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Galangin ameliorates changes of membrane-bound enzymes in rats with streptozotocin-induced hyperglycemia

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ABSTRACT

Objective: To assess the protective effect of galangin on membrane bound enzymes in rats with streptozotocin-induced diabetes.

Methods: A single low dose of streptozotocin was injected to adult male albino rats to induce hyperglycemia. Galangin (8 mg/kg) or glibenclamide 600 μg/kg as a standard drug was given orally once daily for 45 days by gavage. Membrane-bound adenosine triphosphatases were determined including total ATPase, sodium-potassium-ATPase, calcium-ATPase and magnesium-ATPase in erythrocytes and tissues (kidney, liver, and heart).

Results: The levels of total ATPases, sodium-potassium-ATPase, calcium-ATPase and magnesium-ATPase in erythrocytes and tissues were significantly altered in diabetic rats as compared to that in normal rats. After 45 days of treatment with galangin or glibenclamide, the levels of these enzymes were similar to that of normal control rats.

Conclusions: Oral administration of galangin or glibenclamide can improve activities of these membrane-bound ATPases towards normal levels. Mechanism of galangin needs to be further explored in future.

1. Introduction

Diabetes is a chronic progressive disease and one of leading causes of death in developed and developing countries[1]. In the past three decades, the occurrences of type 2 diabetic have augmented dramatically in developing countries. Over time, diabetes causes various complications: It starts by damaging blood vessels, reducing the blood flow, with sequelae that may be macrovascular (heart attack, stroke, and heart failure)[2]. About 422 million people have suffered from diabetes worldwide and the number will increase by 418 million in near future[3]. Clinical and epidemiological investigations have confirmed that chronic hyperglycemia and oxidative damages are the major causative factors of diabetes by activating polyol pathway, stimulating glucose auto-oxidation and glycating proteins[4].

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The membrane-bound adenosine triphosphatases play an important role in ion transport across cell membrane, which helps to regulate osmotic pressure, cellular volume and membrane diffusion[4]. Sustained hyperglycemia has generated major causative factor of superoxide anion by glucose autooxidation in chemically induced diabetic rats and diabetic patients[5]. Oxidative injury (superoxide radicals) causes membrane phospholipid peroxidation, resulting in membrane damage and cellular dysfunction. Membrane peroxidation also alters membrane-bound adenosine triphosphatases such as sodium-potassium-ATPase, calcium-ATPase, and magnesium ATPase[6,7].

Food beverage or plants act as major therapeutic agents in modern and traditional medicines. A wide variety of traditional plants products have been used as antidiabetic agents by researchers because they have few side effects and can act as a great auxiliary therapeutic drug[8]. Natural phenolic compound of flavonoids is widely found in plants. Flavonoids have several clinical properties including antidiabetic[9,10], antihypertensive[11], anticancer[12] and anti-hepatotoxicity[13]. Galangin is one such bioflavonoid found in *Alpinia officinarum*. Several medicinal benefits of galangin have been confirmed by researchers that include the hyperglycemic lowering activity[14], preventive or inhibitory role of cells inflammation[15], reduction of the tissues or cells damages by anti-oxidant effective role[16], and inhibition on different types of cancer growth by anti-apoptotic and proliferative effects. Our previous study also confirmed that the galangin had antihyperglycemic and antioxidant properties[14]. It is also reported that galangin could stimulate the ATPase enzyme activities[17] and inhibit H^+/K^+/ATPase[18].

Streptozotocin causes insulin deficiency and hyperglycemia by destruction of pancreatic β-cell in animals, and is widely used to establish diabetic animal model[19]. So we selected streptozotocin for modeling in our study. To the best of our knowledge, no studies have been done on the effect of galangin on membrane-bound enzymes. Therefore, our aim was to study the protective effect of galangin on membrane bound enzymes in rats with streptozotocin induced hyperglycemia.

2. Materials and methods

2.1. Animals

Albino Wistar rats (Male only), weighing 180 to 220 g, were brought from Central Animal House, King Saud University. These animals were accommodated in polypropylene cage in air-conditioned room at (25 ± 1) ℃ and exposed with 12-hour dark-light cycle. The animals were provided (throughout experiments) food *ad libitum* in normal pellet diet for laboratory use. Rats were treated caringly as per the guidance provided in the Guide for the Care and Use of Laboratory Animals [National Institutes of Health (NIH 85-23; 1985)].

2.2. Chemicals and treated drugs purchasing

Galangin and streptozotocin were acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.). The rest of other used chemicals such as ATP, $MgCl_2$, $CaCl_2$, $MgSO_4$, KCl and NaCl *etc*. were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), laboratory supplier in analytical grade. Glibenclamide is an antidiabetic drug for type 2 diabetic disease. It can improve the insulin activities by protecting the pancreatic cells damages, thereby reducing the higher blood glucose ranges. Hence, glibenclamide was used as a standard drug for this experimental study.

2.3. Hyperglycemia induction in animals

Animals (overnight fasted) were injected (intraperitoneally) with low and single dosage of streptozotocin (40 mg) prepared with 0.1 M citrate buffer (pH 4.5) to induce hyperglycemia[15]. To prevent mortality due to streptozotocin, 20% of glucose was administered for one day. Ninety-six hours after streptozotocin-induction, diabetes was confirmed by estimating the glucose level. Animals with plasma glucose above 180 mg/dL were selected for further experiments.

2.4. Grouping

A total of 42 rats were randomly allocated into seven groups 6 rats per group. In our earlier study where the acute oral toxicity study of galangin was reported and it had no signs of toxicity even at the highest dose of 320 mg/kg[14]. Hence, in this study, galangin at 4, 8 and 16 mg/kg was selected and this drug or glibenclamide at 600 μg/kg mixed with dimethyl sulfoxide (5%) was given orally once daily for 45 d.

Group Ⅰ: Control rats received only 5% dimethyl sulfoxide
Group Ⅱ: Normal rats received 16 mg/kg of galangin
Group Ⅲ: Hyperglycemic rats
Group Ⅳ: Hyperglycemic rats received 4 mg/kg of galangin
Group Ⅴ: Hyperglycemic rats received 8 mg/kg of galangin
Group Ⅵ: Hyperglycemic rats received 16 mg/kg of galangin
Group Ⅶ: Hyperglycemic rats received 600 μg/kg of glibenclamide

Before treatment of 0th day and after treatment of 45th day, the body weight was calculated by using normal weighing balance. After 45 days of treatment, rats were fasted for 12 h, then anaesthetized by 24 mg/kg ketamine (intramuscular injection) and sacrificed by cervical dislocation. Blood sample was received in EDTA tube for the assessment of glucose by using available glucose kit (Trinder reagent Kit method).

2.5. Grouping for further study

Thirty rats were randomly separated into five groups (each group

having 6 rats). Galangin at 8 mg/kg or 600 μg/kg glibenclamide (mixed with dimethyl sulfoxide 5%) was treated daily once for 45 d. The 8 mg/kg of galangin showed optimized activity of glucose lowering than other two doses such as 4 and 16 mg/kg[14]. Hence, this active dose of 8 mg/kg was used for further experiments.

Group Ⅰ: Normal (5% dimethyl sulfoxide)
Group Ⅱ: Normal plus 8 mg/kg of galangin
Group Ⅲ: Hyperglycemic rats
Group Ⅳ: Hyperglycemic plus 8 mg/kg of galangin
Group Ⅴ: Hyperglycemic plus 600 μg/kg of glibenclamide

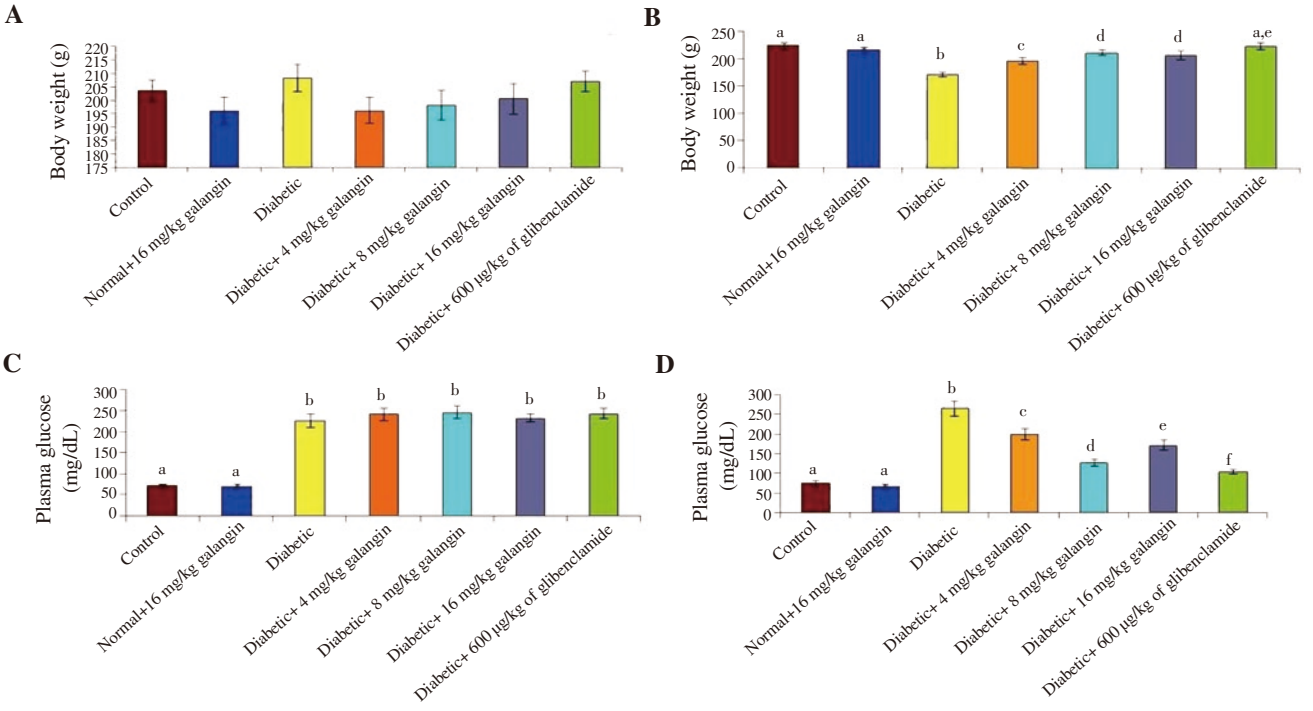

Figure 1. Effect of galangin on body weight (A: 0th day) and (B: 45th day) and plasma glucose (C: 0th day) and (D: 45th day) in diabetic rats. Values are expressed as mean ± SD (n=6). [a,b,c,d,e,f]Values not sharing same superscript differ significantly at P<0.05 by Duncan's Multiple Range Test.

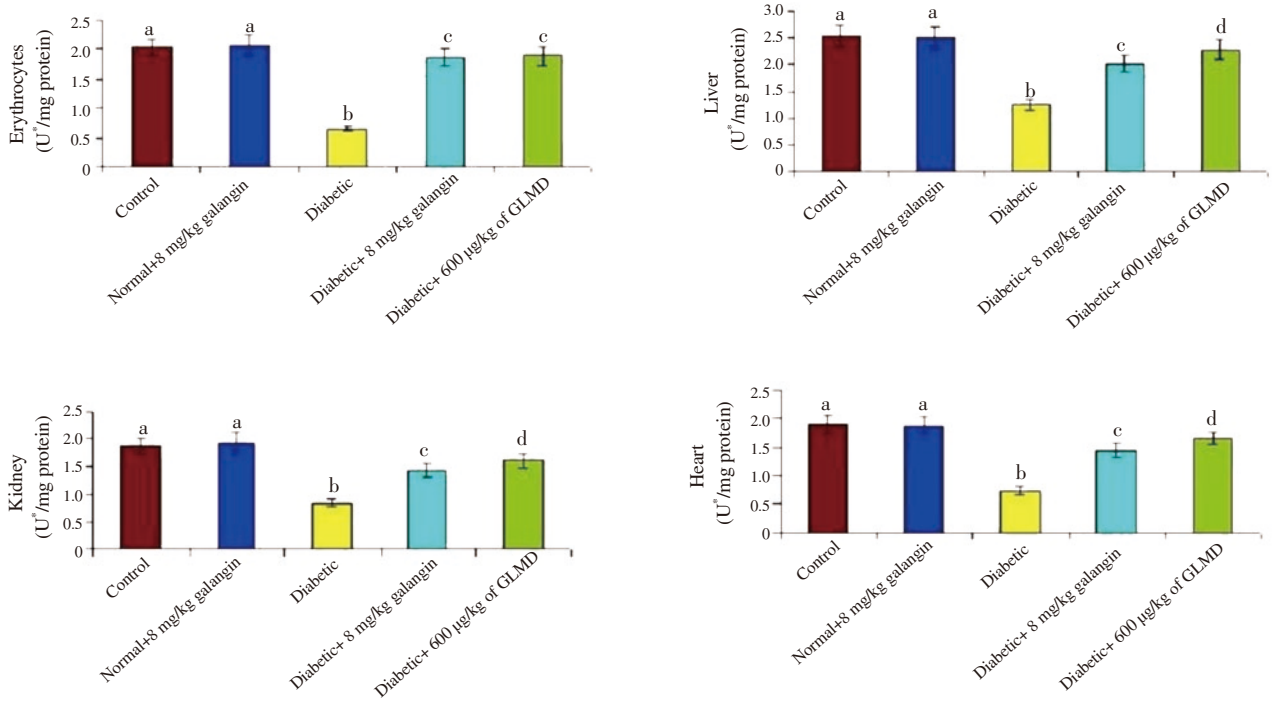

Figure 2. Effect of galangin on the activity of total ATPases in diabetic rats. Values are expressed as mean ± SD (n=6). [a,b,c,d]Values not sharing a common superscript differ significantly at P<0.05 by Duncan's Multiple Range Test. *-μmol of Pi liberated per hour. GLMD: glibenclamide.

After 45 days of treatment, rats were fasted for 12 h, then sacrificed by cervical dislocation. Blood collected and erythrocytes were isolated as the following procedure. The buffy coat was collected after the plasma isolation and then the white cells were detached. The remaining section of erythrocytes was thoroughly cleaned with physiological saline. Afterwards, erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was isolated by 2 500 rpm centrifugation for 10 min and the supernatant was used for the measurement of membrane bound ATPases. Moreover, liver, kidney and heart were collected for the measurement of membrane bound ATPases.

2.6. Biochemical assays

Plasma glucose was measured by the Trinder reagent kit method[20]. Total protein from tissues was measured by Lowry *et al*[21] method. Erythrocytes, liver, kidney and heart total ATPase activity was measured using Evans method[22], and sodium-potassium-ATPase activity based on Bonting method[23]. Moreover, calcium-ATPase and magnesium-ATPase activities were determined by Hjerten and Pan[24] and Ohnishi *et al*[25], respectively.

2.7. Statistical analysis

Results were calculated by one-way analysis (ANOVA) of Duncan's multiple range test (DMRT) with the commercially available statistical analysis SPSS software (9.05). Results were expressed as mean ± SD. P values < 0.05 were considered significant.

3. Results

3.1. Effect of galangin on glucose and body weight

Body weight was decreased and plasma glucose increased in rats with streptozotocin-induced hyperglycemia. Meanwhile, galangin or glibenclamide improved these alterations to almost normal level. Rats treated with 8 mg/kg of galangin showed the better result of glucose and body weight than the other two doses (Figure 1).

3.2. Effect galangin on ATPase activities

Figures 2-5 show that activities of total ATPase, sodium-potassium-ATPase, calcium-ATPase and magnesium-ATPase in tissues (liver, kidney, and heart) and erythrocytes all decreased in rats with streptozotocin-induced hyperglycemia. But galangin or glibenclamide significantly increased the activity of these enzymes.

4. Discussion

Chronic hyperglycemia accompanied by insulin resistance with or without insulin deficiency (type-2 diabetics) is associated with many diabetic complications as an outcome of oxidative damage to organs. Increased oxidative stress and reduced antioxidants occur during diabetes by glucose autoxidation and protein glycosylation[26,27]. Sodium-potassium-ATPase, calcium-ATPase, and magnesium-

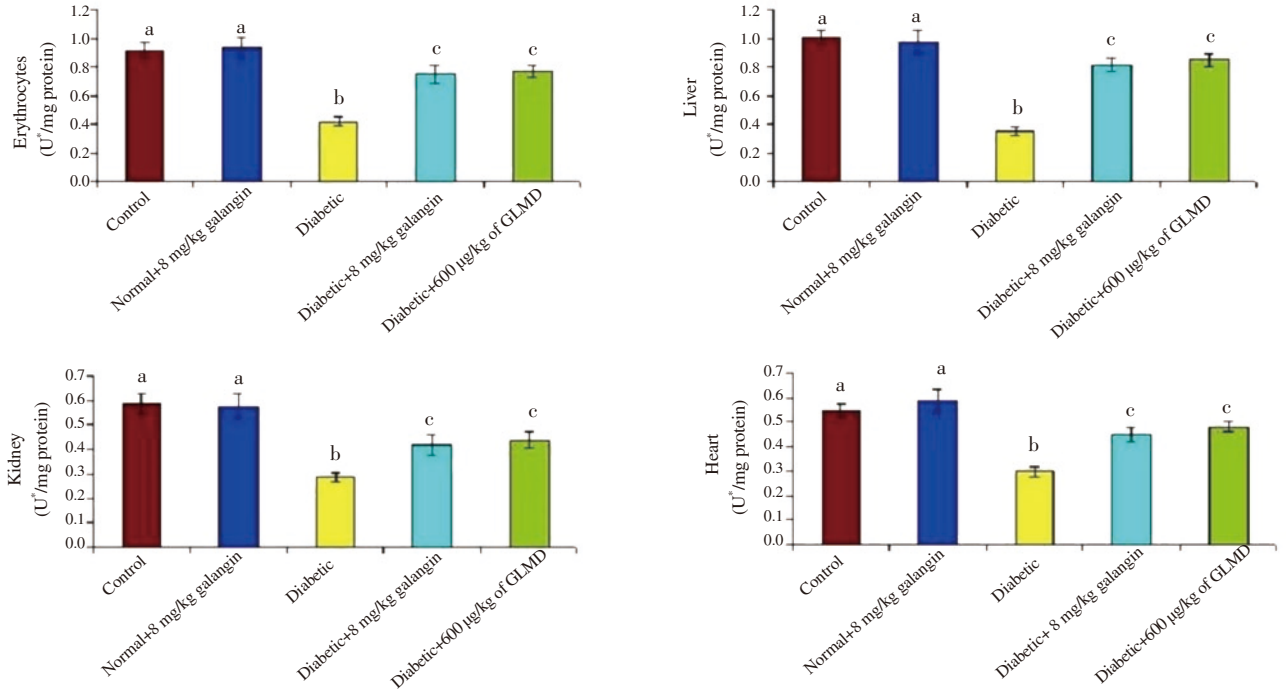

Figure 3. Effect of galangin on the activity of Na^+/K^+-ATPase in diabetic rats. Values are expressed as mean ± SD (n=6). [a,b,c]Values not sharing a common superscript differ significantly at P<0.05 by Duncan's Multiple Range Test. *-μmol of Pi liberated per hour. GLMD: glibenclamide.

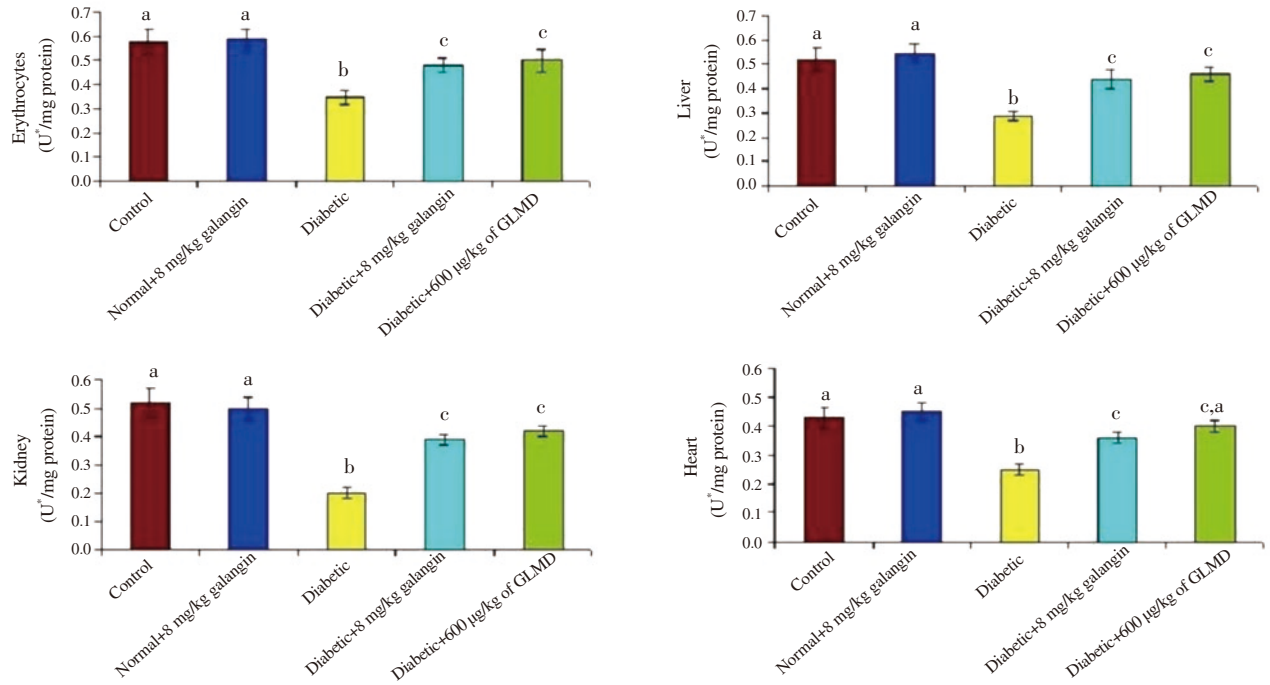

Figure 4. Effect of galangin on the activity of Ca^{2+}-ATPase in diabetic rats. Values are expressed as mean ± SD (n=6). [a,b,c]Values not sharing a common superscript differ significantly at P<0.05 by Duncan's Multiple Range Test. *- μmol of Pi liberated per hour. GLMD: glibenclamide.

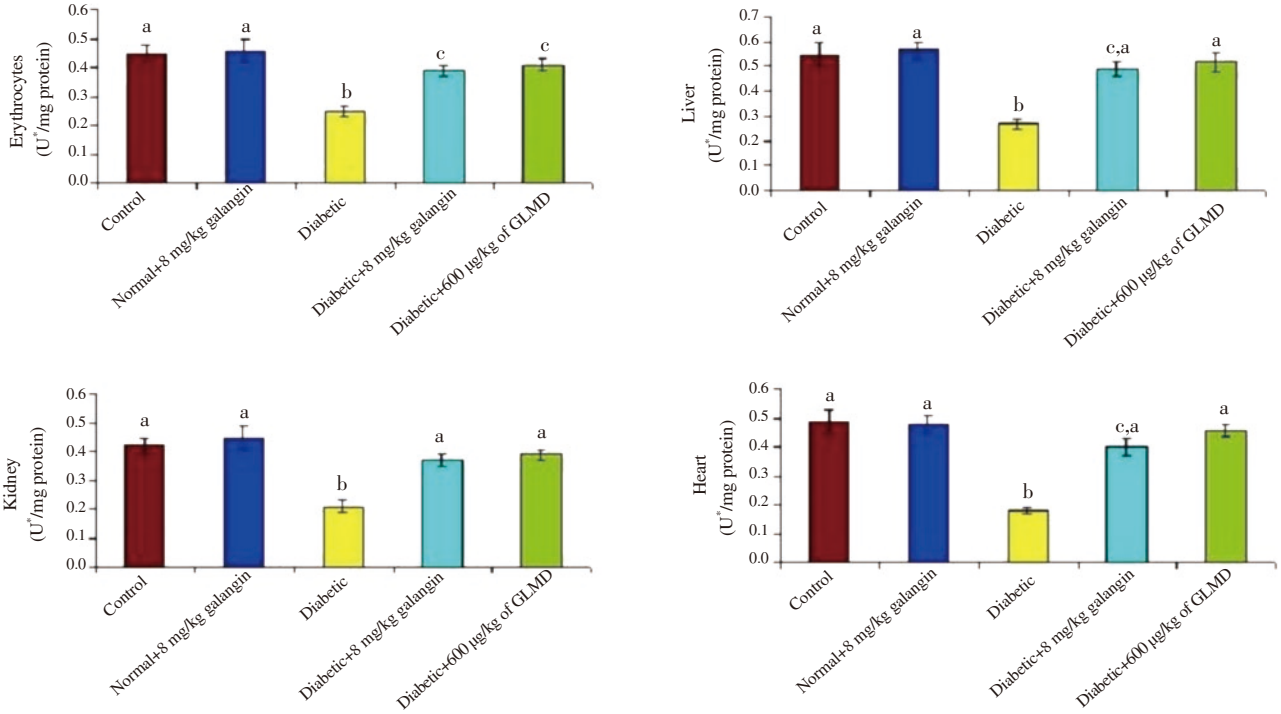

Figure 5. Effect of galangin on the activity of Mg^{2+}-ATPase in diabetic rats. Values are expressed as mean ± SD (n=6). [a,b,c]Values not sharing a common superscript differ significantly at P<0.05 by Duncan's Multiple Range Test. *-μmol of Pi liberated per hour. GLMD: glibenclamide.

ATPase play a major role in the maintenance of ion transport, electrolyte balance and cell volume regulations[28,29]. The membrane-bound adenosine triphosphatases activities (*i.e.* Na^+/K^+, Ca^{2+}, Mg^{2+}-ATPase) are shown to be altered in the hyperglycemic state due to membrane lipid peroxidation[30]. Erythrocytes and tissues are more sensitive to peroxidative damage due to high content of unsaturated fatty acid in membrane. Hence, membrane bound ATPases have been used as markers for hyperglycemic damage.

Sodium-potassium-ATPase moves two potassium ions into cell and carries three sodium ions out of the cell, which played an important role in cellular homeostasis[31]. Insulin plays an active role in biosynthesis of sodium-potassium-ATPase[32]. A recent study has reported that the sodium potassium-ATPase activity decreases significantly in liver, heart and erythrocytes of chemically-

induced hyperglycemia in rats[33]. The cellular membrane damage caused by free radicals results in reduction of sodium-potassium-ATPase activity. Reduced activity of sodium-potassium-ATPase may contribute to the pathophysiology of diabetic complicated risks[34]. Oxidation of thiol groups is abundant in sodium-potassium-ATPase, which could be involved in control of sodium-potassium-ATPase activity. Lipid and protein changes in the cellular membrane caused sodium-potassium-ATPase activity in chronic hyperglycemic patients[35]. In this current study, decreased activity of sodium-potassium-ATPase was observed in chemically streptozotocin-induced hyperglycemia in rats. Treatment with galangin and glibenclamide brought sodium-potassium-ATPase activity towards normalcy, by insulin secretion effect and/or by preventing damage to membrane lipids by oxidative stress. Moreover, Gupta *et al*[36] have discovered that insulin treatment with chronic hyperglycemic state partially restored sodium-potassium-ATPase activity.

Calcium and magnesium ATPases are found in cellular membranes, which are dependable for preserving calcium gradient[37]. Further, insulin plays an important role for regulating cellular Ca^{2+} involving Ca^{2+}-Mg^{2+}-ATPase. Ca^{2+}-Mg^{2+}-ATPase is involved in the Ca^{2+} reabsorption in proximal tubules[38]. Ca^{2+} and Mg^{2+}-ATPases activities were significantly decreased in experimental diabetic rats. The results of this finding are consistent with previous report[39]. This decreased enzymes' activity could be due to reduced secretion or action of insulin, which regulates these enzymes. Further, increased lipid peroxidation also could have diminished the activity of Ca^{2+} and Mg^{2+}-ATPases in uncontrolled high blood glucose status[40]. Oral therapy of galangin and glibenclamide had restored the Ca^{2+} and Mg^{2+}-ATPases activities towards normal levels. Thus improved ATPase activity could be due to it increased secretion or activity of insulin, reduced membrane phospholipids damages and improved glycaemic control.

Galangin treatment enhanced the functions of total ATPase, sodium-potassium-ATPase, calcium-ATPase and magnesium-ATPase in diabetic rats, which could be attributed to enhanced insulin secretion, antioxidant status and the glucose lowering activity. In our previous reports, we claimed that galangin significantly lowered the glucose and increased the insulin secretion in diabetic rats[14]. Antioxidant is a therapeutic agent for many disorders complication including diabetes, hypertension and cancer. It's also associated with the protective effect on abnormal ATPase activities through the diminished oxidative stress in streptozotocin induced diabetic rats. Galangin with the antioxidant potential possibly decreases the membrane lipid peroxides. Our previous study showed the galangin acts as a potent antioxidant in diabetic rats[14]. The active metabolites of galangin such as kaempferol and quercetin are identified by *in vitro* studies, which could be responsible for the several biological effects including anti-diabetic, anti-oxidant and membrane bound enzyme activities[41].

In conclusion, galangin ameliorates membrane-bound ATPase changes in tissues (liver, kidney, and heart) and erythrocytes in diabetic rats. Mechanism of galangin could be further evaluated in future.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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