in the mice fed with *P. spicigera* were comparable to those of controls well as those treated with insulin. The results confirm the anti-diabetic activity of *P. spicigera* leaf extracts.

KEY WORDS: *Screening, Prosopis spicigera, Antidiabetic, Mice.*

INTRODUCTION:

Diabetes mellitus affects a large number of people throughout the world. India leads the way with its largest number of diabetic subjects (Senthil kumar *et al*. 2006) A worldwide projection suggests that more than 300 million people will have diabetes by the year 2025 and the global cost of treating diabetes & its complications could reach 1 trillion US dollars annually (Valinathan 1998). Clinically diabetes is associated with a number of chronic complications including neuropathy, retinopathy and cardiovascular diseases (Mahdi *et. al.*, 2003). In view of the undesirable side effects of synthetic drugs WHO (1980) has recommended evaluation of plants effective in the treatment of Diabetes. In recent times, herbal remedies are gaining prominence, because of the observation that the efficacy of allopathic medicines, which were once universally effective is on the wane . (Rajendra, *et. al*, 2007) the ability of herbal medicine to affect body systems on the chemical constituents it contains. Natural compounds derived from plants with anti diabetic activity includes complex carbohydmicees, alkaloids, glycopeptides steroids, flavonoids, lipids, coumarines, sulphur compounds (Marles,1996).

Many Indian medicinal plant have been found to be useful is successfully managing diabetes and from some of them active compounds have been isolated. (Shukla *et. al*,2000). Anti-diabetic effect of Murraya Koenigii leaves on streptozotocin induced diabetic mice was reported Yadav *et.*

al .(2006). The major merits of herbal medicine seem to be then efficacy, low incidence of side effects and cost effectiveness.

Prospis spicegera is a commonly occurring plant along the semi dry regions. *P.spicigera* is an erect prickly tree or shrubs, with two pinnate leaves, leaflets are narrow, flower are penta merous usually sessile, in narrow spike fliform. Since leaves do not form food for herbivores, this plant is usually available throughout the year. Though the plant is used by locals as medicine, there is no documental work (personal communication with locals). Hence an attempt has been made to scientifically verify the medicinal properties of *P.spicigera*

MATERIAL AND METHODS:

Streptozotocin and bovine serum albumin (BSA) were procured from sigma chemical cost Louis,MO,USA. All other chemicals were used of analytical grade and purchased locally.

Plant material:-leaves of *P.spicigera* leaves were collected from semi dry region of Satara district of Maharashtra state.

Prepamiceion of plant extract:-The *P.spicigera* leaves were dried at room temp. the leaves were powdered in an electric grinder and stored at 5 degree C until further use. The powder was mixed with 95% ethanol and the extraction procedure was carried out in soxhlet appamiceus.ethnol was evapomiceed in rotator evapomiceor at 40 to 45degree C under reduced pressure. The yield of extract was 45gm.

Animals:-wistar mice weighing 25-30gms of either sex were used. Animal were housed in standard environmental conditions.

Experimental induction of diabetes: Diabetes was induced using streptozotocin (55mg/kg/body weight single i.p. injection in0.1M citmicee buffer pH4.5

Streptozotocin (STZ) is a glucosamine nitrosurea compound that how elective cytotoxicity to pancreatic B cells and is used to induce type I diabetes in experimental animals. The mechanism of the induction of the diabetes is not clear, but it is proposed that could be related to the genemiceion of free radical such as super oxide, hydrogen peroxide, nitric oxide which results in DNA fragmentation.

EXPERMENTAL DESIGN

After a `basal reading at 4th week of streptozotocin injection control and diabetic mice were randomly selected.

The mice were divided into following three groups of six each:

Group 1: Healthy control mice receiving 0.1M citmicee buffer (pH 4.5).

Group 2: Diabetic control.

Group 3: Diabetic mice given ethanol extract of *P.spicigera*(200mg/kg/day) in aqueous solution orally once daily for 30 days.

Group 4: Diabetic mice treated with protamine-zine Insulin ip injection (6 units/kg/day) for 30 days

Mice in all groups were provided with food and water and *ad libitum*.

At the end of experimental period, the animal were anaesthetized and sacrificed. Blood sample were collected in tubes containing potassium oxalate and sodium floride . The plasma was stored at -4^{0}C until analysis was completed. The liver was excised, rinsed in ice-cold saline,cut into small pieces and homogenized with Potter-Elvehjem glass-Teflon homogenizer in Tris-Hcl buffer (pH 4.5).The homogenate was centrifuged at 1000 rpm 10 min.Supernatant was used for various measurements. The following analyses were carried out:Total erythrocytes count,Total leucocytes count, Differential count, and Haemoglobin content ,Total serum protein,Blood glucose and lipid profile from blood plasma. In biochemical studies from liver: Alkaline phosphatase (ALP),Aspartase amino tranferase (AST),Alkaline amino tranferase (ALT) and Lactate dehydrogenese (LDH).

RESULT AND DISCUSSION:

The result of blood parameters are given in the table no.1and level of liver enzymes in table no.2.& fig.no.1 .Increase in total count of erythrocytes and leucocytes was observed in STZ induced diabetic mice i.e.12 million/mm^{3}and17 million thousand/mm^{3}respectively as compared to values of 9 million/mm^{3}and14 million thousand/mm^{3}respectively in control mice. A similar trend was observed incase of differential count leucocytes and hemoglobin content.

The trend observed in the HB content in the present study is not accordance with that reported by Boappana *et.al,*(1997) and Senthil Kumar *et.al,*(2006) where the HB content decreased in diabetic mice.

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over production of glucose due to glycogenolysis and gluconeogenesis as well decreased utilization of glucose by the tissue Lantner,(1958).The oral administmiceion of *P.spicigera* significantly lowered the blood glucose levels. The effective reduction of elevated levels of cholesterol in *P.spicigera* treated mice is in accordance with Boappana *et.al,*(1997)

The conversion of glucose to glycogen in the liver cells is dependent on the extra cellular glucose concentmiceion and on the availability of insulin which stimulates glycogen synthesis over wide range of glucose concentmiceions (Stalmans *et.al* 1997).Liver plays an important role in the maintenance blood glucose level by regulating its metabolism. Hexokinase which brings about the first phosphorylation step of glucose metabolism, is reduced in diabetic mice (Nehal and Baquer,1989).Lactate dehydrogenase in anaerobic glycolysis, catalyses the conversion of pyruvate to lactate which is subsequently converted to glucose along with amino acids and glycerol in the gluconeogenic flux. There are the two important events that balance the glucose load in our body

(Bhavapriya and Govindasamy,2000).Although a number of studies about LDH levels in the diabetes mellitus have been carried out the results are contradictory. The present study indicated a decrease in LDH levels in diabetes mellitus than those in normal cases (Rajendran *et.al.*2007). It has been suggested that the increase in enzyme levels may be resulting from the influence of insulin on liver and muscle (Lehninger,1982).The present study indicates the need for further investigation for ascertaining the cause of elevated levels of enzymes.However,from the present study it can be concluded that ethanolic extracts of *Prosopis spicigera* exhibited antidiabetic activity. This may be due to differential constituents of the leaf acting synergistically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes.

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Table no 1 Effect of treatment of ethnol (PSEE) of *Prosopis spicigera* **leaves on some blood parameters in STZ induced diabetic mice.**(PSEE – *Prosopis spicigera* Ethanol extract)

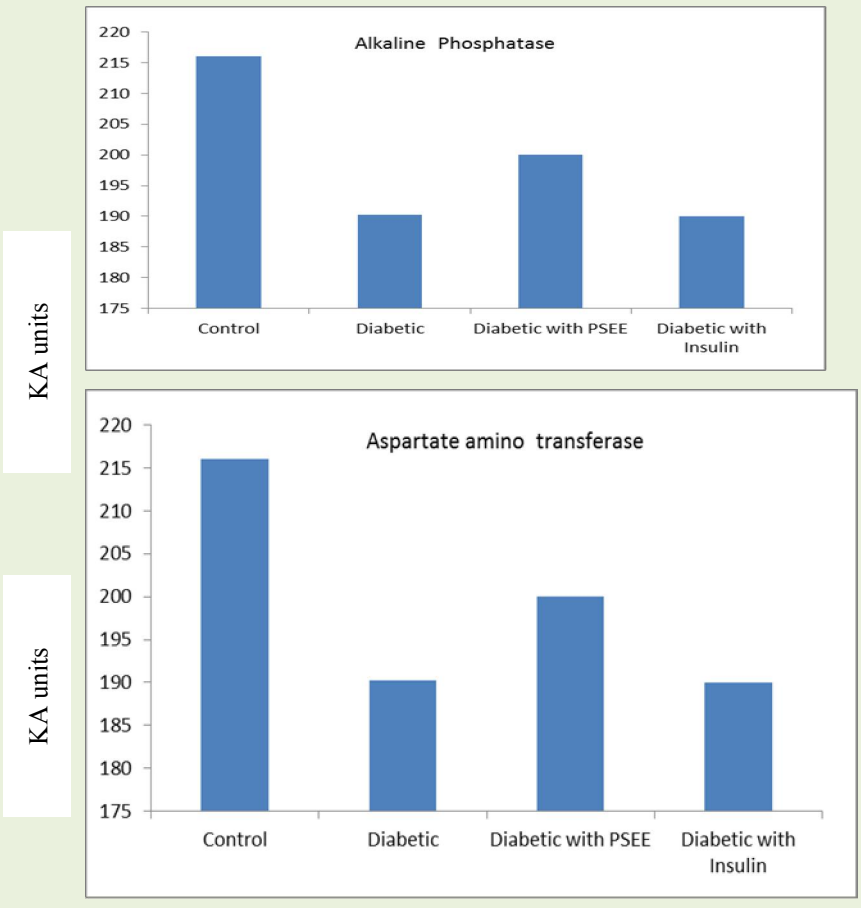
Sr.no	Blood parameter	Control	Diabetic	Diabetic with PSEE	Diabetic with Insulin
1	Total erythrocyte count	9 million/mm^3	12 million/mm^3	10 million/mm^3	11 million/mm^3
2	Total leucocyte count	14 thousand/mm^3	17 thousand/mm^3	15 thousand/mm^3	14 thousand/mm^3
3	Defferential count				
	Neutrophil	20%	22%	23%	22.5%
	Lymphocyte	60%	70%	65%	64%
	Monocyte	5%	2%	5%	5%
	Esosinophil	2%	5%	6%	5%
	Basophil	1%	1%	1%	1%
4	Haemoglobin content	15 gm%	18 gm%	16 gm%	15 gm%
5	Blood glucose	85.2 mg%±8	250 mg%±12	100 mg%±7	110 mg%±6
6	Total serum protein	7.10g/dl±12	6.2g/dl±15	6.8g/dl±2	7 g/dl±3
7	Total cholesterol	150.5 mg/dl	185.4mg/dl	162 mg/dl	163mg/dl

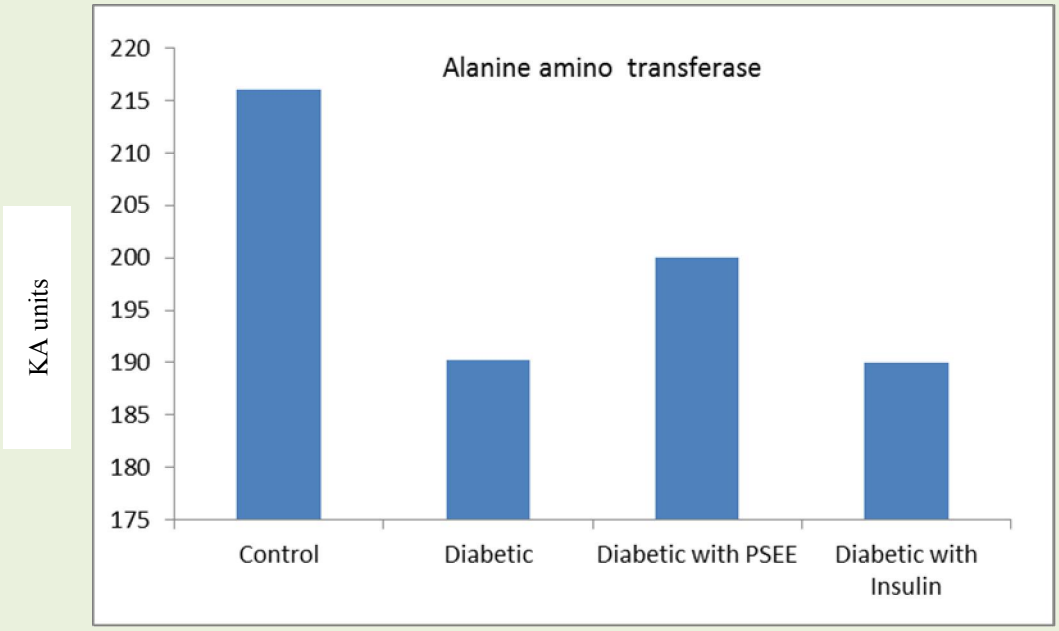
N=6 In each group, Values are mean + - S.D.

Table no 2 Effect of treatment of *Prosopis spicigera* leaves ethanolic extract (PSEE) on some biochemical parameter in STZ induced diabetic mice liver.

Sr .no	Parameter	Control	Diabetic	Diabetic with PSEE	Diabetic with Insulin
1	ALP	103.2±6.2 KA units	130.2 ±9 KA units	112.8 ±1 KA units	110 ±1 KA units
2	AST	85 ±5 KA units	140.5±7 KA units	100.2±6 KA units	102±5 KA units
3	ALT	40.3±3 KA units	60.3±8 KA units	55.4±7 KA units	54±6 KA units
4	LDH	216±4 IU/gm	190.3±5 IU/gm	200±4 IU/gm	190±4 IU/gm

Fig.no.1 Effect of treatment of *Prosopis spicigera* leaves ethanolic extract (PSEE) on some biochemical parameter in STZ induced diabetic mice liver.

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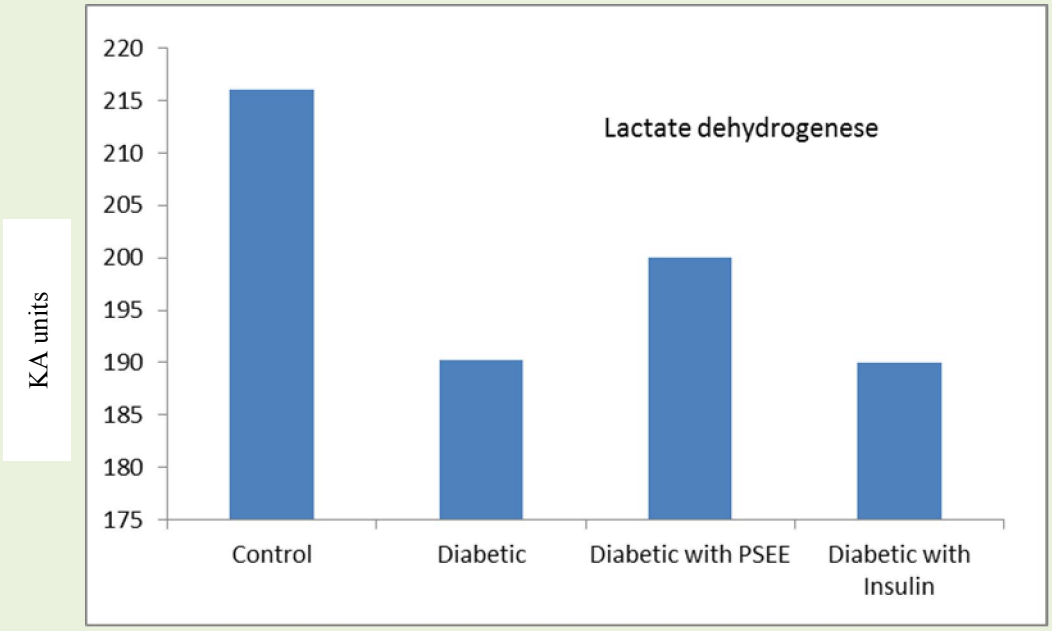
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