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Protective Effect of *Alpinia galanga* in STZ Induced Diabetic Nephropathy

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Abstract: The activity of the alcoholic extract of the rhizomes of *Alpinia galanga* was studied for the treatment of diabetes-induced nephropathy in rats. Wistar rats received a single intraperitoneal streptozotocin injection (60 mg kg^{-1} b.wt.) to induce diabetes. Rats were considered diabetic if blood glucose concentration increased up to 200 or more mg dL^{-1}. The rats were orally administered alcoholic extract of *Alpinia galanga* (50, 100 and 200 mg kg^{-1}), once daily for 40 days. Body weight, blood glucose, urinary albumin, glycosylated haemoglobin, Blood Urea Nitrogen (BUN), creatinine, lipids profile, Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH) and Catalase (CAT) were then evaluated. After 40 days of treatment, *Alpinia galanga* significantly ($p<0.05$) decreased glycaemia, Blood Urea Nitrogen (BUN), urinar albumin and increased body weight in diabetes-nephropathic rats. The extract (200 mg kg^{-1}) decreased MDA significantly ($p<0.01$); GSH ($p<0.05$), increased SOD ($p<0.05$) and CAT ($p<0.05$) in the rats, compared with nephropathic control. The extract (100 and 200 mg kg^{-1}, respectively) lowered ($p<0.05$) total cholesterolemia, blood triglycerides ($p<0.05$), blood LDL cholesterol ($p<0.05$), but increased blood HDL cholesterol ($p<0.01$). Overall, atherogenic index was decreased significantly ($p<0.05$). In the present study, the rhizomes of *Alpinia galanga* demonstrated significant nephro-protective activities in the tested models. The alcoholic extract of the rhizomes of *Alpinia galanga* holds promise for the development of a standardized phytomedicine for diabetes mellitus and kidney disease treatment.

Key words: Diabetic nephropathy, *Alpinia galanga*, rhizome, oxidative stress, STZ

INTRODUCTION

Diabetic nephropathy the most common cause of end-stage renal failure is one of the most serious complications of diabetes. At present, 15-25% of type I diabetes patients (Hovind *et al*., 2003) and 30-40% of patients with type II diabetes are diagnosed with diabetic kidney disease (Yokoyama *et al*., 2000). In diabetic nephropathy there is specific renal morphological and functional alterations. In high glucose induced renal injury ROS play an important role (Ha and Lee, 2000; Iglesias-de la Cruz *et al*., 2001). Flavonoids are abundant plant phenolic compounds. There are about 6000 plants which are reported to possess hypoglycemic, antioxidant and antidiabetic activities (Sharma *et al*., 2008).

Alpinia galanga wild (Family-Zingiberaceae) syn. Languas galangal (Rao *et al*., 2010) known as Greater galangal in English, Kulanjan in Hindi (Jaju *et al*., 2009) and Barakulanjan in Hindi (Indrayan *et al*., 2009), have been widely cultivated in Sri Lanka, India, Malaysia, Indonesia, Egypt, Saudi Arabia (Arambewela *et al*., 2007) and is found abundantly in Thailand (Trakranrungsie *et al*., 2008). Ayurveda and Siddha medicine system employ *Alpinia galanga* to care for various kinds of disease together with diabetes mellitus (Jaju *et al*., 2009). *Alpinia galanga* is rich in Phenolic compounds such as flavonoids and phenolic acids (Mayachiew and Devahastin, 2008). Rhizomes are lowest in fat but richest in carbohydrate (Indrayan *et al*., 2009). The dominant components isolated from rhizome were galangoisoflavonoid (Jaju *et al*., 2009), β-sitosterol diglucosyl caprate (Jaju *et al*., 2009, 2010), methyleugenol, p-coumaryl diacetate, 1'-acetoxyeugenol acetate, trans-p-acetoxycinnamyl alcohol, trans-3,4-dimethoxycinnamyl alcohol, p-hydroxybenzaldehyde, phydroxycinnamaldehyde, trans-p-coumaryl alcohol, galangin, trans-p-coumaric acid, acetoxychavicol acetate (ACA), hydroxychavicol acetate (HCA) (Min *et al*., 2009) and galanganol B (Kaur *et al*., 2010) .

Based on traditional use and chemical constituent preset therein we decided to evaluate the role of alcoholic extract of *Alpinia galanga* in diabetic nephropathy.

MATERIALS AND METHODS

Identification of plant material: Dried rhizomes of greater galangal (*Alpinia galanga*) were purchased from a local market of Gurgaon (Haryana). Rhizomes were botanically authentification was from NISCAIR, New Delhi (References ID: NISCAIR/RHMD/Consult/-2011-12/1709/09).

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Preparation of extract: The coarsely powered plant material was extracted with petroleum ether (60-80°C) in soxhlet apparatus until it become colorless. The defatted plant material then extracted with alcohol until it become colorless. The alcoholic extract was dried and kept at 4°C for future evaluation.

Phytochemical screening: The ethanolic extract of *Alpinia galanga* was subjected to various phytochemical screening (Khandelwal, 2008; Kokate *et al*., 2006).

Experimental animal: Male Sprague dowley rat weighing between 200-250 g were purchased from disease free animal house of NIPER, Mohali. They were kept in the Animal House of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra and housed at standard conditions of temperature (22±1°) and 12/12 h light/dark cycle. They were fed with standard pellet diet (Ashirwad industries, Ropar, Punjab) and had free access to water. Fasting animals were used during the experiment. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies: Acute toxicity study was performed according to OECD, 2001-423 guideline (acute toxic class method). Swiss male mice (20-25 g) were selected by random sampling technique.

Induction of diabetes: Type 1 diabetes was induced by i.p. injection of STZ (dissolve in citrate buffer 0.1 mol L^{-1}, pH 4.2) in a dose of 60 mg kg^{-1} body weight for three successive days. Rats were considered diabetic if blood glucose concentration increased up to 200 or more mg dL^{-1} (Abo-Salem *et al*., 2009).

Experimental protocol: Fifty rats were used in this study and classified into 5 groups (10 animals/group) as follows:

- **Group I:** Received vehicle (normal saline) and served as control group
- **Group II:** Injected with STZ, i.p. in a dose of 60 mg kg^{-1} body weight for 3 successive days and served as diabetic group
- **Group III-V:** Received alcoholic extract of *Alpinia galanga* at different doses level of 50, 100 and 200 mg kg^{-1} b.wt., respectively via oral gavage daily for 40 days, starting after 3 days of STZ injection

Body weight: The changes in body weight were calculated at the time of STZ treatment and at 40th day of experiment using an automatic electronic balance (A and D Co.Ltd. Japan).

Changes in kidney weight: The weight of the left kidney at sacrifice was measured in grams and was regarded as absolute weight.

Biochemical analyses: Blood samples for the measurement of blood chemistry were drawn into pre chilled EDTA containing tubes and immediately placed on ice. Blood samples were centrifuged at 2300 g for separation of serums and stored at -70°C until assay. Serums were used for the estimation of glucose, 24-h urea protein, Blood Urea Nitrogen (BUN) and creatinine as described previously (Tikoo *et al*., 2007a, b). The levels of serum Triacylglycerol (TG), Total Cholesterol (TC), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were measured as previously described (Yahagi *et al*., 2002). Glycosylated Hb (Hba1c) was estimated by the methods (Nayak and Pattabiraman, 1981).

Antioxidant measurement: Rats were scarified by cervical dislocation. Kidneys were removed, washed with physiological saline, removed fatty tissue and weighed. They were homogenized in ice cold 20 mM Tris-HCl buffer (pH 7.4) and the homogenates were then centrifuged at 10,000 g for 10 min at 4°C (Montilla *et al*., 2005). The supernatant was collected and used for assessment of GSH (Beutler *et al*., 1963), SOD (Kono, 1978), CAT (Luck, 1971) and MDA (Wills, 1965).

Histopathological examination: Kidney removed from the all animal were cleaned and fixed in 10% buffered formalin solution. Then they were embedded in paraffin and stained with hematoxylin-eosin for histopathological studies. All sections were evaluated for the degree of tubular and glomerular injury and necrosis (Makni *et al*., 2010).

Statistical analysis: The data were analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests to determine level of significance using GraphPad InStat version 3.05 for Windows, (GraphPad Software, San Diego California USA). A value of $p<0.01$ was considered significant results are expressed as Mean±SD for ten rats in each group.

RESULTS

Phytochemical screening: The ethanolic extract of *Alpinia galanga* showed the presence of alkaloids, glycosides, flavanoids and tannins.

Table 1: Effect of *Alpinia galanga* extract on body weight, sugar level, glycosylated haemoglobin and kidney weight of diabetic rats

	Body weight (g)			Sugar level (mg dL^{-1})		
Groups	1st day	40th day	%Increase or decrease in body	1st day	40th day	HbA1C (%)
1	306±0.66	392±0.32^{a1,b1}	21.93±0.42^{a1,b1}	94±0.75^{b1}	103.4±0.07^{a1,b1}	4.86±0.14^{b1}
2	272±0.14	240±0.64^{a1}	13.33±0.78*	428±0.89^{a1}	524.2±0.23	6.34±0.11^{a1}
3	280±0.15	323±0.28	13.33±0.73	332.8±0.15^{a}	215±0.39^{b1}	5.9±0.09^{a1}
4	252±0.17^{a}	302±0.62^{a}	16.55±0.32	259.8±0.91	201±0.14^{b1}	5.56±0.09^{a1,b1}
5	258±0.74^{a}	330±0.95^{b}	21.88±0.94^{a}	456.8±0.81^{a1}	203.6±0.45^{b1}	5.26±0.1^{b1}

The data were expressed by Means±SEM of 10 rats /group, *represent% decrease in body weight, ^{a}: Significantly different from control group, ^{b}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Dunnett's t-test at $p<0.05$, ^{a1}: Significantly different from control group, ^{b1}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Dunnett's t-test at $p<0.01$

Acute toxicity studies: Result of acute toxicity studies showed that alcoholic extract of *Alpinia galanga* rhizome was safe only up to a dose of 2000 mg kg^{-1} b.wt. Three doses i.e., 50, 100 and 200 mg kg^{-1} b.wt. for oral administration of *Alpinia galanga* were selected for further Pharmacological studies.

Body weight: There was marked reduction in the body weight of diabetic animal's group (Group II) as compared to that of the control and extract treated groups (Table 1). The mean body weight of control animals (Group I) was 306.00±0.66 g on 1st day and increased to 392±0.32 g at the end of the study. The% increase in body weight of control animals was 21.93±0.42%. There was a significant ($p<0.01$) and sustained decrease in body weight of group 2 animals i.e., Mean±SEM 272±0.14 g on the 1st day and decreased to 240±0.64 g on 40th day, respectively. Oral administration of *Alpinia galanga* extract at a dose of 200 mg kg^{-1} b.wt. (group IV) caused significant increment (21.88%) in body weight of STZ induced diabetic rats. The initial body weight of diabetic rats treated with 200 mg kg^{-1} b.wt. dose was Mean±SEM 258±0.74 g and increased to 330±0.95 g at the end of the study.

Changes in urinary albumin levels: Urinary albumin excretion was significantly increased in the diabetic group (group II) as compared to the control (group I) (Table 3). *Alpinia galanga* in dose of 200 mg kg^{-1} b.wt. showed marked reduction in urinary albumin level was 0.09±0.07 g dL^{-1}.

Changes in blood glucose levels: The plasma glucose concentration in Diabetic control group increased significantly in comparison to the control and *Alpinia galanga* treated group. The mean blood glucose level in control animals was 94±0.75 mg dL^{-1} on the 1st day and 103.4±0.07 mg dL^{-1} on 40th day. There was a significant ($p<0.01$) and sustained increase in blood glucose level in Diabetic control group. In Diabetic control group, mean±SEM blood glucose level on the 1st day was 428±0.89 mg dL^{-1} and increased to 524.2±0.23 mg dL^{-1} on 40th day. Oral administration of *Alpinia galanga* extract to diabetic rats also caused significant decrease in plasma glucose level in comparison to the result obtained from diabetic group. *Alpinia galanga* at a dose of 200 mg kg^{-1} b.wt. (Group V) caused marked decreased in sugar level of diabetic rats. (Table 1).

Changes in glycosylated haemoglobin (HbA1C) levels: Glycosylated haemoglobin (HbA1C) level increased significantly in diabetic control group with Mean±SEM was 6.34±0.11% as compare to control animals with Mean±SEM was 4.86±0.14. Oral administration of *Alpinia galanga* extract at a dose of 200 mg kg^{-1} b.wt. produced a marked decreased in HbA1C i.e., 5.26±0.18%. HbA1c levels were significantly higher in diabetic animal's group when compared with control animals (Group I). *Alpinia galanga* treatment significantly reduced HbA1c in diabetic rats relative to untreated diabetic animals (Group II) ($p<0.01$). Moreover, treatment with extract led to significant reduction in kidney enlargement in a dose dependent manner, i.e., 3.33, 18.52 and 26.66%, respectively (Table 1).

Change in BUN and creatinine levels: Serum Blood Urea Nitrogen (BUN) and creatinine were significantly elevated in the diabetic group as compared to that of control and *Alpinia galanga* treated rats. The mean plasma BUN level in control animals was 39.19±0.68 mg dL^{-1}. There was a significant ($p<0.05$) and sustained increase in BUN level of diabetic group rats (group II) i.e., Mean±SEM was 406.12±0.2 mg dL^{-1}. The administration of *Alpinia galanga* extract to diabetic rats resulted in significant decrease of plasma BUN level in comparison to that obtained from diabetic group rats (group II). BUN level of animals treated with *Alpinia galanga* at dose of 200 mg kg^{-1} b.wt. (group V) was 74.68±0.07 mg dL^{-1}. The mean plasma creatinine level in control animals was 0.97±0.12 mg dL^{-1}. There was a significant ($p<0.01$) and sustained increased in creatinine level of diabetic group with Mean±SEM was 11.74±0.08. Creatinine level of group V decreased significantly to 0.98±0.04 mg dL^{-1} (Table 2).

Table 2: Change in biochemical parameters after 40 days of *Alpinia galanga* administration in diabetic rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
BUN(mg dL^{-1})	39.19±0.68^{b}	406.12±0.20^{a}	206.82±0.69	100.92±0.14	74.68±0.07^{b}
Creatinine (mg dL^{-1})	0.97±0.12^{b1}	11.74±0.08^{a1}	6.57±0.49	4.10±0.74^{b}	0.98±0.04^{b1}
Urinary albumin(g dL^{-1})	0.12±0.05^{b}	0.39±0.02^{a}	0.23±0.04	0.21±0.09	0.09±0.07^{b1}
Total cholesterol (mg dL^{-1})	49.21±0.10^{b}	86.57±0.82^{a}	68.30±0.52	59.59±0.89	50.56±0.83^{b}
HDL-C(mg dL^{-1})	45.16±0.62^{b1}	16.42±0.68^{a1,b1}	23.72±0.52^{b1}	24.49±0.26^{a1,b1}	47.08±0.48^{a1,b1}
TG (mg dL^{-1})	45.74±0.98^{b}	100.27±0.98	78.48±0.98	69.02±0.45	51.79±0.05^{b}
MDA (umoles/mg protein)	2.64±0.64^{b1}	54.0±0.960^{a1}	45.3±0.700^{a1}	5.3±0.780^{b1}	3.2±0.540^{b1}
LDL-C(mg dL^{-1})	3.83±0.07^{b}	50.09±0.56^{a}	28.88±0.46	21.29±0.51	5.68±0.18^{b}
Atherogenic index(AI)	0.089±0.18^{b}	4.27±0.98^{a}	1.88±0.56	1.43±0.45	0.074±0.20^{b}

The data were expressed by Means±SEM of 10 rats /group, ^{a}: Significantly different from control group, ^{b}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Dunnett's t-test at $p<0.05$, ^{a1}: Significantly different from control group, ^{b1}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Duunett's t-test at $p<0.01$

Table 3: Effect of *Alpinia galanga* extract on antioxidant parameters in renal tissue

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
MDA (umoles/mg protein)	0.04±0.01^{b1}	16.17±0.65^{a1}	10.04±0.21	6.10±0.09	0.12±0.06^{b1}
GSH (μ moles mg^{-1} protein)	1.64±0.23^{b1}	0.26±0.13^{a1}	0.53±0.13^{a1}	0.69±0.5^{a1}	0.87±0.12^{b}
SOD(units mg^{-1} protein)	45.09±0.76^{b1}	14.92±0.28^{a1}	18.87±0.03^{a1}	19.28±0.55^{a}	39.65±0.17^{b}
CAT (μ moles of H_2O_2/min/mg protein)	2.96±0.84^{b}	0.94±0.24^{a1}	1.10±0.05	1.14±0.59^{a}	1.34±0.14^{a}

The data were expressed by Means±SEM of 10 rats/group, ^{a}: Significantly different from control group, ^{b}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Dunnett's t-test at $p<0.05$, ^{a1}: Significantly different from control group, ^{b1}: Significantly different from STZ-induced diabetic group, using one-way ANOVA with Duunett's t-test at $p<0.01$

Changes in lipid profile: Serum cholesterol, LDL-C, triglycerides (TG), Atherogenic index (AI) and MDA level of the diabetic group were significantly higher while HDL-C was lower as compared to the control normal rats. However, administration of *Alpinia galanga* significantly improved these parameters in a dose-dependent manner as shown in (Table 2).

Changes in lipid peroxidation and antioxidant defence systems in the renal tissue: MDA concentration in kidney of diabetic group significantly increased in comparison to the control animals (Table 3). The mean MDA level in control animals was 0.04±0.01 nmoles mg^{-1} protein. There was a significant ($p<0.05$) and sustained increased in MDA level of diabetic group rats (group II) was 16.17±0.65 nmoles mg^{-1} protein. *Alpinia galanga* extract in different doses produced marked reduction in the elevated MDA level in kidney of diabetic group in a dose dependent manner i.e., 10.04±0.21 nmoles mg^{-1} protein (50 mg kg^{-1} b.wt.), 6.10±0.09 nmoles mg^{-1} protein (100 mg kg^{-1} b.wt.) and 0.12±0.06 nmoles mg^{-1} protein (200 mg kg^{-1} b.wt.), respectively.

GSH levels and antioxidant enzyme activities (CAT and SOD) in kidney of control and tested groups were shown in Table 3. GSH concentration in kidney of diabetic group significantly increased in comparison to the control group. The mean GSH level in control animals was 1.64±0.23 μ moles mg^{-1} protein. There was a significant ($p<0.05$) and sustained decreased in GSH level of diabetic group rats with Mean±SEM was 0.26±0.13 μ moles mg^{-1} protein. *Alpinia galanga* extract in doses of 50, 100 and 200 mg kg^{-1} body weight produced marked increased in GSH level in a dose dependent manner i.e., 0.53±0.13 μ moles mg^{-1} protein, 0.69±0.5 μ moles mg^{-1} protein and 0.87±0.12 μ moles mg^{-1} protein, respectively.

SOD concentration in kidney of group 2 (diabetic group animals) significantly increased in comparison to control group. The mean SOD level in control animals was 45.09±0.76 units mg^{-1} protein. There was a significant ($p<0.05$) and sustained decrease in SOD level of diabetic group rats. In STZ induced diabetic rats, Mean±SEM SOD level was 14.92±0.28 units mg^{-1} protein. *Alpinia galanga* extract in doses of 50, 100 and 200 mg kg^{-1} body weight produced marked increased in SOD level in a dose dependent manner i.e. 18.87±0.03 units mg^{-1} protein, 19.28±0.55 units mg^{-1} protein and 39.65±0.17 units mg^{-1} protein, respectively (Table 3).

CAT concentration in kidney of diabetic group animals significantly increased in comparison the control. The mean CAT level in group 1 was 2.96±0.84 μ moles of H_2O_2/min/mg protein. There was a significant ($p<0.05$) and sustained decrease in CAT level of diabetic group rats with Mean±SEM was 0.94±0.24 μ moles of H_2O_2/min/mg protein. *Alpinia galanga* extract in doses of 50, 100 and 200 mg kg^{-1} b.wt. produced marked elevation in MDA level in kidney of diabetic group in a dose dependent manner i.e., 1.10±0.05 μ moles of H_2O_2/min/mg protein, 1.14±0.59 μ moles of H_2O_2/min/mg protein and 1.34±0.14 μ moles of H_2O_2/min/mg protein, respectively (Table 3).

Histological changes in renal tissue: Histological examination of control and extract treated groups showed normal cells structure of kidney. STZ treatment elicited significant morphological changes in kidney of diabetic

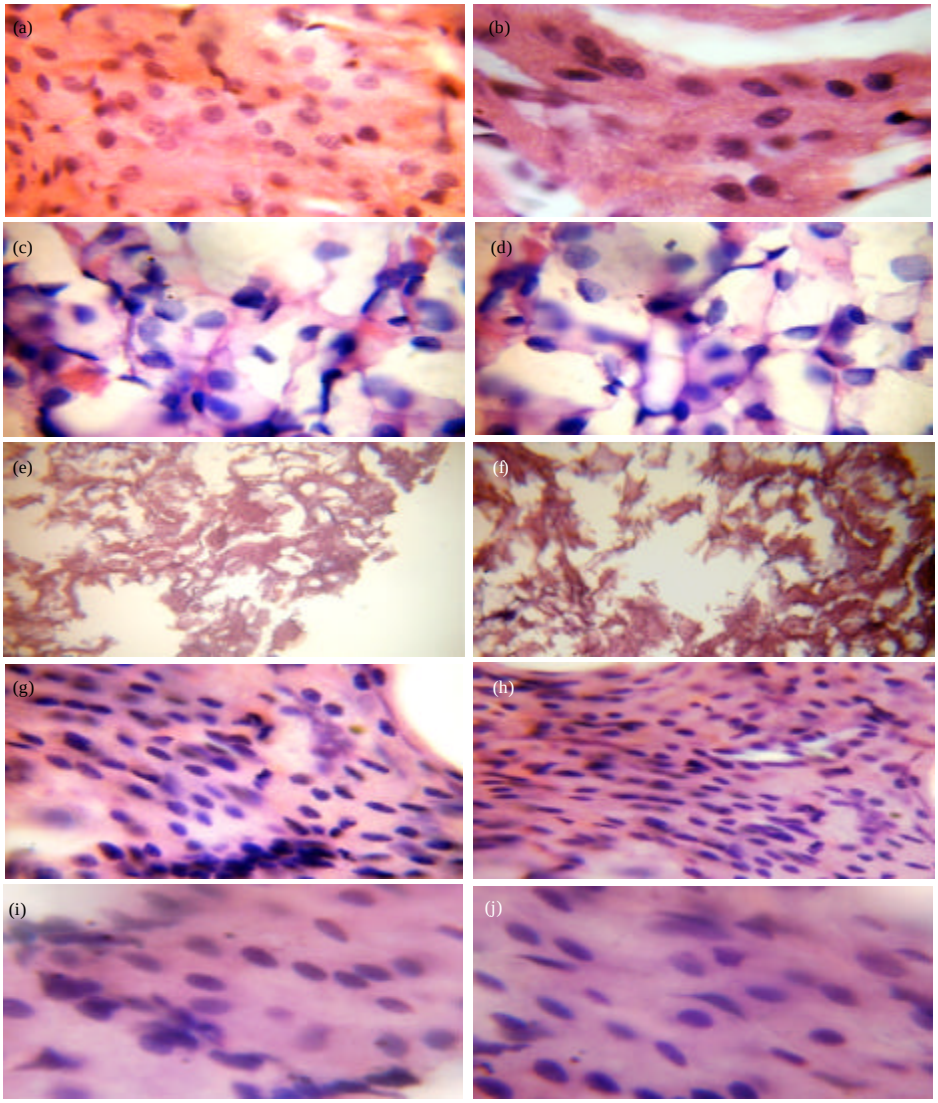

Fig. 1(a-j): Photomicrograph showing kidney cells of control animal stained with haematoxylin and eosin healthy cells having spherical shape and granulated cytoplasm. X1000, (c-d): Photomicrograph showing diabetic group animal kidney cells stained with haematoxylin and eosin showing swelling, ruptured cell wall and distorted cytoplasm with increased vacuoles. X1000, (e-f): Photomicrograph showing kidney cells treated with 50 mg kg^{-1} b.wt. of *Alpinia galanga* stained with haematoxylin and eosin showing less swelling, regeneration of cell wall with decrease in vacuoles size. X1000, (g-h): Photomicrograph showing kidney cells treated with 100 mg kg^{-1} body weight of *Alpinia galanga* stained with haematoxylin and eosin showing intermediate preventive effect showing circular and oval shaped cells, less granular cytoplasm, regeneration of cell wall with less vacuoles size.15×40X, (i-j): Photomicrograph showing kidney cells treated with 200 mg kg^{-1} b.wt. of *Alpinia galanga* stained with haematoxylin and eosin showing maximum preventive effect showing oval shaped cells, granular cytoplasm, regenerated cell wall and intermediate vacuoles. 15×40X

group rats with severe renal injury. Kidneys of control animals showed spherical shape cells with granulated cytoplasm (Fig. 1a-b) but in kidney of diabetic group animals showed swelling, ruptured cell wall and distorted cytoplasm with increased vacuolization (Fig. 1c-d). These changes were significantly inhibited in *Alpinia galanga*

treated groups (100 or 200 mg kg^{-1} b.wt.). Treatment of diabetic animals with *Alpinia galanga* (100 or 200 mg kg^{-1} b.wt.) significantly caused preventive effect on cells i.e., cell wall regeneration with less vacuoles size and granular cytoplasm (Fig.1g-h, i-j).

DISCUSSION

Streptozotocin-induced diabetes mellitus causes the destruction of beta cells of islets of Langerhans (Kavalali *et al*., 2003) which leads to the reduction in insulin release. An insufficient release of insulin causes high blood glucose level namely hyperglycaemia, which results in development of diabetic complications (Donnini *et al*., 1996) because of oxidative damage cause by the generation of Reactive Oxygen Species (ROS) (Mohamed *et al*., 1999). STZ-induced diabetic animals may exhibit many other diabetic complications such as myocardial, cardiovascular, gastrointestinal, nervous and urinary bladder dysfunctions (Ozturk *et al*., 1996).

Our data revealed that there was marked reduction in the total body weight as well as elevation in the kidney weight of the diabetic group animals as compared to that of the control normal group. *Alpinia galanga* treatment showed a significant amelioration in both body and kidney weights in a dose dependent manner.

In diabetic nephropathy, significantly higher plasma concentration of Very LOW Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL) and triglyceride level but lower level of high density lipoprotein cholesterol (HDL) was found (Hovind *et al*., 2001). Achuthan founded that ethanolic extract of *Alpinia galanga* caused a reduction in serum and tissue level of total cholesterol, triglycerides and phospholipids and significantly increase the serum level of high density lipoprotein in arteriosclerosis (Bonnet and Cooper, 2000). The results of this study showed that serum level of glucose, total cholesterol, LDL-C, TG and MDA were significantly elevated in diabetic rats as compared to that of the normal control rats. Administration of *Alpinia galanga* significantly improved these parameters in a dose-dependent manner. Moreover, the highest dose of extract (200 mg kg^{-1}) was able to reduce the blood glucose to normal level. Moreover, the serum level of HDL-C, which was significantly decreased in diabetic rats, was also improved by *Alpinia galanga* in a dose-dependent manner. Atherogenic index (AI) is the only definite way to assess potential atherogenicity of LDL particles i.e. increased AI factor causes dense LDL size, more risk of atherosclerosis (Achuthan and Padikkala, 1997). In this study, diabetic group animals showed higher value of AI. Treatment with 200 mg kg^{-1} dose of *Alpinia galanga* caused marked reduction in AI value as compared to both diabetic and control group. These findings may indicate that *Alpinia galanga* can improved the lipid profile of diabetic rats.

The presented results suggested that *Alpinia galanga* extract exhibit significant hypoglycaemic, hypolipidemic and nephroprotective effects in STZ induced diabetic rats. Fasting blood glucose level in diabetic rats was important basal parameters for monitoring diabetes. This finding suggests that *Alpinia galanga* may improved the disturbed metabolism associated with diabetes. Alcoholic extract of *Alpinia galanga* in different doses showed significant reduction in blood glucose and glycosylated haemoglobin (Hb1Ac) in diabetic rats. Glycosylated haemoglobin (HbA1c) which is an index of long term glycaemic control in diabetic patient (Viswanathan *et al*., 2009). It has also been shown that the lower the HbA1c, the lower risk for nephropathy, i.e., no threshold for beneficial effects exists (Beckman *et al*., 2002). A greater decline in GFR has also been associated with higher HbA1c levels (Beckman *et al*., 2002). Regression and remission of microalbuminuria defined as 50% reduction in the urine albumin excretion rate and later presence of normoalbuminuria have also been associated with lower HbA1c levels (Araki *et al*., 2005). Moreover, results showed that serum BUN and creatinine level were significantly elevated in diabetic group animals as compared to that of the control normal rats. BUN declined significantly in the *Alpinia galanga* treated groups, however, creatinine level decreased only with the highest dose of *Alpinia galanga*. BUN reached to the normal levels in the group treated with the highest dose of *Alpinia galanga* (200 mg kg^{-1} b.wt.). Moreover, urinary albumin excretion (a marker of early diabetic nephropathy) was improved after treatment with *Alpinia galanga* in a dose-dependent manner.

Treatment of rats with *Alpinia galanga* extract at different doses significantly reduced the kidney MDA level and conversely increased GSH, SOD and CAT activities as compared to that of diabetic group. *Alpinia galanga* normalized MDA activity at all tested doses. However GSH, SOD and CAT normalized only at doses of 200 mg kg^{-1} b.wt. These data may indicate that the protective effect of AG extract against renal damage in diabetic rats is dose-dependent. These results indicated that *Alpinia galanga* prevent renal damage in diabetic nephropathy (shown in histopathological results).

CONCLUSION

Phenolic compounds can act as free radical scavengers, removing Reactive Oxygen Species (ROS) which can be initiators of oxidative stress and chronic inflammation (Dobiasova and Frohlich, 2001). Novel bioactive flavonoid compounds which are currently in trial for combating many oxidative stress-related diseases such as diabetes, cancer, arthritis, alzheimer's and parkinson's diseases (Schinella *et al*., 2002). A variety of phenolic and flavanoids have been purified from the rhizomes of *Alpinia galanga* such as eugenol, chavicol analogues, cinnamic, coumaric acid derivatives which show ability to inhibit Nitric Oxide (NO) or Reactive Oxygen Species (ROS) production (Min *et al*., 2009).

Hence it can be concluded that the alcoholic extract from the rhizomes of *Alpinia galanga* possessed antioxidant activity, making it a plant to be used in treatment of diabetic nephropathy disorders.

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