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Effect of astragalin on the activities of membrane bound enzymes on leadacetate toxicity in albino rats

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ABSTRACT

In vivo effect oflead on membrane bound enzymes like Na^+ and K^+ ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase, total ATPases were studied. on RBC membrane exposed to 90 days to a sublethal concentration (160 mg/kgb.wt/day) of lead. The activity of membrane bound enzymes in RBC membrane of lead acetate treated rats was found to be decreased as compared to those of control rats. Asignificant ($p<0.05$) increase in the enzyme activity was observed on treatment with astragalin on lead acetate supplementation as compared to those of the untreated induced fed rats.

Key words: Astragfalin, Membrane bound enzymes, Lead acetate.

INTRODUCTION

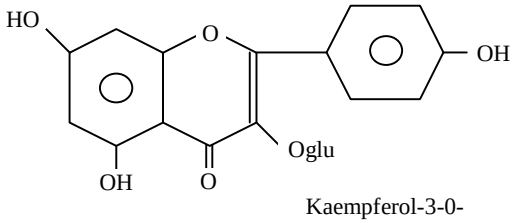
Erythrocytes are highly sensitive to peroxidative damage probably due to the high content of unsaturated fatty acid in their membrane. Therefore, the fair degree of ATPases activities in erythrocyte membrane could be serving as simple, safe and useful marker of intracellular damage (Ohta *et al.*,1989). Na^+, K^+-ATPase is an energy utilizing transmembrane enzyme, which is responsible for the maintenance of ionic gradients of Na^+ and K^+ ATPase has been shown to very susceptible to free radicals and membrane lipid peroxidation (Mishra *et al.*, 1989).Lipid peroxidation has been shown to alter Na^+,K^+-ATPase function by modification at specific active sites in a selective manner (Qayyum *et al.*, 2001). Depletion of glutathione and other protective antioxidants by RNS may greatly contribute to increasing amount of reactive species, which may also account for impaired activity of Na^+,K^+-ATPase (D'Ambrossio *et al.*, 2001). To evaluate the

possible effects of astragalin on chronic lead acetate induced abnormalities of membarane bound enzyme Na^+/K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase, total ATPases on RBC membrane

EXPERIMENTAL SECTION

Collection of Plant Materials

The fresh flowers of *Pongamia pinnata* was collected from the local gardens of STET Women's College, Mannargudi, Tamilnadu, India and voucher specimen are deposited submitted in the STET Herbarium at the Department of Botany and Microbiology, S.T.E.T. Women's College, Mannargudi, Tamil Nadu, India. The flowers were extracted with 85% EtOH (4 x 500 ml) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was successively fractionated with benezene (3 x 250 ml), peroxide – free Et_2O (3 x 250 ml) and EtOAc (4x 250 ml).By ^1H and ^{13}C – NMR, the identity of the pigments obtained from EtOAc fraction was found to be astragalin.



Kaempferol-3-0-glucoside (astragalin)

Source of chemical

All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai and Himedia Laborateries Pvt.Ltd., Mumbai, India.

Experimental Animals

Healthy adult male albino rats were used for the experiment. The weight of the animals ranged (160-180 g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 hours dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India).

Experimental design

Rats were divided into the following groups.

Group 1: *Control rats.*

Group 2: *Rats continued to receive lead acetate and considered as toxic control.*

Group 3:*Rats were administered astragalin (20 mg/kg b.wt/day) along with lead acetate.*

After 90 days of treatment, the animals were fasted for 12 h and sacrificed by cervical dislocation. Blood was collected in a cleaned tube with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters.

Erythrocyte preparation

After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline. A known volume of

erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2500 rpm for 10 min and the supernatant was used for the estimation of membrane bound enzymes.

Isolation of the Erythrocyte Membrane

The erythrocyte membrane was isolated according to the modified procedure of Dodge *et al.,* (1963).

Ion-Transort Enzymes

The activity of Na^+/K^+-ATPase was assayed according to the method of Bonting, (1970).Ca^{2+} ATPase (Hjertan and Pan, 1983), Mg^{2+} -ATPase (Ohinishi *et al.,1*982) and the enzyme was assayed by the modified method of Evans, (1969). Inorganic phosphorus was estimated by the method of Fiske and Subbarow, (1925).

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using the statistics software package SPSS for Windows, v. 13.0; Chicago, IL, USA.

RESULTS

Na^+/K^+-AT Pase

The administration of astragalin, MeOH extract of *P. pinnata* and carvedilol shown remarkable changes in the activities of erythrocyte membrane bound Na^+/K^+-ATPase of normal and experimental rats (Table 1). In lead acetate alone treated rats shown a decrease in the activity of Na^+/K^+-ATPase than compared to normal rats. Administration of astragalin on lead acetate treated rats significantly elevated the activity of Na^+/ K^+- ATPase as compared to lead acetate alone treated control rats.

Ca^{2+}-ATPase

The activity of Ca^{2+}-ATPase of the normal and treated rats are shown a remarkable changes, (Table 1). After 90 days of lead acetate treatment, resulted a significant decrease by 80%. In contrast, astragalin treated rats. The activity of the Ca^{2+}-ATPase increased significantly.

Table 1: Changes in the concentration of Na^+K^+ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase and total ATPases in RBC membrane of control and experimental animals

Groups	Na^+K^+ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase	Total ATPase
Group I	0.80 ± 0.07^a	0.87 ± 0.08^a	0.72 ± 0.07^a	2.23 ± 0.21^a
Group II	0.47 ± 0.04^b	0.59 ± 0.05^b	0.54 ± 0.05^b	39 ± 0.13^b
Group III	0.71 ± 0.06^a	0.75 ± 0.07^a	0.63 ± 0.06^a	2.01 ± 0.19^a

Values are expressed as means ± S.D. for six rats in each group. In each rows, means with different letter superscript differ significantly at p<0.05 (DMRT).

Na^+K^+ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase: µg of phosphorus liberated /min/mg protein

Total ATPase : µmole of Phosphorus liberated/min/mg protein

Mg^{2+}-ATPase

The level of Mg^{2+}-ATPase in the erythrocyte membrane, was found to be decreased in lead acetate treated rats. Administration of astragalin showed a significant elevation in the level of Mg^{2+}-ATPase when compared with lead intoxicated rats (Table 1).

Total ATPase

A significant decrease observed in the total ATPase activity in erythrocyte membrane of in lead treated rats when compared with control rats. Administration of astragalin resulted a significant increase in the total ATPase activity than lead acetate intoxicated rats (Table 1).

DISCUSSION

Total ATPase, Sodium potassium ATPase (Na^+K^+ATPase), calcium ATPase (Ca^{2+}ATPase) and magnesium ATPase (Mg^{2+}ATPase) are responsible for the transport of Na^+, K^+, Ca^{2+}, Mg^{2+}ions, respectively, across the membrane at the expense of ATP hydrolysis (Stekhoven and Bonting, 1981).The treatment with astragalin to lead acetate fed rats increased the activities of Na^+/K^+ATPase in RBC membrane as compared to the unsupplemented lead acetate fed rats may be because astragalin supplementation significantly prevented the accumulation of cholesterol in plasma and tissues there by maintaining the fluidity and integrity of the bilayer resulting in the optimal activity of erythrocyte membrane Na^+/K^+ATPase. Astragalin supplementation may also have a direct stimulatory effect of Na^+/K^+ATPase. Lead acetate intoxication in lead acetate treated rats caused a significant decrease in the activities of total ATPases, Ca^{2+} ATPase and Mg^{2+}ATPase. Mg^{2+}ATPase is involved in energy requiring processes in the cell. The marked loss of total ATPases, Ca^{2+} ATPase and Mg^{2+}ATPase in lead acetate intoxicated rats may be due to the loss of protein-SH, a consequence of lipid peroxidative damage that is observed in lead acetate intoxication. Treatment with astragalin in rats prevented the lead acetate -induced alterations in the total and Mg^{2+}ATPase activities probably by lipid peroxide lowering effect. Supplementation with astragalin to lead acetate treated rats resulted in near normal erythrocyte membrane levels of total ATPase, Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase.

The present findings of decreased erythrocyte membrane-bound enzyme activities can be attributed to altered membrane fluidity, enhanced lipid peroxidation and declined antioxidant defense status during the course of lead acetate administration. Lipid peroxidation of biological membrane results in changes in its fluidity and in the activities of membrane-bound enzymes (Janic and Boure, 1990). A diminished membrane Na^+/K^+ATPase activity associated with increased lipid peroxidation could be related to an impairment in the optimal interaction of Na^+/K^+ATPase with membrane phospholipids considering that its activity is modulated by the physicochemical properties of the membrane into which it is inserted .The activity of Na^+/K^+ATPase was decreased in the RBC membranes of lead acetate treated rats as compared to the control.

The activity of membrane bound enzymes Na^+/K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, total ATPase activities in RBC membrane of lead acetate treated rats was found to be decreased as compared to those of control rats. The Na^+/K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, total ATPase activity were increased significantly on treatment with astragalin on lead acetate

supplementated rats when compared to those of the unsupplemented rats. Further investigation are needed to demonstrate the binding of lead with –SH groups of the enzyme.

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