ASSESSMENT OF ANTIHYPERGLYCAEMIC AND ANTIOXIDANT POTENTIAL OF LEAVES OF *SOLANUM NIGRUM LINN.* IN ALLOXAN INDUCED DIABETIC RATS

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Summary

The present study was designed to investigate the possible actions of aqueous extract of leaves of *Solanum nigrum Linn* (ALSN)*,* on glucose homeostasis and antioxidant defense in rats. The test was performed in both normoglycemic and alloxan induced hyperglycemic rats under treatment of the extract at 50 and 100 mg/kg dose levels for 30 days, keeping glibenclamide (2.5 mg/kg) as standard drug. The biochemical parameters used in the study are blood glucose, plasma-insulin, lipid profiles, and anti-oxidant enzyme activity, using standard experimental procedure. The test result revealed that in normoglycemic rats the blood glucose level reduces to a significant ($p<0.05$) extent in a dose-dependent manner, while the hyperglycemic rats showed a progressive fall of blood sugar level in a significant extent ($p<0.05$ to 0.001) with an increase of plasma insulin level. The extract at the tested dose levels, significantly ($p<0.05$) increases the peripheral utilization of glucose by isolated rat hemi-diaphragm.

The extract, inhibit the diabetic induced lipid peroxidation and improve the antioxidant defense in diabetic rats and recovered the marked alterations in antioxidant enzyme levels in liver as well. It was concluded that, the remarkable antidiabetogenic effect exerted by the leaf extract of *Solanum nigrum*, may be due to its potent antioxidant properties.
Keywords: *Solanum nigrum*, antihyperglycaemic, antioxidant, lipid peroxidation, alloxan.

Introduction

Diabetes mellitus (DM) is a major heterogeneous endocrine and metabolic disorder, characterized by altered metabolisms of carbohydrate, lipid and protein, which not only lead to hyperglycaemia but also cause many complications, such as hyperlipidemia, hypertension and atherosclerosis (1). The increased glucose level tends to glucose auto oxidation and auto oxidative glycosylation of proteins, which leads to oxidative stress, contributes to the development and progression of diabetes along with secondary complications (2, 3). Antioxidants have been shown to prevent the destruction of β-cells (4) by inhibiting the peroxidation chain reaction and thus, may provide protection against the development of diabetes (5). Abnormally high levels of free radicals cause membrane damage due to lipid peroxidation and protein glycation and the simultaneous decline or disturbance of antioxidant defense mechanisms in diabetes leads to cell and tissue damage (6). As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. It has been postulated that many of the negative effect of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamin E, C and other non-nutrient antioxidant such as phenolic compounds and flavonoids (7). Plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β-cell function and prevent diabetes induced ROS formation (8) and many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM (9). Despite this, few traditionally used antidiabetic plants have received proper scientific screening (1). The World Health Organization (WHO) has recommended that this area warrants further evaluation (10).

Solanum nigrum Linn. (Solanaceae) commonly known as Black Berried Nightshade is a fairly common herb or short-lived perennial shrub, found in disturbed habitats, distributed throughout India, Ceylon and all temperate and tropical regions of the world. The leaves are known to be used to treat headache & diseases of nose (11), ringworm (12), heart & liver ailments, wounds & burns (13), toothache (14). The ethnomedical information cited that hot aqueous extract of dried leaves is used for its antidiabetic (15), antiviral (16), antipyretic, anticonvulsant, sedative, antimalarial, antispasmodic & diaphoretic (17), molluscicidal (18), anti-bronchitis & anti-gastralgia (19) activities. The different parts of the plant is reported for treating diabetes, convulsions, apoplexy, sore mouth, common cold, uterine cancer, dermatitis, tonsillitis, acute gastritis and pain caused by trauma, dislocations & boils (20). The *Kondh* tribes of Orissa, India use the hot aqueous extract of the fruits and leaves as a folk medicine for the treatment of diabetes mellitus. The seed diet has been reported to possess significant hypoglycemic and hypocholesterolemic activity in rats (21). The leaves are reported to contain several constituents e.g. flavonols like Quercetin, Hyperoside (22), Steroids and alkaloids like Sitosterol, Solamargine, Stigmastrol, Campesterol, Cholesterol (23), Solasodine (24) and Sapogenin like Tigogenin (25).

In our earlier study, it is reported that the aqueous extract of leaves of *Solanum nigrum* endowed with hypoglycemic and antidiabetic activity in single dose administration models only (26). In continuation of our earlier work, the objective of the present study aims at extensive evaluation of the anti-hyperglycemic effect of the leaves of *Solanum nigrum* in chronic diabetic models in rats and to validate the folklore claim of the activity of the plant in a more scientific manner.

Material and Methods

Plant Materials

Fresh and mature plant of *Solanum nigrum linn.* was collected from Konark, Orissa, India and authenticated by the taxonomist, Dr. A. K. Pradhan, Professor, Department of Botany, PPD Mahavidyalaya, Tigiria, Cuttack, Orissa, India. A voucher specimen (Regdn. No. SPS/SOAU/2009/008) has been preserved in the institution herbarium of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University for future reference. After due

authentication, fresh matured leaves were collected in bulk, cleaned thoroughly with distilled water, followed by shade drying for 12 days. The shade dried leaves were coarsely powdered in an electrical grinder and preserved in a nylon bag in a deep freezer, till further use.

Preparation of the extract

Powdered plant material (550 g) was refluxed with 1500 ml of distilled water for 48 h, after defatting with petroleum ether (60-80 ^{0}C). The solvent with plant residue were filtered and concentrated in a rotary evaporator, a dark brownish viscous residue was obtained (yield: 21.52% (w/w) with respect to dried plant material).

Preparation of the test samples

The measured quantity of aqueous extract of leaves of *Solanum nigrum* (ALSN) and glibenclamide (2.5 mg/kg) was suspended in 25% Tween 20 in distilled water and used as test drug for oral administration.

Maintenance of Animals and approval of protocol

Healthy male albino wistar rats, weighing 150–200 g body weight were collected from the Institutional animal house for the study. The selected animals were housed in acrylic cages in standard environmental conditions (temp: 20–25 ^{0}C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rodent diet for one week in order to adapt to the laboratory conditions and water *ad libitum.* The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. 1171/C/08/CPCSEA).

Determination of blood glucose levels

Fasting blood glucose concentration was measured, using a Glucomonitor (Optium make), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns (27, 28).

Screening for glucose lowering effects of test extract

The Screening for antihyperglycaemic activity was performed as per the standard procedures (29).

In multi dose treated normoglycaemic animals

The animals were fasted for 12 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), the rats were then divided into four groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (2.5 mg/kg) and served as reference control. Groups III and IV received the test extract at a dose of 50 and 100 mg/kg, respectively, in a similar manner. The test extract, standard drug and solvent were administered to respective group once daily for 30 days. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30 day of treatment.

In multi dose treated alloxan induced diabetic animals

The animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After 1 h, the animals were provided rodent-feed *ad libitum*. The blood glucose level was measured 72 h after administration of alloxan. The animals showing blood glucose level beyond 200 mg/dl, were considered for the study. The diabetic animals were segregated into four groups of six rats each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route. Group II received glibenclamide (2.5 mg/kg); Groups III and IV received the test extract at doses of 50 and 100 mg/kg respectively in a similar manner, for 30 days. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30th day of treatment.

Study of glucose utilization on isolated rat hemidiaphragm

The rats' hemi diaphragms were isolated from the selected healthy albino rats immediately after killing the animals by decapitation. The diaphragms were divided into two halves. The hemidiaphragms were then placed in culture tubes containing 2ml tyrode solution with 2g% glucose and incubated for 30 min at 37 ^{0}C in an atmosphere of 95% O_2 – 5% CO_2 with shaking. Six sets of similar experiments were performed, in which, (I) corresponds to diabetic control (II) reference standard insulin (0.25 IU/ml), (III) ALSN (50 mg/ml), (IV) ALSN (100 mg/ml), (V) insulin (0.25 IU/ml + extract (50 mg/ml)) and (VI) insulin (0.25 IU/ml + extract (100 mg/ml)). Following incubation, the hemidiaphragms were taken out

and weighed. The glucose content of the incubated medium was measured. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium (30).

Study of the test extract on Plasma Insulin levels

Four groups of rats were taken out of which Group I served as diabetic control, Group II, Group III and Group IV animals received oral daily dose of glibenclamide (2.5 mg/kg), ALSN (50mg/kg) and ALSN (100mg/kg) respectively. Blood was collected at 0, 5, 10, 20 & 30th day and plasma insulin was measured by following the method of Radio Immunoassay (RIA), employing double antibody technique using insulin kit (31). Insulin values were expressed as μU/ml.

Effect of ALSN on serum lipid levels

At the end of 30 days of treatment with the test extract, the animals were sacrificed by decapitation under ether anesthesia and blood samples were collected from test, standard and solvent treated groups including normal animal as reference. The serum supernatant was separated out by centrifugation and was subjected for the determination of the lipid profile studies such as total lipids, phospholipids, total cholesterol, triglycerides, HDL, LDL, VLDL and free fatty acids (32).

Effect of ALSN on antioxidant status

After sacrificing the animals on 30th day, the liver tissue from various groups of animals were removed carefully followed by washing thoroughly with ice cold saline, 0.5 gms of the wet tissue was weighed exactly and homogenized in 0.1M Tris–HCl buffer, pH 7.4 at 4 ^{0}C in a Remi homogenizer with a Teflon pestle rotated at 600 rpm for 30 min. The homogenate was centrifuged at 2500 rpm for 10 min at 4 ^{0}C using refrigerated centrifuge. The supernatant was used for the assay of lipid peroxidation products and antioxidant enzymes such as thiobarbituric acid reacting substances (TBARS) (33), hydroperoxides (HP) (34, 35), malondialdehyde (MDA) (36), conjugated dienes (CD) (37), reduced glutathione (GSH) (38, 39), glutathione peroxidase (GSH-Px) (40), glutathione reductase (GR) (41), superoxide dismutase (SOD) (42), catalase (CAT) (43).

Acute oral toxicity studies

Acute oral toxicity studies on the plant reported that the aqueous extract of leaves of the plant *Solanum nigrum*, is slightly toxic in a dose level of 763 mg/kg (44). The dose of the extract for animal studies has been fixed at 50 mg/kg and 100 mg/kg body weight.

Statistical analysis

All the results were analyzed statistically evaluated using one-way analysis of variance followed by Dunnet's t-test. A *p*-value less than 0.05 are considered significant. All the results are expressed as Mean ± SEM, for six animals in each group.

Results

Effects of ALSN on blood glucose levels

Effects of ALSN on multi- dose treated normoglycemic animals

The results of ALSN on blood sugar level of normoglycemic rats are depicted in Table 1. The test result indicates that, there is a significant reduction ($p < 0.05$ to $p < 0.01$) in blood glucose level from 15th day onwards, and registered 21.7 and 33.9% reduction at the end of 30 days, in animals treated with 50 and 100 mg/kg of the test extract. However the standard drug glibenclamide at the same day reduces the blood glucose 33.80% with $p < 0.001$, when compared with solvent control group.

Effects of ALSN on multi- dose treated alloxan induced diabetic rats

The results illustrated in Table 2, of the study reveals that, the extract reduces the blood glucose level to an extent of 46.49% and 59.23% at 50mg/kg and 100mg/kg body weight respectively at the end of the 30th day of the study, where as the standard drug glibenclamide registered 66.79% of reduction at the same day of the study. However the individual data shows a statistical significance ranges between $p < 0.05$ to $p < 0.001$, throughout the experiment when compared with solvent control and analysis of variance registered $p < 0.01$ level of significance.

Effects of ALSN on glucose utilization by isolated rat hemidiaphragm

The results of study on glucose uptake by isolated rat hemidiaphragm are shown in Table 3, which reveals that the test extract at 50 mg/ml and 100mg/ml concentration exhibited uptake of 3.71 and 4.73 mg/g/30min respectively, while only insulin showed 6.36mg/g/30min. However, insulin and test extract combination respond to 6.33 and 7.16mg/g uptake of glucose at the same time. The extent of glucose uptake differ significantly ranges from $p < 0.05$ to $p < 0.001$ when compared with diabetic control group.

Effects of ALSN on Plasma Insulin Levels

The results of the test depicted in Table 4. The *Solanum nigrum* extract at both the tested dose levels could significantly ($p < 0.001$) increase insulin concentrations in a progressive manner in diabetic treated rats when compared with diabetic control. The extract at the dose level of 50 and 100 mg/kg recorded a maximum increase in insulin concentration of 103.5 and 117.66 µU/ml respectively on 30th day. On the other hand, glibenclamide showed maximum plasma insulin concentration of 181.83 µU/ml at the end of 20th day, while on 30th day it registered 119.66 µU/ml of plasma insulin with statistical significance of $p < 0.001$.

Effects of ALSN on serum lipid profile

The Table 5 illustrate the levels of serum lipid profile such as total lipids, total cholesterol, phospholipids, triglycerides, HDL, LDL, VLDL and free fatty acids on 30th day of the study. The diabetic rats showed significant ($p < 0.001$) increase level of all tested lipid profiles except HDL, which showed decrease value in a significant ($p < 0.05$) extent. The extract at both the dose levels showed a dose dependent and significant ($P < 0.05$ to $p < 0.001$) reduction in total lipids, triglycerides, LDL, VLDL and free fatty acids, however a marked decrease in the levels of total cholesterol and phospholipids were also been recorded, when compared to diabetic control group, while the HDL levels were approaching almost normal values when compared to without treatment normal control group.

Study of effect on serum lipid peroxidation products and reduced glutathione contents

Table 6 presents the estimated concentrations of liver Thiobarbituric acid reactive substances (TBARS), Hydroperoxides (HP), Malondialdehyde (MDA) and Conjugated dienes (CD) on 30th

day of the study of both test and standard drug. The extract treated alloxan induced diabetic animals, the lipid peroxidation products such as TBARS, HP, MDA and CD levels are declained with an extent of 46.71, 20.85, 27.19, 23.72% respectively in case of 50mg/kg dose, while in 100mg/kg body weight dose level, the % decrease become 57.88, 25.36,32.62, 31.81 respectively, with statistical significance of p<0.001.In a similar way the standard drug also showed a reduced value of 64.49, 34.15, 35.46, 37.69 in percentage wise, with statistical significance in the same experiment.

The enzymes like Reduced glutathione (GSH), Glutathione peroxidase (GSH-Px), Glutathione reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT) values are lowered significantly ($p < 0.001$) in diabetic rats as compared with normal control rats. The test extract showed an elevated value of an extent of 13.27, 6.89, 11.37, 15.25, 29.62% in case of 50mg/kg dose, while in 100mg/kg dose level, it showed 28.45, 27.58, 20.40, 43.69, 50.21%, respectively as per the above mentioned order of enzyme, with a statistical significance ($p < 0.001$). However the standard drug glibenclamide, at the same time, registered an increased % of 47.69, 44.82, 48.10, 64.03, and 64.07 respectively with statistical significance ($p < 0.001$).

Discussion

The limitations of currently available pharmacological agents for control of blood glucose have encouraged the researcher to rethink the development of novel antidiabetic agents with different mechanism of action. In our earlier work, we have reported hypoglycemic and antidiabetic activity of aqueous extract of *Solanum nigrum* leaves (ALSN) in normoglycemic and alloxan induced hyperglycemic rats by single dose treatment only (26). In continuation of our earlier work, the present study aims at extensive evaluation of aqueous extract of *Solanum nigrum* leaves towards a mechanistic hypoglycemic and antioxidant potential upon 30 days of study.

The conclusion derived from these data revealed a defined role of aqueous extract in normoglycemic, and alloxan-induced diabetic rats, the aqueous extract of leaves of Solanum nigrum, found to possess dose dependent suppression of glucose level, with prolonged hypoglycemia at higher dose of 100mg/kg, which is almost same effect as that of synthetic drug glibenclamide. All these

glucose lowering effect of the extract may possibly due to the insulinotropic effect at the islet beta cell level as evidenced by the increased plasma insulin levels which can be comparable with that of standard glibenclamide and all these parameters are widely accepted as markers of insulinotropic effect (31). Though there is no clear understanding on the mechanism of insulinotropic effect of sulfonyl ureas till yet, but thought to be due to its binding to pancreatic beta receptors (45) or ionophoretic (ATP sensitive K^+ channel) modulation (46, 47) or facilitating effect of Ca^{2+} inflow into beta cells (48, 49). The activity of the extract in increasing peripheral utilization of glucose by isolated rat hemidiaphragm, suggest that the extract may contribute to the insulinotropic effect or direct insulin like activity and extra pancreatic effect. (30).

Alloxan, a beta-cytotoxin, induces "chemical diabetes" by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation (50). This damages a large number of β-cells, resulting in decrease in endogenous insulin release, which leads to decreased utilization of glucose by the tissue (51). The results depicted in this study revealed that the sub-acute antidiabetic, hypoglycemic and insulintropic effects of *ALSN* were similar to those of glibenclamide. The possible mechanism, by which the plant extract mediates its antidiabetic action, is potentiation of pancreatic secretion of insulin from existing residual β-cell of islets and due to enhanced utilization of blood glucose by peripheral tissues as well.

Free radicals e.g. superoxide radical, hydroxyl radical, peroxy radical and singlet oxygen radicals have been implicated in many disease conditions. Herbal drugs containing radical scavengers are gaining importance in treating such conditions. Many plants possess dynamic antioxidant properties owing to their phenolic and flavonoid contents (52). In our previous study, the said plant extract is reported to possess the phenolic content (33.83 μg of pyrocatechol equivalent /500mg) and flavonoid content (5.86 mg equivalent of quercetin /gm), which may be partly responsible for its possible antioxidant activity (26).

It has been reported that the increase in glucose levels in alloxan-induced diabetic rats is associated with dislipidemia characterized by elevated serum triglycerides and total cholesterol levels. The improvement of blood glucose levels caused by most hypoglycaemic treatments is associated with a reduction of serum

triglycerides and total cholesterol (53, 54). The significant reduction in the levels of LDL, VLDL, TC, TG, FFA, phospholipids & total lipids and increase in the levels of HDL demonstrates that, the extract may have property to enhance the transcription of lipoprotein lipase similar to that of insulin.

Hyperglycemia induces the generation of free radicals which can affect antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation (55). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (1). Reactions of oxygen free radicals with all biological substances especially with polyunsaturated fatty acids lead to increased lipid peroxidation (LPO) (56) resulting in impairment of membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (57). The present investigation resulted in significant increase in lipid peroxidation products such as TBARS, CD, MDA and HP in liver of diabetic rats which suggest that peroxidative injury may be involved in the development of diabetes. The extract treated diabetic animals, showed a significant reduction in liver lipid peroxidation products, which indicate that ALSN is having, potential to inhibit the oxidative damage of liver tissues. Moreover, GSH is mainly involved in the synthesis of important macromolecules and in the protection against reactive oxygen compounds (58). A marked decrease in liver GSH is observed in diabetic rats, which contributes a factor in the pathogenesis of diabetes. In the present study the test extract showed a significant decrease of liver GSH level, which may be one more contributing property of the extract towards its antidiabetic potential.

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS (57). SOD is one of the most important enzymes and scavenges O_2- anion (which is the first product of O_2 radicals) to form H_2O_2 in the enzymatic antioxidant defence system and hence abolishes the toxic effects due to this radical or other free radicals derived from secondary reactions (58). The O_2- anion is reported to inactivate CAT and GSH-Px (59). Catalase has been recognized as a major determinant of hepatic and cardiac antioxidant status (60) and is known to be involved in detoxification of H_2O_2 concentrations (61), whereas GSH-Px is sensitive to lower concentrations of H_2O_2. In

Diabetes, the alloxan-generated ROS causes non-enzymatic glycosylation and oxidation resulting in the inactivation and inhibition of antioxidant enzymes such as SOD and CAT (7). In the present study, it was observed that long term treatment with the extract had reverse the activities of these enzymatic antioxidants (SOD, CAT, GPx and GR), by significantly increasing the activity of such enzymes.

Conclusion

The present study report clearly depicted that the *Solanum nigrum* extract endowed with hypoglycaemic and antihyperglycaemic activity due to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism as evidenced by insulinotropic and antioxidant properties of the extract.

Acknowledgements

Table 1: Effect of *ALSN* on blood glucose in multi-dose treated on normoglycemic rats in oral route.

Groups & Treatment	Blood Glucose Levels(mg/dl)							% decrease at 30th day
	0th day	5th day	10th day	15th day	20th day	25th day	30th day	
I. Solvent Control (Tween + Water)	99.5 ± 4.80	98.66 ± 4.66	96.5 ± 4.77	101.16 ± 4.04	96.83 ± 4.15	97.83 ± 5.60	102.16 ± 4.90	-
II. Glibencamide (2.5mg/kg)	92.5 ± 2.68	86.83 ± 2.75	79.83 ± 2.95[a]	59.5 ± 2.77[c]	62.33 ± 2.33[c]	61.16 ± 3.29[c]	56.16 ± 2.68[c]	33.88
III. ALSN (50mg/kg)	101.83 ± 4.20	98.66 ± 4.98	93.33 ± 5.37	89.5 ± 4.88	88.16 ± 4.53	84.66 ± 4.74	79.66 ± 6.50[a]	21.77
IV. ALSN (100mg/kg)	87.5 ± 6.41	85.16 ± 5.22	81.5 ± 6.30	76.66 ± 5.45[b]	74.33 ± 3.26[b]	72.83 ± 6.26[b]	57.83 ± 6.60[b]	33.90
F (3,20)	1.93	2.65	2.78	16.43**	17.14**	12.50**	10.74**	-

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test,

(F-value denotes statistical significance at *$p<0.05$, **$p<0.01$),

(t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to solvent control group.)

Table 2: Effect of *ALSN* on blood glucose in multi-dose treated in alloxan induced diabetic rats in oral route.

Groups & Treatment	Blood Glucose Levels(mg/dl)							% decrease at 30th day
	0th day	5th day	10th day	15th day	20th day	25th day	30th day	
I. Solvent Control (Tween + Water)	285.66 ± 12.71	279.83 ± 11.81	257.66 ±11.67	246.83 ±11.80	239.33 ±10.39	235.83 ± 9.76	231.83 ± 6.30	-
II. Glibencamide (2.5mg/kg)	296.66 ± 13.07	199.66 ±10.21[c]	138.16 ±10.33[c]	116.83 ± 6.60[c]	115.16 ± 5.24[c]	103.83 ± 7.46[c]	98.5 ± 6.82[c]	66.79
III. ALSN (50mg/kg)	278.16 ± 10.51	248.16 ±13.33	210.66 ±11.83[a]	178.16 ±12.95[b]	172.33 ±10.84[c]	157.5 ± 7.39[c]	148.83 ± 7.17[c]	46.49
IV. ALSN (100mg/kg)	272.66 ± 13.92	208.16 ±13.53[b]	157.16 ±12.61[c]	138.33 ±10.09[c]	129.33 ± 9.18[c]	126.33 ± 9.61[c]	111.16 ± 7.35[c]	59.23
F (3,20)	0.67	9.14**	21.54**	28.86**	36.84**	44.57**	70.70**	-

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *$p<0.05$, **$p<0.01$)

(t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to group-I.

Table 3: Effect of *ALSN* on Peripheral glucose-uptake by isolated rat hemi-diaphragm.

Incubation medium	Glucose uptake (mg/g/30 min)
I. Tyrode solution with glucose (2 g%) – Diabetic Control	3.21 ± 0.18
II. Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml)	6.36 ± 0.62^c
III. Tyrode solution with glucose (2 g%) + ALSN (50 mg/ml)	3.71 ± 0.55
IV. Tyrode solution with glucose (2 g%) + ALSN (100 mg/ml)	4.73 ± 0.26^a
V. Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml + ALSN (50 mg/ml)	6.33 ± 0.39^c
VI. Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml + ALSN (100 mg/ml)	7.16 ± 0.42^c
F (5,30)	18.28**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *$p<0.05$, **$p<0.01$)

(t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to group-I).

Table 4: Effect of *ALSN* on Plasma Insulin Levels

Group and Treatment	Plasma Insulin (µU/ml)				
	0th day	5th day	10th day	20th day	30th day
I. Diabetic Solvent Control	21.66 ± 3.75	23.33 ± 4.79	21.25 ± 2.80	26.66 ± 3.44	23.83 ± 2.73
II. Glibencamide (2.5mg/kg)	33.83 ± 3.87[a]	74.16 ± 7.00[c]	169.83 ± 11.71[c]	181.83 ± 9.28[c]	119.66 ± 7.64[c]
III. ALSN (50mg/kg)	23.18 ± 4.16	43.13 ± 2.41	78.83 ± 7.33 [c]	91.66 ± 8.25 [c]	103.5 ± 8.31 [c]
IV. ALSN (100mg/kg)	30. 83 ± 4.55	59.66 ± 5.13[c]	112.33 ± 8.99[c]	114.5 ± 7.92[c]	117.66 ± 7.21[c]
F (3,20)	2.41	20.90**	71.25**	112.95**	62.79**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *$p < 0.05$, **$p < 0.01$)

(t-value denotes statistical significance at [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ respectively, in comparison to '0' hr result.

Table 5: Effect of *ALSN* on serum lipid profile in alloxanised rats on 30th day of study

Groups and Treatment	Serum Lipid profile							
	Total Lipids (mg/dl)	Total Cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Free Fatty Acids (mg/dl)
I. Normal	113.66 ± 7.76[c]	75.65 ± 5.90[c]	105.5 ± 8.10[c]	62.08 ± 6.93[c]	51.5 ± 4.68[a]	27.5 ± 6.86[c]	16.33 ± 1.40[c]	412.41 ± 77.47[c]
II. Diabetic Control (Tween + Water)	393.16 ± 23.54	188.16 ± 16.15	212.91 ± 15.42	189.08 ± 20.19	32.66 ± 3.43	63.73 ± 6.21	45.06 ± 1.94	1324.58 ± 137.14
III. Glibencamide (2.5mg/kg)	141.83 ± 17.37[c]	96.75 ± 10.31[b]	127.75 ± 13.40[b]	78.33 ± 6.44[c]	54.11 ± 6.11[b]	35.08 ± 3.35[b]	18.75 ± 1.84[c]	675.41 ± 80.69[c]
IV. ALSN (50mg/kg)	221.5 ± 15.16[c]	166.41 ± 15.99	189.5 ± 18.83	113.83 ± 10.25[b]	32.66 ± 4.49	49.83 ± 6.23	33.25 ± 2.34[b]	917.08 ± 70.60[b]
V. ALSN (100mg/kg)	216.83 ± 28.62[c]	147.06 ± 21.36	173.33 ±15.37	97.83 ± 13.67[c]	44.66 ± 3.36	41.58 ± 2.84[a]	24.25 ± 2.42[c]	796.33 ± 56.85[b]
F (4,25)	30.11**	10.02**	9.12**	15.35**	5.01**	6.77**	33.70**	14.27**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at $*p<0.05$, $**p<0.01$)

(t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to group-II).

Table 6: Determination of concentration of Thiobarbituric acid reactive substances (TBARS), Hydroperoxides (HP), Malondialdehyde (MDA), Conjugated dienes (CD) and activities of Reduced glutathione (GSH), Glutathione peroxidase (GSH-Px), Glutathione reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT) in the extract treated rat liver.

Group and Treatment	TBARS (μM/100g wet tissue)	HP (μM/100g wet tissue)	MDA (μM/100g wet tissue)	CD (μM/100g wet tissue)	GSH (μM/g wet tissue)	GSH-Px (μM/g wet tissue)	GR (μM/g wet tissue)	SOD (Units/mg protein)	CAT (Units/mg protein)
I. Normal Control	4.41 ± 0.71^c	15.66 ± 0.84^c	0.84 ± 0.05^c	55.41 ± 2.55^c	21.66 ± 1.13^c	0.47 ± 0.04^c	5.41 ± 0.10^c	9.11 ± 0.13^c	8.38 ± 0.23^c
II. Diabetic Control (Tween+Water)	26.5 ± 2.06	29.25 ± 1.04	1.41 ± 0.05	94.83 ± 3.01	12.58 ± 0.89	0.29 ± 0.02	3.43 ± 0.09	5.31 ± 0.17	4.76 ± 0.18
III. Glibencamide (2.5 mg/kg/day)	9.41 ± 1.16^c	19.26 ± 0.94^c	0.91 ± 0.04^c	59.08 ± 2.54^c	18.58 ± 0.86^c	0.42 ± 0.01^b	5.08 ± 0.20^c	8.71 ± 0.34^c	7.81 ± 0.36^c
IV. ALSN (50mg/kg/day)	14.12 ± 0.41^c	23.15 ± 0.97^c	1.10 ± 0.06^b	72.33 ± 3.63^a	14.25 ± 0.91^a	0.31 ± 0.01	3.82 ± 0.16^a	6.12 ± 0.09^b	6.17 ± 0.42^c
V. ALSN (100mg/kg/day)	11.16 ± 0.83^c	21.83 ± 1.13^c	0.95 ± 0.03^c	64.66 ± 2.60^c	16.16 ± 0.94^a	0.37 ± 0.02	4.13 ± 0.18^a	7.63 ± 0.12^c	7.15 ± 0.53^c
F (4, 25)	48.45**	38.39**	13.35**	32.32**	14.99**	6.91**	23.67**	46.82**	15.92**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at $*p<0.05$, $**p<0.01$) and (t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to group-II.)

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