

Hypoglycemic Effect of *Spinacia oleracea* in Alloxan Induced Diabetic Rat

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Abstract: Green leafy vegetables is a rich source of dietary fibres, nutraceuticals and vitamins, folic acid and minerals. Hypoglycemic effect of 70% ethanolic *Spinacia oleracea* extract were studied in normal and alloxan induced diabetic rats. Animals treated with extract showed much lowered serum glucose level; 54 % (P<0.001) on 9th day. The level of serum triglycerides and cholesterol increased significantly in diabetic rat as compared to normal rats. The body weight of the treated diabetic rats was restored near normal level. There was a fall in albumin level in the control diabetics leading to a low A:G ratio. The urea level in treated animals was 19±2.280 mg/dL versus 18±2.757 mg/dL (control). The level of creatinine in Group II (Diabetic induced) and Group V (Diabetic + Glibenclamide) did not show any significant variation when compared to control. The alloxan induced rates treated with *Spinacia oleracea* showed decreased SGOT and SGPT level significantly (P<0.01). Reduction was found to be slightly higher as compared with control. In conclusion, these results suggest that *Spinacia oleracea* posses antidiabetic principle and can be useful for treatment of diabetes. Further studies to fractionate the active principle and to elucidate the exact mechanism of action are, therefore, required to be undertaken.

Key work: Alloxan induced diabetic rat • Hypoglycemic effect • *Spinacia oleracea*

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1]. Diabetes was discovered as early as 700-200 BD; until the time insulin was invented, this disorder was managed principally by the traditional practices by using medicinal plants. [2]

In the treatment of *diabetic mellitus*, non-pharmacologic measures (e.g. diet, exercise and weight loss) remains a critical component of therapy.

Dietary management includes the use of traditional medicines that are mainly derived from plants [3]. Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (garlic), *Azadirachta indica* (Neem); *Vinca rosea* (Nayantara) [4] and *Ocimum santum* (Tulsi). However this was reported that these are less effective in lowering glucose levels in severe diabetes. [5]

Spinacea oleracea is an edible plant commonly used by all man kind. In a preliminary study, it was observed that *Spinacea oleracea* extract, which is traditionally used in the food to reduce the serum glucose level, had significant antioxidant activity *in vitro* as it found to significantly reduce lipid peroxidation, scavenge hydroxyl radical and superoxide radicals *in vitro*. [6] Alloxan, has been show to produce diabetes by damaging of islet cells of pancreas by liberated oxygen radicals. In the present study, the hypoglycemic action of *S.oleracea* against alloxan induced diabetic rat was reported.

MATERIALS AND METHODS

Plant Material: The Leaves of *Spinacia oleracea* were collected from areas in and around Pollachi, India and authenticated by Botanical Survey of India, Coimbatore.

Preparation of Plant Extract: Leaves of *Spinacia oleracea* collected were shadow dried, powdered and extracted twice with 70% ethanol overnight. This solvent system can extract the phenolic constituents in the leaves which are mainly responsible for its antioxidant activity.

The extract was evaporated to dryness under vacuum. Extract was resuspended in distilled water and used for the animal experiment. The yield of the extract was 9.8% and the extract was stored at 4°C until use.

Animals and Chemicals: Male Wistar rats weighing 200 - 250g used in the experiment were purchased from Karpagam University, Coimbatore and housed in polypropylene cages at room temperature (25°C-30°C) and had free access to drinking water and basal diet. The entire animal experiments were done as per the guidelines of Institutional Animal Ethical Committee. Alloxan was purchased from sigma chemicals (St Louis, Mo, USA). Nitroblue tetrazolium, oxidized glutathione and deoxy ribose were purchased from SRL Chemicals, Mumbai. All other chemicals used were of analytical reagent grade.

Experimental Procedure: Rats (66) were divided into four groups. Group I, normal untreated animals (6). Group II, control animals (20) received freshly prepared alloxan in normal saline, ip as a single dose of 120mg/kg body weight on day zero. They were further administrated with 1 ml of distilled water (vehicle) orally every day during the experimental period. Group III, animals (20) were treated with alloxan as in group II. Additionally, they were treated with 100 mg/kg body weight of 70% ethanolic extract of *S.oleraceae*, in 1 ml water orally (Once daily), starting from the same day of alloxan administration (Day 1). Group IV animals (20) were treated with alloxan as in group II and were treated with 100 mg/kg body weight of Metformin 1 ml water orally (Once daily), starting from the same day of alloxan administration (Day 1). As the control animals were also found to reduce their blood sugar, experiment was terminated on day 12.

The animals were sacrificed on the 12th day after the drug treatment by chloroform anesthesia. Blood was collected by heart puncture and serum and erythrocytes were separated. The liver was washed thoroughly and kept in -20° C till the analyses were completed. [5]

Biochemical Analysis: Serum glucose levels were estimated by GOD/POD enzymatic method of Trinder [7]. Serum and triglycerides were determined as per Hawk *et al.*, [8]. Protein content of the enzyme was determined by lowrys method [9]. Serum albumin, globulin [10], Serum urea, SGOT and SGPT was estimated by the method of King *et al.* [11].

Statistical Analysis: Statistical analysis was done using the student's 't' test for glucose estimation and *in vivo* antioxidant data were analyzed as per Barrett's test of Anova using one way classification.

RESULTS

The effect of administration of 70% ethanolic *Spinacia oleraceae* extract did not have any significant effect on serum glucose level in alloxan diabetic rats during first five days. However, as compared with untreated controls, animals treated with 70% ethanolic *Spinacia oleracea extract* showed much lowered serum glucose level from day 6 onwards. On 9th day serum glucose in extract treated animals was found to be reduced to 54 % (P<0.001) when compared with that of control diabetic animals (Table 1). In addition hypercholesterolemia and hepertriglyceridemia was observed. The level of serum triglycerides and cholesterol were increased significantly in diabetic rat as compared to normal rats (Table 1). These was significant (P<0.01) reduction in the levels of these on treatment with *Spinacia oleraceae*. At the end of 15 days of treatment the body weight of the untreated diabetic rats was found to be significantly decreased and the administration of *Spinacia oleraceae* to diabetics rats restored the changes in body weight to near normal level. (Table. 2)

The extent of gluconeogenesis and ketogenesis was assessed by estimation of serum cholesterol, Protein, SGOT and SGPT level in normal, diabetic and treated animals after 12th day of test during drug treatment.

All the protein parameters viz., total protein, albumin, globulin and A:G ratio were tested and the results we recorded. (Table 3) The parameters did not show any deviation from normal range in treated diabetic rats. On the other hand, there was a fall in albumin level in the control diabetics leading to a narrow A:G ratio.

The level of urea, creatinine, SGOT and SGPT in normal, diabetic and treated animals were shown in Table 4. The normal function of the kidney was assessed as blood urea level. The urea level in diabetic was found to be 37±1.414 mg/dL, it was altered from treated animals of 19±2.280 mg/dL against 18±2.757 mg/dL (Control). The level of creatinine in Group II and Group V did not show any significant variation when compared to control. In diabetic animals the change in levels of serum enzymes are directly related to change in the metabolism in which the enzyme is involved. Hence the improvements noticed in the level of enzymes SGOT and SGPT.

Table 1: Concentration of Glucose, Triglycerides, Cholesterol in Serum of Control and Experimental groups

Particulars	Control	Diabetic induced	Diabetic +Plant extract treatment (1 ml)	Diabetic+ treated (2 ml) Plant extract	Diabetic+ Glibenclamide (1 ml)	Plant extract Treated (1 ml)
	I	II	III	IV	V	VI
Glucose (mg/dl)	120±4.690	250±3.286 ^{***}	152±1.095 ^{b***}	141±2.449 ^{***}	110±4.147 ^{d***eNS}	118±2.449 ^{NS}
Triglycerides (mg/dl)	84±3.286	220±3.286 ^{***}	83±2.098 ^{b***}	80±2.449 ^{***}	79±0.33d ^{***eNS}	80±1.677 ^{gNS}
Cholesterol (mg/dl)	129±2.449	270±3.286 ^{***}	120±3.286 ^{b***}	116±2.449 ^{***}	115±2.449 ^{d***eNS}	118±2.280 ^{***}

Value are expressed as mean ± S.D. Values are taken as a mean of five individuals experiments

a. Group II compared with Group I

b. Group III compared with Group II

c. Group IV compared with Group II

d. Group V compared with Group II

e. Group V compared with Group III

f. Group VI compared with Group I

***P<0.001 *p<0.05

**P<0.01NSNon Significant

Table 2: Change of body weight in control and experimental animals

Groups	Body Weight	
	Initial Weight	Final Weight
Normal	150.97±1.610	165.072±3.551 ^{***}
Diabetic Control	163.77±2.898	156.84±2.542 ^{***}
Diabetic + Plant extract (1 ml)	161±1.885	161.6±6.558 ^{NS}
Diabetic + Plant extract (2 ml)	152.366±1.921	157.3±8.271 ^{NS}
Plant extract alone	154.6±1.775	158.78±7.966 ^{NS}

**p<0.001

NSNon Significant

Table 3: The Concentration of Total Protein, Albumin, Globulin and A/G ratio in Serum of Control and Experimental groups

Particulars	Control	Diabetic induced	Diabetic +Plant extract treatment (1 ml)	Diabetic+ treated (2 ml) Plant extract	Diabetic+ Glibenclamide (1 ml)	Plant extract Treated (1 ml)
	I	II	III	IV	V	VI
Total Protein (g/dl)	8.2±0.245	4.5±0.245 ^{***}	7.9±0.245 ^{b***}	7.5±0.276 ^{***}	8.0±0.31d ^{***eNS}	8.4±0.303 ^{NS}
Albumin (g/dl)	5.1±0.329	2.5±0.329 ^{***}	4.5±0.329 ^{b***}	4.6±0.2 ^{***}	4.6±0.31d ^{***eNS}	4.7±0.245 ^{fNS}
Globulin	3.1±0.395	2.0±0.509 ^{***}	3.8±2.12 ^{bNS}	1.9±0.98 ^{eNS}	3.4±1.69 ^{dNSeNS}	3.7±0.415 ^{NS}
A/G Ratio	1.25±0.83	1.49±0.76 ^{NS}	1.86±1.57 ^{bNS}	2.33±1.62 ^{eNS}	2.00±1.54 ^{dNSeNS}	1.29±0.201 ^{eNS}

Value is expressed as mean ± S.D. Values are taken as a mean of five individuals experiments

a. Group II compared with Group I

d. Group V compared with Group II

b. Group III compared with Group II

e. Group V compared with Group III

c. Group IV compared with Group II

f. Group VI compared with Group I

***P<0.001 *p<0.05

**P<0.01NSNon Significant

Table 4 The Concentration of Urea, Creatinine, SGOT and SGPT in Serum of Control and Experimental groups

Particulars	Control	Diabetic induced	Diabetic +Plant extract treatment (1 ml)	Diabetic+ treated (2 ml) Plant extract	Diabetic+ Glibenclamide (1 ml)	Plant extract Treated (1 ml)
	I	II	III	IV	V	VI
Urea (mg/dl)	18±2.757	37±1.414 ^{a***}	21±1.414 ^{b***}	19±2.280 ^{c***}	20±2.280 ^{d***eNS}	17±2.098 ^{fNS}
Creatinine (mg/dl)	0.6±0.245	1.7±0.245 ^{***}	0.8±0.245 ^{b***}	0.5±0.141 ^{c***}	0.6±0.228 ^{d***eNS}	0.6±0.2 ^{fNS}
SGOT (U/L)	24±2.449	53±1.673 ^{a***}	33±1.414 ^{b***}	32±2.449 ^{c***}	31±1.414 ^{d***eNS}	28±3.033 ^{fNS}
SGPT (U/L)	42±2.757	59±2.449 ^{a***}	41±1.414 ^{b***}	40±2 ^{c***}	29±2.280 ^{d***eNS}	39±1.414 ^{fNS}

Value is expressed as mean ± S.D. Values are taken as a mean of five individuals experiments

a. Group II compared with Group I

d. Group V compared with Group II

b. Group III compared with Group II

e. Group V compared with Group III

c. Group IV compared with Group II

f. Group VI compared with Group I

***P<0.001 *p<0.05

**P<0.01NSNon Significant

The alloxan induced rats treated by *Spinacia oleracea* showed decreased SGOT and SGPT level significantly ($P < 0.01$). Reduction was found to be slightly higher compared as with control.

DISCUSSION

Management of diabetes with the agent devoid of any side effects is still a challenge in the medical system. This concern has led to an increase and demand for natural products with antihyperglycaemic activity.

The purpose of choosing alloxan monohydrate as the diabetes-inducing agent was known to produce *diabetes mellitus* irreversibly with a single dose administration by selective necrotic action on the beta cells of pancreas leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals viz., increased blood glucose level [12], decreased protein content [4], increased level of cholesterol and triglycerides [13] was reported.

The ethanolic extract of leaves of *S.oleracea* against alloxan induced diabetic rat as studied. *Spinacea oleracea* extract (100mg/kg, body weight) significantly decreased serum glucose level in hyperglycaemic animals. Similarly ethanolic extract of stem bark of *B. aristata* (25 mg/kg, 50mg/kg) and glibenclamide (5g mg/kg) showed reduction in blood sugar level was reported. [5] Similar antidiabetic activity was seen in the ethanol extract of leaves of *C. phlomoidis*. Moreover, improvement of body weight of the extract treated animal further supports the antidiabetic effect of *S.oleracea* as diabetic condition is associated with loss of body weight. This was supported by Semwal *et al.* [5].

It is well known that the level of glycemic control is the major determinant of serum level of triglycerides. Several investigators demonstrated that near normalization of the blood glucose level resulted in significant reduction in levels of plasma cholesterol and triglycerides level. Similar results were obtained with the seed powder of *Momordica charantia* [14], *Momordica charantia* fruit [15] and jambolan fruit [16].

Many works have reported increases in transaminase activities in liver and serum of diabetics. The increased level of transaminases which is active in absence of insulin because of availability of amino acid in the blood of diabetics are responsible for the increased gluconeogenesis and ketogenesis observed in diabetics. Sumana and Suryawanshi, [4] observed elevation in transaminase activity (SGOT and SGPT) in liver and kidney in diabetic rat. Increased gluconeogenesis and

ketogenesis observed in diabetes may be due to the high level in the activities of these transaminases. The restoration of SGOT and SGPT to their respective normal level after treatment with extract of *S.oleracea* strengthen the antidiabetogenic effect in this extract. Moreover, SGOT and SGPT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal function of liver. Similar result was reported by Semwal *et al.*, [5]. In this context, *Spinacea oleracea* can rightly be mentioned as a plant of considerable importance for *diabetic mellitus*. Further studies to fractionate the active principle and to elucidate the exact mechanism of action are, therefore, required to be undertaken.

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