



Preliminary phytochemical screening and anti diabetic activity of *Zingiber officinale* rhizomes

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Abstract

Herbs are plants which are used in a number of ways including cooking, religious, rituals and medicines. In botany herbs are defined as seed producing plant with non woody stems which weather and die back to the ground after season growth. Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. It is characterized by hyperglycaemia expressed as abnormal glucose value, which is due to insulin deficiency and/or insulin resistance which results in decrease utilization of carbohydrate and excessive glycogenolysis and gluconeogenesis from amino acid by fatty acids. The literature survey exposed *Zingiber officinale* possessing anti-arthritis, anti-migraine, anti-thrombotic, anti-inflammatory, hypolipidaemic, hypocholesterolaemic, anti-nausea properties. Thus in the present study, an attempt was made to investigate the various phytochemicals present in the petroleum ether and ethanol extract of the rhizomes of *zinigiber officinalis* and also the study has been undertaken to corroborate the anti-diabetic property of ethanolic extract of *Zingiber officinale* in alloxan induced diabetic rats. The results thus obtained were comparable with the standard drug, glibenclamide.

Key-Words: Herbs, Diabetes, *Zingiber officinale*, Alloxan, Glibenclamide, Anti diabetic activity.

Introduction

Herbs are plants which are used in a number of ways including cooking, religious, rituals and medicines. In botany herbs are defined as seed producing plant with non woody stems which weather and die back to the ground after season growth. Regarding the health use herbs are source of pharmacologically active substance which affect the living organism. There are some herbal products which can both food and drug^{1, 2}. Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. It is characterized by hyperglycaemia expressed as abnormal glucose value, which is due to insulin deficiency and/or insulin resistance which results in decrease utilization of carbohydrate and excessive glycogenolysis and gluconeogenesis from amino acid by fatty acids. It has been defined by the world health organization (WHO) on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.3 mmol/L (140mg/dl) or greater than 11.1 mmol/L (200mg/dl) two hours after carbohydrate meal or two hours after oral ingestion of the equivalent of 7.5g of glucose^{3, 4}.

Ginger is said to be native of South East Asia, but is cultivated in Jamaica, Taiwan and India. More than 35% of the world's Zinger production is from India⁵. Oleo resin (5.3 – 8.6%) comprising of non-volatile pungent principles (gingerols-mainly [6]-gingerol). Non pungent substance(fats and waxes) and volatile oil. Volatile oil (1.5 - 2.2 %) containing sesquiterpene hydrocarbon viz; α -zingiberen, β -sesquiphellandrene and α -curcumene as major constituents. The literature survey exposed *Zingiber officinale* possessing anti-arthritis, anti-migraine, anti-thrombotic, anti-inflammatory, hypolipidaemic, hypocholesterolaemic, anti-nausea properties⁶. Thus in the present study, an attempt was made to investigate the various phytochemicals present in the petroleum ether and ethanol extract of the rhizomes of *zinigiber officinalis* and also the study has been undertaken to corroborate the anti-diabetic property of ethanolic extract of *Zingiber officinale* in alloxan induced diabetic rats. This attempt will provide and shed light on validating the use of *Zingiber officinale* in its traditional and general use.

Material and methods

Collection of Plant Material

The fresh rhizomes of *Zingiber officinale* was collected in the Chittoor of Andhra Pradesh, botanically

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identified by department of Pharmacognosy, SLN college of Pharmacy, Pallur, and a voucher specimen (NVK/2009/01) was stored in department of Pharmacognosy, SLN college of Pharmacy, Pallur and was made free of earthy matter and foreign matter and shade dried.

Preparation of Extract

About 500gms of completely dried material was powdered in a mechanical grinder and finely sifted using Sieve-30 and subjected to continuous hot percolation process using soxhlet apparatus, by using solvents petroleum ether (60-80°C), ethanol. The extracts thus obtained were concentrated to a thick brownish yellow semi-solid mass using Rotary vacuum evaporator. The petroleum ether, ethanol of *Zingiber officinale* Rose thus obtained extracts were denoted as PEZO and EEZO for petroleum and ethanolic extracts respectively and were used for Phytochemical and pharmacological evaluations^{7,8}.

Preliminary phytochemical screening^{7,8,9}

Both the extracts that are PEZO and EEZO were screened for various phytochemicals using standard procedures and the results were tabulated.

Test for carbohydrates

A small quantity of various extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's test:

The filtrate was treated with 2-3 drops of 1% alcoholic α -naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Iodine Test:

To the filtrate add 2ml of Iodine solution. Appearance of blue colour shows the presence of starch.

Fehling's test:

The filtrate was treated with 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates. Another portion of extracts were hydrolyzed with dilute hydrochloric acid for few hours on a water bath and hydrolysate was subjected to the following tests to detect the presence of glycosides.

Legal's test:

To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

Detection of fixed oils and fats

Filter paper test:

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicates the presence of fixed oils.

Saponification test:

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Detection of proteins and free amino acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

Millon's test:

The above-prepared extracts were treated with Millon's reagent. Red colour formed shows the presence of proteins and free amino acids.

Biuret test:

To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.

Ninhydrine test:

The extracts were treated with Ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids.

Detection of saponins

The extracts were diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Detection of tannins and phenolic compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1) 5% Ferric chloride solution -
violet colour

2) 1% solution of gelatin containing 10% NaCl -
white precipitate

3) 10% lead acetate solution -
white precipitate

Detection of phytosterols

Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then this chloroform

solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski test:

To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

Liebermann Burchard test:

The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection of alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

- 1) Mayer's reagent cream precipitate
- 2) Dragendorff's reagent orange brown precipitate
- 3) Hager's reagent yellow precipitate
- 4) Wagner's reagent reddish brown precipitate

Detection of gums and mucilages

A small quantity of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilages.

Detection of flavonoids

1. Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of Flavonoids.
2. To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of Flavonoids.
3. **Shinoda's test:** Small quantities of the extracts were dissolved in alcohol. To those pieces of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of Flavonoids.

Pharmacological screening of anti diabetic activity

Animals: Wistar albino rats weighing about 150-200gms of either sex were used for the pharmacological studies. The animals were kept under standard conditions(day/night rhythm)8.00am to 8.00pm, $22 \pm 2^\circ\text{C}$ room temperature, 50-60% relative humidity standard pellet diet(Hindustan lever, Bangalore)and water *ad libitum*. The animals were housed for one week in poly propylene cages prior to the experiments to acclimatize to laboratory conditions. It is randomly distributed into three different groups

with six animals in each group under identical conditions throughout the experiments. The experimental protocol was approved by The Institutional Animal Ethics Committee (IAEC) of SLN College of pharmacy, pallur. As the ethanolic extract was showing presence of more number of phytoconstituents, and comparatively more extractive yield, it was selected for the pharmacological screening at dose of 200mg/kg BW. Animals were divided into three groups into six rats each.

Group – I (Control) - Alloxan injected (150mg/kg of body weight) intra peritonially and kept without any treatment to study the diabetic nature, Group – II (Standard) - Alloxan injected (150mg/kg of body weight) intra peritonially and Glibenclamide (10 mg/kg of body weight), and

Group – III (Ethanolic Extract) - Alloxan injected (150mg/kg of body weight) intra peritonially and EEZO (200mg/kg of body weight).

Method

The acclimatized animals were kept fasting for 24 hrs with water *ad libitum* and injected intraperitonially at a dose of 150mg/kg of body weight of alloxan monohydrate freshly prepared in normal saline (0.9 % w/v) solution. Before starting the experiment animals were separated according to their body weight. After one hour of Alloxan administration, animals were given feed *ad libitum* and 1 ml of (100ml/dL) glucose I.P to combat encountering severe hypo glycaemia. After 72 hours of alloxan injection, the animals were tested for evidence of diabetes by estimating their blood glucose level by using glucometer. The blood glucose levels were more than 140 mg/dl was criteria for diabetes according to world health organization. To the animals the EEZO (200 mg/kg of body weight, intra peritonially) and standard drug glibenclamide (10 mg/kg body weight) were administered by dissolving in 2% Tween 80, water and normal saline respectively. The blood glucose levels were monitored at interval of initial (zero hour), 1st, 3rd, 5th and 7th day of administration of single dose for acute and prolong action was studied respectively. The body weight of the animals from all the groups was recorded and all the parameters were tabulated^{10, 11}.

Statistical analysis

The data represents mean \pm SEM results were analysed statistically by ANOVA followed by Dunnett's 't' test using computer fitted Graph pad prism software student's version 5.0. The difference was considered significant when $P < 0.05$.

Results and Conclusion

Extraction

The Ethanolic extract and petroleum ether of *Zingiber Officinalis* was extracted from the rhizomes and the percentage yields of the extracts were shown in the table – 1.

Preliminary Phytochemical screening

The preliminary phytochemical screening of the PEZO and EEO showed the presence various phytoconstituents which were showed in table No. 2

Anti diabetic activity

The data of the blood glucose level of rats treated with Alloxan (100mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed above 150 mg/dl. (Table No. 4). According to the world health organization the blood glucose level above 150 mg/dl indicates the Hyperglycaemic activity. Severe thirst, reduction in body weight were also noticed (Table-3). The administration of *Zingiber officinale* ethanolic extract at a dose of 200 mg/kg body weight showed significant anti-hyperglycaemic effect which was evident from the 1st day on wards. The decrease in blood glucose was highly significant on the 7th day. The anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in table –4 and fig. No.2. the fasting blood glucose level of diabetic animal significantly reduced from 245 mg/dl to 87 mg/dl on 7th day after the administration of the ethanolic extract of *Zingiber officinale*. The decreasing blood glucose levels are comparable to that of 10 mg/kg of Glibenclamide.

The present study was undertaken to evaluate the anti-diabetic properties of an ethanolic extract of ginger in alloxan induced diabetic rats. Alloxan damage to the islet tissue was confined to the insulin secreting pancreatic beta cells (*Dunn Js kekpatric*) through a direct effect (*Hellman Diderholn*). The alpha cells being resistant to Alloxan (*Dunnis Js Duffing*). Thus Alloxan proved to be a suitable compound for inducing experimental diabetes with typical symptoms such as mentioned above. Considering the problems associated with marketed hypoglycaemic, health care providers have been looking for a safe substitute for them. Currently several herbal medications with different mechanism of action have been found to have hypoglycaemic effect. The blood glucose levels of the Anti-diabetic Activity of Alloxan Induced Diabetic Rats were shown in table – IV. It represents that decrease in blood glucose levels. The Glibenclamide (10 mg/kg body weight) shows significant ($P^*<0.05$) effect on 3rd hr compare to the initial and more significant ($P^{**}<0.01$) effect on the 5th hr & 7th Day

compare to the initial. The ethanolic extract (200mg/kg body weight) of the *Zingiber officinale* shows significant ($P^*<0.05$), effect on 5th hr of the administration of ethanolic extract and more significant ($P^{**}<0.01$) effect on the 7th day after administration of the ethanolic extract of *Zingiber officinale*.

The blood glucose levels of the Anti-diabetic Activity of Alloxan Induced Diabetic Rats were shown in table – IV. It represents that decrease in blood glucose levels. The glibenclamide (10 mg/kg body weight) shows significant ($P^*<0.05$) effect on 3rd hr compare to the initial and more significant ($P^{**}<0.01$) effect on the 5th hr & 7th Day compare to the initial. The ethanolic extract (200mg/kg body weight) of the *Zingiber officinale* shows significant ($P^*<0.05$), effect on 5th hr of the administration of ethanolic extract and more significant ($P^{**}<0.01$) effect on the 7th day after administration of the ethanolic extract of *Zingiber officinale*.

Based on our current results, it appears that ethanolic extract of *Zingiber officinale* shows pronounced blood glucose – lowering in alloxan induced diabetic rats. Further studies are required to identity the extract mechanism of action of the anti-diabetic activity of ethanolic extract of *Zingiber officinale*.

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Table 1: Extractive values of *Zingiber officinalis*

Plant Name	Part used	Method	Percentage yield (w/w)	
			Petroleum Ether extract	Ethanol Extract
<i>Zingiber officinale</i>	Rhizome	Continuous hot percolation	1.5%	2.9%

Table 2: Preliminary phytochemical screening of Petroleum ether and ethanolic extract of *Zingiber officinale*

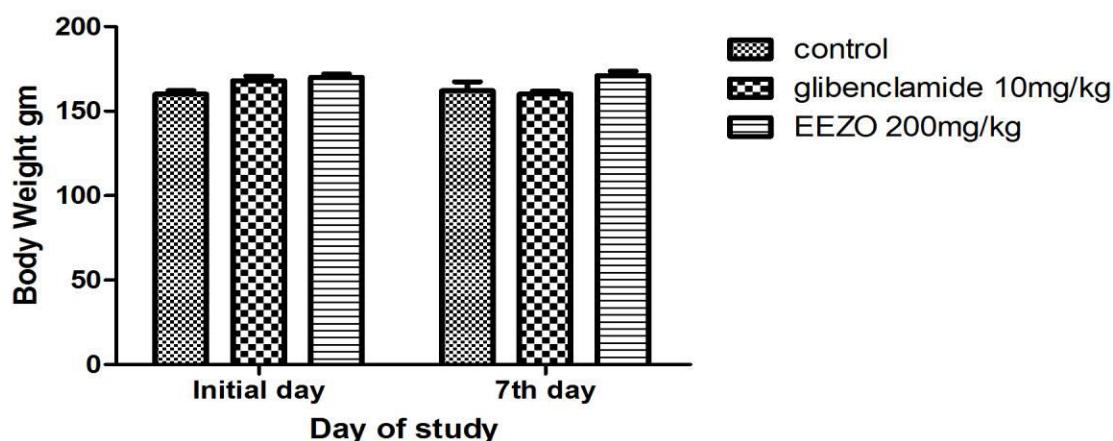
S/No.	Phytoconstituents	EEZO	PEZO
1	Alkaloids	+	+
2	Carbohydrates	+	-
3	Glycosides	+	-
4	Saponins , proteins & Amino acids	+	+
5	Phenolic compounds	+	-
6	Tannins	+	+
7	Gums and mucillages	-	+
8	Flavonoids	+	-
9	Fixed oils& fats	-	+
10	Volatile oils	-	-
11	Tri terpenoids	+	-
12	Phytosterols	+	-

“+” Present, “-” Absent

Table 3: Effect of *Zingiber officinale* on body weight in Alloxan induced diabetic rats

Treatment	Dose	Initial body weight	7 th Day Body weight
Control	Alloxan 150 mg/kg	160 \pm 2.2	162 \pm 5.4
Glibenclamide	Glibenclamide 10 mg/kg	168 \pm 2.8	160 \pm 1.84*
Ethanolic extract	<i>Zingiber officinale</i> 200 mg /kg	170+2.10	171+2.75**

N = 6 Animals in each group, Values are expressed as mean \pm SEM, P* $<$ 0.05 Significant, P** $<$ 0.01 highly Significant Vs Control

Fig. 1: Effect of *Zingiber officinale* on body weight in Alloxan induced diabetic ratsTable 4: Effect of *Zingiber officinale* on fasting blood glucose level in Alloxan induced diabetic rats

Treatment	Dose	Blood Glucose Level (mg/dL) (Mean \pm SEM)					
		Normal	After Alloxan Induction Initial	1 st day	3 rd day	5 th day	7 th Day
Control	Alloxan 150 mg/kg	90 \pm 1.09	199 \pm 0.96	206 \pm 1.13	220 \pm 2.02	240 \pm 2.4	260 \pm 2.4
Glibenclamide	Glibenclamide 150 mg/kg	69 \pm 1.5	260 \pm 1.3	121 \pm 1.90	105 \pm 1.90*	83 \pm 1.5**	81 \pm 0.69 **
Ethanoic extract	<i>Zingiber officinale</i> 200 mg /kg	63 \pm 1.39	245 \pm 1.19	217 \pm 0.88	117 \pm 0.93	101 \pm 1.3*	87 \pm 0.9 **

N = 6 Animals in each group, Values are expressed as mean \pm SEM, P* < 0.05 Significant, P** < 0.01 More Significant Vs Control, SEM = Standard Error Mean.

Fig. 2: Effect of *Zingiber officinale* on fasting blood glucose levels