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RESEARCH ARTICLE

SYNERGISTIC ANTIDIABETIC ACTIVITY OF LIQUORICE AND JATAMANSI IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder that is rapidly becoming a major threat to population all over the globe. Diabetes is due to either the pancreas not producing enough insulin or because cells of the body do not respond properly to the insulin that is produced. Antidiabetics from natural source play a key role in treatment of diabetes. Herbal plants are power house of sources for anti-diabetic principles. The present study aimed the anti-diabetic activity of combined extract of liquorice and jatamansi. Liquorice previously proven for anti-diabetic activity. But in this study carried Synergistic ant diabetic actions of combination extract liquorise and jatamansi in Alloxan induced diabetic rats. The overnight fasted non diabetic rats are treated with alloxan monohydrate 50mg|kg body weight among them rats with plasma glucose level >150mg|dl were selected for diabetic study. The liquorice & jatamansi extract which is prepared in solvent ether is administered for test group along with glibenclamide for standard group which is sulfonyl urea's for about 7days. Test sample showed significant decrease in blood glucose level of diabetic rats at the dose 200mg|kg body wt. compared to diabetic controlled rats. Glibenclamide also showed decrease in blood glucose level at the dose 5mg/kg body wt. Hence our present study revealed the synergistic action of liquorice & jatamansi near to the standard and minimal side effects and less cost with easy available of drugs

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INTRODUCTION

Diabetes mellitus

Diabetes mellitus (DM) or simply diabetes is a group of metabolic diseases in which a person has high blood sugar. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated diabetes can cause many complications. Acute complications include diabetic keto acidosis and non ketotic hyperosmolar coma. Serious long-term complications include heart disease, kidney failure, and damage to the eyes. Diabetes is due to either the pancreas not producing enough insulin, or because cells of the body do not respond properly to the insulin that is produced. There are three main types of diabetes mellitus, Type 1 DM results from the body's failure to produce insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes", Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes also with an absolute insulin deficiency. This form was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes", Gestational diabetes, is the third main form and occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. Prevention and treatment often involve a healthy diet, physical exercise, not using tobacco, and being a normal body weight. Blood pressure control and proper care also important for people with the disease. Type 1 diabetes must be managed with insulin injections. Type 2 diabetes may be treated with medications with or without insulin. Gestational diabetes usually resolves after the birth of the baby.

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Herbal formulations

Herbal formulation shall be labeled to comply with relevant labeling requirements under food or drug or cosmetics laws as applicable. Additionally, adequate information shall be provided on label of such formulations to include the name of the herb, parts used, nature and type of extract or processed

herb used, extraction ratios, quantity per unit dose or Preserving, name(s) of inert excipients used and any preservatives added shall be provided on the label.

Advantages of Herbal Medicine

Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicines for the world growing population. Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity. Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy. Herbal medicine has provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude from and as a pure chemical upon which modern medicines are structured.

Drug profiles

Jatamansi

Botanical source Nardostachys Jatamansi,
Family Name - Valerianaceae, Habitat - Found in India, Nepal and Bhutan Active constituents are Valerononе, Virolin, Jatamansic acid.

1. Jatamansi can be used internally as well as externally.
2. It is a well-known brain tonic and imparts peace to mind.
3. The powder helps in treating mental retardation and mental disorders.

Liquorice

Biological source - *Glycyrrhiza* glabra, Active constituents are Glycyrrhizin, Glycyrrhizic acid, Glycyrrhizinate, It is used in the treatment of peptic ulcer, upper respiratory infections**,** Anti-diabetic, Gastric irritation, anti-inflammatory activity, viral infections.

Glibenclamide

Glibenclamide is an anti-diabetic drug. It is sulfonylureas, closely related to sulfa drugs.Under the trade names Diabeta, Glynase and Micronase in the United States.

Mechanism of action: Inhibits ATP sensitive potassium channels, this inhibition causes cell membrane depolarization opening voltage dependent calcium channel. This results in an increase in intracellular concentration of calcium in the beta cell and subsequent stimulation of insulin release.

Objective of the study

The present study aimed the synergistic action of anti-diabetic activity combined herbal extract Liquorice and Jatamansi also previously not proven for anti-diabetic activity. Our study evaluating the anti-diabetic activity. Most of the existing anti diabetics produces side effects such as hypo glycemia, hepato toxicity, constipation, nausea, and tachycardia. Anti-diabetics from the natural sources may play a key role in the treatment of Diabetes

Experimental

MATERIALS

Table 1.

S.no:	Name of the chemical / glassware	Purity / specification	Source
1.	Jatamansi	Rhizome	Anantapuramu
2.	Liquorice	Root	Anantapuramu
3.	Ethanol	95%	Loba chemicals ltd
4.	Glass wares	borosilicate	JSW

Glass wares were cleaned whenever necessary.

Collection of herbs and extraction

Collection of herbs was from Anantapuramu market. It was authentified by D*r. J. Raveendra Reddy, M.Pharm., Ph.D.,*

Morphological and powder microscopy characters

Color: blackish brown, Taste- Acrid, sour, odor- aromatic

Powder microscopy

Take the powder sample in a watch glass, to it add 1ml of chloral hydrate and 1ml of dilute Hydrochloric acid and heat it for 60 seconds. Then transfers the material into another watch glass, to it's add 1ml of phloroglucinol. Take little quantity on a slide with the help of brush place a drop of glycerin, cover it with cover slip. Fix under the microscope adjust it and identify the characters.

Purification of solvents

The solvents obtained commercially (LR-Grade Extra pure) were purified by distillation methods prior to use for extraction and for Phyto-chemical investigation. Required quantity of ground powder was weighed and then it was extracted with solvent Ethanol by using cold maceration technique or by using continuous hot percolation method (Soxhlet method).

PREPARATION OF EXTRACTS

Continuous extraction method by using Soxhlet apparatus

Freshly collected plant material was dried in shade, and then coarsely powdered in a blender. The coarse powder (500 gm) was extracted successively with Ethanol, each 300ml in a soxhlet apparatus for 6hrs temperature not exceeding 45^0c.All the extracts were filtered through Watts's man No.41 filter paper and evaporated on a water bath and finally dried in vacuum. The residues obtained were used for screening the preliminary phyto chemical and anti-diabetic activity.

Preliminary Phyto-chemical screening

The chemical tests for various phyto constituents in the ethanolic extract were carried out as described below.

(A) Test for alkaloids

(i) Dragendroffs test

In a test tube containing 1 ml of extract, few drops of Dragen droffs reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of alkaloids.

(ii) Wagner's test

To the extract, 2ml of Wagner reagent was added formation of reddish Brown precipitate indicated the presence of alkaloids.

(iii) Mayer's test

To the extract, 2ml of Mayer's reagent was added dull yellow precipitate is revealed the presence of alkaloids.

(iv) Hager's test

To the extract, 2ml of Hager's reagent was added, a dull yellow precipitate revealed the presence of alkaloids.

B) Test for carbohydrates (sugar)

(i) Molisch's test

To the powder 1ml of α- Napthol solution and concentrated sulfuric acid through the sides of test tube were added. Purple or reddish violet color at junction of the two liquids revealed the presence of carbohydrates.

(ii) Fehling's test

To the powder, equal quantities of Fehling's solution A and B were added and on heating, formation of a brick red precipitate indicated the presence of carbohydrates.

C) Test for Saponin

About 1 g of powder is mixed with distilled water to 20ml and shaken in a graduated cylinder for 15min. Layer of foam formed indicates the presence of saponin.

D) Test for proteins

(i) Biurette test

To the extract, 1ml of 40% sodium hydroxide solution and two drops of 1% copper sulphate solution were added. Formation of violet color indicated the presence of proteins.

(ii) Xantho protein test

To the extract, 1ml of concentrated nitric acid was added. As a white precipitate was formed, it is boiled and cooled. Orange color indicates the presence of aromatic nitric acids.

(iii) Tannic acid test

To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

E) Test for Terpinoid (Noller's test)

To the 1ml extract, tin (one bit) and thionyl chloride (1ml) were added. Appearance of pink color indicates the presence of terpinoids.

F) Test for steroids

i) Liebermann-Buchard's test

The powder was dissolved in 2ml chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicated the presence of steroids.

ii) Salkowski test

The powder was dissolved in chloroform and equal volumes of concentrated Sulphuric acid. Formation of bluish red to cherry red color in chloroform layer and green fluorescence in the acid layer represented the steroid components in the test samples.

G) Test for fixed oil

A small quantity of powder extract was pressed between the filter papers. Formation of grease spot indicates the presence of fixed oils and fats.

Physico chemical parameters

Determination of Foreign matter

Weigh 100 to 500gm of the drug sample to be examined or the minimum quantity prescribed in the monograph and spread it out in a thin layer. The foreign matter should be detected by inspection with unaided eye or by the use of lens (6 xs). Separate and weigh it and calculate the percentage present.

Determination of Bulk density

A sample of about 50gm which is previously has been passed through sieveno: 20 introduced Ina 100ml graduated cylinder. The cylinder is dropped at 2 sec intervals into a hard wooden surface 3 times from a height of 1 inch. The final bulk volume is determined and bulk density calculated.

Determination of Total ash

2 to 3 gm powder weigh accurately and placed on crucible at temperature not exceeding 450^0C cool and weigh. If a carbon free ash cannot be obtained. Exhaust the charred mass with hot water, collect residue on an ash less filter paper. Add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450^0c. Calculate percentage of total ash.

Determination of Acid insoluble ash

The ash obtained in above step is boiled for 5minutes with 25ml of dilute hydrochloric acid. Collect the insoluble matter in ash less filter paper, wash with hot water and ignite to constant weight. Calculate percentage of acid insoluble ash.

Extractive values

Powder was extracted in alcohol, water 50ml each separately by cold maceration method and their extractive values are determined as per the method given in Ayurvedic pharmacopoeia of India.

Alcohol soluble extractive

Macerate 5gms of the coarsely powdered drug with 100ml of alcohol in a closed flask for 24hours, shaking frequently during 6 hours and following to stand for 18hours. Filter rapidly taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tare flat bottomed shallow dish and dry at $105^0$to constant weight and weigh. Calculate percentage.

Water soluble extractive

Macerate 5gms of the coarsely powdered drug with 100ml of water and add 5ml of chloroform in a closed flask for 24hours, shaking frequently during 6 hours and following to stand for 18 hours. Filter rapidly taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tare flat bottomed shallow dish and dry at 105^0 to constant weight and weigh. Calculate percentage.

Loss on drying

Place about 10 gms of drug after accurately weighing. Evaporating dish dry at 105^0 for 5hours and weigh. Continue the drying and weighing at one hour interval until difference between 2 successive weightings corresponds to not more than 0.25%. Constant weight is reached when 2 consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, NMT 0.01g difference.

Foaming index

1g ofdrug weigh accurately and transfer to a 500ml conical flask containing 100ml of boiling water and maintain at moderate boiling for 30 minutes, cool and filter into a 100ml volumetric flask and add sufficient water through the filter to dilute the volume to 100ml. Place the above decoction into 10 stoppered test tubes (height 16cm and diameter 16mm) in a series of successive portions of 1,2,3,4 up to 10ml and adjust the volume of the liquid in each tube with water to 10 ml. stopper the tubes and shake them in a length wise motion for 15 seconds, 2 frequencies per second. Allow to stand for 15 minutes. Note 1cm height of the foam and calculate foaming index.

Foaming index=1000/a

Where is the volume in ml, of decoction used for preparing dilution in tube where foaming is observed.

Anti-diabetic activity

Animals used

Wister albino rats (150-200gm) were procured from Indian institute of sciences, Bangalore India. Before and during the experiment rats were fed with standard diet. After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7days under Standard environmental conditions of temperature and relative humidity.

Induction of diabetes

The non-diabetic rats were randomly divided into four groups. Each group contain 6 rats, Group I rats considered as normal control, Group II rats considered as diabetic control , Group III rats considered as standard and Group IV considered as test. Diabetes was induced by intra peritoneal injection of 120 mg/ kg of alloxan monohydrate. The alloxanized rats were kept for 7days with free access food and water. On the 8^{th}, the rats were fasted for 12 hours and their blood sugar glucose levels were determined using Semi auto analyzer. Rats with glucose levels above 250 mg/dl were used for the study

Mechanism of Alloxan

- Alloxan is selectively toxic to insulin –producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction.
- The beta cell toxic action of Alloxan is initiated by free radicals formed in this redox reaction.
- The uric acid derivative initiates free radical damage to DNA in the beta cells of the pancreas, causing the cell to malfunction and die.

Table 2. Treatment protocol

No	Groups	Treatment
1.	Group I (normal control)	Normal control rats received 1%cmc for 7 days.
2.	Group II(diabetic control)	Diabetic control animals received alloxan.
3.	Group III (alloxan + standard)	Alloxan + standard drug(5mg/kg body weight)for 7 days
4.	Group IV (alloxan+extract)	Alloxan+extract200mg/kg body

Table 3. Estimation of blood glucose level

Pipette into test tube labeled as	Blank	Standard	Test
Sample	---	---	10µl
Standard	---	10µl	---
Enzyme reagent	1ml	1ml	1ml

RESULTS

Macroscopic and microscopic characters

Table 4.

S.no:	Macroscopic and microscopic characters	Result (Yes/No)
1.	Color:	Blackish brown
2.	Taste:	Acrid, sour
3	Odor:	Aromatic
4	Cork cells	Yes
5	Phloem fibers	No
6	Large vessels	Yes
7	Starch grains	Yes
8	Xylem vessels	No

Powdermicroscopy

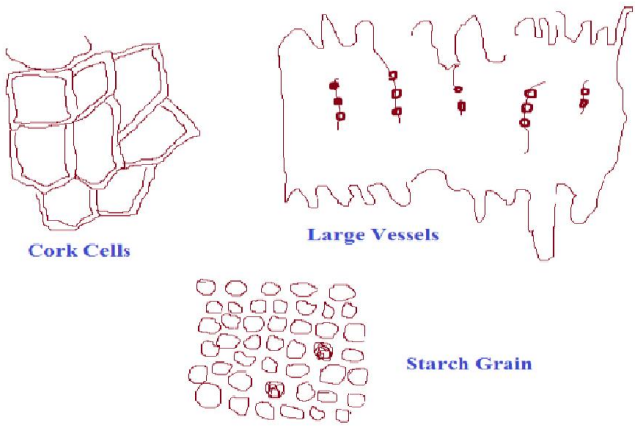

Figure 1.

Preliminary Photochemical Screening of herbal extract

Table 5

S.no	Particulars	Alcohol extract
1.	Alkaloids	+
2.	Gum	-
3.	Carbohydrates	+
4.	Proteins	+
5.	Terpinoids	-
6.	Saponin	+
7.	Glycoside	+
8.	Fixed oil	-
9.	Steroids	-
10.	Amino acids	-

+ = present; - = absent

Preliminary phyto chemical screening of herbal extracts revealed the presence of alkaloids, glycoside, proteins and The extractive values for various solvents were tabulated in the table.

Physico chemical characteristics

Table 6.

S.No	Parameter	Lead value (percentage)
1.	Total ash	27.5%
2.	Acid insoluble ash	18.5%
3.	Alcohol soluble extract	3.4%
4.	Loss on drying	0.44gms
5.	Water soluble extract	16.6%
6.	Extraction weight	6gms
7.	Foreign matter	NMT 1%
8.	Bulk density	NMT 1%

Foam index

Table 7.

S.no	Foam
1	4mm
2	6mm
3	6.5mm
4	7mm
5	8mm
6	1.1cm
7	1.1cm
8	1cm
9	1cm
10	1.3cm

Anti diabetic activity

Table 8.

S.no	Group	Mean blood glucose level(mg/dl)	
		0 day	7 days
1	Normal control	97.5±7.04	98.33±0.206
2	Diabetic control	100±9.66	213±0.193
3	Standard (Glibenclamide)	100±8.56	94.22±4.94 ***
4	Test (alloxan+ extract)	108.33±9.1	107.33±6.43 ***

All values are expressed as mean ±SEM n=6, ***p<0.01 when compared with disease control

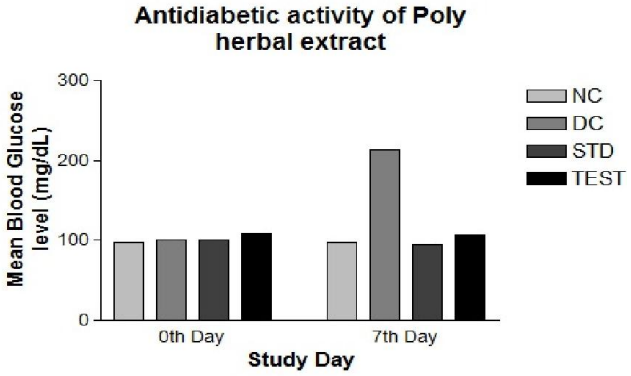

Figure 2.

DISCUSSION

Macroscopic characters include blackish brown color, taste is acrid, and odor is aromatic. Presence of powder microscopic characters like cork cells, large vessels, Starch grains, phloem fibers. Phyto-chemical screening of extract shows the presence of alkaloids, carbohydrates, saponin glycosides and Physico-chemical parameters includes water soluble extract, alcohol soluble extract, loss on drying, Total ash, acid insoluble ash, foam index, foreign matter. In view to the current status on diabetes and its epidemic the present investigation was undertaken. In proceedings to the research approach in search of new anti diabetic drug, the literature survey reveal the toxicity of chemical agent, hence the work has been directed towards the herbal and phyto medicine as choice for diabetes.

The herbal extract were prepared in solvents like ethanol and screened for ant diabetic activity. Prepared extract in the form of 0.1% suspension through oral route, dose dependent on animal body weight as a7 day's treatment and diet followed.

In focus to phyto chemical constituents of herbal for diabetes, it was observed that the glycoside show significant effect from poly herbal extract. In present study, on administration of combined herbal extract and Glibenclamide for 7days, a sustained and significant ($p<0.01$) decreased in blood glucose of the diabetic rats was observed at a dose of 200 mg/kg as compared to the diabetic control group. Glibenclamide also showed a significant ($p<0.01$) decrease in blood glucose dose of 5mg/kg.

Conclusion

- From the present study it was concluded, that the combined herbal extract exhibit anti diabetic activity, which is proven experimentally.

- Compared to synthetic drugs, combined herbal extract exhibits similar activity with less (or) may be no side effects.
- The present study revealed the synergistic activity of Liquorice and Jatamansi.

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