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Research Article

Effect of *Kaempferia galanga* Rhizome Extract on Haematological Parameters in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder that is characterized by elevated blood glucose levels, altered lipids, carbohydrates, and protein metabolism. The present investigation was undertaken to determine the hypoglycemic potential of hydroethanolic extract of *Kaempferia galanga* rhizome, and to assess hematological profile in diabetic rats. Adult male albino rats of Wistar strain were induced with diabetes by intraperitoneal injection of streptozotocin (STZ) (60 mg/kg body weight). Changes in hematological profile reported by STZ-induced diabetic rats were found to be restored to normal conditions upon administration of *K. galanga* extract in treated groups indicating hematoprotective effect of the extract. Diabetic rats exhibited an increased level of serum glucose and reduced body weight compared to normal control rats. The *K. galanga* rhizome extract-treated animals exhibited a significant increase in body weight and reduced level of blood glucose, thereby restoring to near-normal levels. The present findings indicate that *K. galanga* rhizome can be considered for treating diabetes-induced complications.

Introduction

The DM is one of the chronic metabolic disorders, exemplified by hyperglycemia. It results in defect in the secretion of endocrine hormone, insulin. As per the World Health Organization (WHO), currently, there are about 422 million diabetic people, and by 2040, this count may increase up to 600 million.[1] DM ranks third next to malignant tumors and cardiovascular diseases, creating threat to health of humans.[2] It is also directly associated with protein damage and pathological conditions, such as, cardiomyopathy, retinopathy, nephropathy, and neuropathy.[3] Type 2 diabetes is found to be associated with an array of interrelated plasma lipid abnormalities, which include an elevated level of triglyceride and low density lipoprotein (LDL) with a diminished high density lipoprotein (HDL) level.[4]

In order to treat diabetes, a plethora of medicinal herbs, and different herbal formulations are being exploited[5] in spite of enormous synthetic medications commercially available. As adverse complications were exerted by oral hypoglycemic drugs, immense consideration is given to herbal plants with hypoglycemic assets.[6] *K. galanga* L. is one of the most valuable medicinal plants belonging to the Zingiberaceae family. It is often added as an ingredient in formulation of *Ayurvedic* drugs. In addition to antihypertensive, anticancer, anti-inflammatory activity, it is also used to treat various clinical conditions, like asthma, spleenic abnormalities, bronchitis, wounds, etc.[7] Since the anti-diabetic study *in vivo* has not been investigated, the recent research was proposed to evaluate the antihyperglycemic potential of hydroethanolic rhizome extract of *K. galanga* and hematological parameters in STZ-induced diabetic animals.

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Materials and Methods

Collection of Plant Material

The *K. galanga* rhizome was used for the present study. It was collected from I-AIM (FRLHT), Bangalore. The genus and species were identified and certified by the Botanical Survey of India, Tamil Nadu Agriculture University Campus, Coimbatore with the voucher number BSI/SRC/5/23/2018/Tech/3455.

Preparation of Hydroethanolic Rhizome Extract

The rhizome portion of *K. galanga* was used for the present study. The rhizome portion was washed thoroughly in freshwater and shade dried. It was then ground to coarse powder, followed by extraction with 50% hydro ethanol in the Soxhlet apparatus. The extract obtained was concentrated and evaporated to dryness. It was then used for further treatment.

Procurement of Animals

Male albino Wistar rats (6 weeks old), weighing 170 to 200 grams, were utilized for the present investigation. The experimental animals were kept in well-ventilated polypropylene cages and were provided with 12 hours light/dark schedule throughout the period of study. A standard diet and clean drinking water was provided to the animals, and a sterile condition was maintained. The research was done following the guidelines of the ethical committee for the reason of utilization of lab animals for experiments. The ethical clearance number assigned was IAEC No. KMCRET/Ph.D/03/2016-17.

Acute Toxicity Study

Acute oral toxicity study was performed for *K. galanga* as per the Organization for Economic Cooperation and Development (OECD) guidelines 423. Healthy male albino Wistar rats were kept in fasting overnight and were fed orally with the rhizome extract at an increasing concentration of 100, 500, 1,000, and 2,000 mg/kg bw. After the oral administration of hydroethanolic extract *K. galanga,* the animals were examined for behavioral changes and mortality during the first half an hour, 1, 2, and 6 hours, and regularly for the first 24 hours with special consideration given for the first 4 hours. The observation was prolonged for 14 days regularly for toxicity determination of *K. galanga*.[8]

DM in Experimental Rats

The animals were kept overnight fasted and were induced with diabetes by a single injection of STZ intraperitoneally at a concentration of 60 mg/kg body weight dissolved in 0.1 M cold citrate buffer (pH 4.5). After 6 hours of STZ injection, rats were given a 10% dextrose solution in order to prevent the STZ-induced hypoglycemia. Progression of diabetes was ensured after 72 hours by estimating blood glucose levels. Animals showing blood glucose level of more than 250 mg/dL were regarded as diabetic and were utilized for further studies.[9]

Design of the Study

Experimental rats were segregated into six different groups, each consisting of six animals, and the study period was 28 days.

Group I: Normal healthy control rats received saline (0.9% w/v);

Group II: Diabetic rats injected intraperitoneally with STZ (60 mg/kg bw);

Group III: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (250 mg/kg bw) orally for 28 days;

Group IV: Diabetic rats treated with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw) orally for 28 days;

Group V: Diabetic rats administered with glibenclamide (600 µg/kg bw) orally for 28 days;

Group VI: Normal healthy rats administered with hydroethanolic extract of *K. galanga* rhizome (500 mg/kg bw) orally for 28 days;

Measurement of Body Weight of the Animal

To determine the effect of *K. galanga* on animal body weight, the experimental rats were weighed regularly during weeks 1 to 4, and expressed in grams.

Collection of Blood Sample from the Experimental Rats

The rats were sacrificed, and blood sample was collected from the experimental rats by cardiac puncture at the end of treatment period, after subjecting the animal to fast overnight. The serum was then separated by centrifugation and used for the study.

Analysis of Hematological Parameters

Enumeration of red blood cells was done using hemocytometer, packed cell volume (PCV) by centrifugation method, and the level of hemoglobin (Hb) was estimated by cyanmethemoglobin method.[10] Mean corpuscular hemoglobin in the red blood indices was determined by computation method.[11] Total white blood cell and the differential count was determined by Turk's fluid method and Leishman stain method.[12]

Estimation of Serum Glucose and Protein

The level of serum glucose was determined by following Trinder (1969)[13] and serum protein by Lowry's method.[14]

Statistical Analysis

The data of all the parameters were analyzed using the software GraphPad Prism 5. Analysis of variance (ANOVA), one way ANOVA followed by Dunnet's test was performed. The data were expressed as mean ± standard error of mean (SEM).

Results and Discussion

Acute Toxicity Studies

Acute toxicity studies showed no discernible behavior changes upon oral administration of hydroethanolic extract of *K. galanga* rhizome up to 2,000 mg/kg bw. Mortality at this dose during 72 hours observation period was found to be nil.

Effect of *K. galanga* on Body Weight of Experimental Rats

As shown in Table 1 and Fig. 1, a significant reduction in body weight was reported in Group II animals from 2nd to 4th week, when compared to Group I, i.e., normal control rats. These findings are in line with the reports of Kumar *et al.*,[15] where a reduced body weight in STZ-induced hyperglycemic animals was noticed. This change might be due to dysfunction of gastrointestinal motor and sensory neurons that often occur in diabetic conditions,[16] which in turn lead to reduction in intake of food linked with protein catabolism and resultant weight loss.[17]

Upon treatment with the extract of *K. galanga,* Group III and Group IV rats exhibited a significant increase in body weight, ensuring the property of *K. galanga* in managing hyperglycemia, thereby controlling muscle wasting and induce adipocyte formation. This is in accordance with Oyedemi *et al.*[18] and Chhanda *et al.*,[19] who reported that the bodyweight of diabetic rats was elevated upon administration of stem bark of *Afzelia Africana* (Smith) and *Eugenia jambolana,* and extract of *Musa paradisiaca* root to diabetic models.

Effect of *K. galanga* on Serum Glucose Level in Experimental Rats

Serum glucose level in control and treated animals is shown in Table 2 and Fig. 2.

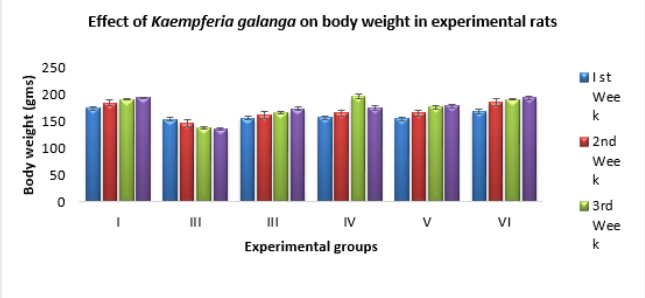

Fig. 1: Effect of *K. galanga* on body weight of experimental rats

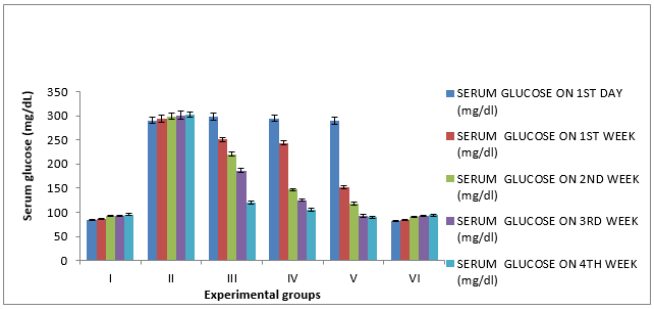

Fig. 2: Effect of *K. galanga* on serum glucose level in experimental rats

Table 1: Effect of *K. galanga* on body weight of experimental rats

Groups	*Body weight (g) Week 1*	*Body weight (g) Week 2*	*Body weight (g) Week 3*	*Body weight (g) Week 4*
I	174 ± 2.69	184 ± 2.02	190.8 ± 1.65	193.4 ± 1.77
II	153.8 ± 2.27^{a}	146.6 ± 2.59^{a}	137.6 ± 2.47^{a}	135 ± 2.03^{a}
III	155.8 ± 4.21^{b}	161.2 ± 3.43^{b}	166.6 ± 2.41^{b}	172.8 ± 2.97^{b}
IV	156.4 ± 4.77^{bc}	165 ± 3.67^{b}	169.2 ± 4.12^{b}	174.6 ± 3.71^{b}
V	154.2 ± 4.11^{b}	165.2 ± 2.81^{b}	176 ± 2.81^{b}	178.2 ± 3.43^{b}
VI	168.1 ± 3.51	185.4 ± 4.20	190.9 ± 1.52	193.2 ± 2.67

Data expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's; $^{b}p < 0.01$; $^{a}p < 0.05$ calculated by comparing the treated group with induced group; $^{bc}p < 0.05$ significant among treated group

Table 2: Effect of *K. galanga* on serum glucose level in experimental rats

Groups	*Serum glucose on 1st day (mg/dL)*	*Serum glucose on 1st week (mg/dL)*	*Serum glucose on 2nd week (mg/dL)*	*Serum glucose on 3rd week (mg/dL)*	*Serum glucose on 4th week (mg/dL)*
I	85.82 ± 0.947	87.5 ± 0.992	88.5 ± 0.442	83.17 ± 1.529	91.17 ± 2.368
II	283.17 ± 6.28	288.33 ± 7.28^{a}	298.73 ± 5.7^{a}	340.67 ± 8.48^{a}	388.02 ± 5.24^{a}
III	296.53 ± 7.63	260.33 ± 3.75^{b}	248.33 ± 4.71^{b}	186.5 ± 3.58^{b}	140.5 ± 2.65^{b}
IV	293.34 ± 5.84	253.65 ± 3.96^{b}	212.81 ± 2.04^{bc}	165.23 ± 2.98^{bc}	104.67 ± 3.29^{bc}
V	288.52 ± 7.38	210.11 ± 3.64^{b}	169.83 ± 3.57^{b}	143.32 ± 3.04^{b}	96.66 ± 2.45^{b}
VI	82.33 ± 0.02	86.37 ± 1.12	87.33 ± 1.4	83.11 ± 0.02	91.89 ± 1.8

Data expressed as the mean ± SD; Statistical significance calculated by one way ANOVA followed by Dunnett's; $^{b}p < 0.01$; $^{a}p < 0.05$ calculated by comparing treated group with induced group; $^{bc}p < 0.05$ significant among treated groups

Table 3: Effect of *K. galanga* on red blood cells, hemoglobin, mean corpuscular haemoglobin, and packed cell volume in experimental rats

Groups	*Total RBC count 1*1,000,000/mm^3*	*Hemoglobin (g/dL)*	*Mean corpuscular haemoglobin (picograms/cell)*	*Packed cell volume (L/L)*
I	6.44 ± 1.71	12.83 ± 2.403	19.92 ± 2.902	0.52 ± 0.02
II	4.5 ± 1.682^a	9.27 ± 1.595^a	21.57 ± 4.253^a	0.36 ± 0.03^a
III	4.96 ± 1.249^b	10.7 ± 1.493^b	20.63 ± 4.055^b	0.49 ± 0.02^b
IV	5.89 ± 0.69^b	11.57 ± 0.473^b	20.22 ± 1.217^b	0.5 ± 0.05^b
V	5.54 ± 0.144^b	11.2 ± 0.781^{bc}	19.64 ± 2.36^b	0.51 ± 0.02^{bc}
VI	6.09 ± 0.148	12.21 ± 0.428	19.33 ± 1.117	0.51 ± 0.01

Data expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's; $^b p < 0.01$; $^a p < 0.05$ calculated by comparing treated group with induced group; $^{bc} p < 0.05$ significant among treated groups

Table 4: Effect of *K. galanga* on total and differential leukocyte count in experimental rats

Groups	*Total WBC count 1*1,000/mm^3*	*Lymphocytes (%)*	*Monocytes (%)*	*Neutrophils (%)*	*Eosinophils (%)*
I	26.8 ± 0.57	65.67 ± 3.58	3.02 ± 1.15	24.6 ± 0.11	2 ± 1.54
II	9.47 ± 1.38^a	50 ± 1.21^a	0.23 ± 0.57^a	14.2 ± 1.07^a	0.67 ± 0.57^a
III	11.07 ± 1.32^b	57.33 ± 0.5^b	1.33 ± 0.57^b	16.33 ± 1.59^b	1 ± 0.02^b
IV	11.7 ± 1.11^b	58.67 ± 1.52^b	1.6 ± 0.57^b	19.67 ± 1.57^b	1.33 ± 0.3^b
V	12.2 ± 0.11^{bc}	62 ± 1.73^{bc}	2.65 ± 1.52^{bc}	22.57 ± 1.52^{bc}	1.67 ± 0.52^{bc}
VI	25.25 ± 1	63.84 ± 2.14	2.92 ± 0.08	24.14 ± 0.97	1.77 ± 0.1

Values are expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's; $^b p < 0.01$; $^a p < 0.05$ calculated by comparing treated group with induced group; $^{bc} p < 0.05$ significant among treated groups

Streptozotocin (STZ) induces diabetic condition by its selective cytotoxic potential on β-cells of pancreatic islets.[20] It acts at molecular level by alkylating DNA, which will result in reduction in secretion of insulin hormone by pancreatic cells. Hence, an imbalance in synthesis and utilization of glucose occurs, resulting in hyperglycemia.[21] In the present study, the administration of hydroethanolic extract of *K. galanga* rhizome (250 and 500 mg/kg bw) exhibited a significant decrease in serum glucose levels from week one to week four, when compared to that of diabetic control rats. The *K. galanga* at a dose of 500 mg/kg bw showed a better hypoglycemic effect than that of 250 mg/kg bw dose. Similar results were observed by Sayantan and Abhishek.[22]

Effect of *K. galanga* on Haematological Parameters in Experimental Rats

The deleterious effect of any compounds on the blood constituents could be revealed by assessing hematological parameters. Evaluation of hematological parameters would reflect any abnormalities in biomolecules and organ pathophysiology.[23]

Glycosylation of hemoglobin which arises due to increased blood glucose levels during diabetes, results in anaemic condition.[18] Also, generation of free radicals due to oxidation of these proteins leads to hemolysis of Red blood cell (RBC).[24]

In the present hematological investigation, as shown in Tables 3 and 4, values of RBC, white blood cell (WBC), and PCV had reduced significantly, indicating a positive correlation with previous literature that anemia is familiar pathophysiology connected with diabetes mellitus.[25] Colak *et al.*[26] also reported that hypochronic anemia is developed due to oxidative stress associated with diabetes. Alterations in the hematological profile are commonly seen in the diabetic state.[27] Administration of *K. galanga* extract upturned these abnormal situations and re-established the normal condition suggesting hematoprotective potential of the *K. galanga* rhizome extract. Similar findings were also described with *Solanum lycocarpum* on hematological parameters in diabetic rats.[28]

Conclusion

The present investigation revealed a significant reduction in body weight in diabetic animals compared to control groups. Treatment of diabetic rats with hydroethanolic extract of *K. galanga* significantly increased the body weight of the animals. Increase in blood glucose level reported by diabetic rats was reverted to near normal levels in treated groups ensuring the potential of *K. galanga* in management of obesity and hyperglycemia. Hematological studies revealed significant changes in the levels of RBC, WBC, PCV, and Hb in treated groups compared to diabetic groups, indicating hematoprotective effect of hydroethanolic extract of *K. galanga* rhizome.

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