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Research Article

Anti-Diabetic Effect of Zingiber Officinale on Sprague Dawley Rats

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ABSTRACT

Objective: To investigate the anti-diabetic effect of aqueous extract of *Zingiber officinale* against Streptozotocin induced diabetes in Sprague Dawley rats. Methods: SD rats were divide into six of five groups and allowed to acclimatize for one week. Diabetes mellitus was induced in rats by single intraperitoneal injection of STZ (60 mg/kg body weight). Group 1 served as the normal control rats which received normal saline. Group 2 served as the diabetic control which was allowed free food and water. Group 3 received 200mg/kg aqueous extract of ginger. Group 4 received 400mg/kg aqueous extract of ginger. Group 5 received 0.5mg/kg glibenclamide which was used as reference drug for hyperglycaemia. Fasting blood glucose level were measured on every 7th day for the period of 7 weeks. Results: The STZ-treated rats exhibited hyperglycaemia accompanied with weight loss, indicating their diabetic condition. Aqueous extracts of ginger at a dose of 200 and 400 mg/kg, were significantly effective in lowering blood glucose levels ($P < 0.05$). Conclusion: The present study indicates that aqueous extract of *Zingiber officinale* possesses hypoglycaemic properties.

Keywords: Sprague Dawley rats, Streptozotocin, *Zingiber officinale*, Hypoglycaemia.

INTRODUCTION

Epidemiological studies have shown that vegetables and fruits consumption can protect humans against oxidative damage by inhibiting or reducing free radicals and reactive oxygen species (ROS)[1,2]. Many plants including vegetables and fruits are bases of natural antioxidants that can combat against oxidative stress and play a key part in the chemoprevention of diseases that have their aetiology and pathophysiology in (ROS)[3,4]. These positive effects are believed to be attributed to the antioxidants like flavonoids, lycopene, carotenoids, β-carotene and phenolic[5] Diabetes mellitus (DM) is categorized by chronic hyperglycaemia with disorders of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or a metabolic disorder of multiple aetiology[6,7]. The effect of (DM) includes long term destruction, dysfunction and failure of various organs. The characteristics signs of (DM) are polyuria, thirst, blurring of vision and weight loss. The most severe forms of DM are ketoacidosis or non-ketotic hyperosmolar state which may lead to stupor, coma and death in the absence of effective treatment[8,9]. DM is characterized by persistent hyperglycaemia and other signs, as distinct from a single illness or condition[10].

The genus *Zingiber* comprises of about 85 species of aromatic herbs from tropical Australia and East Asia. *Zingiber officinalis* Roscoe, generally known as ginger belongs to family *Zingiberaceae.* It is consumed globally as a spice and flavouring agent and attributed to have numerous medicinal properties. Herbal compendium of British reported its action as carminative, anti-emetic, peripheral circulatory stimulant, spasmolytic and anti-inflammatory[8]. Several studies have also proved the antitumor effects of ginger by evaluating apoptosis rate and cell cycle progression status[5,8]. The ameliorative effects of ginger extract have also been extensively explained by researchers[11].

MATERIALS AND METHODS

Collection and authentication of plant material

In the present study, *Zingiber officinale* Rhizomes were collected during March – April 2016 from Sungai petani vegetable market Kedah, Malaysia. The plant material was taxonomically identified by a botanist from faculty of Biotechnology at AIMST University Malaysia. (Halia in Malay; Scientifically as *Z.officinale*). The voucher specimen was maintained in AIMST University, Faculty of Medicine research laboratory for future reference. *Z.officinale* rhizome were collected and aqueous extract for use in the study.

Preparation of extracts

Aqueous ginger extract was prepared from locally available Malaysian ginger roots. Ginger roots (20g) were peeled and was cut in to small pieces and homogenized in 75ml cold, sterile 0.9% NaCl solution and 25ml ice cold water to make the volume 100ml. The homogenization was carried out in a blender for 12 minutes. The homogenized mixture was filtered three times through cheese cloth. The filtrate was centrifuged at 2000rpm for 10 min and the clear supernatant fraction was separated and volume made up to 100ml with normal saline. The concentration of this ginger preparation was calculated to have 200mg/ml on the basis of the weight of the starting material[12]. The aqueous extract was stored in sample tubes at -20°C until fed to rats.

Acute toxicity

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The acute oral toxicity of aqueous extract of *Z.officinale*, in female Sprague Dawley rats was studied as per reported method[13]. These extracts were given to three groups (n = 6) of rats at concentrations 1000, 1500 and 2000 mg/kg body weight. The treated animals were kept under observation for 2 days, for mortality and general behaviour. No toxic effects were observed till the end of the study.

Experimental animals

Sprague Dawley rats of female sex weighing 160-200 g were procured from registered breeders (Universiti Sains Malaysia) and were housed in a clean polypropylene cages with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 12/12 h) at AIMST University animal house. They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory conditions for 10 days prior to experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Induction of diabetes

Streptozotocin (STZ) induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents. After overnight fasting (deprived of food for 16 hours, had been allowed free access to water), diabetes was induced in rats by single intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer, pH 4.5, at a dose of 60 mg/kg body weight. After the injection they have free access to food and water. The animals were allowed to drink 10 % glucose solution overnight to overcome the hypoglycaemic shock. The development of diabetes was confirmed after 48 h of the STZ injection. The animals having fasting blood glucose levels more than 200mg/dL were considered as diabetic rats and used for experimentation.

Experimental protocol

The rats were divided into five groups (n = 5). Except group I which served as normal non diabetic control all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III, IV received plant extracts 200mg/kg and 400mg/kg b.w orally. Group V received the reference drug glibenclamide (0.5 mg/kg b.w.) daily for 42days. (Das, et al. 2011) Fasting blood glucose (FBG) level of each rat was measured on 0, 7th, 14th, 21st 28th, 35th and 42 days by using a one touch glucometer (Accu-check).

RESULTS AND DISCUSSION

To investigate the anti-diabetic activity of aqueous extract of *Z.officinale* on STZ induced diabetic in SD rats, the rats were grouped into five groups and groups 3 and 4 were administered with the extract orally and the results were compared with group 1 and 2. The following results in (Table 1) shows the hypoglycaemic effect of aqueous extracts of *Z.officinale.* The effect of aqueous extract of *Z.officinale* in STZ treated rats is depicted in Table 1. The blood glucose levels of animals treated with aqueous extracts was 160.40± 46.91 and 191.40 ± 43.60 compared to untreated 315.40 ± 19.09. The blood glucose level of the animals treated with drug glibenclmade at the end of the experiment decreased to (106.80± 15.67). The normal control group at the end of the experiment shows a value of 98.60± 7.27).

The results in Table 2 shows the effect of aqueous extracts of *Z.officinale* on body weight of STZ induced diabetic rats. The effect of aqueous extract of *Z.officinale* on bodyweight of STZ treated rats is depicted in Table 4.5. The average body weight of animals treated with aqueous extracts was 187.00±6.25 and 181.20±5.28 compared to untreated 175.80±13.93. The body weight of the animals treated with drug glibenclamide at the end of the experiment increased to (187.60±6.16). The normal control group at the end of the experiment shows a value of 211.00±8.63.

The results in Table 3 shows the effect of aqueous extracts of *Z.officinale* on food intake of STZ induced diabetic rats. The effect of aqueous extract of *Z.officinale* on food intake of STZ treated rats is depicted in Table 4.6. The food intake of animals treated with aqueous extracts was 134.14±2.55 and 136.14±2.03 compared to untreated 146.00±2.55. The food intake of the animals treated with drug glibenclamide at the end of the experiment increased to (135.91±3.18). The normal control group at the end of the experiment shows a value of 94.31±2.15.

The results in Table 4 shows the effect of aqueous extracts of *Z.officinale* on water intake of STZ induced diabetic rats. The effect of aqueous extract of *Z.officinale* on water intake of STZ treated rats is depicted in Table 4. The water intake of animals treated with aqueous extracts was 211.4±5.5 and 207.1±5.6 compared to untreated 154.0±0.5. The water intake of the animals treated with drug glibenclmade at the end of the experiment decreased to (148.6±8.1). The normal control group at the end of the experiment shows a value of 113.1±0.6.

To assess the safety of the plant material being used we have carried out the toxicity assay with the *Z.officinale* aqueous extracts. The acute oral toxicity of aqueous extract of *Z.officinale*, in female Sprague Dawley rats was studied with concentrations 1000, 1500 and 2000 mg/kg body weight the treated animals were kept under observation for 2 days, for mortality and general behaviour. No toxic effects were observed till the end of the study.

In our study, it is observed that the blood glucose levels of the STZ treated (Diabetic control) animals continued to increase during the 7 weeks of the experiment period compared to the non STZ treated animals (Control group). Diabetic control animals showed a significant increases of blood glucose levels (P<0.001). At the end of the study, animals treated with aqueous extracts of *Z.officinale* (200 and 400 mg/ kg) showed significant decrease in blood glucose levels (Table 1).The aqueous extracts of ginger have shown to decrease the blood glucose levels from the 2nd week on words. Glibenclamide (0.5mg/kg) was used as positive control. Glibenclamide has completely reduced the STZ induced hyperglycemia in animals at the end of 7th week when

Table 1: Effect of aqueous extract of *Z.officinale* on blood glucose levels in STZ induced diabetic rats

Treatment	Blood glucose level mg/dl											
Groups	Pre-study	0th week		1st week		2nd week		3rd week		4th week		5th week
Group 1	97.80 ± 3.90	100.40 ± 4.67		101.20 ± 4.55		101.00 ± 5.45		104.00 ± 5.67		96.60 ± 7.70		98.60 ± 7.27
Group 2	95.00 ± 3.46	327.00 ± 28.78[d]		313.40 ± 47.85[c]		294.00 ± 17.00[c]		311.60 ± 20.91[d]		302.20 ± 24.67[d]		315.40 ± 19.09[e]
Group 3	96.70 ± 3.80	333.40 ± 22.29[d]		314.20 ± 33.77[c]		128.00 ± 25.08[b]		149.00 ± 38.27[a]		156.00 ± 48.19[a]		160.40 ± 46.91[a]
Group 4	97.18 ± 4.15	319.80 ± 37.06[d]		337.60 ± 34.49[c]		192.20 ± 64.25		183.00 ± 59.89		190.80 ± 51.75		191.40 ± 43.60
Group 5	99.66 ± 3.44	327.80 ± 59.10[d]		295.00 ± 75.79[c]		171.00 ± 58.84		172.40 ± 40.49		109.00 ± 15.35[a]		106.80 ± 15.67[a]

All the values are mean ± SEM (n =5). [a]P < 0.05 and [b]P < 0.01 compared to pre-study day; [c]P < 0.05, [d]P < 0.01 and [e]P < 0.001 compared to group I (One-way Anova followed by Tukey's *post-hoc* test was applied). Group 1= Normal control rats; Group 2= diabetic control rats; Group 3= 200mg/kg *Z.officinale* aqueous extract was administered orally; Group 4=400mg/kg *Z.officinale* aqueous extract was administered orally; Group 5= Glibenclamide 0.5mg/kg was administered orally.

Table 2: Effect of aqueous extract of *Z.officinale* on body weight of STZ induced diabetic rats

Treatment	Bodyweight in grams					
Groups	Pre-study	1st week	2nd week	3rd week	4th week	5th week
Group1	192.80 ± 4.06	204.20 ± 9.79	205.60 ± 8.27	213.40 ± 10.36	212.80 ± 9.86	211.00 ± 8.63
Group2	194.80 ± 2.43	185.80 ± 10.96	184.40 ± 14.05	181.60 ± 12.17	175.40 ± 14.44	175.80 ± 13.93
Group3	190.00 ± 6.34	182.20 ± 6.05	180.40 ± 6.77	183.00 ± 6.90	184.00 ± 7.57	187.00 ± 6.25
Group4	187.80 ± 9.09	173.00 ± 11.45	174.00 ± 10.61	176.80 ± 8.70	181.00 ± 8.55	181.20 ± 5.28
Group5	188.80 ± 4.36	170.20 ± 10.37	180.00 ± 6.65	181.20 ± 8.39	183.40 ± 9.23	187.60 ± 6.16

All the values are mean ± SEM (n=5). Group 1= Normal control rats; Group 2= diabetic control rats; Group 3= 200mg/kg *Z.officinale* aqueous extract was administered orally; Group 4=400mg/kg *Z.officinale* aqueous extract was administered orally; Group 5= Glibenclamide 0.5mg/kg was administered orally.

Table 3: Effect of aqueous extract of *Z.officinale* on food intake of STZ induced diabetic rats

Treatment	Food intake in grams	
Groups	1st week	5th week
Group1	107.00±9.31	94.31±2.15
Group2	102.31±5.33	146.00±2.55
Group3	110.14±1.86	134.14±2.55
Group4	107.14±7.66	136.14±2.03
Group5	110.00±3.95	135.91±3.18

All the values are mean ± SEM (n=5). Group 1= Normal control rats; Group 2= diabetic control rats; Group 3= 200mg/kg *Z.officinale* aqueous extract was administered orally; Group 4=400mg/kg *Z.officinale* aqueous extract was administered orally; Group 5= Glibenclamide 0.5mg/kg was administered orally.

compared to partial reduction after treatment with aqueous extracts of ginger (Table 1). After 7 weeks of treatment with ginger extract, the blood glucose levels of the ginger-treated diabetic rats were significantly reduced (P <0.001) in comparison with the control diabetic rats. The present results clearly show that an aqueous extract of raw ginger effectively lowers blood glucose levels in diabetic rats. Active compounds in plant extracts like flavonoids, terpenoids, alkaloids, and glycosides have antioxidant activity and claimed to possess antidiabetic effect.

In our study, we have observed loss of body weight in STZ-induced diabetic rats and the loss in body weight was controlled by treatment with aqueous extract of *Z.officinale*. Administration of aqueous extract of *Z.officinale* (200mg/kg and 400mg/kg) and diabetic control drug glibenclamide (0.5mg/kg) to diabetic rats resulted in an increase in body weight compared to diabetic rats during the experimental period. The present study findings suggested that *Z.officinale* treatment has positive effect on maintaining body weights in diabetic rats. The protective effect of plant fraction on body weight of diabetic rats may be due to its ability to reduce hyperglycemia. A gradual increase in body weights of glibenclamide treated groups was similar to that of normal control rats as compared to pre-study. STZ-induced diabetes mellitus was characterized by severe loss of body weight due to increased muscle wasting in diabetes[14]. STZ is a glucosamine-nitrosourea derived from *Streptomyces achromogenes* (gram-positive bacterium), and it is used for the treatment of pancreatic beta cell carcinoma. STZ inducing diabetes,

Table 4: Effect of aqueous extract of *Z.officinale* on water intake of STZ induced diabetic rats

Treatment	Water intake in(ml)		
Groups	Pre-study	1st week	5th week
Group 1	114.3±3.3	101.1±15.1	113.1±0.6
Group 2	113.6±2.4	165.2±9.2	154.0±0.5
Group 3	113.4±2.1	217.1±2.9	211.4±5.5
Group 4	110.3±3.6	217.1±2.8	207.1±5.6
Group 5	110.4±3.6	193.6±7.7	148.6±8.1

All the values are mean ± SEM (n=5). Group 1= Normal control rats; Group 2= diabetic control rats; Group 3= 200mg/kg *Z.officinale* aqueous extract was administered orally; Group 4=400mg/kg *Z.officinale* aqueous extract was administered orally; Group 5= Glibenclamide 0.5mg/kg was administered orally.

hyperinsulinemia, or hyperglycemia by damaging the pancreatic beta cells[15,16]. Table 3 and 4 shows STZ causes, hyperphagia and polydypsia in diabetic rats. A gradual increase in food and water intake in diabetic treated groups were observed over the period of the study. This report was in contrast with the work of (Akhani et al. 2004) who found out that *Z.officinale* treatment did not produce any change in hyperphagia and polydypsia in diabetic rats. This may be due to different method of extraction of *Z.officinale.*

CONCLUSION

It can be concluded from these studies that aqueous extract of *Z.officinale* has significant potential in the treatment of diabetes. Further studies are required to identify and characterize the active compounds in *Z.officinale*, specifically the Malaysian variety of ginger taken up for this study.

CONFLICT OF INTERESTS

Declared none

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