Therapeutic Implications of *Gymnadenia Orchidis* Lindl Root Salep Against Induced-Diabetes

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ABSTRACT

Diabetes, the world largest metabolic disorder has become a serious threat to public health. The management of diabetes by synthetic drugs causes many unwanted complications. Hence this study was designed to explore the root Salep of *Gymnadenia orchidis* Lindl against type-2 diabetes to achieve a complications free herbal treatment for the disease. The Streptozotocin (STZ) induced-diabetic rats were supplemented with root Salep orally daily at an effective dose (200 mg/ g of body weight). The body weights and fasting blood glucose levels were measured periodically for 32 days. After treatment period, the animals were sacrificed and glycosylated haemoglobin, lipid profiles, antioxidant enzymes levels, liver function enzymes etc. were determined. Phytochemically determined terpenoids was extracted from the root and orally supplemented (4 mg/g body weight) to the induced-diabetic animals. Normalization of fasting blood glucose levels, significant (P<0.001) decrement of glycosylated haemoglobin percentage, liver enzymes activities and increase body weights and anti-oxidants levels were noted for the Salep supplemented diabetic rats. Terpenoids played the key role in such observations. The root Salep of *Gymnadenia orchidis* Lindl or its terpenoids may be used as potentially herbal therapeutic agent for long term and effective solution against type-2 diabetes mellitus.

Keywords: Anti-oxidants, Diabetes, *Gymnadenia orchidis* Lindl, Lipid peroxidation, Toxicity.

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. It is the fastest growing metabolic disorder that causes a serious threat to public health. The number of diabetic patients has risen from 108 million in 1980 to 422 million in 2014.[1] The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% to 8.5% within this period. About 1.5 million deaths were caused directly by diabetes and another 2.2 million deaths were due to high blood glucose in 2012. Also WHO projects that diabetes will be the 7th leading cause of death within 2030.[2] In India, the number of people suffering from diabetes is about 35 million, which is a gross estimate as many people are unaware of whether they are carrying the disease or not.[3] These alarming figures show that diabetes could be more severe in India in the near future.

Diabetes is a major cause of blindness, kidney failure; heart attacks, stroke and lower limb amputation etc. Adults with diabetes have a 2-3-fold increased risk of heart attacks and strokes. [4] Combined with reduced blood flow, neuropathy (nerve damage) in the foot increases the chance of foot ulcers, infection and eventual need for limb amputation. Diabetic retinopathy is a vital cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. About 2.6% of global blindness can be attributed to diabetes.[5] Diabetes is among the leading causes of kidney failure.[6]

The general trend for the treatment of diabetes is to use synthetic drugs and insulin therapy. But synthetic drugs or insulin therapy for the management of diabetes mellitus causes numerous drawbacks, like insulin resistance,[7-8] anorexia nervosa, brain atrophy, fatty liver [9] and so on. Also this type of management for diabetes is not only causing many side effects but also very costly and furthermore, those synthetic drugs alone will not be able to fulfill the needs of the growing number of diabetic patients in the near future. Hence the alternative solutions to this problem need to be explored from abundance of natural medicinal plants which have shown the ability to control diabetes. [10-12] However, these medicinal plants are mostly used only locally and very little scientific work has been done on these life saving plants to identify their active compounds responsible for the management of blood glucose level.

One of such medicinal plants that could have the ability to reduce hyperglycemia is *Gymnadenia orchidis* Lindl. The plant belongs to the family *Orchidaceae* and is found in the Himalayan region from Pakistan to South–East Tibet at an altitude range of 2400 – 4000 m. This perennial herb has a tuberous root which is divided into 2 or 3 lobes. The roots of this plant when grinded and mixed

with water form a thick 'Salep' which is traditionally used by the people of Bhootia community to get some relief against diabetes.[13] Our study has established that the *Gymnadenia orchidis* Lindl, root Salep is a compatible, safe and complication free agent against type-2 diabetes whose terpenoids component plays the active role against the disease.

MATERIALS AND METHOD

Materials and chemicals

The fibrous roots of *Gymnadenia orchidis* Lindl was collected from the local market in Darjeeling, West Bengal, India. The freshly picked roots were immediately transferred into a container with dry ice and were brought to the laboratory and stored in a -20 ^{o}C refrigerator until used. Streptozotocin was purchased from Sigma Chemical Company Inc. USA. All other analytical grade chemicals were purchased from standard chemical companies. Glycosylated haemoglobin kit was provided by Biosystems, Barcelona, Spain. Cholesterol and Acid phosphatase (ACP) kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. Urea, Alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) kits were supplied by Piramal Healthcare Limited, Mumbai, India. Triglyceride and creatinine were measured by using the kits provided by Merckotest®, Merck, Goa, India.

Authentication of the plant and phytochemical analysis of the root Salep

The plant whose root used in the study was submitted to Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok, India for phytochemical analysis. Dr. D. K. Agarwal identified the root and Dr. M. Gangopadhayay authenticated the plant as *Gymnadenia orchidis* Lindl of the Orchidaceae family (Accession No.: 0046 dated 26.09.2014. V. No. SHRC- 5/02/2012 - Tech. - 195).

The method described by Harbone (1973), [14] and also by Trease and Evans (1983), [15] was used for identification of different phytochemicals in the root Salep of *Gymnadenia orchidis* Lindl. Aqueous extract of the dried roots was made for three different concentrations (1 mg/ml, 10 mg/ml and 25 mg/ml) and analyzed in triplicate for each concentration in HPLC to get statistical average. The presence of flavonoids, alkaloids, terpenoids, steroids, cardiac glycosides, saponin, tannin, polyphenols, vitamin C, carbohydrate(s), protein(s) and free amino acid etc. were determined by the standard laboratory methods.

Toxicity study of the root Salep

Dust root powder was prepared from the tuberous root by grinding the dry roots in a mortar placed in ice bath and different concentrations of Salep as per requirement was freshly prepared with sterile deionized water to the powder root before use.

Adult female albino rats (30 in number) of Wistar strain having standard body weights (130 – 150 g) and age (4 – 5 months) were procured at a time from the animal housing facility of Jadavpur University. The animals were acclimatized under standard conditions of temperature and humidity with 12 h light/dark cycles. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Jadavpur University, Kolkata, India (constituted as per the "Gazette of India "notification part II Sec. 3 (ii) 17 of the Ministry of Environment & Forestry, Government of India, dated 8th September 1998 for the "Prevention to cruelty to animal 1968"). They were housed to polypropylene cages and fed normal protein diet (18% casein, 70% carbohydrate, 7% fat, 4 % salt mixture and 1% vitamin mixture).[16] The animals were divided into 6 groups having 5 rats in each for toxicological study as follows.

Group 1 – The animals in this group were maintained with normal protein diet but not supplemented with root Salep. This group served as control group.

Group 2 to Group 6 – The animals in these groups maintained with normal protein diet and received graded dose of root Salep containing 0.1 g, 0.2 g, 0.3 g, 0.5 g and 1 g of dry root powder/ kg body weight/day respectively for 10 days. After oral administration of the root Salep, the gross activity, posture and tone, eye ball movement, reaction and reflexes of the animals were noted in every day. On the 11th day the rats were sacrificed with mild anesthesia and the blood was collected from the heart. Liver function enzymes such as acid phosphatase (ACP), alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) and kidney function test (urea and creatinine) were determined from serum by using the supplied kits for toxicological analysis of the Salep treated animals.

Extraction of terpenoids from root Salep

A 40 g of root sample was taken and crushed in mortar and pestle and kept soaked in 200 ml of ethanol for 6-8 days. Then it was filtered in filter paper and the filtrate was taken in a conical flask. The ethanol was removed completely by heating the sample over water bath. The sample was suspended in 20 ml of distilled water and subsequently 100 ml of petroleum ether in a separating funnel and shaken vigorously for 1 min and kept for standing time about 10 min for separation of two layers. The expected terpenoids that came to the pet ether layer was eluted. Again some amount of pet ether was mixed with the rest of the solution, shaken vigorously and kept for some

time until two layers separated. In this way by repeating the process 10-12 times, terpenoid was eluted in the pet ether layer. The pet ether was removed from the sample by distillation process. Finally crude terpenoid obtained was kept in 4 ℃ for further analysis. The presence of terpenoid was confirmed by thin layer chromatographic process with two known standards menthol and limoneme. Approximately 30 mg crude terpenoid was obtained from 40 g dry root.

Experimental design on diabetic rats by using root Salep

Female albino rats of Wistar strain (40 in number and having fasting blood sugar levels < 110 mg/dl) were procured similarly from animal housing facility. Out of 40 animals, 10 were taken in the control group. The rest of the animals were intraperitoneally injected with streptozotocin (STZ) (60 mg/kg body weight dissolved in 0.1M citrate buffer at pH 4.5) to induce diabetes (Eliza et al., 2009)[17]. The induced diabetes was confirmed by measuring the fasting blood glucose level after 3 days. Those induced animals were then clustered into two experimental groups (STZ-induced diabetic group and STZ-induced diabetic with Salep supplemented group) having 10 rats in each whose fasting blood sugar levels were > 150 mg/dl. The experiment was thus conducted on those 3 groups of rats as follows:

Control group – Animals having fasting blood glucose level < 110 mg/dl and not given any treatment.

STZ-induced diabetic group – Animals having blood glucose level > 150 mg/dl and were not supplemented with root Salep.

STZ-induced diabetic with Salep supplemented group – Animals in this group having blood glucose level > 150 mg/dl and supplemented with root Salep (200 mg powder root/ kg body weight for 32 days).

The blood glucose level in 12 hour fasting condition of each rat was measured by making a small incision at the tip of the tail by using digital Breeze2 glucometer supplied by Bayer Healthcare LLC, USA. This measurement was repeated in every 3 days till the completion of the experiment. Body weight and general health conditions were also monitored every day in the fasting state. After the treatment period (32 days) all the rats were sacrificed after mild anesthesia and the blood samples were collected from the heart and stored in normal BD Vacutainer and EDTA (5.4 mM/3ml blood) containing BD Vacutainer for further analysis. Serum was isolated from the blood stored in normal BD Vacutainer.

Biochemical analysis of serum

Glycosylated haemoglobin (Hb1Ac) was measured using the kit and procedure provided by Biosystems, Barcelona, Spain (Bisse and Abraham, 1985)[18]. SGOT and SGPT were determined by

the kits supplied by Piramal Healthcare Limited, Mumbai, India. Total serum protein was measured using the method given by Lowry et al. (1951)[19]. Total Cholesterol was measured using the kit provided by Accurex Biomedical Pvt. Ltd., Mumbai, India. Triglyceride was measured using kit provided by Merckotest®, Merck, Goa, India. Lipid peroxidation was determined from the Thiobarbuturic Acid test (TBA test) with modification by Kumar and Das (1993).[20] Super oxide dismutase (SOD) activity was assayed by the method based on the reduction of nitroblue tetrazolium (NBT) to blue pharmazone by superoxides, produced phytochemically in the reaction system.[21] Reduced glutathione (GSH) was determined by using the method of Davila et al. (1991)[22] and glutathione peroxidase (GPx) was estimated by using the method of Levander et al. (1983).[23]

Experiments on diabetic rats by using extracted terpenoids

In this experiment, 5 groups of female albino rats of Wistar strain having 5 animals in each were taken and maintained as described earlier. Diabetes to the animals was induced by STZ. The groups were as follows:

Control – The fasting blood glucose levels of all the animals was < 110 mg/dl and no treatment was given.

Diabetic – The fasting blood glucose levels of all the animals was > 150 mg/dl and no treatment was given.

Terpenoid (+) – The fasting blood glucose levels of all the animals was > 150 mg/dl and terpenoid (4 mg/ kg bogy weight as a trial dose) extracted from the root of *Gymnadenia orchidis* Lindl was supplemented orally.

Terpenoid (-) – The fasting blood glucose levels of all the animals was > 150 mg/dl. After extraction of terpenoid from the powder root of *Gymnadenia orchidis* Lindl, the powder was washed repeatedly by deionized distilled water, air dried and the supplemented (200 mg/ kg body weight) to the diabetic animals orally.

Metformin – The fasting blood glucose levels of all the animals was > 150 mg/dl and Metformin (100 mg/ kg bogy weight) was supplemented orally.

Different groups of animals were treated with drugs twice a day with 8 h interval for nine doses of 5 days schedule as described by Chakrabarti et al. (2003). [24] Fasting blood glucose levels and liver glycogen estimation were done on the sacrificed animals after the treatment.

Statistical analysis

All experimental set up was repeated twice and data (n = 10 for toxicological test and n = 20 for biochemical analysis) were averaged and given mean ± SD. The statistical analysis of the data obtained from control, diabetic and aqueous roots Salep of *Gymnadenia orchidis* Lindl

supplemented groups was performed by one way analysis of variance (ANOVA). The significant levels of the observed data were determined at *, $P < 0.01$ (significant) and **, $P < 0.001$ (more or highly significant).

RESULTS AND DISCUSSION

Toxicity test

Toxicity results showed that the Salep did not produce any harmful effect on the animals as all the animals were remained alive and healthy even after 10 days of dosing. The body weight of the rats remained steady throughout the toxicity study (Table 1). The overall behaviors of the animals noted regularly were found to be normal after the oral supplementation of the root Salep. Fasting blood glucose levels were found to be the minimum for the Salep (200 mg/kg body wt.) supplemented animal in Group 3 (Table 1). It was observed that there was a trend of increase ACP (Fig.1A) levels (within normal range) in serum of Salep supplemented rats in comparison to control rats. The levels of ALP (Fig. 1B), SGOT (Fig. 1C) and SGPT (Fig. 1D) were initially decreased and then increased (within normal range) with the increased dose of Salep supplementation compared to the normal group. Urea and creatinine (Fig. 2) both levels were produced similar results as seen in SGOT and SGPT. The most normal levels of blood glucose, liver function enzymes and kidney function parameters were observed to the animals belonging in the Group 3 suggesting that 200 mg/kg body weight dose of the Salep could be most suitable for the study of the action against type 2 diabetes mellitus. This was taken as the effective dose of the Salep for treatment against diabetes.

Table1: Body weight and fasting blood glucose levels of the rat under toxicity test

	Body weight (g)		Blood glucose (mg/dl)	
Group	**0 day**	**10 days**	**0 day**	**10 days**
Group 1	153 ± 6.6	158 ± 4.1	94.1 ± 7.8	99.9 ± 9.7
Group 2	160 ± 7.4	165 ± 7.8	99.0 ± 8.6	95.7 ± 10.3
Group 3	160 ± 8.9	163 ± 9.1	95.6 ± 10.1	88.6 ± 5.7
Group 4	161 ± 8.6	164 ± 9.8	94.7 ± 8.8	90.6 ± 10.4
Group 5	157 ± 7.2	156 ± 7.1	92.6 ± 11.2	91.7 ± 7.9
Group 6	150 ± 6.3	152 ± 6.5	95.5 ± 9.8	93.7 ± 8.9

n = 10 for each group. All values are expressed as the Mean $\pm$ S.D
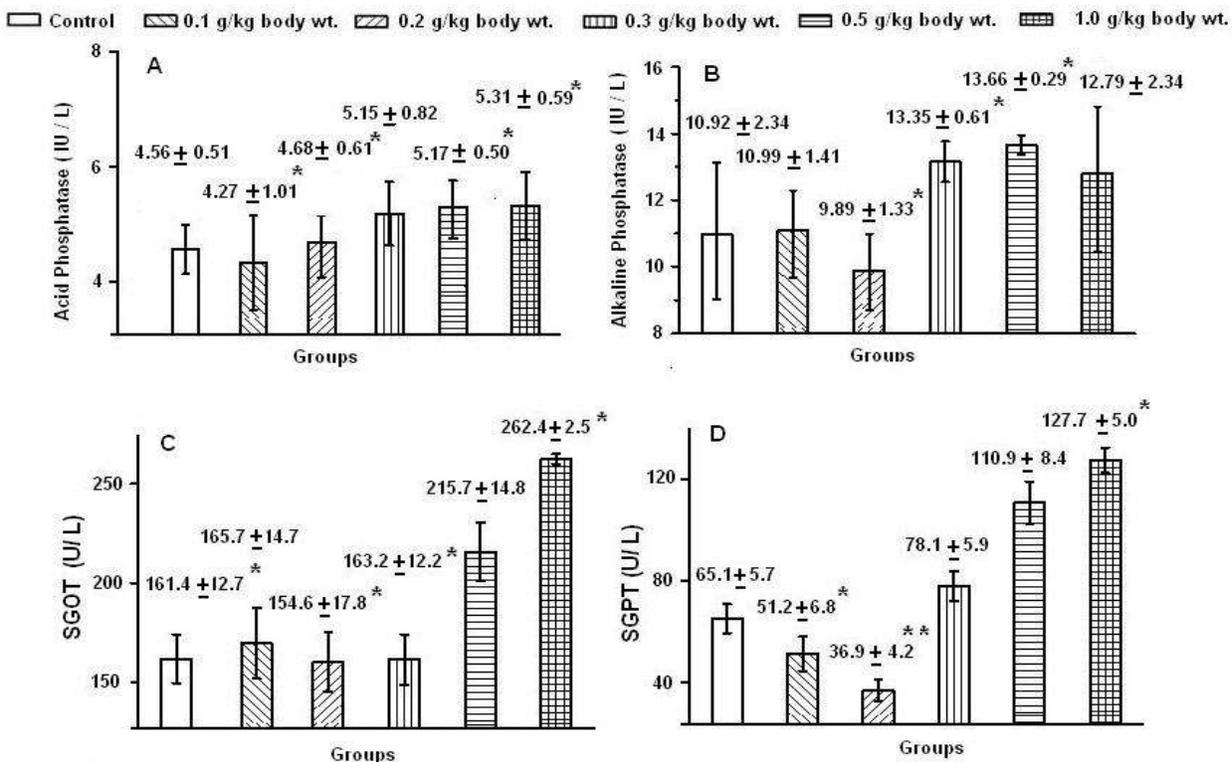
Figure 1: Effect of root Salep at different doses on hepatic enzymes of rat serum, where

A: ACP; B: ALP; C: SGOT; D: SGPT

Data were averaged and presented as mean ± S.D. (N = 10), where * means significant (P < 0.01) and ** implies more significant (P < 0.001).
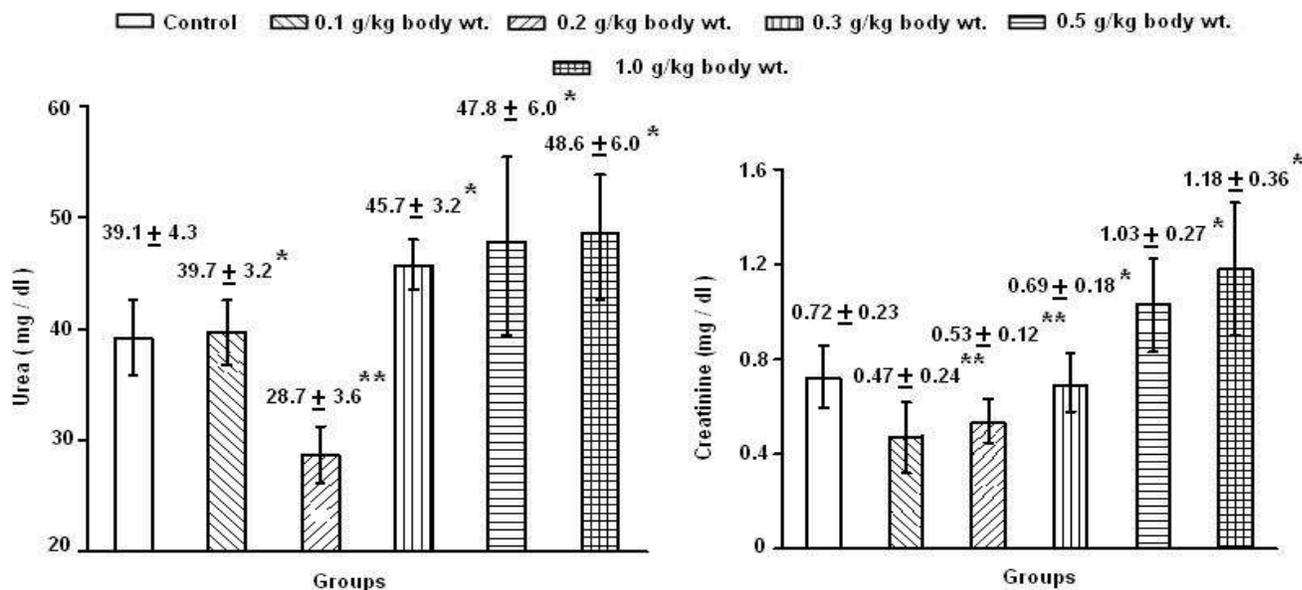
Figure 2: Effect of root Salep at different doses on renal parameters.

Data were averaged and presented as mean ± S.D. (N = 10), where * means significant (P < 0.01) and ** implies more significant (P < 0.001).

Phytochemical analysis

Phytochemical analysis showed that the root Salep of *Gymnadenia orchidis Lindl* contained adequate amount of terpenoids, carbohydrates and proteins and trace amount of tannins, polyphenols, steroids and vitamin C (Table 2). Flavonoids, alkaloids, saponin and free amino acids were not detected in the root Salep.

Table 2: Photochemical compositions of the root Salep

| Phytochemical	Present (+) / Absent (--)
Flavonoids	--
Alkaloids	--
Terpenoids	++
Steroids	+
Cardiac glycosides	+
Tannins	+
Saponins	--
Polyphenols	+
Vitamin C	+
Carbohydrates	++
Proteins	++
Free amino acids	--

++   Adequately present, + Present, - Absent

Effect of the Salep on Diabetic rats

The blood glucose level of the Salep treated (200 mg/kg body wt.) diabetic rats was restored to normal levels after 3 doses of Salep (Fig. 3A). It was noted from Fig. 3B that the percentage of Glycosylated haemoglobin was increased (about 30.5%) in STZ induced diabetic rats compared to control rats. More significant ($P < 0.001$) reduction of Glycosylated haemoglobin percentage was observed on the Salep supplemented diabetic group (about 26.8% lower than that of non-treated diabetic group). The body weights of the induced diabetic rats were decreased compare to the control rats due to the induction of the disease which were restored significantly ($P<0.01$) when the animals received Salep supplementation (Data not supplied).

The activity of liver function enzymes were elevated in case of diabetic rats. SGOT was increased by 69.6% (Fig. 4A) and SGPT was increased by 23.1% (Fig. 4B) with respect to their control rats. The levels of SGOT (about 19.8%) and SGPT (about 32.1%) both were decreased significantly ($P < 0.01$) to the Salep supplemented diabetic rats as compared to the non- treated diabetic rats. Diabetes induction increased both the cholesterol (about 26.6%) and triglycerides (12.4%) levels compared to the control rats (Fig. 4C). Salep supplementation to the diabetic rats reduced the total cholesterol level (about 18.8%) significantly ($P<0.01$) and triglycerides level (about 23.2%) more significantly ($P < 0.001$) compared to the diabetic induced rats. Lipid peroxidation also more

significantly (P < 0.001) decreased to the animals belonging in the Salep supplemented group with respect to the animals of the diabetic induced group (Fig. 4D). It was also observed that the levels of antioxidant enzymes such as SOD, GSH and GP_x were lowered for diabetic rats but restored by the treatment of root Salep (Fig. 5).
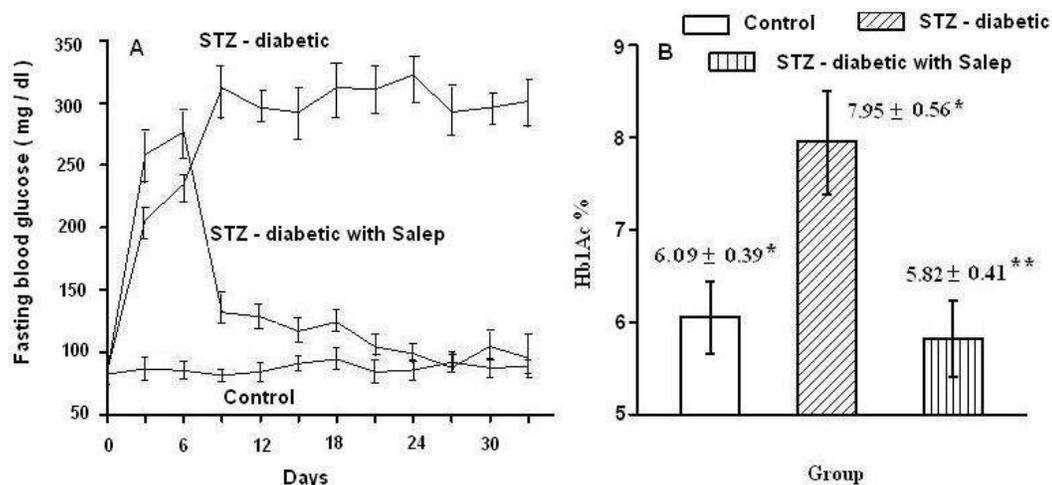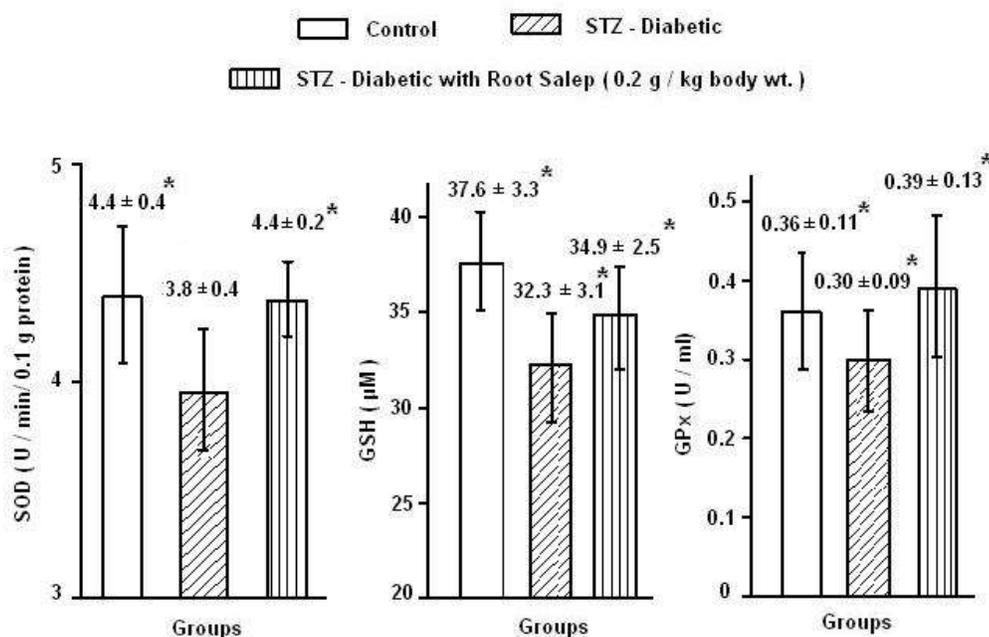

Figure 3: Effect of root Salep (0.2 g/kg body wt.) on diabetic rat. Where A: Blood glucose;

B: Hb1A$_c$

Data were averaged and presented as mean ± S.D. (N = 10), where * means significant (P < 0.01) and ** implies more significant (P < 0.001).
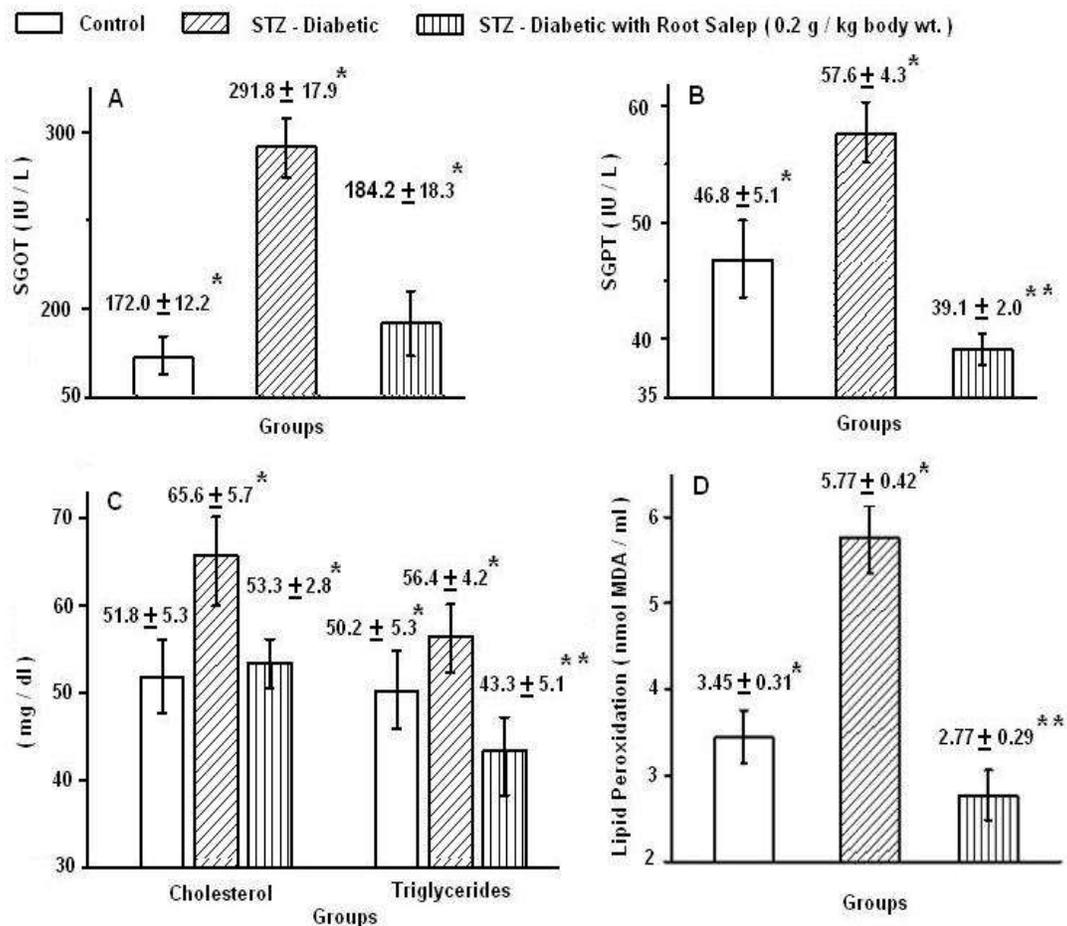
Figure 4: Effect of root Salep (0.2 g/kg body wt.) on diabetic rat. Where A: SGOT; B: SGPT; C: Lipid levels; D: Lipid peroxidation

Data were averaged and presented as mean ± S.D. (N = 10) where, * means significant (P < 0.01) and ** implies more significant (P < 0.001)

Figure 5: Effect of root Salep (0.2 g/kg body wt.) on antioxidant enzymes of diabetic rat.

Data were averaged and presented as mean ± S.D. (N = 10) where, * means significant (P < 0.01) and ** implies more significant (P < 0.001).

Effect of terpenoid on diabetic rats

The blood glucose levels of the STZ induced diabetic animals treated with terpenoid (extracted from the root) were found to be normal (< 110 mg / dl) as seen from Table 3. There was no effect on blood glucose level of the diabetic animals when supplemented root Salep devoid of terpenoid. Liver glycogen content of the diabetic animals also increased with the terpenoid supplementation compared to the control group but remained almost same when terpenoid lacking Salep treated diabetic animals (Fig. 6).

Table 3: Fasting blood glucose levels of the rat under different treatment

Group	Glucose levels	
	1st day	5th day
Control	98.2 ± 7.7[*]	102.2 ± 16.7[*]
Diabetic	196.2± 23.0[*]	206.0 ± 50.4[*]
Diabetic +Terpenoid	180.4 ± 25.3*	108.4 ± 9.7[**]
Diabetic – Terpenoid	215.0 ±25.5[*]	264.2 ± 47.2[*]
Diabetic + Metformin	157.8 ± 6.4[*]	125.0 ± 9.7[*]

n = 10 for each group. All values are expressed as the Mean ± S.D. with Statistical significance as *, P < 0.01 (significant); **, P < 0.001 (more significant)
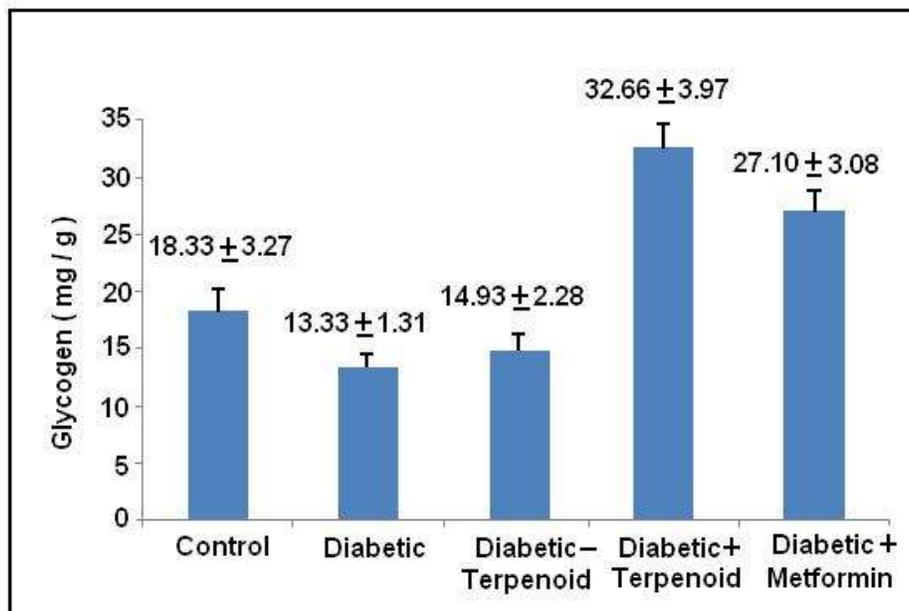
Figure 6: Liver glycogen content of diabetic rat under different treatment conditions

Data were averaged and presented as mean ± S.D. (N = 10).

DISCUSSION

The perennial herb *Gymnadenia orchidis* Lindl is mostly used in locally for the treatment of diabetes but no scientific study was done before on this life saving plant in this respect. This study furnishes to establish the protective role of the tuberous root of *Gymnadenia orchidis* Lindl against diabetes through proper scientific approaches. Toxicological investigation on rats has revealed that the Salep is toxicologically safe and can be used in edible form. The tested biochemical parameters (like ACP, ALP, GPT, GOT, Urea and Creatinine etc.) of the Salep supplemented rats at higher doses (> 200 mg powder root) were found to be slightly higher values with respect to control level but still the values were within the accepted range suggesting that the root Salep did not produce any harmful effects on the normal functions of liver (Fig. 1), kidney (Fig. 2) and general health conditions (Table 1). It was also evident from the studies that the most effective dose of Salep for the treatment of diabetic rats was 200 mg/kg body weight per day.

Our experimental results showed that the fasting blood glucose level was significantly increased and body weight was significantly decreased of the rats when the animals received a single dose injection of STZ. Single dose injection of STZ causes severe type 2 diabetes to the animals.[25] It is generally assumed that STZ is toxic to the insulin-producing β cells which is taken up via the cell membrane GLUT2 glucose transporter and causes DNA alkylation and eventually causes β cell death.[26-28] Our findings clearly showed that Salep supplementation to the diabetic induced rats normalized the fasting blood glucose levels (Fig. 3A) and increased the body weight (data not

supplied) in comparison to the normal group. Glycosylated haemoglobin (HbA$_{1c}$) is a well known marker for confirmation of diabetes to the patients. Higher levels of HbA$_{1c}$ are found in people with persistently elevated blood glucose, as in diabetes mellitus. The International Diabetes Federation and American College of Endocrinology recommend HbA$_{1c}$ values below 6.5% is the reference range for non diabetic patient, while American Diabetes Association recommends that the HbA$_{1c}$ be below 7.0% for most patients.[28] Our findings showed that the HbA$_{1c}$ percentage of STZ treated induced diabetic rats were quite higher (7.95) than that of the control rats (6.09) as seen from Fig. 3B. The percentage of HbA$_{1c}$ was more significantly (P < 0.001) reduced in Salep supplemented induced diabetic rats (5.82) confirming the protective role of the herb *Gymnadenia orchidis* Lindl against type 2 diabetes.

Diabetes caused dysfunction as well as damage of the liver cells, [29] due to which the liver function enzymes SGOT and SGPT levels were increased in STZ treated induced diabetic rats. Oral supplementation of the root Salep reduced significantly (P < 0.01) the levels of SGOT and SGPT levels as observed in experimental observations (Fig. 4A & 4B). Diabetes also causes disorder in carbohydrate metabolism that leads to disorder in lipid metabolism because carbohydrates and lipid metabolism are interrelated to each other.[30] Diabetes thus increased the concentrations of cholesterol and triglycerides levels (Fig. 4C) within the blood. Increment of serum lipid peroxidation (Fig. 4D) associated with diabetic induced rats was in agreement with the earlier findings.[30, 31] Lowered levels of cholesterol, triglyceride and lipid peroxidation of the Salep supplemented animals suggested that the lipid profile was very well regulated by the *Gymnadenia orchidis* Lindl root Salep.

In diabetes mellitus, increased formation of reactive oxygen species (ROS) due to high level of glucose in both blood plasma and tissues creates oxidative stress that damages the tissues.[32] Enzymatic antioxidants like SOD, GSH and GPx constitute a mutually supportive team of defense against ROS that were found to decrease in diabetic induced condition (Fig. 5). SOD, the mitochondrial enzyme and usually found in plasma membrane, is a ubiquitous enzyme and protects aerobic cells against ROS.[33] GP$_x$ is a seleno enzyme that catalyzes the reaction of hydroperoxides with GSH to form glutathione disulfide (GSSG) and the reduced product of the hydroperoxides.[34] Depletion in the activities of SOD, GSH and GPx in the serum of diabetic induced rats might be due to the increased utilization of these antioxidants to counter lipid peroxidation. Glutathione is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stress. Glutathione reacts directly with ROS and electrophilic metabolites, for several enzymes.[33] Hepatic GSH plays a crucial role in both

scavenging ROS and detoxification of drugs.[34] Thus, a decrease in GSH activity (Fig. 5) not only impairs cell defense against diabetes, but also results in enhanced oxidative stress and tissue damage. The root Salep of *Gymnadenia orchidis* Lindlincreased the activity of SOD, GSH (P < 0.01) and GPx (P < 0.01) of diabetic induced rats and therefore played an important role in cellular defense against type 2 diabetes.

Phytochemical analysis showed that the root Salep contained various amount of terpenoids, tannins, polyphenols, vitamin-c, steroids and proteins (Table 2). The effects of dehydroabietic acid (DAA), a diterpene, on glucose and lipid metabolism were examined using obese diabetic KK-Ay mice by Kang et al. (2009).[35] They have shown that DAA treatment decreased not only plasma glucose and insulin levels but also plasma triglyceride (TG) and hepatic TG levels. Nazaruk and Borzym-Kluczyk (2015),[36] have shown that triterpenes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels. Santos et al. (2012)[37] demonstrated the antihyperglycemic and hypolipidemic effects of *α, β*-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice.

The decrease of blood glucose and lipid profile levels and increase of liver glycogen content (Fig. 6) of the Salep supplemented rats may be thus explained on the basis of terpenoids present in the root Salep of *Gymnadenia orchidis* Lindl. The antioxidant potency of polyphenols,[38] vitamin C and tannins,[39] present in the root Salep may also take some definite role to combat against diabetes. The structural and functional properties of these compound could be lost during extraction of terpenoids from the root for which the therapeutic effect of those components could not be observed in our results.

CONCLUSION

Our results thus suggested that the root Salep could be an effective herbal therapeutic measure in controlling the blood glucose and regulating normal metabolism for patients having type 2 diabetes mellitus. Terpenoid present in the root Salep should have some definite role to counter the effect of diabetes on rats. Further studies will enable us for identification of highly effective key component(s) present in the complication free *Gymnadenia orchidis* Lindl for clinical trials to prevent or treat the life threatening diabetes disease over a long period of time.

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