Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2271-2274
Received: 06-03-2019
Accepted: 07-04-2019

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Cardio protective efficacy of fisetin on glycoprotein and membrane bound enzymes against isoproterenol induced cardiotoxicity in wistar rats

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Abstract
This study evaluates the preventive effect of fisetin on membrane bound ATP ases and glycoprotein in isoproterenol induced myocardial infarcted rats. Male wistar rats were pretreated with fisetin (30 mg/kg) orally daily for the period of 30 days. After pretreatment, rats were subcutaneously induced myocardial infarction by isoproterenol (100 mg/kg) at an interval of 24 h for 2 days. The activity of sodium potassium adenosine triphosphatase was decreased and the activities of magnesium adenosine triphosphatase and calcium adenosine triphosphatase were increased in isoproterenol treated rats. The levels of glycoprotein (hexose, hexosamine, fucose and sialic acid) were significantly increased in serum and heart of Isoproterenol-induced rats. Pretreatment with fisetin to isoproterenol treated rats normalized all the biochemical parameters studied. The observed effects are due to their free radical membrane stabilizing property and their strong antioxidant effect.

Keywords: Isoproterenol, myocardial infarction, fisetin, glycoprotein

Introduction
Myocardial infarction is the most important form of Cardiovascular Disease (CVD) [1]. MI is the acute condition of myocardium that occurs due to disruption of imbalance between coronary blood supply and myocardial oxygen demand. MI is still most common disease and affects severe stress in the modern world [2]. The Isoproterenol (ISO) are mediators of β_1 and β_2 adrenoceptors. Both β_1 and β_2 adrenoceptors mediate the positive inotropic and chronotropic effects to β-adrenoceptor agonists [3]. High dose of isoproterenol causes severe damage to myocardial tissue. Nowadays research has been focused on food and medicinal plants that have been found to have certain preventive measures with fewer side effects in the treatment. Fisetin is a plant polyphenol from the flavonoid group, It is found in various fruits and vegetables, mostly present in strawberries, apples, onions, and cucumbers [4]. Berries contain more amount of fisetin as compared to other fruits. It is known for anti-cancerous, anti-diabetic, antioxidant, anti-inflammatory and memory stimulator effects [5]. However no systematic studies were found to be done on effect of Fisetin on experimental animal model of Myocardial infarction. Fisetin significantly reduces lipid peroxidation production and improves cardiac sustainability in myocardial infarcted rats. Hence, this study was undertaken to assess the efficacy of Fisetin in the treatment of Myocardial infarction.

Materials and Methods
Experimental animals and diet
The experiments were carried out according to the guidelines of the Committee and approved by the Institutional animal ethical committee (IAEC/XLI/03/CLBMCP/2017). All the experiments were done with an healthy male albino wistar rats weighing 150-180 g. They were housed in polypropylene cages (47 cm x 34 cm x 20 cm) lined with husk, renewed every 24 h under a 12:12 h light dark cycle at around 22ºC. The rats had free access to tap water and food. The rats were fed on a standard pellet diet.

Drug and chemicals
Fisetin was purchased from Sigma Chemical Company, Deisenhofen, Germany. Isoproterenol hydrochloride, was purchased from Sigma Chemical Co., St. Louis, MO, USA. All the other chemicals used in this study were of analytical grade and obtained from E. Merck and Himedia, India.

Induction of experimental myocardial infarction

Isoproterenol (100 mg/kg) was dissolved in normal saline and subcutaneously injected to rats at an interval of 24 hrs for 2 days.

Experimental design

The animals were grouped into six rats in each group: Group I: Normal control rats; Groups II: Normal rats were treated with Fisetin (30 mg/kg); Group III: ISO-control rats (100 mg/kg) alone; Groups IV: rats were pretreated with Fisetin (30 mg/kg) and then subcutaneously injected with Isoproterenol (100mg/kg). Fisetin was dissolved in 0.5% dimethyl sulfoxide (DMSO) and orally administrated using an intragastric tube daily for a period of 30 days. On the 31st day at 9.00 am, the first dose of isoproterenol was subcutaneously induced to rats. Twelve hours after the second dose of ISO-injection, all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and serum and plasma were separated by centrifugation. Heart tissue were excised immediately and rinsed in ice-chilled normal saline and the whole height was weighed.

Biochemical estimations

Glycoprotein components (Hexose, hexosamine, sialic acid and fucose) were estimated in serum and heart. Protein-bound hexose was estimated by the method of Dubois and Gilles [6]. Hexosamine was estimated by the method of Wagner [7]. Fucose was estimated by the method of Dische and Shettle [8]. Sialic acid was estimated by the method of Warren [9]. The activity of Na+/K+-ATPase was assayed according to the procedure of Bonting [10]. The activity of Ca2+-ATPase was assayed according to the method of Hjerten and Pan [11]. The activity of Mg2+-ATPase was assayed by the method of Ohnishi et al. [12]

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 17.00. Results were expressed as mean ± SD for six rats in each group. *P* values< 0.05 were considered significant.

Results

The level of glycoprotein (hexose, hexosamine, fucose and sialic acid) in serum and heart of normal and ISO-induced rats is shown in Figures 1& 2. Significantly elevated levels of glycoproteins were observed in serum and heart of ISO-induced rats, when compared with normal control rats. Pretreatment with fisetin significantly lowers the levels of glycoprotein in serum and heart of ISO-induced rats, when compared with ISO-alone induced rats.

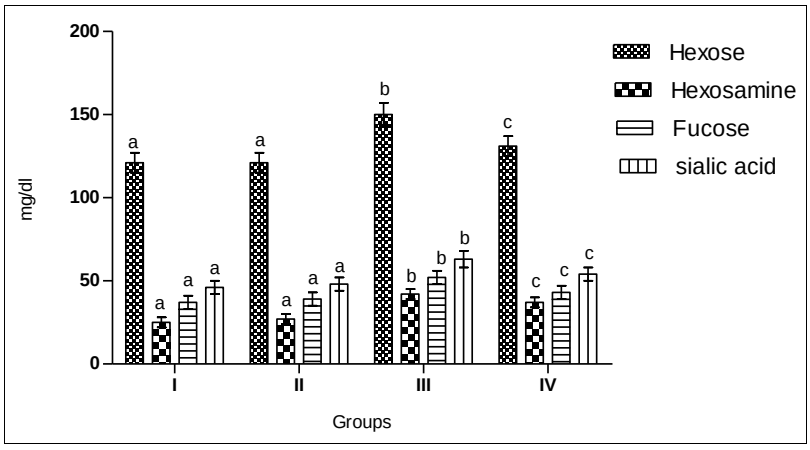

Fig 1: Effect of Fisetin on the levels of hexose, hexosamine, fucose and sialic acid in serum of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean ± SD for six rats in each groups; values not sharing a common superscript (a, b, c) differ significantly with each other (*P*< 0.05, DMRT)

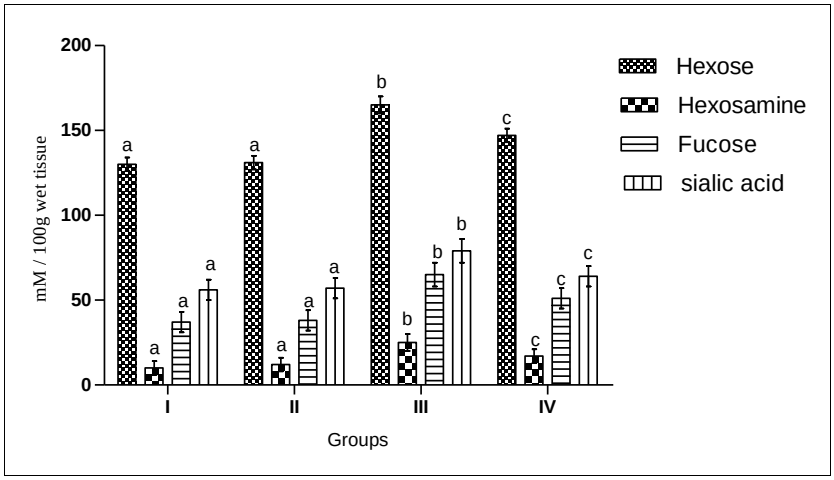

Fig 2: Effect of Fisetin on the levels of hexose, hexosamine, fucose and sialic acid in heart of normal and isoproterenol (ISO)-induced myocardial infarction in rats. Each value is mean ± SD for six rats in each groups; values not sharing a common superscript (a, b, c) differ significantly with each other (*P*< 0.05, DMRT)

Table 1- illustrate the effect of fisetin on the activities of sodium/potassium dependent adenosine tri phosphatase (Na^+/K^+-ATPase), calcium and magnesium dependent adenosine tri phosphatase Ca^{2+} and Mg^{2+}-ATPases in normal and ISO-induced rats. The activity of Na^+/K^+-ATPase show decreased significantly and the activities of Ca^{2+} and Mg^{2+}-ATPases significantly increased in the heart of ISO-induced rats, when compared to normal control rats. Fisetin pretreatment to ISO-induced rats significantly increased the activity of Na^+/K^+-ATPase and lowers the activities of $Ca^{2+}$and Mg^{2+}-ATPases in the heart when compared to ISO-alone induced rats.

Table 1: Effect of Fisetin on the activities of Na^+/K^+, Mg^{2+} and Ca^{2+}-ATPases in the hearts of normal and isoproterenol (ISO) induced myocardial infarcted (MI) rats.

Groups	Normal control	Normal + Fisetin (30mg/ kg)	ISO (100mg /kg) alone	Fisetin (30mg/kg) + ISO
Na^+ / K^+ ATPase (µmol of Pi/min/mg of protein)	0.732 ± 0.06^a	0.735 ± 0.06^a	0.215 ± 0.02^b	0.632 ± 0.06^c
Mg^{2+} ATPase (µmol of Pi/min/mg of protein)	3.654 ± 0.32^a	3.657 ± 0.31^a	6.432 ± 0.58^b	4.543 ± 0.42^c
Ca^{2+} ATPase (µmol of Pi/min/mg of protein)	0.851 ± 0.08^a	0.856 ± 0.08^a	2.521 ± 0.26^b	1.652 ± 0.13^c

Each value is mean ± SD for six rats in each groups; values not sharing a common superscript (a, b, c) differ significantly with each other ($P< 0.05$, DMRT)

Discussion

Hexose, hexosamine, fucose and sialic acid are the basic components of glycoproteins. The levels of glycoproteins are stated to be significantly elevated in myocardial infarction [13]. An increase in glycoprotein components has been reported to related to the duration, severity and existence of degenerative vascular diseases [14]. The functions of glycoprotein stabilize the tissue may be involved in maintaining the structural stability of collagen fibrils. Our study clearly shows an increase in the levels of hexose, hexosamine, fucose, and sialic acid in ISO-induced rats. The elevated levels of serum glycoprotein components might be due to secretion from cell membrane glycoconjugates into the circulation [15]. Increase in glycoprotein level could also be due to increased synthesis to repair the damaged membrane structure by peroxidation. The Fisetin pretreated rats showed a significant decrease in these glycoprotein levels compared to ISO-administered rats. Maintenance of ambient levels of heart glycoprotein of fisetin treated rats might be due to the free radicals scavenging levels and decrease the lipid peroxidation process by their antioxidant property.

ATPases are closely connected with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium [16]. Determination of membrane associated enzyme activities like ATPases indicate the alterations in membrane under pathological conditions. The abnormalities in sodium/potassium dependent adenosine tri phosphatase (Na^+/K^+-ATPase) and calcium dependent adenosine tri phosphatase (Ca^{2+}-ATPase) activities with accompanied increase in base line sodium and calcium concentration are well documented in cardiac dysfunction. In this study, we observed lowered activities of Na^+/K^+-ATPase and increased activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase in ISO-induced rats. Na^+/K^+-ATPase, located in the cardiac sarcolemma is considered to be involved in the maintenance of intracellular Na^+ and K^+ concentrations in the myocardium. Na^+/K^+-ATPase inactivation occurs due to enhanced lipid peroxidation by experimentally induced isoproterenol. The inhibition of Na^+/K^+-ATPase activates the Na^+ and Ca^{2+} exchange mechanism, which play a role in regulating the cellular calcium levels [17]. Calcium is essential for normal cardiac function, for the maintenance of cell membrane integrity and for coagulation of blood. In the heart, cytosolic calcium is carefully controlled and Ca^{2+} is the key ion for normal activity of many enzymes [18]. Membrane Ca^{2+}-ATPase is responsible for fine tuning of intracellular calcium as well as the contractility and excitability properties of muscles. Ca^{2+}-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels. Pretreatment with Fisetin increased the activity of Na^+/K^+-ATPase and decreased the activities of Ca^{2+} and Mg^{2+}-ATPases in ISO-induced rats. Increased Na^+/K^+-ATPase activity due to pretreatment of fisetin could regulate the intracellular Ca^{2+} levels, thereby protecting the myocardium from excess damage by maintaining the membrane integrity. Fisetin has effective scavenger of free radicals under *in vivo* conditions, Antioxidant activities are mainly due to its hydroxyl groups at C-3, C-3′, C-4′ and C-7 positions and are enriched by carbonyl group at C-4. The presence of double bond between C-2 and C-3 conjugated with the 4-oxo group also eases higher electron delocalization. Pretreatment with fisetin has been previously reported to reduce H_2O_2 induced cell death and it protects cells against cell damage even at a low to high concentration,The potent free radical scavenging activity of Fisetin was attributed to the presence of the C ring group.The observation was also made that the more hydroxyl groups in the Fisetin possesses, more effective free radical scavenger the Fisetin becomes [19]. These effects show membrane stabilizing property of Fisetin.

Conclusion

The protective effect of fisetin in preventing free radical mediated myocardial damage and thereby eliminating the acute fatal complications by protecting the membrane damage against ISO-induced infarction. Fisetin pretreatment also shows the necrosis inhibition and reduced inflammation in ISO induced rats. The free radical scavenging, antioxidant, lowering of lipid and membrane stabilizing properties of fisetin could responsible for these effects on histology of the myocardium. Epidemiological studies suggest that diet rich in herbs and medicinal plants are protective against cardiovascular disease (CVD). It could be concluded that regular consumption of medicinal plants like fisetin could offers protection to the heart. Further clinical trials are warranted before fisetin could be developed as a drug for the cardiovascular disorders.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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