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## Phytochemical and Anti-hyperglycemic study of *Solanum nigrum*

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### Abstract

Phytochemical study of *Solanum nigrum* revealed the isolation of known fifteen compounds where compound 1 identified as spirost-5-ene-3 $\beta$ ,12 $\beta$ -diol on the basis of IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral study. Other fourteen known compounds are characterized on the basis of Co-TLC elemental analysis and M.P.

The hypoglycemic potential of aqueous extract (50, 100 mg/kg) of leaves of *Solanum nigrum* Linn (Solanaceae) was evaluated by normoglycemic rats followed by alloxan and glucose loaded hyperglycemic rats by single oral administration. The plant extract was subjected to the study of presence of different phytoconstituents by using standard qualitative chemical methods. The preliminary antioxidant potential of the extract was evaluated by spectrophotometric method. The objective of this study is to induce experimental diabetes mellitus using Alloxan monohydrate in normal adult albino rats and study the anti-diabetic activity of changes in body weight, consumption of food and water, volume of urine and levels of glucose between normal and diabetic rats. Diabetes mellitus (DM) is a common endocrine disorder. Hypoglycemic agents from natural and synthetic sources are available for treatment of diabetes. Indian medicinal plants have been found to be useful to successfully manage diabetes. The effect of methanolic and water extract of *S. nigrum* leaves was investigated in normal, glucose load conditions and Alloxan monohydrate induced diabetic rats. Significant hypoglycemic activity was exhibited by the poly herbal formulation.

**Key words:** *Solanum nigrum*, Alloxan, Hypoglycemic, Diabetes mellitus, Alloxan.

### Introduction:

The selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from medicinal plants. Numbers of scientific and popular literatures has reported more than 1200 plants as hypoglycemic agents as plant drugs are frequency considered to be less toxic with lesser or rare side effects than those of synthetic ones<sup>1</sup>. *Solanum nigrum* Linn. (Solanaceae) commonly known as Black Berried Nightshade is a fairly

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common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats.

Diabetes mellitus (DM) is a common endocrine disorder affecting more than 150 million people worldwide and this number is likely to increase to 300 million by the year 2025, out of which more than one-fifth are Indians. According to the International Diabetes Federation, India has been declared as the diabetes capital of the world. Medicinal plants have been used as sources of drugs for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population<sup>2</sup>. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have no side effects.

World Health Organization has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which we lack safe modern medicine. The objective of present study is to investigate the effect of methanolic and water extract of *Solanum nigrum* leaves was investigated in glucose load conditions in normal rats and alloxan monohydrate induced diabetic rats. Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4-5%) all over the world. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts<sup>3</sup>. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as ‘madhumeha’ and some of them have been experimentally evaluated and the active principles compound isolated.

Hyperglycaemia or diabetes mellitus is caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentration of glucose in the blood, which in turn damage many of the body systems in particular the blood vessels and nerves<sup>4</sup>. Chronic hyperglycaemia during diabetes causes glycation of body proteins that in turn lead to secondary complications effecting eyes, kidneys, nerves and arteries. A part from currently available therapeutic options many herbal medicines have been recommended for the treatment of diabetes medicinal plants have the

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advantage of having no side-effects. Some of them are be used in traditional systems of medicines from hundreds of the years in many countries of the world<sup>5</sup>.

Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypo lipidemic agents. Flavonoids and polyphenols are being used to treat diabetes and dyslipidemia. This is based on the fact that, excessive oxidative stress is implicated in the pathology and complications of DM and polyphenols with antioxidant properties exert beneficial antidiabetic effect by correcting the disturbed oxidative milieu in diabetic conditions. *S. nigrum* contain both the organic and inorganic constituents<sup>6</sup>. Abundant research work has been carried out on the organic constituents of the *S. nigrum* while little attention has been paid on the role of inorganic elements in the medicinal use of these plants. Most of these plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicine. Trace elements play a very important role in the formation of the active chemical constituents present in *S. nigrum* and are therefore responsible for their medicinal as well as toxic properties.

Nowadays, Diabetes mellitus has become a real problem of public health in developing countries. It is actually a chronic disorders related to abnormality of carbohydrate, fat and protein metabolism<sup>7</sup>. This is due to defective or deficient insulin secretary response. This results into impaired glucose use, which is a characteristic feature of diabetes mellitus i.e. resultant hyperglycemia. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes. Plants are rich sources of antidiabetic, antihyperlipedemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenol. Several drugs are used to control diabetes, however perfect glycemic control is rarely achieved<sup>8</sup>. Diabetic women often use aqueous extracts of plants during pregnancy without any concern as to their possible outcomes. The effects of many of these plants have already been proven experimentally in animals and humans while others require further investigations.

Diabetes mellitus is now recognized as a serious global health problem. Westernized cultures and populations experiencing rapid acculturation are showing a sharp rise in non-insulin-dependent diabetes mellitus (NIDDM). The prevalence of NIDDM is increasing exponentially<sup>9</sup>. Nutritional factors including antioxidants have great influence in the management of diabetes mellitus and its complications; an imbalance between oxidative stress and antioxidative defense

mechanisms in diabetics can result in cell and tissue damage and accelerate diabetic complications<sup>10</sup>. Administration of appropriate antioxidants could prevent or retard diabetic complications to some extent<sup>11</sup>.

*Solanum nigrum* can be highly toxic to livestock and humans, and it is considered a weed. Nonetheless, ripe berries and cooked leaves are used as food in some locales; and plant parts are used as a traditional medicine. There is a tendency in literature to collectively refer to many of the black nightshade species as *Solanum nigrum*<sup>6</sup>."

### **Materials and Methods:**

**Plant Material:** Leaves of *Solanum nigrum* were collected from local habit of Ayodhyay, U.P., India, and dried in shade till total moisture is removed from the plant.

**Extraction:** Leaves of *Solanum nigrum* were coarsely powdered and extracted. The hot extraction process was done with the help of Soxhlet apparatus for 72 hrs. Methanol and water solvents were used for extraction. It was concentrated by distillation and water bath. Extracts were kept in desiccators for the removal of remaining moisture.

**Methanolic Extract:** 15gm of dry powder was subjected to Soxhlet extraction with 300 ml methanol (95%) as solvent, extraction was carried out for 10 cycles and temperature was maintained at 65°C. Color of extract was dark green. The yield was 1.02gm.

**Water Extract:** 15gm of dry powder was subjected to Soxhlet extraction with 300 ml water as solvent, extraction was carried out for 10 cycles and temperature was maintained at 100°C. Color of extract was dark green having yield of 1.87gm.

**Animals:** Male albino rats weighing about 125 to 150 gram (45-60 days old) maintained under standard experimental conditions (Temperature 27±2°C, relative humidity 60±5% and 12 hours light/dark cycle) were housed in standard environmental conditions.

**Acute Toxicity Studies:** Healthy adult Wistar albino rats of either sex, starved overnight were divided into five groups and were orally fed with the methanol and aqueous extract of *Solanum nigrum* in increasing dose levels of 100 and 200, mg/kg body weight. The rats were observed continuously for 2hrs for behavioral, neurological and autonomic profiles and after a period of 24 and 72 hrs for any lethality or death<sup>12</sup>.

**Induction of diabetes:** Hyperglycemia was induced by injecting alloxan monohydrate at a dose of 120 mg/kg intraperitoneally. The animals were kept under observation and after 48 hrs were tested for hyperglycemia using glucometer.

**Group I (control):** This group was kept as normal control animals without any treatment.

**Group II (Diabetic control):** This group was taken as diabetic control with the injection of Alloxan monohydrate.

**Group III:** This group was taken as Standard control with the injection of Alloxan and the Standard glibenclamide.

**Group IV:** This group was treated with Solanum nigrum methanol extract was mixed with rat feed.

**Group V:** This group was treated with Solanum nigrum water extract was mixed with rat feed.

**Oral Glucose Tolerance Test in normal rats (OGTT):** Rats were divided into five groups and were administered normal saline and dose of 500 mg/kg oral of aqueous extract. Glucose solution 2 g/kg was administered 30 min after the administration of the extract. Blood samples were withdrawn from retro-orbital at intervals of 30, 60 and 120 min of glucose administration and the level of blood glucose was measured.

**Experimental Design:** Body weights and blood glucose level of individual animals were recorded before and the period of study in week interval. The end of the 21<sup>st</sup> day, the experimental animals were sacrificed along with their control group. The animals were fasted overnight, sacrificed by decapitation and blood was collected from individual animals and the serum was obtained from the clotted blood samples for Biochemical studies. The Liver and other organs were immediately excised, after perfusing with physiological saline, the organs were blotted dried and it is used for further analysis.

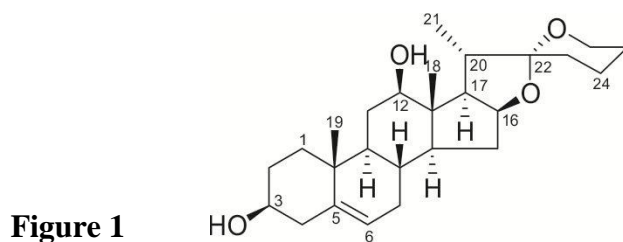
**Collection of Blood Sample:** Blood samples were collected from the tail vein puncture of treated rats. The samples were taken in to tubes with anticoagulant. From these samples blood sugar was estimated by a standard method.

**Estimation of total protein in Blood sample:** Serum globulin was calculated as the difference between total protein and albumin. The estimation of serum total cholesterol was done by Wx beng and Pileggi's method by using cholesterol kit manufactured by M/s Mediprobe Laboratories Pvt. Ltd., Hyderabad, India.

## Results and Discussion:

### Phytochemicals study:

The 50% EtOH extract of the fruits of *S. nigrum* was partitioned successively into ethyl acetate and water. The ethyl acetate-soluble fraction was purified by silica gel, reversedphase C18 gel column chromatography yielded one new compound 1 together with 14 known compounds, N-trans-feruloyltyramine (2),<sup>13</sup> (R)-3-(4-hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (3),<sup>14</sup> (E)-ethyl caffeate (4),<sup>15</sup> ethyl 4-hydroxy-3-methoxycinnamate (5),<sup>16</sup> guaiacylglycerol- $\beta$ -ferulic acid ether (6),<sup>17</sup> 3-O-acetylbetulinic acid (7),<sup>18</sup> chlorogenic acid (8),<sup>19</sup> caffeic acid (9),<sup>20</sup> methylsinapate (10),<sup>21</sup>  $\beta$ -sitosterol (11),<sup>22</sup> drummondol (12),<sup>23,24</sup> 2 $\alpha$ ,9-dihydroxy-1,8-cineole (13),<sup>25</sup> tryptophol acetate (14),<sup>26</sup> and 4-amino-3-methoxyphenol (15)<sup>27</sup> (Figure 1).



**Figure 1**

Compound 1 was obtained as an amorphous powder and its molecular formula was assigned as C<sub>26</sub>H<sub>40</sub>O<sub>4</sub>, based on the [M-H]<sup>-</sup> peak at m/z 415.2859 (calcd for C<sub>26</sub>H<sub>39</sub>O<sub>4</sub> 415.2848) in the HRESIMS. The <sup>1</sup>H NMR spectrum showed two tertiary methyl groups at  $\delta$ H 0.78 (3H, s, H-18) and 1.06 (3H, s, H-19), one secondary methyl at  $\delta$ H 1.03 (3H, d, J = 6.4 Hz, H-21), an olefinic proton at  $\delta$ H 5.35 (1H, d, J = 5.1 Hz, H-6), assignable to a spirostane moiety.<sup>27</sup> The <sup>13</sup>C and DEPT NMR spectroscopic data showed three methyls at  $\delta$ C 11.6, 14.0, and 19.9, two olefinic carbons at  $\delta$ C 122.4 and 142.3, three oxygenated methines at  $\delta$ C 72.4, 80.4, and 82.3, one oxygenated methylene at  $\delta$ C 63.3, and one spiroketal carbon at  $\delta$ C 110.6. From <sup>1</sup>H, <sup>13</sup>C, and DEPT NMR data, it was observed that a methyl group corresponding to C-27 in F ring was absent in this molecule when compared with the typical spirostane-type compound.<sup>28</sup> In the HMBC experiment, the presence of a double bond ( $\Delta$ 5,6) in the B ring was determined by the observation of long range correlations between the methyl proton at  $\delta$ H 1.06 (Me-19) and the olefinic carbon at  $\delta$ C 142.3 (C-5), as well as the olefinic proton at  $\delta$ H 5.35 (H-6) and  $\delta$ C 38.0 (C-10) as shown in Figure 2. HMBC correlations of H-18 ( $\delta$ H 0.78) to both of C-12 and C-13 were able to locate a hydroxy group on C-12. The relative configuration of the hydroxy group at C-12 was assigned as

$\beta$  orientation, based on the observed ROESY correlations of H-12 to H-14 and H-17, and H-17 to H-12, H-14 and H3-21. The ROESY correlations of H2-26 to H-16, and H-16 to H-17, and H-17 to H-12 confirmed the relative configuration of the C-22 position as R\*. By comparing the coupling constant of H-3 ( $W_{1/2} = 24$  Hz) with the published value ( $W_{1/2} = 24$  Hz),<sup>28</sup> the hydroxy group on C-3 was found to be  $\beta$ -oriented. Thus, the structure of **1** was determined to be a spirost-5-ene-3 $\beta$ ,12 $\beta$ -diol.



**Figure 2.** Key  $^1\text{H}$ - $^1\text{H}$  COSY ( ), HMBC (H C) and ROESY ( ) correlations of compound **1**.

**Table 1.**  $^1\text{H}$ - (400 MHz), and  $^{13}\text{C}$ -NMR (100 MHz) Chemical Shifts of Compound **1** in  $\text{MeOH-}d_4$

position	<b>1</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
1	38.6 t	1.08, 1.86 (m)
2	32.3 t	1.53, 1.81 (m)
3	72.4 d	3.39 (m $W_2$ 24)
4	43.0 t	2.24 (m)
5	142.3 s	□ -
6	122.4 d	5.35 (d, 5.1)
7	31.8 t	1.62 (m)
8	31.9 d	1.46 (m)
9	51.4 d	1.08 (m)
10	38.0 s	□ -
11	32.4 t	1.39 (m)
12	80.4 d	3.28 (dd, 6.7, 4.5)
13	47.0 s	□ -
14	56.5 d	1.10 (m)
15	33.0 t	2.01 (m)
16	82.3 d	4.41 (q, 7.3, 7.3, 7.3)
17	63.3 d	1.90 (m)
18	11.6 q	0.78 (s)
19	19.9 q	1.06 (s)
20	43.8 d	1.89 (m)
21	14.0 q	1.03 (d, 6.4)
22	110.6 s	-
23	31.7 t	1.71 (m)
24	31.7 t	1.71 (m)
25	24.9 t	1.92 (m)
26	63.0 t	3.76 (m)

Assignments were based on  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC experiments.

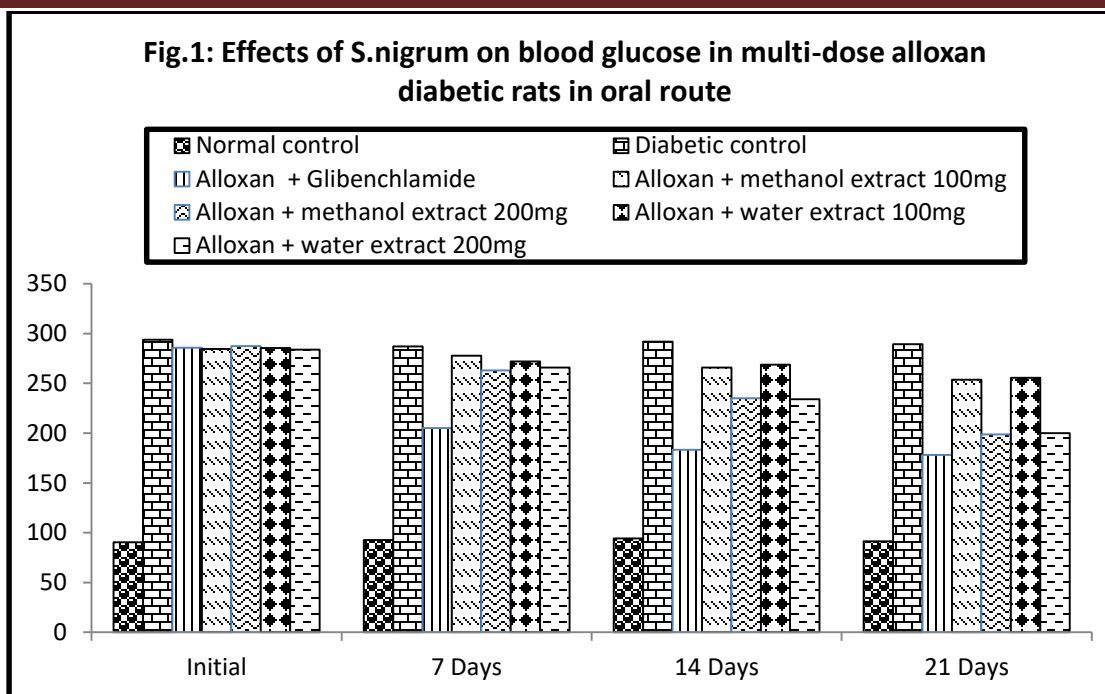
**Biological Study:**

The effect of single oral administration of water and methanolic extracts of *Solanum nigrum* are shown in (Table-1). An experimental study reveals that the methanolic and water extracts from *Solanum nigrum* (100 and 200 mg/kg) orally administered produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats<sup>29</sup>. Methanol extract is effective even at a higher dose (200 mg/kg) in decreasing blood sugar level in alloxan treated rats. The plant extract almost brought down blood glucose level by 50 % in diabetic animals.

**Table-1: Effect of *Solanum* plant extract on blood glucose in multi-dose treated in alloxan induced diabetic rats in oral route**

Groups	Treatment		Blood Glucose Level (mg/dl)			
			Initial	Day 7	Day 14	Day 21
I	Normal control		90.46±3.80	92.82±1.92	94.32±1.73	91.29±3.44
II	Diabetic control		293.8±5.27	286.91±5.05	291.8±5.41	289.41±9.75
III	Alloxan + Glibenclamide		285.86±6.92	205.25±7.06	183.18±6.15	178.13±9.75
IV	Alloxan + methanol extract	100mg	284.48±5.32	277.76±5.65	265.76±2.60	253.75±0.19
		200mg	287.48±5.32	262.98±7.08	234.90±32.0	198.70±60.99
V	Alloxan + water extract	100mg	285.48±5.32	271.90±7.6	268.87±90.07	255.67±0.70
		200mg	283.86±6.92	265.90±6.98	233.90±7.67	200.02±0.23

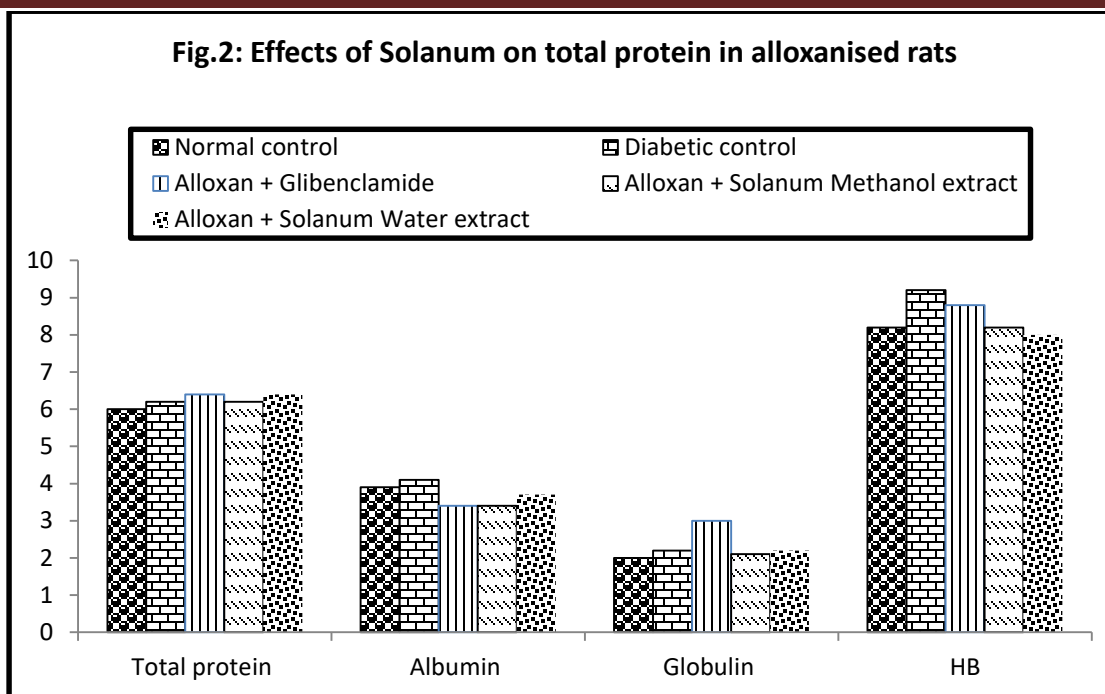
**Note:** Values given as means±S.E.M from six rats in each group. \*\*\*p<0.001 significant from normal and diabetic control animals.



Thus, it may be effective like tolbutamide. This study reports for the first time, the anti hyperglycemic effect of *Solanum nigrum* suggests that the active principle from this plant could be effective in the treatment of diabetes. It was found that blood glucose level decreases 293 mg/dl to 253.75 mg/dl and 198.7 mg/dl in alloxan+methanolic extract treated (100mg+200mg) treated albino rats for 21 days. It suggests that *S. nigrum* act as good hypoglycemic agent<sup>30</sup>. Table-2 demonstrates that the effect of the methanol and water extract on total protein. Methanol extract water even at a higher dose (200 mg/kg) in decreasing level in alloxan treated rats.

**Table-2: Effect of *Solanum* plant extract on total protein in Alloxanised rats on 21st Day of study**

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	HB (%)
A	Normal control	6.0	3.9	2.0	8.2
B	Diabetic control	6.2	4.1	2.2	9.2
C	Alloxan + Glibenclamide	6.4	3.4	3.0	8.8
D	Alloxan + <i>Solanum</i> Methanol extract	6.2	3.4	2.1	8.2
E	Alloxan + <i>Solanum</i> Water extract	6.4	3.7	2.2	8.0



In this study, we discussed about the hypoglycemic and antidiabetic effects of the *Solanum nigrum* on normal and Alloxan-induced-diabetic rats. Acute toxicity studies revealed the non toxic nature of the *Solanum nigrum* plant extract. The total protein increases in *nigrum* water extract<sup>31</sup> 6.0 g/dl to 6.4 g/dl in alloxan+solanum water extract.

The serum biochemical liver investigation report (Table-3) containing estimation of marker enzyme in alloxanised showed, the enzymes like ASAT, ALAT and ALP which are considered to be good indices of liver and kidney damage are significantly reduced by the extract and therefore it may be presumed that the extract protects cellular damage. The other serum biochemical parameters like Total bilirubin, Albumin, Total protein, globulin were found insignificant different when compared with reference animal group<sup>32</sup>.

**Table-3: Effect of aqueous leave extract of Solanum nigrum (ALSN) on serum biochemical parameters in alloxan induced rats on 30th day of study**

Groups and Treatments	Serum Biochemical parameters							
	ASAT (u/l)	ALAT (u/l)	ALP (u/l)	TB (mg/dl)	DB (mg/dl)	Albumin (gm/dl)	Total Protein (gm/dl)	Globulin (gm/dl)
I. Normal	22.83± 2.42	28.91± 2.41	105.35± 11.12	0.91± 0.14	0.25± 0.07	3.71± 1.00	6.61± 0.64	1.96± 0.34*
II. Solvent Control (Tween+ Water)	42.08± 2.80	57.16± 4.14	258.5± 15.64	1.55± 0.38	0.31± 0.03	5.33± 1.20	4.38± 0.68	1.01± 0.25
III. Glibenclamide (2.5 mg/kg)	24.2± 3.14*	29.41± 3.58*	135.25± 13.15*	0.74± 0.12*	0.22± 0.01	4.31± 1.01	5.95± 0.71	1.26± 0.26
IV. ALSN (50 mg/kg)	39.75± 2.50	42.41± 4.08*	113.16± 10.84	1.30± 0.11	0.41± 0.04	5.08± 1.15	4.26± 0.69	0.73± 0.13
V. ALSN (100 mg/kg)	32.25± 2.30*	40.78± 4.12*	138.91± 11.01*	1.22± 0.17*	0.38± 0.04*	4.53± 1.15	4.81± 0.93	1.11± 0.24
F(4,25)	10.88**	9.62**	22.15*	3.10*	0.33	1.93	3.17*	F(4,25)

There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The dose of the test drug has been selected on the basis of dose calibration curve<sup>33</sup>. The results of the study have shown that the methanol extract of Solanum nigrum at a dose of 200 mg/kg body weight has a marked hypoglycemic activity by improvement of the glucose tolerance test in normoglycemic rats and by lowering the blood glucose levels in Alloxan-induced-diabetic rats<sup>34</sup>.

The results of the study have shown a significant difference between the initial and final fasting glucose levels of methanol extract Solanum nigrum and glibenclamide treated groups.

Induction of diabetes by alloxan leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins<sup>35</sup>. The results obtained with the methanol extract treatment in chronic diabetic model further clarified the antidiabetic effect of the extract. After 21 days of plant extract treatment, gain in the body weight was observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide. Methanol extract of *Solanum nigrum* showed significant increase in serum insulin level<sup>36</sup>.

The methanol extract of *Solanum nigrum* has beneficial effects, in reducing the elevated blood glucose level and lipid profile of alloxan-induced-diabetic rats, but has no effect on normal rats. From this preliminary investigation, it has been concluded that the leaves of *S. nigrum* have significant hypoglycemic activity<sup>37</sup>.

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