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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACTS OF *GLYCYRRHIZA GLABRA* IN STREPTOZOTOCIN INDUCED ALBINO RAT

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ABSTRACT

Glycyrrhiza glabra (Phytochemical - Glycyrrhiza - Flavonol A) is herbal plant uses in different countries to treat diabetes mellitus. *Glycyrrhiza glabra* is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder. Babul plant is therapeutic used as Anti-cancer, anti tumours, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains and diarrhea, Nerve stimulant, Cold, Congestion, Coughs, Dysenter, Fever, Hemorrhages, Leucorrhea, Ophthalmia and Sclerosis. The toxicity study has showed that the 24-Methyl cycloartan compound has no toxic effect on red blood cells; therefore we suggest that this compound can be successfully and safely use to treat diabetes mellitus instead of insulin.

KEYWORDS

Glycyrrhiza glabra, Isolation, Anti-diabetic activity, Terpenoids, Diabetes mellitus, Glucose, and Streptozotocin.

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INTRODUCTON

Incontrovertibly there are worldwide changes in healthcare industry within the third millennium. Ayurvedic system of healthcare has gained importance and is becoming popular. It is a comprehensive system of healthcare that originated in India. Because of the effectiveness and fewer adverse reactions compared to the synthetic chemicals, Ayurvedic system has attained popularity globally. The classical text of Ayurveda mentions number of plants for the management of several diseases. Undoubtedly several researchers had given their contributions for locating hidden

therapeutic potentials of number of Ayurvedic drugs, but still number of plants need a comprehensive study on them. Therefore this study is concentrated on one such very effective and potent medicinal herb- licorice[1]. Glycyrrhiza glabra Linn is one among the foremost extensively used medicinal herb from the traditional medical record of Ayurveda. It is also used as a flavoring herb. The word Glycyrrhiza springs from the Greek term glykos (meaning sweet) and rhiza (meaning root). Glycyrrhiza glabra Linn, commonly referred to as 'liquorice' and 'sweet wood' belongs to Leguminosae family. Vernacular names for liquorice are Jeshthamadh (Marathi), Jothi-madh (Hindi), Yashtimadhu, Madhuka (Sanskrit), Jashtimadhu, Jaishbomodhu (Bengali), Atimadhuram, Yashtimadhukam (Telugu), Jethimadhu (Gujarati) and Atimadhuram (Tamil)[2]. In traditional medicine, liquorice has been recommended as a prophylactic agent for gastric and duodenal ulcers. It is employed in dyspepsia as an anti-inflammatory agent during allergenic reactions[3]. It is used as a contraceptive, laxative, anti-asthmatic, emmenagogue, galactagogue, antiviral in folk therapy[4]. Glycyrrhiza roots are useful for treating cough due to its demulcent and expectorant property[5]. It is also effective against anemia, gout, pharyngitis, tonsillitis, flatulence, sexual debility, hyperdypsia, fever, skin diseases, swellings. Liquorice is effectively utilized in acidity, leucorrhoea, bleeding, jaundice, hiccough, hoarseness, bronchitis, vitiated conditions of Vata dosha, gastralgia, diarrhea, fever with delirium and anuria[6,7]. It is an important ingredient in medicinal oils used for the treatment of rheumatism, hemorrhagic diseases, epilepsy and paralysis[7]. It has been proved by several years of research that, glycyrrhizin breaks down within the gut and exerts anti-inflammatory action almost like hydrocortisone and other corticosteroid hormones. The effect is due to stimulation of hormone production by adrenal glands and reduction in the breakdown of steroids by the liver and kidneys. Effectiveness of glycyrrhizin within the treatment of chronic hepatitis and liver cirrhosis is proved[8]. Glycyrrhiza glabra is taken into account together of the simplest remedies for relieving pain and other symptoms like discomfort caused by acrid matter within the stomach. It alleviates the irritating effects of acids in a better way than alkalies[9]. It is a superb tonic and is additionally used as demulcent in catarrh of the genitourinary passages[10]. The effect of liquorice extract on serum lipid profile and liver enzymes was studied in albino mice. Root extract of Glycyrrhiza glabra was found to have anti-lipidemic and antihyperglycemic activity at low doses[11].

METRIAL AND METHODS

Preparation of Extract

The dried pulverized material was extracted with fuel in very Soxhlet extraction equipment. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 7.5 %). The methanolic extract was concentrated to dryness in vaccum at 35^{o}C. Active constituents from the dried extracts were separated by column chromatography with different solvent ratio.

Preliminary Chemical Tests

The extract was subjected to preliminary screening, for numerous active phytochemical constituents like alkaloids, carbohydrates, steroids, protein, tannins, phenols, flavonoids, gum and mucilage, glycosides, saponins and terpins.

Isolation of Alkaloids

Fresh Seeds (1kg fresh wt) of Glycyrrhiza glabra (Sw.) DC. were soaked in MeOH for 2 weeks at room temp with the extract filtered and concentrated to dryness in vaccuo at 35^{o}C, with the concentrate (20g) was then partitioned between water and ethyl acetate with the aqueous layer was basified with ammonium hydroxide (pH 9) and extracted repeatedly with chloroform. The combined chloroform layers were concentrated to dryness in vacuo at 35^{o}C with the concentrate (5g) separated by column chromatography into various fractions of MeOH- H2OTFA (trifluoroacetic acid) (80: 20: 0.1, v/v), MeOH-TFA (100: 0.1, v/v) and CH3Cl-TFA (100: 0.1, v/v), respectively. The MeOH-H2O-TFA fraction was concentrated to dryness in vacuo at 35 °C. The combined fractions were identified by TLC and recrystallized with methanol.

Selection of Dose

Acute oral toxicity test was carried out according to the OECD guideline No.423. Wistar unusual person Rats were unbroken for nightlong abstinence before drug administration. A total of 3 animals were used, which received a single oral dose in 2000mg/kg, body weight of different extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality, if any, determined over a amount of two weeks. Hence in our studies we selected 1/10 and 1/5th dose i.e. 200 and 400mg/kg dose.

Preparation of Doses

Doses equivalent to 200mg and 400mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Streptozotocin (STZ) induced diabetes in rats

After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was observed by moderate Polydipsia and marked Polyuria. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250mg/dl were selected for further study. (Ali *et al*, 2009)[12].

Experimental Design of antidiabetic study of *Glycyrrhiza glabra*

In order to assess the anti-diabetic activity, the animals were divided in fifteen groups of six animals in each group.

Group 1: Normal control, 0.9% NaCl-treated animals

Group 2: Diabetic control, STZ -treated rats (40mg/kg body weight)

Group 3: Treated with Pet. Ether extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 4: Treated with Pet. Ether extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 5: Treated with chloroform extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 6: Treated with chloroform extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 7: Treated with ethyl acetate extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 8: Treated with ethyl acetate extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 9: Treated with ethanolic extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 10: Treated with ethanolic extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 11: Treated with methanolic extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 12: Treated with methanolic extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 13: Treated with aqueous extract of Glycyrrhiza glabra (200mg/kg body weight)

Group 14: Treated with aqueous extract of Glycyrrhiza glabra (400mg/kg body weight)

Group 15: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Blood collection and biochemical estimations in serum

On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer (Easy Gluco, Morepen Laboratories Ltd, New Delhi). (Tripathi and Chandra, 2010)[13].

Statistical Analysis

Data were expressed as the mean standard error of mean (S.E.M.) of the means and statistical analysis was carried out employing one-way ANOVA. Differences between the data were considered significant at $P < 0.05$.

Antidiabetic study of *Glycyrrhiza glabra*

Effect on Blood glucose level

The induction of diabetes with streptozotacin increases the blood glucose level significantly ($p<0.001$) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly

with the $p<0.001$ in 14 days whereas methanolic extract (200 and 400mg/kg) reduced the glucose level moderately and highly significant with $p<0.01$ and $p<0.001$. Petroleum ether, chloroform, ethyl acetate, ethanolic extracts had moderately significant effects ($p<0.01$) on 14th and 21st days. However, aqueous extracts didn't show any significant decrease in glucose levels. The results are shown in Table No.1 and Figure No.1.

Table No.1: Effect of different extracts on glucose level in Streptozotocin induced diabetic rats

Group No	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	81.6 ± 0.51	84.1 ± 0.25	82.7 ± 0.67	82.7 ± 0.66	81.37 ± 0.33
II	Diabetic control	83.7 ±0.67	245.3 ± 1.89	269.7 ± 1.37	271.3 ±3.08	288.9 ± 0.31
III	Pet. Ether extract (200mg/kg)	81.7 ± 0.81	247.3 ± 1.31	241.1 ± 2.09	236.3± 2.77	231.3 ± 0.27
IV	Pet. Ether extract (400mg/kg)	82.81 ± 1.03	251.4 ± 2.97	225.5 ± 2.91	216.9± 2.99	202.7 ± 0.56
V	Chloroform extract (200mg/kg)	82.7 ± 0.84	246.7 ± 1.39	226.7 ± 2.37	218.3± 3.07	214.9 ± 2.03
VI	Chloroform extract (400mg/kg)	80.3 ± 0.37	246.3 ± 1.41	224.7 ± 2.36	216.5± 3.31	215.7 ± 2.99
VII	Ethyl acetate extract (200mg/kg)	79.3 ± 0.9	244.4 ± 1.43	266.1 ± 2.36	257.3 ± 3.03	259.7 ± 0.32
VIII	Ethyl acetate extract (400mg/kg)	79.1 ± 0.92	241.5 ± 1.37	258.41 ± 3.02	253.3 ± 3.11	249.6 ± 0.27
IX	Ethanolic extract (200mg/kg)	78.4 ± 0.88	241.7 ± 1.77	224.3 ± 1.47	219.4 ±2.98	217.5 ± 0.44
X	Ethanolic extract (400mg/kg)	81.3 ± 0.87	244.7 ± 1.56	225.1 ± 2.19	218.9± 3.02	214.7 ± 2.55
XI	Methanolic extract (200mg/kg)	83.47 ± 1.19	246.3 ± 2.99	221.6 ± 3.13	208.9±3.11	201.7± 0.25
XII	Methanolic extract (400mg/kg)	86.87 ± 1.11	245.6 ± 3.09	208.2 ± 2.79	194.6±3.02	178.3± 0.82
XIII	Aqueous extract (200mg/kg)	80.9 ±0.81	240.7 ± 1.49	271.8 ± 1.33	271.3 ±3.12	283.1 ± 0.34
XIV	Aqueous extract (400mg/kg)	84.7 ±0.77	241.7 ± 1.89	268.2± 1.33	265.3 ±3.11	262.1 ± 1.35
XV	Glibernclamide (5mg/kg)	84.9 ± 0.99	248.9 ± 2.71	203.3 ± 2.89	181.7± 3.02	171.2± 0.37

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control Vs treated groups

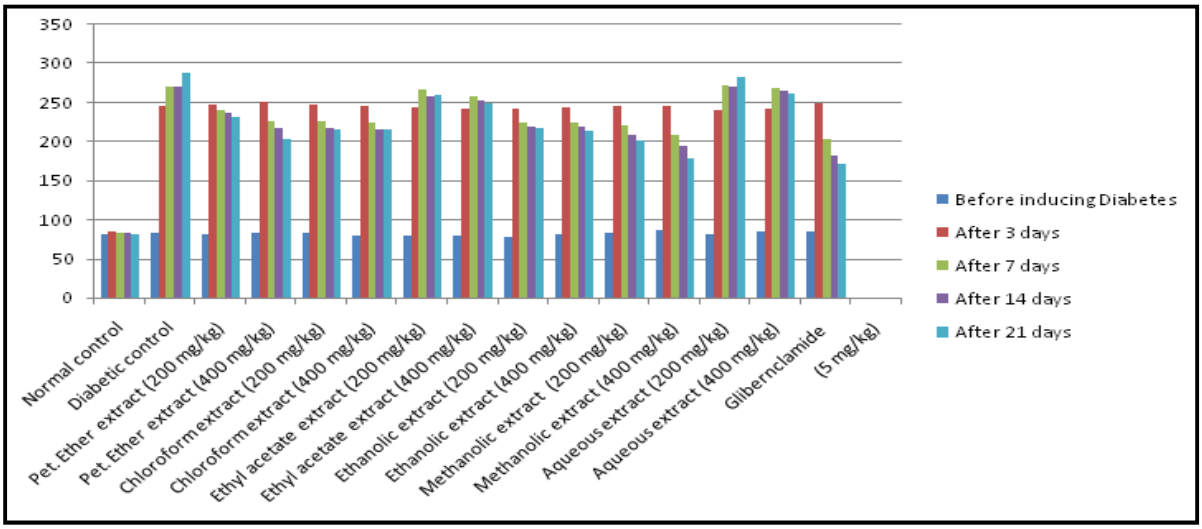

Figure No.1: Effect of different extracts on glucose level in Streptozotocin induced diabetic rats

CONCLUSION

Non-insulin dependent diabetes mellitus is a multifactorial sickness, which is characterized by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to be responsible for damage to cell membranes through non-enzymatic glycosylation of proteins, auto-oxidation of glucose or increase metabolism of glucose by the sorbitol-polyol pathway. Cell damages will in turn, result in elevated production of reactive oxygen species. Predictably, insulin dependent diabetes is treated with exogenous insulin and noninsulin dependent diabetes with synthetic oral hypoglycemic agents like sulphonylureas and biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatropy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in kidneys and severe vascular complications. Similarly, oral hypoglycemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and hemolytic anemia's, generalized allergic reactions, dermatological reaction etc. Traditionally, there are various herbs are being used for the treatment of diabetes mellitus, from which merely some have been evaluated as per the modern system of medicine. From these plants only plant extracts have been prepared and evaluated for its Antihyperglycaemic activity. Most of the reported plants seem to act directly on pancreas and stimulate insulin release in the blood. Some will favorably alter the activities of regulatory enzymes of glycolysis, gluconeogenesis and other pathways by acting directly on tissues like liver, muscle and fat (extra-pancreatic effect). Chemical constituents of these plants are known to possess wide range of medicinal properties. The research was envisaged for antidiabetic activity of different extracts of *Glycyrrhiza glabra* procured by successive extraction methods and to find out or isolate the most possible active compounds from the active extracts showing the best activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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