Research article

Dose-dependent effect of galangin on fructose-mediated insulin resistance and oxidative events in rat kidney

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Galangin is an antioxidant flavonol present in high concentrations in the rhizome of *Alpinia galanga*. We investigated the effect of galangin on whole-body insulin resistance and kidney oxidative stress in a fructose-induced rat model of metabolic syndrome. Male albino Wistar rats were divided into 6 groups containing six animals each. Groups I and VI received a starch-based control diet, while groups II, III, IV and V were fed a high fructose diet (60 g/100 g). Groups III, IV and V additionally received galangin (50, 100 and 200 μg/kg body weight, respectively) while group VI received 200 μg galangin/kg body weight. At the end of 60 days, fructose-fed rats exhibited insulin resistance, increased levels of peroxidation end products and diminished antioxidant status. galangin, dose-dependently normalized blood glucose and insulin levels. The minimum effective dose was 100 μg galangin/kg body weight. At this dose, galangin also prevented the development of insulin resistance and the exaggerated the response to oral glucose challenge. The oxidant–antioxidant balance was maintained by galangin. Micro-albuminuria and tubular and glomerular changes observed in fructose-treated rats were significantly prevented by galangin (100 μg/kg body weight). These findings imply that galangin potentiates insulin sensitivity and antioxidant capacity and reduces renal damage in this dietary model of metabolic syndrome.

Keywords: fructose, galangin, insulin resistance, kidney, antioxidants

Introduction

Metabolic syndrome, formerly known as syndrome X, is a multifactorial complex condition that encompasses several clinical and biochemical disorders such as abdominal obesity, insulin resistance, impaired glucose tolerance, hypertension and dyslipidemia.[1] These disorders are secondary to hyperinsulinemia and decreased insulin responsiveness in the target tissues.[2] Metabolic syndrome and insulin resistance are significant due to the fact that they predispose to diseases of high morbidity like type 2 diabetes, cardiovascular and renal diseases.[3] Experimentally, rats fed high-fructose diet (60 g/100 g) exhibit all the above features and form a well-known rodent model of metabolic syndrome.[4] Fructose feeding is also

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Received 8 April 2010, revised manuscript accepted 17 July 2010

DOI 10.1179/135100010X12826446921545

associated with a decline in antioxidants and oxidative damage to proteins and lipids.[5] Studies involving supplementation of antioxidants to fructose-fed rats have witnessed improved insulin sensitivity,[5–7] suggesting that oxidative stress is an instigator of insulin resistance in this model.

Flavonoids are a diverse class of naturally occurring polyphenolic plant compounds that have a variety of important biological activities useful for human health. Galangin (galangin; 3,5,7-trihydroxyflavone) is a flavonol (a class of flavanoids) constituent of *Alpinia galanga* (Zingiberaceae) rhizome and propolis, a natural beehive extract. The dried rhizome of the *Alpinia galanga* is used as a condiment and as an antimicrobial agent. It is used as a local medicine for cough, cold, stomach-ache and diarrhea. This well-known medicinal plant has been used in Asian (Unani) traditional medicine for treating diabetes mellitus.[8] Galangin has been shown to possess antimutagenic, antitumor, anticlastrogenic and antiviral properties. Further, metabolic-modulating effects of galangin are also found in the literature.[9] Such a broad spectrum of action might stem from its antioxidant and anti-inflammatory activities.[10] Although galangin has been subject to such extensive studies, there is a lack of information on the ability of galangin to promote insulin sensitivity and combat oxidative stress in the insulin resistance state. In view of this, we wanted to investigate whether galangin has any beneficial effects on insulin sensitivity and oxidant and antioxidant balance in fructose-fed rat kidney.

Materials and methods

Diet

A control diet containing starch and a test diet containing fructose were prepared in our laboratory. The diets were prepared fresh each day.

Control diet contained 60% corn starch, 20% casein, 0.7% methionine, 5% ground nut oil, 10.5% wheat bran, 3.5% salt mixture and 0.2% vitamin mixture. The fructose diet contained all these ingredients except corn starch which was replaced by an equal quantity of fructose.

Animals and treatment

Male Wistar rats of body weight 150–180 g were obtained from the Central Animal House, Raja Muthiah Medical College, Annamalai Nagar, India. They were housed in an animal house under controlled conditions on a 12-h light/12-h dark cycle. The animals received a standard pellet diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee of Animal Care, Raja Muthiah Medical College, Annamalai Nagar.

Experimental design

The animals were divided into six groups (n = 6) and were maintained as follows:

Group 1 CON, animals received the control diet containing starch.

Group 2 FRU, received the high fructose diet.

Group 3 FRU + GA1, received the fructose diet and galangin (50 μg/kg body weight).

Group 4 FRU + GA2, received the fructose diet and galangin (100 μg/kg body weight).

Group 5 FRU + GA3, received the fructose diet and galangin (200 μg/kg body weight).

Group 6 CON + GA3, received the control diet and galangin (200 μg/kg body weight).

Food and water were provided *ad libitum* to the animals. Galangin was administered by oral intubation. Food intake and body weight were determined. Animals were maintained in the respective groups for 60 days. On the 59th day of the experimental period, the rats were fasted overnight. Blood samples were collected by sinoocular puncture. Samples were again collected at 120 min after administration of glucose (2 g/kg body weight). The blood glucose concentration was quantitated using a kit from Agappe Diagnostics Pvt. Ltd, Kerala, India. Insulin was assayed using an ELISA kit (Accubind, Monobind Chemicals Ltd, CA, USA). The area-under-curve (AUC) values for glucose and insulin were calculated. Insulin sensitivity was assessed by homeostatic model assessment (HOMA)[11] after species specific adjustment and quantitative insulin check index (QUICKI).[12] The formulae used are given below:

$$\text{HOMA} = \frac{\text{Insulin (}\mu\text{U/dl)} \times \text{Glucose (}\mu\text{U/ml)}}{2430} \quad \text{Eq. 1}$$

$$\text{QUICKI} = \frac{1}{[\log(\text{Glucose mg/dl}) + \log(\text{Insulin }\mu\text{U/ml})]} \quad \text{Eq. 2}$$

On the 60th day of experimental period, the animals were fasted overnight and sacrificed by cervical decapitation the next day under anesthesia

Table 1 Initial and final body weight of experimental animals

Groups	CON	FRU	FRU + GA_1	FRU + GA_2	FRU + GA_3	CON + GA_3
Initial body weight (g)	180.3 ± 11.2	175.4 ± 10.8	173.6 ± 11.5	175.2 ± 10.7	178.7 ± 11.2	180.8 ± 11.4
Final body weight (g)	220.5 ± 10.7	250.7 ± 10.7	245.8 ± 9.4	225.9 ± 10.4	235.2 ± 10.5	225.8 ± 11.7

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA_1, fructose-fed rats treated with galangin (50 μg/kg body weight/day); FRU + GA_2, fructose-fed rats treated with galangin (100 μg/kg body weight/day); FRU + GA_3, fructose-fed rats treated with galangin (200 μg/kg body weight/day); CON + GA_3, control rats treated with galangin (200 μg/kg body weight/day).

Table 2 Oral glucose tolerance test in control and experimental animals

Groups	CON	FRU	FRU + GA_1	FRU + GA_2	FRU + GA_3	CON + GA_3
0 min	59.9 ± 4.2	122.3 ± 8. 7[a]	98.0 ± 4.3	62.6 ± 3.7[b]	73.4 ± 4.2	63.4 ± 3.3
60 min	131.7 ± 8.3	181.5 ± 11.2[a]	169.2 ± 9.3	133.3 ± 9.2[b]	146.6 ± 9.3	135.5 ± 8.6
120 min	64.9 ± 3.8	128.41 ± 7.6[a]	104.59 ± 7.4	67.6 ± 4.6[b]	75.4 ± 5.5	67.4 ± 5.5

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA_1, fructose-fed rats treated with galangin (50 μg/kg body weight/day); FRU + GA_2, fructose-fed rats treated with galangin (100 μg/kg body weight/day); FRU + GA_3, fructose-fed rats treated with galangin (200 μg/kg body weight/day); CON + GA_3, control rats treated with galangin (200 μg/kg body weight/day).
[a]Significant as compared to control rats ($P < 0.05$; DMRT).
[b]Significant as compared to fructose-fed rats ($P < 0.05$; DMRT).

(ketamine hydrochloride, 35 mg/kg body weight, i.p.). Blood was collected in tubes containing ethylene diamine tetra acetate (EDTA). The body was cut open and the kidneys excised, washed in ice-cold saline and the homogenate was prepared in cold 0.1 M phosphate buffer, pH 7.4. Plasma was separated by centrifugation at 1500 *g* for 10 min. The red blood cells were washed thrice in physiological saline and used for the preparation of the hemolysate by the method of Dodge *et al*.[13] Assays were done in plasma, hemolysate and kidney homogenate. Animals from each group ($n = 6$) were kept individually in metabolic cages and 24-h urine samples were collected in sealed beakers that contained 0.2 ml of 10 N hydrochloric acid for analysis of proteins.

Biochemical assays

Measurement of oxidative stress markers

The level of thiobarbituric acid reactive substances (TBARS) in kidney homogenate was estimated by the method of Niehaus and Samuelsson[14] and that of lipid hydroperoxides (LHPs) by the method of Jiang *et al*.[15] Protein carbonyl (PC) content was determined by the method of Levine *et al*.[16] and urinary protein was measured using a kit from Qualigens (Mumbai, India). Hemoglobin in hemolysate[17] and protein[18] in kidney were assayed.

Measurement of antioxidants

The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the levels of reduced glutathione (GSH), ascorbic acid (vitamin C), α-tocopherol (vitamin E) and protein were assayed in the kidney homogenate by methods outlined elsewhere.[19]

Histopathology

For histopathology, the kidneys were removed and fixed in 10% buffered formalin. They were later processed, sectioned to a thickness of 4–5 μm and stained with hematoxylin and eosin.

Statistical analysis

Data are expressed as mean ± SD ($n = 6$). Statistical evaluation was carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Initial and final body weights of animals are shown in Table 1. Weight gain was observed in all groups at the end of the experimental period. However, the final body weights did not significantly vary between the groups. Food intake and fluid intake during the experimental period were similar between the groups.

Table 2 shows the blood glucose values in response to the oral glucose load in each group. The glucose levels in fructose-fed rats were higher than all the

Table 3 Glucose, insulin, homeostatic model assessment (HOMA), quantitative insulin check index (QUICKI), $AUC_{glucose}$ and $AUC_{insulin}$ in experimental animals

Parameters	CON	FRU	FRU + GA1	FRU + GA2	FRU + GA3	CON + GA3
Glucose (mg/dl)	69.8 ± 3.6	130.6 ± 12.5[a]	105.3 ± 7.4	73.8 ± 5.7[b]	86.6 ± 6.4	70.5 ± 4.2
Insulin (U/ml)	30.2 ± 3.0	68.6 ± 5.8[a]	52.4 ± 4.6	33.5 ± 2.4[b]	43.6 ± 2.6	30.3 ± 2.7
HOMA	1.04 ± 0.06	3.1 ± 0.2[a]	2.25 ± 0.4	1.05 ± 0.06[b]	1.55 ± 0.12	0.94 ± 0.05
QUICKI	0.30 ± 0.02	0.25 ± 0.01[a]	0.26 ± 0.01	0.29 ± 0.01[b]	0.28 ± 0.01	0.30 ± 0.02
$AUC_{glucose}$ (mg/ml/min)	131.7 ± 7.5	181.5 ± 8.5[a]	169.2 ± 6.9	135.5 ± 5.8[b]	146.6 ± 6.7	133.3 ± 7.6
$AUC_{insulin}$ (μU/ml/min)	43.6 ± 3.1	86.5 ± 6.5[a]	75.3 ± 5.7	55.3 ± 3.8[b]	64.3 ± 4.8	42.3 ± 2.6

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA1, fructose-fed rats treated with galangin (50 μg/kg body weight/day); FRU + GA2, fructose-fed rats treated with galangin (100 μg/kg body weight/day); FRU + GA3, fructose-fed rats treated with galangin (200 μg/kg body weight/day).
[a]Significant as compared with control rats ($P < 0.05$; DMRT).
[b]Significant as compared with fructose-fed rats ($P < 0.05$; DMRT).
AUC, area under the curve, HOMA and QUICKI are calculated by formulae given in Materials and Methods.

Table 4 Lipid hydroperoxides (LHP), thiobarbituric acid reactive substances (TBARS), protein carbonyl (PC) in plasma and kidney

Parameters		CON	FRU	FRU + GA	CON + GA
LHP*					
	Plasma	0.67 ± 0.08	1.11 ± 0.14[a]	0.76 ± 0.05[b]	0.66 ± 0.04
	Kidney	1.72 ± 0.07	3.02 ± 0.21	1.81 ± 0.15[b]	1.39 ± 0.05
TBARS*					
	Plasma	3.06 ± 0.25	5.92 ± 0.29[a]	3.95 ± 0.21[b]	3.04 ± 0.27
	Kidney	1.89 ± 0.12	3.17 ± 0.21[a]	2.04 ± 0.11[b]	1.85 ± 0.09
PC*					
	Plasma	1.89 ± 0.09	3.79± 0.15[a]	2.08 ± 0.18[b]	1.87 ± 0.07
	Kidney	1.74 ± 0.06	2.32 ± 0.11[a]	1.81 ± 0.56[b]	1.72 ± 0.06

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA, fructose-fed rats treated with galangin (100 μg/kg body weight/day); CON + GA, control rats treated with galangin (100 μg/kg body weight/day).
[a]Significant as compared with control rats ($P < 0.05$; DMRT).
[b]Significant as compared with fructose-fed rats ($P < 0.05$; DMRT).
*mmol/dl for plasma; μmol/mg protein for kidney.

other groups. Here again, we observed that GA_2 and GA_3 had more promising effects than GA_1 in reducing glucose levels.

Table 3 shows glucose, insulin, HOMA, QUICKI and $AUC_{glucose}$ and $AUC_{insulin}$ values. Insulin resistance was observed in fructose-fed rats as measured by HOMA and QUICKI values which were significantly altered in fructose-fed rats when compared to control rats. The values were close to normal in GA_2 and GA_3 treated fructose-fed rats. Both $AUC_{glucose}$ and $AUC_{insulin}$ levels were significantly higher in fructose-fed rats than in control rats. GA_2-treated fructose-fed rats showed significantly reduced values of $AUC_{glucose}$ and $AUC_{insulin}$ as compared to those of untreated fructose-fed rats (Table 3). Fructose-diet induced hyper-insulinemia and hyperglycemia were completely normalized in GA_2 and GA_3 groups. As we found that GA_2 and GA_3 were equally effective, we fixed the minimum dosage of galangin (GA_2, 100 μg) for further study.

Oxidative stress parameters, TBARS, HP and PC in plasma and kidney are listed in Table 4. The levels of TBARS, HP and PC were significantly higher in animals fed fructose diet than those fed control diet. However, galangin-treated fructose-fed rats showed significantly lower levels as compared to fructose-fed rats.

The activities of SOD, CAT and GPx in the hemolysate and kidney and the levels of GSH, vitamins C and E in circulation and kidney of fructose diet-fed animals were significantly decreased ($P < 0.05$) as compared to control animals Tables 5 and 6. However, galangin treatment prevented the detrimental effects of fructose by uplifting the levels of antioxidants.

The levels of urinary protein (mg/dl) were CON, 1.05 ± 0.07; FRU, 2.00 ± 0.2; FRU + GA, 1.10 ± 0.07 and CON + GA, 1.04 ± 0.05. Treatment of fructose-fed animals with galangin reduced proteinuria

Figure 1 (A–J) shows the histology of kidney of

Table 5 Levels of enzymatic antioxidants in hemolysate, kidney

Parameters		CON	FRU	FRU + GA	CON + GA
SOD[A]					
	Hemolysate	3.8 ± 0.26	2.5 ± 0.18[a]	3.1 ± 0.18[b]	3.8 ± 0.25
	Kidney	4.1 ± 0.32	2.8 ± 0.18[a]	3.9 ± 0.29[b]	4.2 ± 0.30
CAT[B]					
	Hemolysate	187.9 ± 1.4	141.9 ± 1.5[a]	182.5 ± 10.3[b]	188.4 ± 11.4
	Kidney	62.4 ± 6.5	47.3 ± 2.4[a]	58.4 ± 4.4[b]	63.7 ± 1.5
GPx[C]					
	Hemolysate	6.7 ± 0.36	4.2 ± 0.43[a]	6.1 ± 0.52[b]	6.7 ± 0.62
	Kidney	10.6 ± 1.3	6.4 ± 0.3[a]	9.6 ± 0.9[b]	10.9 ± 0.9

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA, fructose-fed rats treated with galangin (100 μg/kg body weight/day); CON + GA, control rats treated with galangin (100 μg/kg body weight/day).
[a]Significant as compared with control rats ($P < 0.05$; DMRT).
[b]Significant as compared with fructose-fed rats ($P < 0.05$; DMRT).
[A]Unit/mg Hb for hemolysate; amount of enzymes which gave 50% inhibition NBT reduction/mg protein.
[B]μmol of H_2O_2 consumed/min/mg Hb for hemolysate; μmol of H_2O_2 consumed/min/mg protein for kidney.
[C]μmol of GSH utilized/min/mg Hb for hemolysate; μmol of GSH utilized/min/mg protein for kidney.

Table 6 Levels of non-enzymatic antioxidants in plasma and kidney of experimental groups

Parameters		CON	FRU	FRU + GA	CON + GA
Vitamin C*					
	Plasma	171.2 ± 9.4	108.7 ± 8.9[a]	160.3 ± 11.1[b]	172.1 ± 11.5
	Kidney	45.3 ± 3.2	24.7 ± 1.2[a]	38.7 ± 1.4[b]	45.9 ± 0.05
Vitamin E*					
	Plasma	30.4 ± 1.4	18.8 ± 1.5[a]	26.4 ± 1.8[b]	29.2 ± 1.6
	Kidney	26.5 ± 1.8	15.4 ± 0.2[a]	22.5 ± 1.9[b]	26.9 ± 1.4
GSH*					
	Plasma	898.5 ± 6.2	582.5 ± 4.2[a]	793.5 ± 6.2[b]	908.8 ± 7.5
	Kidney	6.3 ± 0.5	4.5 ± 0.3[a]	5.6 ± 0.3[b]	6.8 ± 0.4

Values are mean ± SD of 6 rats from each group. CON, control rats; FRU, fructose-fed rats; FRU + GA, fructose-fed rats treated with galangin (100 μg/kg body weight/day); CON + GA, control rats treated with galangin (100 μg/kg body weight/day).
[a]Significant as compared with control rats ($P < 0.05$; DMRT).
[b]Significant as compared with fructose-fed rats ($P < 0.05$; DMRT).
*μmol/l for plasma; μmol/mg protein for kidney.

experimental animals. The sections from control rats exhibit normal glomeruli and tubules (A,B). Fatty infiltration is absent. Figure 1C,D represents kidney sections from fructose-fed group, which reveals segmental glomerulonephritis, proteinaceous material, vessel wall thickening and inflammatory cell infiltration in the parenchyma. Sections of fructose-treated animals show extensive changes involving almost all the glomeruli (C,D). Renal damage is only partially mitigated in FRU+GA_1 groups (E,F). The severity of renal injury induced by fructose is lowered by galangin (100 μg/kg body weight; Fig. 1G,H). About 75% of the glomeruli appear normal. Fatty infiltration and inflammation are reduced. Despite being a high dose, GA_3 was not as effective as GA_2 in controlling the glomerular changes. Renal damage is still noticed involving more than 50% of the glomeruli in FRU+GA_3 (Fig. 1I). However, GA_3 administration

Figure 1 *(see opposite page)* **Histopathology of kidney using hematoxylin and eosin (A–J; ×20). Sections from control rat show glomeruli surrounded by Bowman's space and capsules; tubules are within normal limits (A,B). (C,D) represent kidney sections of high fructose-fed rats. The arrows in (C) show focal segmental glomerulonephritis and the glomerulus containing proteinaceous material. In (D), the arrows show fat accumulation in the cells lining the tubules. (E,F) represent the kidney sections of high fructose diet-treated rats with low dose (F+GA_1); The arrows show fat accumulation in tubules (E). In (F), the arrows show hyaline cast within the tubules. (G,H) represent sections from rats treated with middle dose (F+GA_2). Accumulation of fat vacuoles is minimized within tubular cells. (H) shows near normal glomeruli. (I) Kidney section from animals treated with high dose (F+GA_3); the arrow shows the focal segmental glomerulonephritis and inflammatory cell infiltrate around Bowman's space. (J) Section of a control rat treated with high dose (C+GA_3) featuring normal glomeruli and tubules**

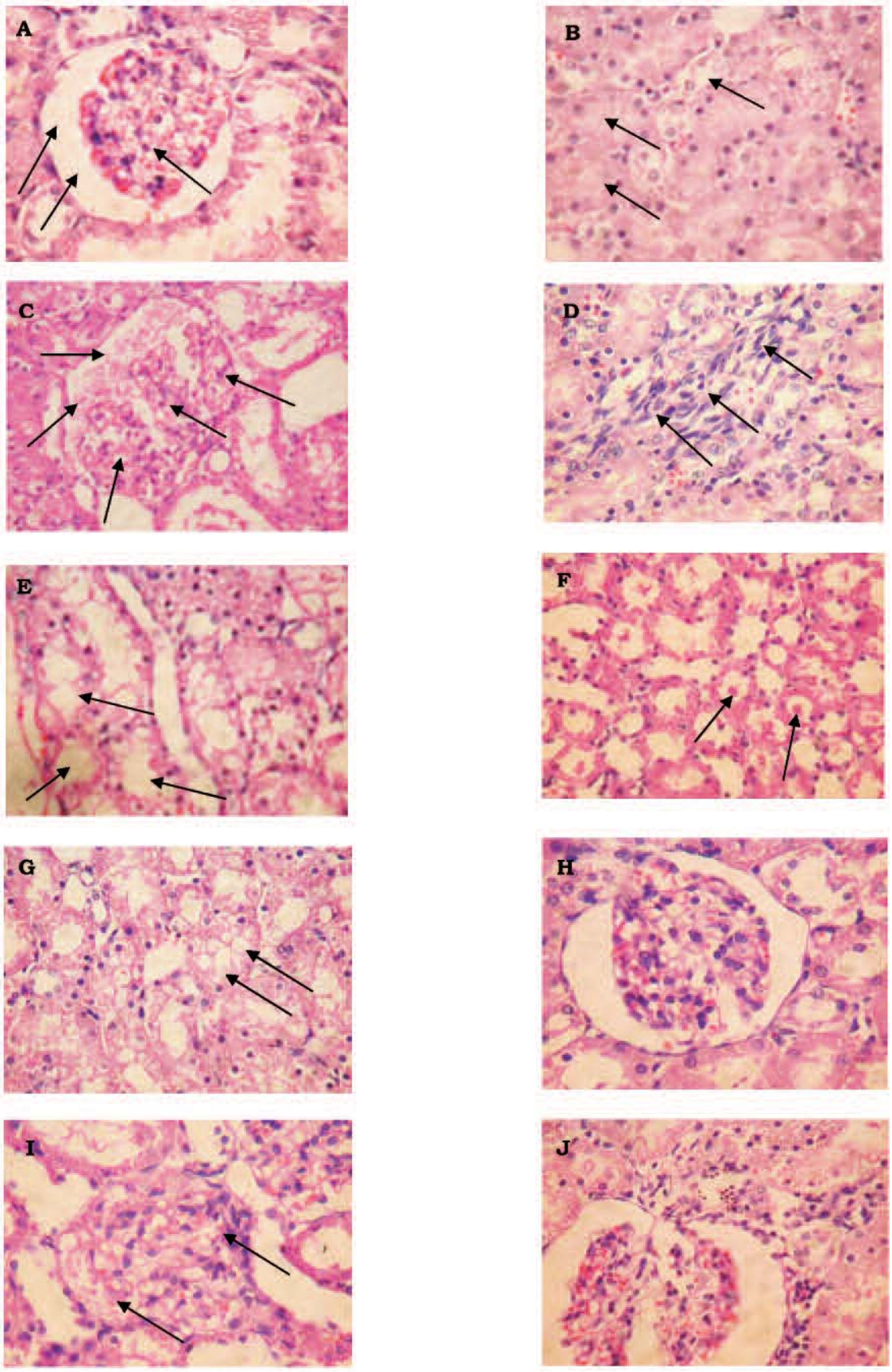

Figure 1 *(see next page)*

to control rat has no deleterious effect on renal histology (Fig. 1J).

Discussion

Literature survey revealed that the insulin-sensitizing action and antioxidant-rejuvenating potential of galangin in fructose-fed rats have not been explored. In this vein, the results of the present study are the first evidence to demonstrate that galangin effectively controls fructose-induced metabolic changes, promotes insulin sensitivity and simultaneously activates the antioxidant system.

The development of insulin resistance in fructose-fed rats is well-documented in the literature.[4–7] Fructose-induced pathology in rodents has been recommended as an experimental model to assess the therapeutic efficacy of putative insulin sensitizers from natural sources.[6,20] Confirming earlier studies,[6,7] high fructose feeding in rats for 2 months led to fasting hyperglycemia, hypertriglyceridemia, hyperinsulinemia, insulin resistance, glucose intolerance and oxidative stress in this study. Defects in insulin receptor gene expression and post-receptor events are noted in fructose-fed rats which are responsible for the development of insulin resistance.[21] Since kidney is a target organ for insulin action, we chose this tissue for our study.

Fructose-fed rats showed increased oxidative stress and inefficient antioxidant system as compared to control rats. This finding is consistent with the documented literature.[5–7] The development of oxidative stress is attributed to the rise in glucose level and formation of glucose derived-free radicals by processes like auto-oxidation and non-enzymatic glycation.[22] Further, an increase in the catabolism of fructose could be associated with the cellular ATP depletion that can increase the susceptibility of cells to lipid peroxidation.

Reduction in antioxidant defense mechanisms and altered redox ratio (GSH/GSSG) have also been reported in fructose-fed rats that contribute to oxidative stress. Reduction in antioxidants could be due to increased utilization to scavenge the free radicals, and/or decreased regeneration from their oxidized forms. Some investigators[23] have observed increase in vitamin E levels while reduction has also been observed.[24] These differences may be due to variation in the duration of treatment or animal strains used. From our results, we suggest that depletion in vitamin E is due to increased utilization and is responsible for the increased susceptibility to lipid peroxidation. Reactive oxygen species (ROS) can damage and reduce the activity of antioxidant enzymes such as CAT and GPx.[25] Decreased SOD activity in fructose-fed rats may be due to enhanced glycation of enzyme protein.[26]

Improved whole body insulin sensitivity and antioxidant status by galangin are the major outcome of the study. Galangin was effective in controlling hyperglycemia and hyperinsulinemia at all three doses tested. A dose-dependent effect at a concentrations of 50 and 100 μg/kg body weight was observed. However, at 200 μg/kg body weight, a similar effect as that of 100 μg/kg body weight was observed. Histological data on renal architecture are consistent with the dose-dependent effect on glycemia and insulin sensitivity parameters. Thus GA_2 was fixed as the minimum effective concentration for studies thereafter.

Flavonoid antioxidants generally act in biological system via their radical-scavenging ability which terminate the chain reaction of lipid peroxidation. A number of publications on the antioxidative effects of galangin are available.[27–30] Galangin is a unique antioxidant in that it has free radical scavenging effect without a pro-oxidant effect. Galangin has a 2,3-double bond, a 3-hydroxy group in the C-ring and two hydroxyl groups in the A-ring but no hydroxyl in the B-ring. These chemical attributes of galangin account for its potent anti-oxidative and ROS-scavenging activities. Galangin is potent scavenger of singlet oxygen and superoxide anion due to its redox properties.[31] Being a low toxic dietary compound, galangin is suggested to be an adjuvant in treating malignancies.[32]

As oxidative stress has been suggested as one of the detrimental effects of fructose, the antioxidant potential may be a mechanism by which galangin prevented fructose-induced insulin resistance. Therapy with antioxidants like vitamin E, lipoic acid and taurine promotes insulin sensitivity in fructose-fed rats,[5,7,8] while treatment with an insulin sensitizer, metformin, improves the antioxidant potential and reduces oxidative stress.[33]

In this study, galangin appears to have a parallel effect on both insulin sensitivity and antioxidant rejuvenation. This suggests that both these processes are inter-related mechanisms. This could be true, since recent reports indicate that ROS can disturb the insulin signal cascade by activation of transcription factors, pro-inflammatory cytokines and C-Jun N-terminal amino kinase pathways.[34] These, in turn, activate serine phosphorylation rather than tyrosine phosphorylation of insulin receptor and insulin

receptor substrates and thereby the downstream events are affected. Further, Araki *et al*.[35] noted that insulin receptor gene expression requires activation of certain transcription factors that depend on GSH and redox homeostasis for efficient action. Thus, we suggest that galangin, by uplifting the antioxidant concentration, could not only protect the cells from ROS and but also might activate insulin receptor gene expression and, thereby, insulin signaling.

Although galangin seems to be a useful therapeutic agent, galangin is shown to be a substrate of cytochrome P450 that could be transformed successively to kempferol and then to quercetin and could exert toxicity in V79 Chinese hamster cells.[36] Whether galangin exerts such mutagenicity *in vivo* at these doses needs to be tested in order to establish the net effect of this compound.

The antioxidant action of galangin, could be one mechanism for its insulin sensitivity effects. However, further studies on the effects of galangin on other aspects such as insulin signaling and lipid and glucose metabolism must also be considered in future. It is of interest to note that Matsui *et al*.[37] reported that galangin has inhibitory action to intestinal maltase activity and this activity is responsible for the hypoglycemic activity of Rhei Rhizoma extract, in which galangin is the major constituent. Thus, studies in this area are necessary to explore the multiple mechanisms of galangin action in this model.

For the first time, we show that galangin (100 μg/kg body weight) reduces fructose-induced insulin resistance and oxidative damage in kidney. This was verified by improved insulin sensitivity indices, reduced lipid and protein damage and enriched antioxidant potential. One of the important effects of insulin on kidney is sodium re-absorption in proximal tubules and defect in renal sodium handling has been documented in fructose-fed rats.[38] Additional studies on the effect of galangin on renal tubular function and structure and blood pressure are currently underway in our laboratory.

Conclusions

The present study provides new data on the promising potential of galangin as an adjuvant for the prevention and/or management of the metabolic syndrome and also offers credentials to the traditional claim on the anti-diabetic efficacy of *Alpinia galanga* rhizome.

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Addendum

DOI 10.1179/135100010X12826446921824

The information that the work published in 'Neuzil J et al. (2007) Mitochondria transmit apoptotic signals in cardiomyocyte-like cells and isolated hearts exposed to experimental ischemia-reperfusion injury. Redox Report **12**, 148–162', and 'Zobalova R et al. (2008) Daxx inhibits stress-induced apoptosis in cardiac myocytes. *Redox Rep* **13**, 263-270' were supported in part by a grant from the Grant Agency of the Czech Republic (305/07/1008) was accidentally omitted.