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## **Antidiabetic activity of leaves of *Spinacia oleracea* Linn. in Alloxan-induced diabetic rats**

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### **ABSTRACT**

*To evaluate the hypoglycaemic activity of ethanolic and aqueous extract of *Spinacia oleracea* in normal and alloxan induced diabetic rats. The ethanolic and aqueous extract of leaves of *spinacia oleraceae* were orally tested at the dose of (200&400mg\kg) for hypoglycaemic effect in normal and alloxan-induced diabetic rats. In addition, changes in body weight, serum cholesterol, triglyceride assessed in the aqueous and ethanolic extract treated diabetic rats, were compared with diabetic control and normal animals histopathological observations during 12 days treatment were else evaluated. Ethanolic and aqueous extract of *spinacia oleraceae* produced a significant reduction in fasting blood glucose levels in the normal and significant reduction in fasting blood glucose levels in the alloxan-induced diabetic rats significant differences were observed in serum lipid profiles (Cholesterol and triglyceride) and changes in body weight by both ethanolic and aqueous treated diabetic animals concurrent histopathological studies of the pancreas of these animals showed comparable regeneration by extract which were earlier necrosed by alloxan.*

**Key words:** Anti-diabetic activity, *Spinacia oleraceae*, Alloxan.

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### **INDRODUCTION**

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycaemic, altered metabolism of carbohydrates, protein and lipids, and an increased risk of vascular complication(1). In conventional therapy, type I diabetes is managed with exogenous insulin and type II with oral hypoglycaemic agents (sulphonylureas, biguanides etc).

In traditional practice medicinal plants are used in many countries to control diabetes mellitus. Diabetes mellitus has recently been identified by Indian council of medical research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. A large number of plant preparations have been reported to possess anti-diabetic activity over last several decades. *Spinacia oleraceae* Linn of family chenopodiaceae an erect, annual herb, 30-60cm, high cultivated throughout India upto an altitude of 2100M leaves alternate, ovate –oblong, obtuse or acute, variously lobed, smooth, soft, succulent.

Different parts of this plant have been reported to possess anti-inflammatory, anti-oxidant and CNS depressive activity. The leaves are cooling, emollient, anti-pyretic, hypoglycaemic, diuretic, laxative, digestible, anthelmintic, urinary concretions, inflammation of large and bowels, sorethroats, pain in the joints, flatulence throat.

In view of alleged anti-diabetic potential of *Spinacia oleraceae* different extract of the plant on fasting blood sugar levels and biochemical parameters such as serum cholesterol, and triglyceride were investigated. Histological examination was also carried out on pancreatic tissue of experimental animals.

## EXPERIMENTAL SECTION

### Plant Material

The leaves of *Spinacia oleraceae* have been collected from Kaaripatti, Salem district, Tamil Nadu, with the help of field botanist. The plant of *Spinacia oleraceae* have been authenticated by Prof. A. Balasubramanian, horticulturist, director of ABS Botanical Conservation, Research and Training Centre, Kaaripatti, Salem district, Tamil Nadu, India (Ref. ABSRTC/08/A-4069). The whole plant was dried initially under shade. It was preserved in a tightly closed container and powdered as per requirements.

### Preparation of Extracts

The dried leaves was subjected to size reduction to a coarse product by using dry grinder and passed through size no:30. About 150gm of this powder was packed into soxhlet apparatus by a hot percolation method. First defatted with petroleum ether, the defatted powder material (marc) thus obtained further extracted with alcohol and fresh powder was used for aqueous extraction by cold maceration method. The solvent was recovered by distillation in vacuum and extracts were stored in desicator used for subsequent experiments.

### Preliminary Phytochemical screening:

Extracts obtained from *Spinacia oleraceae* were subjected to various qualitative tests for the identification of various plant constituents present in this species.

### Pharmacological studies:

#### Procurements of Experimental Animals:

Swiss albino mice (20-25 g) and wistar albino rats (150-200 g) of either sex and of approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The

animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-P.Col./39/2008) after scrutinization. The animals received the drug treatments by oral route as well as intra peritoneal.

### Acute oral Toxicity study

Different doses of 5,50,300,2000mg/kg of ethanolic and aqueous extracts of leaves of *Spinacia oleracea* were administered orally. The alcoholic and aqueous extract of leaves of *Spinacia oleracea* were screened for acute toxicity study by OECD guidelines for determining the LD<sub>50</sub>. The results showed that LD<sub>50</sub> was found to be 2000mg/kg. Therefore dose was fixed on 200mg/kg.

### Assessment of Extracts of *Spinacia oleracea* on Normal Fasted Rats

For the normoglycemic study, rats were divided into five groups (n=6) and were administered 2% gm acacia solution, Glibenclamide (0.5mg/kg) alcohol extract (200&400mg/kg) and aqueous extract (200&400mg/kg) respectively. The blood samples were collected for the measurement of blood glucose level from the tail vein at 0,1,2,3 hrs. The blood glucose level was determined using an electronic glucometer.

The results were recorded and represented in Table:1

**Table 1: Effect ethanolic and Aqueous extracts of leaves of *Spinacia oleracea* linn after 3 hours treatment by using normal fasted rats**

Group	Treatment	BLOOD GLUCOSE LEVEL AT DIFFERENT HOURS IN mg/dl			
		0 hour	1hours	2 hours	3 hours
I	Normal control 5% CMC solution 10 ml/kg	82.6±5.56	81.83±2.87	83.5±3.38	82.5±2.83
II	Standard treated glibenclamide (5mg/kg)	84.4±3.6	83.5±3.65*	82.6±1.16***	84.1±2.26***
III	Alcoholic Ext (200 mg/kg)	79.6±4.31	78.83±4.8*	78.1±3.2**	78.8±1.33**
IV	Alcoholic Ext (400 mg/kg)	80.1±6.21	81.6±3.9*	82.1±2.6***	80.7±2.1***
V	Aqueous Ext. (200 mg/kg)	83.6±2.7	82.3±2.93*	84.8±1.88***	82.7±2.1**
VI	Aqueous Ext. (400mg/kg)	79.7±1.4	80.3±3.48*	79.6±2.61***	83.6±1.07***

Values are represented as mean ± SD (n=6)  
Newmann's keul comparison test (p < 0.01) is used  
p < 0.05 and \*\* p < 0.01 and \*\*\* p < 0.001 vs control

### Assessment of Extracts of *Spinacia oleracea* on Alloxan induced Diabetic Rats

Diabetes was induced in rats by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to overnight fasted rats. The rats were then kept for the next 24 h on 10% glucose solution bottles, in their cages to prevent hypoglycemia.

S. No	Constituents	Tests	Pet ether	Alcohol	Aqueous
1	ALKALOIDS	Mayer's test Dragondraff's test Hager's test Wagner's test	- - - -	- - - -	- - - -
2.	STEROLS	Liebermann's Burchard test Salkowski's	- -	- -	- -
3.	CARBOHYDRATES	Molisch's test Fehling's test Benedict's test Borntrager's test	+ + + +	+ + + +	+ + + +
4.	GLYCOSIDES	legal test keller kiallani test	+ +	+ +	+ +
5.	FIXED OILS AND FATS	Spot test Saponification test	- -	+ +	+ +
6	PHENOLIC COMPOUND	Ferric chloride	-	+	+
7.	PROTEIN & AMINO ACIDS	Biuret test Ninhydrin test Xanthoprotein test Millon's test	- - - -	+ + + +	+ + + +
8.	TERPENOIDS & SAPONINS	Foam test Haemolysis test	- -	- -	- -
9.	TANNINS	Gelatin test FeCl <sub>3</sub> test	- -	- -	- -
10.	GUMS & MUCILAGE	Precipitation to 90% alcohol	- -	+ -	+ -
11.	FLAVONOIDS	Aqueous NaOH Conc. H <sub>2</sub> SO <sub>4</sub>	+ +	+ +	+ +

After 72 h of injection, rats with marked hyperglycemia (fasting blood glucose > 250 mg/dl) were selected and used for the study. The selected diabetic animals were divided into five groups (n = 6). And one more group of normal non-alloxanized animals was also added in the study. Group I (normal control or non-alloxanized rats) and group II (untreated diabetic control rats) received a single oral dose of 0.5 ml/100g of the vehicle. Group III-Glibenclamide(5mg/kg)

Experimental procedure for alloxan induced diabetic model:

- Group 1 - Diabetic rats treated with 5 mg/kg of Glibenclamide
- Group 2 - Diabetic rats treated with 200mg/kg of Alcohol extract
- Group 3 - Diabetic rats treated with 400mg/kg of Alcohol extract
- Group 4 - Diabetic rats treated with 200 mg/kg of aqueous extract
- Group 5 - Diabetic rats treated with 400 mg/kg of aqueous extract

#### **Estimation of Biochemical parameters:**

On 12<sup>th</sup> day, blood was collected from the tail vein kept wide for 1\2 hrs for clotting serum was separated by centrifuge the sample at 6000rpm for 20min. The serum was analysed for cholesterol, LDL, HDL, and triglycerides.

#### **Histopathological Studies:**

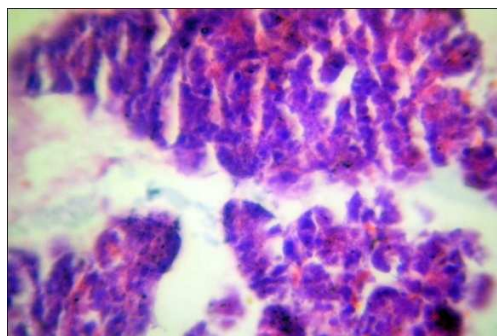
Pancreatic tissues and kidney from all group Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5  $\mu$ m thickness were cut and stained by hematoxylin and eosin (H and E) for histological examination.

#### **Statistical Analysis:**

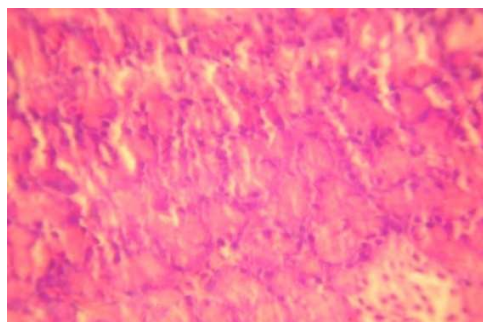
All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean  $\pm$  standard error of mean (S.E.M.). The results are analyzed for statistical significance using one-way ANOVA followed by Newmanns level comparison test.  $P < 0.05$  was considered significant.

### **HISTOPATHOLOGY OF RATS PANCREAS**

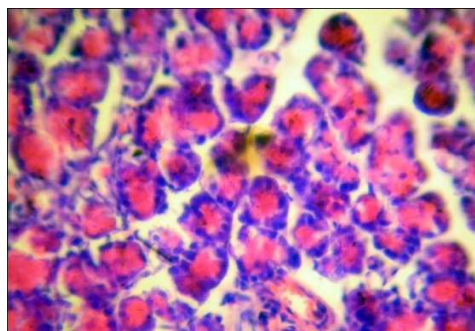
#### **A.NORMAL CONTROL**



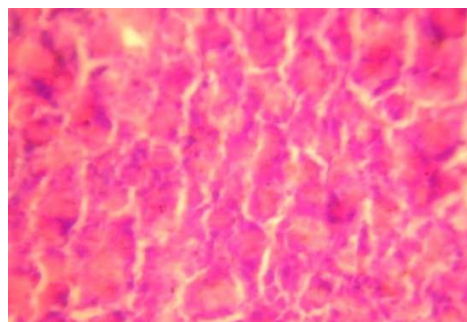
#### **B.DIABETIC CONTROL**



#### **C.STANDARD**

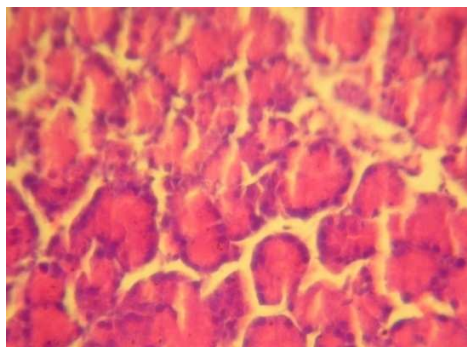


#### **D.ALCOHOLIC EXTRACT 200mg/kg**

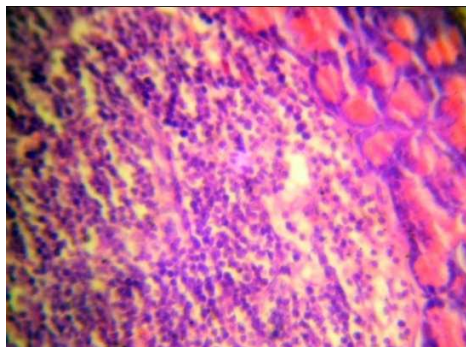




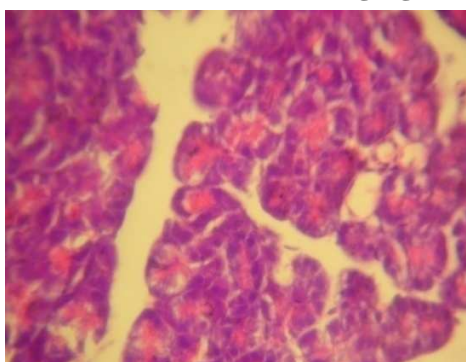
**E.AQUEOUS EXT 200mg/kg**



**F. ALCOHOLIC EXTRACT 400mg/kg**

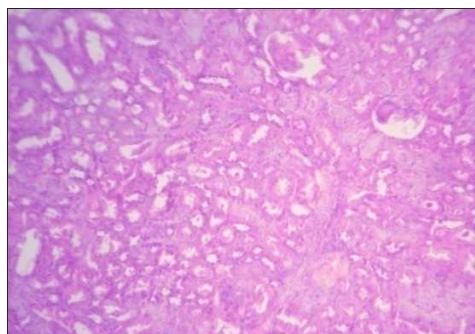


**G.AQUEOUS EXT 400mg/kg**

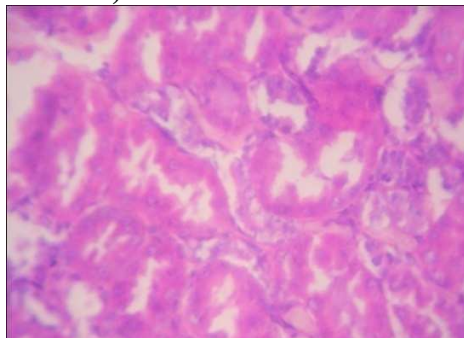


**HISTOPATHOLOGY OF RATS KIDNEY**

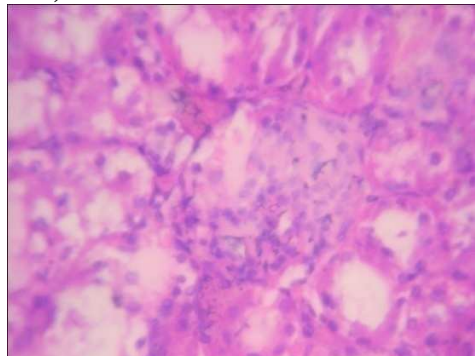
**A1) NORMAL CONTROL**



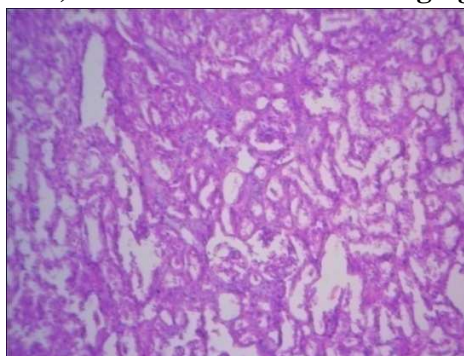
**B1)DIABETICCONTROL**

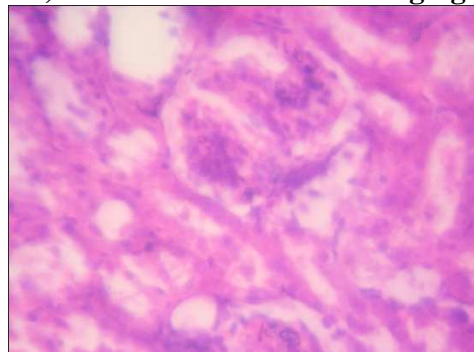
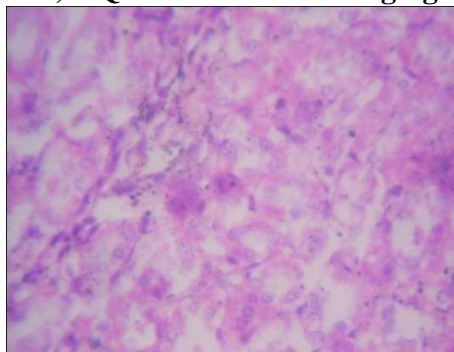


**C1) STANDARD**



**D1)ALCOHOLIC EXT 200mg/kg**



**E1) ALCOHOLIC EXT 400mg/kg****F1) AQUEOUS EXT 200mg/kg**

## RESULT

### **Preliminary phytochemical Screening:**

Preliminary phytochemical screening of the extract of *Spinacia oleraceae* revealed the presence of alkaloids, glycosides, proteins and amino acids, sterols, carbohydrates, phenolic compounds, flavonoids, saponins, and tannins.

### **Acute Toxicity Studies:**

All aqueous-treated rats showed no discernible behavioral changes up to 500 mg/kg by oral route. No mortality was observed at this dose during 72 h observation period.

### **Antihyperglycemic activity screening in normal and Alloxan induced diabetic Rats:**

The anti-diabetic effects of alcoholic and aqueous extract of *Spinacia oleraceae* on the fasting blood sugar level of normal and diabetic rats are shown in tables 1 and 2. In normal animals, significant ( $P < 0.05$ ,  $P < 0.01$ ) reduction in the blood glucose level was observed by both alcoholic and aqueous extract as compared to the control.

Both alcoholic and aqueous extract treatment of the *Spinacia oleraceae* (200&400mg/kg) in alloxan-induced diabetic rats. Resulted in a significant decrease in the elevated blood glucose levels as compared to the control.

On 12<sup>th</sup>, Both the extracts, showed significant anti-hyperglycemic activity.

### **Biochemical Parameters:**

Significant differences were observed in serum lipid profiles (Cholesterol and triglyceride) in alcoholic and aqueous extract treated diabetic animals.

### **Body weight test:**

The Body weight of experimental rats were measured during the period of study of antihyperglycemic activity. In diabetes mellitus the body weight loss was common symptoms. Diabetic control group animals lose their body weight continuously but the treated group after some time they start to regain their body weight. Both alcoholic and aqueous extracts show the significant regain of body weight during study period.

**Histopathological Studies:**

- A-Control
- B-Diabetic control
- C-Glibenclamide(0.5mg/kg)
- D-Alcoholic (200mg/kg)
- E-Alcoholic(400mg)
- F-Aqueous(200mg/kg)
- G-Aqueous(400mg/kg)

However both alcoholic and aqueous extract (200&400mg/kg) treated diabetic rats showed particular restoration of normal cellular population and size of islet cells LD.

**DISCUSSION**

Our study indicates that aqueous and ethanolic extract of *Spinacia oleraceae* exhibited significant anti-hyperglycemic activity in normal and alloxan-induced hyperglycemic rats. To prevent the loss of beta-cell function and mass, beta-cell stabilization or regeneration must occur.

In our studies, damage of pancreas was observed in alloxan-treated diabetic control rats B. The glibenclamide treated group showed regeneration of  $\beta$ -cells C. The comparable regeneration was also shown by aqueous extracts of *Spinacia oleraceae* D. Photomicrographs reinforce healing of pancreas by the aqueous extract of *Spinacia oleraceae*, as a plausible mechanism of their antidiabetic activity. The antidiabetic activity of *Spinacia oleraceae* may be due to the presence of flavonoids. It is reported that flavonoids constitute the active biological principles of most medicinal plants with hypoglycemic and antidiabetic properties.

**CONCLUSION**

Both ethanolic and aqueous extract of *Spinacia oleraceae* exhibited significant anti-hyperglycemic activity in normal and alloxan-induced diabetic rats. They also showed improvement in parameters like body weight and serum lipid profile as well as histopathological studies showed regeneration of  $\beta$ -cells of pancreas and so might be of value in diabetes treatment.

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