

Screening of cardioprotective activity of leaves of *Andrographis paniculata* against isoproterenol induced myocardial infarction in rats

Dipendra Kumar Sah* and Nagarathana P.K.M.

Department of Pharmacology, Karnataka College of Pharmacy, Thirumenahalli Bangalore-560064, India

Corresponding author*

Dipendra Kumar Sah
PG Scholar,
Department of Pharmacology
Karnataka College of Pharmacy,
Bangalore-560064 Karnataka- India
E-mail: dipusah20@gmail.com

Abstract

Objective: The objective of the present study was to investigate the cardioprotective effects of methanolic extract of leaves of *Andrographis paniculata* against Isoproterenol-induced myocardial infarction (MI) in rats.

Method: The rats were divided into five experimental groups viz., Normal control, ISO-treated (Disease control), Propranolol (10 mg/kg + ISO), *Andrographis paniculata* (100 mg/kg +ISO) and *Andrographis paniculata* (200 mg/kg + ISO). Myocardial infarction in rats was induced by the administration of isoproterenol at a dose of 85mg/kg i.p., the rats in group IV and group V were pretreated with methanolic extract of *Andrographis paniculata* in the dose of 100mg/kg b.w. and 200mg/kg b.w. through oral route. Cardiac marker enzymes, lipid profile and antioxidant enzymes as biomarker of cardiotoxicity were determined in experimental animals.

Result: Animals treated with flavonoid of leaves of *Andrographis paniculata* showed significant decrease in LDL-Cholesterol, total cholesterol, Triglycerides, AST, ALT, ALP, antioxidant enzymes viz., superoxide dismutase, catalase LPO and increase in HDL-Cholesterol and further was confirmed by histopathological study.

Conclusion: The results of the study demonstrate that *Andrographis paniculata* strongly protected the myocardium against isoproterenol-induced infarction and suggest that the cardioprotective effects could be related to antioxidant activities.

Keywords: Cardioprotection, Isoproterenol, Antioxidant, *Andrographis paniculata*, Myocardial infarction

1. Introduction

Cardioprotection includes "all mechanism and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage [1]. Cardiovascular disease (CVD) remains the principle cause of death in both developed and developing countries. It may present as a typical heart attack, a sudden death or it may be detected at an advanced stage and be described as a silent infarct. CVD includes high blood pressure, coronary heart disease, congestive heart failure, stroke and accounts for 17,000,000 deaths per annum worldwide. The contributing factor for growing burden of CVDs are increase in prevalence of cardiovascular risk factor specially hypertension, dyslipidemia, diabetes, overweight or obesity, physical inactivity and use of tobacco. It is an area where death gains can be made through the implementation of primary care intervention and basic public health measures targeting diet, lifestyles and environment.

According to World health organization data 16.7 million people die each year owing heart attacks. The figure is one-third of number of deaths worldwide. By 2020-30 more deaths will be caused by heart attacks and India will lead in such number of deaths in worldwide.

Myocardial Infarction, commonly known as heart attack is a disease that occurs when blood to a part of heart is interrupted, causing death of heart tissue. It means necrosis of region of myocardium caused by an interruption in the blood supply to the heart usually as a result of occlusion of coronary artery also called as cardiac infarction. Acute myocardial infarction is characterized by varying degree of chest pain, sweating, weakness, vomiting, arrhythmia and cause loss of consciousness and even sudden death. Several factors increasing the risk of heart attack include elevated level of low density lipoprotein, triglycerides, reduces high density lipoproteins level, blood cholesterol and blood pressure. An increased risk of coronary heart disease (CHD) is associated with high levels of serum total cholesterol [2] and low density lipoprotein (LDL) [3] and decreased levels of high density lipoprotein (HDL) [4].

Isoproterenol (ISO), a synthetic adrenoceptor agonist, has been found to induce myocardial injury in rat as a result of disturbance in physiological balance between production of free radicals and anti-oxidative defense system.

Andrographis paniculata (Family; Acanthaceae), popularly known as 'Kalmegh' a common Indian dietary component, has been used in Indian and Chinese traditional medicine [5]. Pharmacological studies have demonstrated its hepatoprotective[6], anti-inflammatory[7], immunostimulant[8], antihyperglycaemic[9] and cardioprotective properties[10,11]. It has phytochemical constituent flavonoid which act as antioxidant thus can be cardioprotective.

2. Materials and Methods

2.1 Collection and authentication of plant materials:

The leaves of *Andrographis paniculata* was collected from thirupati forest region Thirupati district, Andhra Pradesh, India in the month of July. This plant species was authenticated by Prof. Madhavacheety, Botanist Department of pharmacognosy and phytochemistry (Padmavathi mahikakalasalala). The collected plant material was thoroughly washed with water. The leaves were dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered and stored in an airtight container.

2.2 Preparation of extract:

100gm powdered leaves parts were subjected to successive extraction in a soxhlet extractor using methyl alcohol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get constant weight.

2.3 Animals:

In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220gm were included for the study. Animals were housed in polypropylene cages on clean paddy husk bedding. Animals were maintained under controlled temperature at $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ with 12hr light/dark cycle having access to food and water ad libitum.

2.4 Experimental Design:

The rats were divided into 5 groups of 6 animals each as follows:

Group I (Normal control): Rats were administered 0.9 per cent saline (2 ml/kg/day) once daily for 30 days.

Group II (ISO Control): Rats were administered normal saline (0.9%) orally for 30 days and on day on 29th and 30th day injected with ISO (85 mg /kg i.p.)

Group III: Rats were administered standard drug Propranolol(10mg/kg) orally for 30 days and on 29th and 30th day injected with ISO (85 mg /kg i.p.)

Group IV (*Andrographis paniculata* 100 mg/kg) - Rats were administered methanolic extract of *Andrographis paniculata* (100 mg/kg) orally for 30 days and on 29th and 30th day injected with ISO (85 mg /kg i.p.)

Group V (*Andrographis paniculata* 200 mg/kg) - Rats were administered methanolic extract of *Andrographis paniculata* (200 mg/kg) orally for 30 days and on 29th and 30th day injected with ISO (85 mg /kg i.p.)

Twelve hours after the second injection of ISO all the rats were sacrificed by decapitation. Blood was collected and serum separated after centrifugation. Serum was used for various biochemical estimations [12]. The heart was dissected out, washed immediately in ice-chilled saline, blotted and weighed. A known weight (200 mg) of the heart tissue was homogenized in 5 ml of 0.1 M Tris-HCl (pH-7.4) buffer solution. The homogenate was centrifuged at 3000 rpm for 5 min. The supernatant was used for the estimation of various biochemical parameters [13].

2.5 Serum biochemical estimation

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/liter (IU/L). The levels of plasma total cholesterol (TC), LDL, HDL and TG were estimated in the serum using standard commercial kits from (SPAN India, Ltd, Surat, India)

2.5.1 Estimation of LDH:

The reaction mixture contained 2.90ml of 0.2M Tris-buffer, 10 ml tissue supernatant, 100 ml of 30mM sodium pyruvate and 100 ml of NADH. The rate of change in absorbance was measured at 340nm for 2min at 30sec interval. The oxidation of NADH in the above-mentioned reaction was proportional to LDH present in the sample. LDH was determined from a standard curve obtained using commercially available LDH (Sigma Chemicals, USA). LDH levels are expressed as IU/mg protein [14].

2.5.2 Catalase estimation:

Catalase activity was estimated by the method described by Aebi (1974) [15]. To 50ml tissue supernatant, 1.0 ml of 50mM phosphate buffer (pH7) and 0.1ml of 30mM hydrogenperoxide were added and decrease in absorbance at 240nm was measured every 5s for 30s. Catalase content is expressed as U/mg protein.

2.5.3 Superoxide dismutase estimation:

SOD activity was determined by the method of Marklund and Marklund(1974) [16]. To 100 ml of tissue supernatant, 2.85ml of 0.1M phosphate buffer(pH8.4) and 50 ml of 7.5mM pyrogallol were added and absorbance was measured at 420nm for 3min at 30s intervals. SOD levels are expressed as U/mg protein.

2.5.4 Estimation of Lipid Peroxidation:

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) as described by Wilbur [17]. Briefly 1 ml of trichloroacetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 0.67% were added to 2 ml of homogenate supernatant. The absorbance of the mixture was recorded at 530 nm and the values were expressed as η M of MDA formed /mg of protein.

2.6 Statistical analysis:

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's Multiple Range Test. Results were expressed as mean \pm S.E.M. from six rats in each group.

3. Result

In the present study, ISO-treated rats showed significant ($P<0.001$) increase in the activities of LDH, AST, ALT, Cholesterol, TG, ALP but significant ($P<0.001$) decrease in the activity of HDL ISO-treated rats when compared to the control group (Table 1).

ISO and pretreatment with flavonoid of leaves of *Andrographis paniculata* group (100mg/kg, b.w. p.o.) showed significant reduction ($P<0.001$) in LDH, AST, ALT, Cholesterol, TG, ALP but a significant increase in HDL-Cholesterol ($P<0.001$) activities when compared to ISO-treated group.

ISO and pretreatment with flavonoid of leaves of *Andrographis paniculata* group (200mg/kg, b.w. p.o.) showed significant reduction ($P<0.001$) in LDH, AST, ALT, Cholesterol, TG, ALP but a significant increase in HDL-Cholesterol ($P<0.001$) activities when compared to ISO-treated group.

Similarly, ISO-treated rats showed significant ($P<0.001$) increase in the Catalase, SOD and LPO activities when compared to the control group (Table 2).

ISO and pretreatment with flavonoid of leaves of *Andrographis paniculata* group (100mg/kg, b.w. p.o.) showed significant reduction ($P<0.001$) in Catalase, SOD and LPO activities when compared to ISO-treated group.

ISO and pretreatment with flavonoid of leaves of *Andrographis paniculata* group (200mg/kg, b.w. p.o.) showed significant reduction ($P<0.001$) in Catalase, SOD and LPO activities when compared to ISO-treated group.

Histopathologic study was done, in ISO treated group slide showed abundant areas of necrosis, aggregates of acute inflammatory cells and extravasation of erythrocytes due to damaged vascular spaces and abundant areas of necrosis (approximately 70%). In ISO and propranolol treated group slide showed occasional areas of necrosis (approximately 30%). In flavonoid of leaves of *Andrographis paniculata* (100mg/kg) slide showed areas of necrosis (approximately 50%) whereas in flavonoid of leaves of *Andrographis paniculata* (200mg/kg) slide showed occasional areas of necrosis (approximately 30%).

Table 1: Effect of flavonoid of leaves of *Andrographis paniculata* on plasma total cholesterol, TG, LDL, HDL, AST, ALT, ALP levels in ISO induced myocardial necrosis in rats.

Treatment group	LDH(U/L)	TG (mg/dl)	TCH (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (IU/L)	ALT(IU/L)	ALP(IU/L)
Normal control	213.6 \pm 10.58***	73.09 \pm 0.69*	205.9 \pm 1.69***	181.2 \pm 1.51***	51.95 \pm 0.8***	17.01 \pm 1.59***	14.61 \pm 1.54***	95.21 \pm 1.180**
Isoproterenol 8.5 mg/kg	336.6 \pm 2.92***	120.7 \pm 1.18***	264.8 \pm 0.95***	237.7 \pm 0.68***	23.36 \pm 0.0***	51.02 \pm 2.05***	53.34 \pm 1.22***	333.0 \pm 13.26***
Propranolol 10mg/kg	273.3 \pm 4.16	77.68 \pm 1.10	219.4 \pm 2.95	192.1 \pm 3.02	45.77 \pm 1.61	30.89 \pm 1.5	31.76 \pm 0.68	166.6 \pm 20.5
AP100 mg/kg + ISO	331.0 \pm 10.89***	94.31 \pm 0.92**	245.0 \pm 0.81***	220.5 \pm 0.65***	36.67 \pm 0.41**	40.36 \pm 1.2***	49.47 \pm 1.03***	229.5 \pm 10.46**
AP200 mg/kg + ISO	210.4 \pm 8.67***	86.62 \pm 1.14***	210.3 \pm 1.5***	181.0 \pm 1.4***	41.55 \pm 0.51**	16.9 \pm 1.9***	22.42 \pm 1.38***	118.0 \pm 10.24*

Data was analysed using one way ANOVA followed by Dunnett's t test

*** $P<0.001$, ** $P<0.01$, * $P<0.05$. n=6

Table 2: Effect of *Andrographis paniculata* in ISO induced cardiac oxidative stress in rats

Treatment	Catalase μ g/mg of protein	SOD μ g/mg of protein	LPO μ g/mg of protein
Normal Control	2.307 \pm 0.002***	0.062 \pm 0.002***	0.654 \pm 0.014***
Isoproterenol(8.5mg/kg)	5.801 \pm 0.005***	0.028 \pm 0.001***	1.84 \pm 0.026***
Propranolol(10mg/kg)	2.85 \pm 0.03	0.044 \pm 0.001***	0.82 \pm 0.007***
AP(100mg/kg)+ISO	2.53 \pm 0.02***	0.022 \pm 0.002***	1.173 \pm 0.03***
AP(200mg/kg)+ISO	2.185 \pm 0.004***	0.0149 \pm 0.0004***	0.983 \pm 0.116**

3.1 Histological Examination

(A) Normal Control

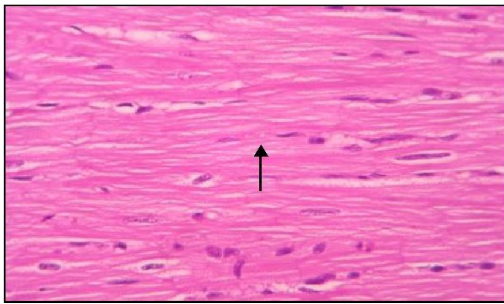


Fig.1[H&E, x400]

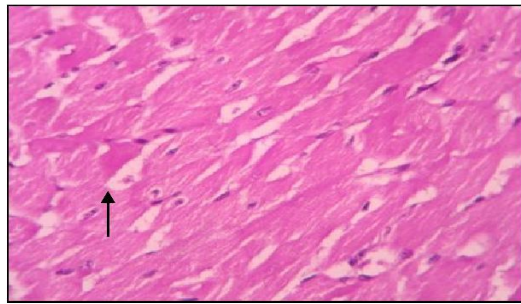


Fig.2[H&E, x400]

(B) Diseased Control

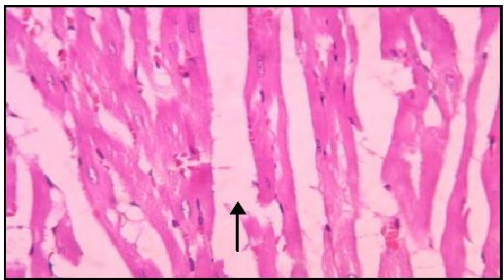


Fig.1[H&E, x400]

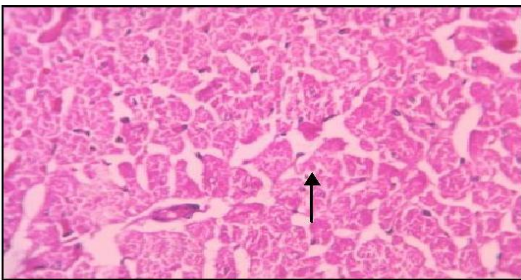


Fig.2[H&E, x400]

(C) Standard group

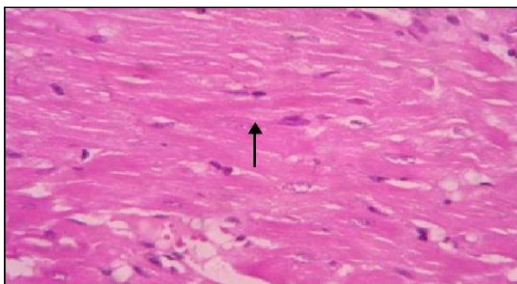


Fig.1[H&E, x400]

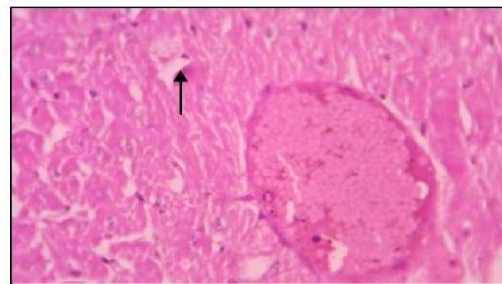


Fig.2[H&E,x400]

(D) Low dose [*Andrographis Paniculata* – 100mg/kg] + Isoproterenol.

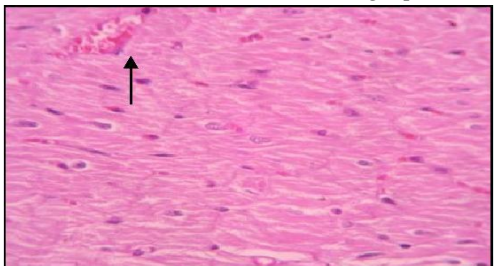


Fig.1[H&E, x400]

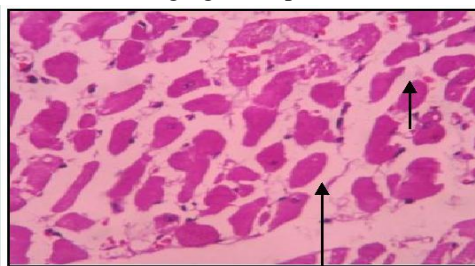


Fig.2[H&E, x400]

(E) High dose [*Andrographis Paniculata* – 200mg/kg] + Isoproterenol.

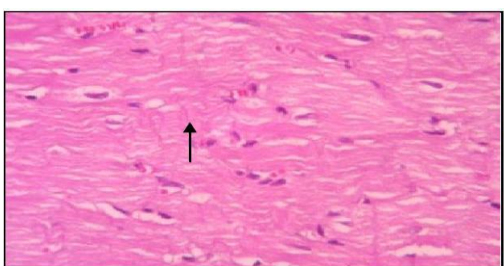


Fig.1[H&E, x400]

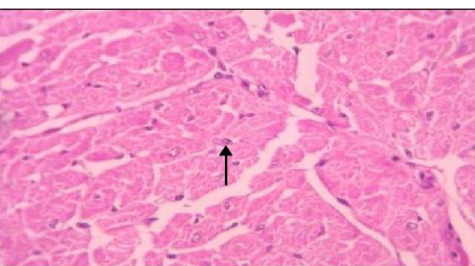


Fig.2[H&E, x400]

4. Discussion

As *Andrographis paniculata* is rich in phytochemical constituents like flavonoids, polyphenols which are said to act as antioxidants. Based on these assumptions leaves of *Andrographis paniculata* was used to study the cardioprotective activity.

Isoproterenol induced myocardial infarction is widely used as a model for evaluating cardioprotective drugs [18]. Radioactive oxygen species (ROS) are formed at an accelerated rate in ISO-treated myocardium. Cardiac myocytes, endothelial cells and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis. 'Infarct-like' lesions are produced in the myocardium when injected with ISO. Myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemmal membrane, followed by stimulation of adenylate cyclase, activation of Ca⁺ and Na⁺ channels, exaggerated calcium inflow and excess of excitation-contraction coupling mechanism leading to energy consumption cellular death. Free radicals generated by ISO initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. The metabolic damage of myocardium results in increase in the contraction of the marker enzymes like LDH, AST, ALP. The CAT and SOD were decreased while LPO increased in the myocardial homogenate of ISO administered rats indicating oxidative stress.

Andrographis paniculata (200mg/kg) prevented the alterations in marker enzymes of myocardial infarction and oxidative stress. Myofibrillar alterations such as myocytosis and myofibrillar degeneration are reported in ISO treated rats. Cardiac sections of the ISO treated animals showed infiltration of inflammatory cells and continuity in the muscle fibre was lacking suggesting an irreversible cell injury. Rats pretreated with *Andrographis paniculata* (flavonoid) showed normal myofibrillar structure with striation and revealed a marked protection by the extract against myocardial necrotic damage. Administration of ISO raised LDL cholesterol and decreased HDL cholesterol level in the serum. An increase in concentration of total cholesterol and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction. High level of circulating cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage. *Andrographis paniculata* elevated HDL level and decreased LDL cholesterol level. Hypertriglyceridemia observed in ISO treated rats clinically reported in ischemic heart disease. Pretreatment with *Andrographis paniculata* prevented the elevation of triglycerides cholesterol and LDL in serum signifying that the myocardial membrane is intact and not damaged. LDH is a cytosolic enzyme which is essentially present in all the tissues involved in glycolysis. From the damaged tissue it is released into the blood stream which becomes a definitive diagnostic and prognostic criterion.

5. Conclusion

From the experimental studies carried out, flavonoid of leaves of *Andrographis paniculata* at two different doses (100mg/kg and 200 mg/kg) showed dose dependent cardioprotective activity. The higher dose 200mg/kg showed significant protection compared to lower dose 100mg/kg.

The cardioprotective effect may be due to the presence of flavonoid. Flavonoid of leaves of *Andrographis paniculata* has shown free radical scavenging activity and the cardioprotective activity may be partially due to this activity. Further studies need to be carried out to isolate the potential chemical constituents of flavonoid of leaves of *Andrographis paniculata* and to find out its mechanism of action in the treatment.

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