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Research Article

Antioxidant and Antidiabetic Activity of Alpinia Galanga

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ABSTRACT
Alpinia galanga belongs to the family Zingiberaceae. Successive extraction was carried out using various solvents. Maximum total phenol and flavonol content were present in Ethanolic extract of Alpinia galanga. Ethanolic extract of Alpinia galanga showed the potent scavenging activity by DPPH method with the IC_{50} value of 69.5±1.375 µg/ml, by lipid per oxidation method with the IC_{50} value of 77±1.876 µg/ml, hydrogen peroxide radical scavenging activity with the IC_{50} value 55±1.59 µg/ml, ABTS radical scavenging method with the IC_{50} value 0.086±1.10 µg/ml. The glucose uptake by rat hemi diaphragm was significantly more in all groups tested compared to control. 400 mg/kg b.wt treated group showed marked increase in body weight. Fluid intake (ml/day) was also increased when compared to the diabetic control Serum glucose level (mg/dl) was found to decrease gradually from the date of administration of the extract to the end of the study when compared to the diabetic control. 400 mg/kg b.wt treated group showed potent serum glucose reducing capacity than 200 mg/kg b.wt treated group. Total protein level was found to increase in the extract treated group when compared to diabetic control. Serum triglyceride level was found to be decreased when compared with diabetic control as well as diabetes treated with Glibenclamide. Total cholesterol was also found to decrease drastically on the administration of the extract when compared with the diabetic control. The Ethanolic extract of Alpinia galanga was found to be effective in inhibiting the α-Glucosidase when compared to Acarbose

Key words: Alpinia galanga, antioxidant, anti diabetic, α-Glucosidase activity, Ethanolic extract.

INTRODUCTION

Increasing evidence in both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Oxidative stress an imbalance between the generation of reaction of oxygen species and antioxidant defense capacity of the body is closely associated with ageing and number of disease including cancer, cardio vascular disease, diabetes and diabetic complications.

Traditional medicine derived from the plant source plays an important role in the management of diabetes mellitus[1,2]. World health organization (WHO) has recommended the evaluation of traditional plant treatment for diabetes as they are effective, non- toxic, with less or no side effects and are considered to be excellent candidates for oral therapy. It was recently known that many medicinal plants possessing experimental and clinical antidiabetic activity are sued in traditional system of medicine. Now a days people prefer herbal medicine rather than synthetic ones therefore there is a need for the search for an effective and safe drug for the treatment of diabetes. It is for this reason that medicinal plants are preferentially evaluated for their therapeutic activity in regulating blood glucose level and oxidative stress induced apoptosis which is pivotal in the pathological process of diabetes mellitus

Alpinia galanga belongs to the family Zingiberaceae has been used as a traditional medicine in china for relieving stomach ache, treating cold, invigorating the circulatory systems and reduced swelling of the many chemical constituent isolated from this plant. Diarylheptanoids are among the characteristic compound which is known to possess antiplatelet, antioxidant, antiproliferative antiemetic activities. So, far this species has not been scientifically evaluated for its antidiabetic activities and hence, it was proposed to evaluate the antidiabetic and antioxidant potential of this plant scientifically. Present study was taken up to evaluate the antidiabetic activity of Alpinia galangal and to establish its therapeutic potential in the treatment of diabetes and its complication [3]

MATERIALS AND METHODS

Collection and authentication

The dried rhizomes of Alpinia galanga were purchased from Abirami botanicals Tuticorin and the same was authenticated by the Abirami botanicals. It was shade dried and coarsely powdered.

Preparation of the plant extract

The coarsely powdered rhizome were subjected to successive soxhlet extraction using solvent petroleum ether, toluene, chloroform, ethyl acetate, acetone, ethanol, water [4].

Qualitative phytochemicals screening

Different qualitative chemical tests were performed for establishing the profiles of the extracts for their nature of chemical composition and for identification of various phytoconsituents [5].

Quantitative phytochemical analysis

Phenol and flavonol are considered to be the most important phytoconsituents that are responsible for the

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Table No-1. Qualitative and quantitative Phyto chemical analysis of Alpinia galanga extracts

Tests	Alpinia galanga extracts						
	Petroleum ether	Toluene	chloroform	Ethyl acetate	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-	+	+
Carbohydrates	-	-	-	-	-	+	+
Phytosterols	+	+	+				
Fixed oil and fats	+	+	+	+	+	-	-
Saponins	-	-	-	-	-	+	+
Tannins	+	+	+	-	-	-	-
Protein and amino acids	+	+	-	+	+	+	+
Glycosides	-	-	-	-	-	+	-
Flavonoids	+	+	+	-	-	+	-
Volatile oils	-	-	-	+	+	-	-
Steroids	-	-	-	+	-	+	-
Terpinoids	+	+	+		-	+	-
Total amount of phenols(%) mg/g of Ascorbic acid	137± 0.78	243± 0.38	82± 1.46	227± 1.03	112± 2.01	254± 1.35	220± 0.87
Total amount of flavonol(%) mg/g) of rutin	119.46± 0.453	157± 1.90	59.86± 0.115	161± 0.45	49.62± 0.342	169.84±0 .145	134± 0.89

pharmacological activities. Total phenol content and total flavonol content were estimated [6].

In- vitro antioxidant evaluation

Antioxidant studies were performed by Diphenyl picryl hydrazyl radical scavenging method[7], ABTS radical scavenging method[8], Lipid per oxidation (LPO) assay [9,10,11] scavenging of hydrogen peroxide radical[12,13].

2.6. Glucose uptake by isolated rat hemi diaphragm

Glucose uptake by rat hemi- diaphragm was estimated by Walaas and Chattapadhyah with some modification. Albino rats of either sex weighed between 160-180 gm were selected. The animals were maintained on a standard pellet diet (water ad libitum) and fasted overnight. The animals were sacrificed by decapitation and diaphragm were dissected out quickly with minimal trauma and divided into two halves. The hemi diaphragm were then rinsed in cold Tyrode solution (without glucose) to remove any blood clot and were placed in small culture tubes containing 2 ml of Tyrode solution with 2 % glucose and incubated for 30 minutes at 37 ^{0} C in an atmosphere of 100 % O_2 with shaking [14,15,16].

Grouping of animals

Group 1- 2 ml fo Tyrode solution with 2 % glucose solution

Group-2- 2 ml of Tyrode solution with 2 % glucose and regular insulin

Group-3- 2 ml of Tyrode solution with 2 % glucose + Ethanolic extract of Alpinia galanga

Table No. 2 Anti oxidant activity of Alpinia galangal extracts

Plant extract	Methods			
	DPPH method	Lipid peroxide method	Hydrogen peroxide radical scavenging method	ABTS radical scavenging method
	IC $_{50}$ Values µg/ml			
Petroleum ether	112.5±0.787	135±1.5	87±1.64	2.138±0.13
Toluene	75.45±0.567	86±1.89	62±1.96	0.91±0.74
Chloroform	287.5±1.178	292±2.01	286±1.098	7.647±1.04
Ethyl acetate	78.54±0.324	97±1.89	65±1.53	0.093±1.47
Acetone	172.15±1.154	187±0.23	110±0.07	3.453±1.09
Ethanol	69.5±1.375	77±1.876	55±1.59	0.086±1.10
Water	85.75±1.567	96±1.45	85±1.63	0.124±1.43
Standard	2.75±0.09		36.16±0.90	11.25±1.43

Group-4- 2 ml of Tyrode solution with 2 % solutions +

Table no.3 Glucose uptake by isolated rate hemi diaphragm

Group	Incubation medium	Glucose uptake (mg/g/30 minutes)
Group-1	Tyrode solution with 2 % glucose (control group)	15.75±0.21
Group-2	Tyrode solution with 2 % glucose + Insulin	17.5±0.34
Group-3	Tyrode solution with 2 % glucose+ Ethanolic extract of Alpinia galangal	22.5±0.16
Group-4	Tyrode solution with 2 % glucose+ Ethanolic extract of Alpinia galanga + Insulin	26.85±0.21

Values are mean ± SEM, n=5, $p<0.01$ as compared to control and standard.

Ethanolic extract of Alpinia Galanga+ Insulin

Two diaphragms from the same animal were not used for the same set of experiment. Following incubation, the hemi diaphragm were taken out and weighed. The glucose content of the incubated medium was measured by GOD-POD method. The uptake of glucose was calculated in mg/g of moist tissue/30 minutes. Glucose uptake per gram of tissue was calculated as the differences between the initial and final glucose content in the incubated medium.

Acute toxicity study

This study was carried out according to the OECD guidelines 423. Female Wistar rats of weight (180-220 g) were taken for the study and kept for over night fasting. Next day, body weight was taken and Ethanolic extract of Alpinia galanga was administered orally at a dose of 2000 mg/kg in distill water. Then the animals were observed for mortality and morbidity at 0, $\frac{1}{2}$, 1, 2,4,6,8,12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked at 6 hr after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weight was taken [17].

α- Glycosidase inhibiting activity

A- Glucosidase inhibitors are among the available glucose lowering medications. This enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharide. The α- Glucosidase inhibitors delay but do not prevent the absorption of ingested carbohydrates but reducing the postprandial glucose and insulin peak. The α-glycosidase inhibitory activity was determined according to Matsui. The assay media contained sodium phosphate buffer (0.1 M, ph 6.8), 4-nitro phenyl α-D glucopyranoside (4-NPGP), 0.1 U α – Glucosidase (from yeast) and plant extract or control drug in the range of 0.2- 200 µg/ml of assay media, in the total volume of 1 ml. The assay was started by addition of 4- NPGP and the change in absorbance at 405nm was measured by spectrophotometer and IC 50 values were calculated [18].

In vivo antidiabetic activity

Animals

Healthy adult Wistar rats of both sexes were obtained from the central animal house from J.S.S college of Pharmacy, Ootacamund, Tamilnadu. The animals were kept in a well ventilated room and animals had exposed to 12 hrs day and night cycle with a temperature between 20 ± 5^0 C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals wee fed with water and rat feed ad libitum, supplied by this institution.

Induction of diabetes in animals

Non- insulin dependent diabetes mellitus was induced in overnight fasted rats by a single peritoneal injection of 50 mg/kg body weight of Streptozotocin[19,20,21]. Hyperglycemia was confirmed by elevated glucose level in plasma, determined at 72 hr. the rats with permanent NIDDM (250-350 mg/dl) were used for the study.

Grouping of animals

Group – 1 – Untreated group
Group-2 - Diabetic control
Group- 3 – Positive control (Glibenclamide 10 mg/kg)
Group-4 – Ethanolic extract of Alpinia galanga (200 mg/kg orally)
Group -5- Ethanolic extract of Alpinia galanga (400 mg/kg orally)

Table No.4 Effect of administration of Ethanolic extract of Alpinia galanga on body weight and fluid intake

Group	Body weight (g)		Fluid intake (ml/day)
	Before treatment	After treatment	
Untreated control	194±1.88	220.5±1.839	22.047±0.247
Diabetic control	222.66±2.33	168.5±2.513	75.288±0.223
Diabetic + Glibenclamide (10 mg/kg)	216.66±1.745	236.33±1.96	53.610±0.375
Diabetic + Ethanolic extract of Alpinia Galanga 200 mg/kg	205.83±2.182	220.83±2.182	57.86±0.373
Diabetic+ Ethanolic extract of Alpinia Galanga 400 mg/kg	216.16±2.056	239.16±2.056	49.84±0.4015

Table No.5 Effect of administration of Ethanolic extract of Alpinia galanga on Glucose Level

Groups	Serum Glucose level (mg/dl)			
	0 day	7th day	14th day	21st Day
Untreated control	92.35± 1.454	91.06± 0.53	89.76± 2.27	88.83± 1.014
Diabetic control	289.62± 3.15#	363.13± 2.35#	402.03± 8.53#	410± 2.045#
Diabetic+ Glibenclamide(10mg/kg)	285.73± 1.25#	167.32± 0.59**	121.49± 1.17**	114.83± 1.302**
Diabetic+ Ethanolic extract of Alpinia Galanga 200 mg/kg	287± 3.0 #	231.83 ± 1,458**	159.49± 5.70**	120.83± 0.345**
Diabetic + Ethanolic extract of Alpinia Galanga 400 mg/kg	284.0± 3.71#	248.15± 4.65**	136.41± 5.11**	115.33± 0.671**

All the values are expressed as mean ± SEM (n=5) # (P>0.05) not significant to standard, ** (P<0.01) significant as compared to standard.

The extract was dissolved in Millipore water and administered for 21 days at a two different dose level i.e 200 mg/kg and 400 mg/kg given orally. The blood was collected from tail vein under light Ketamine/xylazine anesthesia and was centrifuged at 3000 rpm for 10 minutes. Serum glucose, serum triglycerides and serum total protein were analyzed. The parameters such as body weight and fluid intake were also taken into consideration for this study.

Estimation of in- vivo antioxidant enzymes levels

Tissue homogenization

Pancreas was excised by minimal trauma weighed accurately and was collected in ice- cold container containing 10 % potassium chloride solution in tissue homogenizer. Homogenate was taken for further in vivo study.

Estimation antioxidant enzyme level

Homogenate was used to estimate the important antioxidant enzymes such as Catalase, SOD, TBA-RS, and Glutathione [22,23].

RESULT AND DISCUSSIONS

Phytochemical analysis

Phyto chemical analysis for the successive extraction of Alpinia galanga with different solvents showed the presence of various constituents such as alkaloids, carbohydrates, phytosterols, fixed oil and fats, saponins, tannins, protein and amino acids, glycosides, flavonoids, volatile oils, steroids terpinoids etc. Maximum total phenol and flavonol content were present in Ethanolic extract of Alpinia galanga. The results for both qualitative and quantitative Phyto chemical analysis were tabulated in table no -1.

In vitro antioxidant activity

In vitro antioxidant studies revealed that Ethanolic extract of Alpinia galanga showed the potent scavenging activity by DPPH method with the IC_{50} value of 69.5±1.375 µg/ml, by lipid per oxidation method with the IC_{50} value of 77±1.876 µg/ml, hydrogen peroxide radical scavenging activity with the IC_{50} value 55±1.59 µg/ml, ABTS radical scavenging method with the IC_{50} value 0.086±1.10 µg/ml. The results for the in vitro antioxidant activity were tabulated in the table no- 2.

Glucose uptake by isolated rat hemi diaphragm

The glucose uptake by rat hemi diaphragm was significantly more in all groups tested compared to control. The combined effects of the extract and insulin was found to be significantly higher and effect of insulin treated groups significantly higher (p<0.01). The results

Table No-6 In- vivo antidiabetic activity of Ethanolic extract of Alpinia galangal

Group	Serum Glucose level (mg/dl)	Serum Triglycerides(mg/dl)	Total protein (mg/dl)	Total cholesterol (mg/dl)
Untreated control	88.83±1.014	54.83±1.138	6.254±0.28	120.66±1.29
Diabetic control	410±2.045#	85.66±0.88#	3.09±0.12##	292.33±1.64##
Diabetic + Glibenclamide (10mg/kg)	114.83±1.302**	74.83±1.35***	6.11±0.342***	118±1.09***
Diabetic + Ethanolic extract 200mg/kg b.wt	120.83±0.345**	68.33±1.12***	5.56±0.647***	142.58 ±1.4***
Diabetic + Ethanolic extract 400 mg/kg b.wt	115.33±0.671**	64.33±0.80***	5.01±0.174***	129.74±0.18***

All the values are expressed as mean ±SEM (n=5) *** P<0.001 as compared to diabetic control , ##P<0.01 as compared to untreated control.

Table No.7 Effect of administration of Ethanolic extract of Alpinia galanga on GSH, SOD,TBARS.

Groups	GSH (µg/g) of protein	SOD (Unit/ min/ gm tissue)	CAT (µmol of H_2O_2/min/gm tissue)	TBARS nM MDA/mg of tissue
Untreated control	11.54±0.354	9.54±1.09	45.23±1.67	0.317±0.084
Diabetic control	2.17±0.22$^{\#\#}$	4.03±0.28#	28.65±1.98$^{\#}$	0.52±0.02$^{\#\#\#}$
Diabetic+ Glibenclamide (10 mg/kg)	11.01±0.77**	8.78±1.06**	42.62±0.97**	0.301±0.056***
Diabetic+ Ethanolic extract of Alpinia Galanga 200 mg/kg b.wt	5.26±0.85**	5.67±0.66**	30.01±1.78**	0.465±0.354**
Diabetic+ Ethanolic extract of Alpinia Galanga 200 mg/kg b.wt	9.63±0.04**	6.34±0.73**	36.34±0.12**	0.346±0.109***

All values are expressed as mean ± SEM (n=5), ***P<0.001, **P<0.01, *P<0.05, ### P<0.001, ##P< 0.01, #P<0.05 as compared to untreated control

for the glucose uptake by the rat hemi diaphragm were tabulated in the table no.3.

A-Glucosidase activity

Acarbose a carbohydrate inhibitor, when administered showed reduction Acarbose, an- α Glucosidase inhibitors, reduces intestinal absorption of carbohydrates and there by blunt the postprandial rise in plasma glucose in diabetic patients. However flatulence and abdominal bloating due to mal absorption limits its potential as favored medication [24]. The Ethanolic extract of Alpinia galanga was found to be effective in inhibiting the α-Glucosidase when compared to Acarbose

Acute toxicity study

In this study, the Ethanolic extract of Alpinia galanga did not show any signs and symptoms of acute toxicity and on this basis the dose of the extract were decided as 200 mg/kg body weight and 400 mg/kg body weight

In vivo antidiabetic activity

The observations for the in- vivo antidiabetic activity of Ethanolic extract of Alpinia galanga showed significant weight gain in extract treated group when compared to diabetic groups with the dose of 200 mg/kg b.wt. The extract treated group regained its body weight equivalent to that of untreated control group after 20 days administration of the extract where as 400 mg/kg b.wt treated group showed marked increase in body weight. Fluid intake (ml/day) was also increased when compared to the diabetic control. The results for the in vivo antidiabetic activity were tabulated in the table No.4

Serum glucose level (mg/dl) was found to decrease gradually from the date of administration of the extract to the end of the study when compared to the diabetic control. 400 mg/kg b.wt treated group showed potent serum glucose reducing capacity than 200 mg/kg b.wt treated group. Both doses produced almost similar effect to that of diabetes group treated with 10 mg/kg Glibenclamide. The results were tabulated in the table no.5

Serum triglyceride level was found to be decreased when compared with diabetic control as well as diabetes treated with Glibenclamide. The level of serum triglycerides was found to decrease with the increase in the dosage of the extract. Total protein level was found to increase in the extract treated group when compared to diabetic control. Total cholesterol was also found to decrease drastically on the administration of the extract when compared with the diabetic control. All these results were tabulated in the table no.6.

In vivo antioxidant enzyme level

Ethanolic extract of Alpinia galanga at the dose of 400 mg/kg b.wt restored the GSH when compared to untreated control. The GSH was found to be increased when compared to untreated control and with doses. SOD was also found to increase with the increase in dosage when compared t the diabetic control group. SOD was found to be lowered when compared to untreated control and diabetic treated with Glibenclamide. TBARS was also lowered when compared to diabetic control group. At the dose of 400 mg/kg b.wt almost maintained TBARS level equivalent to that of untreated control.

SUMMARY AND CONCLUSION

Regulation of blood glucose level in diabetes can prevent the various complications associated with the disease[25]. The long term maintenance of plasma glucose concentration under a variety of nutritional conditions and energetic demands is one of the most important and closely regulated processes in the mammalian species. Whole body homeostatic is the product of input from three primary tissue, the liver, skeletal muscle and β-cells of pancreas. The liver function as the primary source of endogenous glucose production in the body under conditions of increased peripheral demand through the breakdown of glycogen store (Glycogenolysis) and synthesis of new glucose (Gluconeogenesis) from a variety of precursor molecule. The liver can also take up the glucose carbon as glycogen (Glycogenesis). One of the important sites of glucose uptake is isolated rat hemi diaphragm.

Oxidative stress- the imbalance between the cellular production of oxidants and antioxidant defense within the cells can play an important role in the multifactorial etiology of skeletal muscle, insulin resistance[26,27]. Plasma levels of hydrogen peroxide, one marker of oxidative stress, are higher in subject with type 2 diabetic compared to non- diabetic control. More definite evidence linking oxidative stress and insulin resistance comes from cell cultures and isolated muscle incubation studies. Prolong exposure to a low- grade oxidant stress (H_2O_2) markedly decrease insulin stimulated glucose metabolism.

Medicinal plants are used in several countries to manage diabetes mellitus and are thought to be less toxic than allopathic hypoglycemic drugs plant medicine are also easily available and affordable to many people's [27]. Selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional system of medicine forms the basic for an ideal approach in the development of new drugs from the plant[17]. Ethanolic extract of Alpinia galanga exhibited significant antioxidant and antidiabetic activity in both in vitro and in vivo models. So, it can be used as alternative herbal medicine in the treatment of diabetes and diabetic complication.

Further studies with estimation of insulin and insulin receptors and higher in vivo models five more in sight into the mechanism of antidiabetic and antioxidant activity of Ethanolic extract of Alpinia galanga.

Conflict of interest statement

The authors declare that there are no conflicts of interest

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