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Effectiveness of flavonoid rich leaf extract of Acalypha indica in reversing experimental myocardial ischemia-a biochemical and histopathological evidence

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OBJECTIVE: In this study, we investigated the anti-myocardial ischemia effects of flavonoid rich methanolic leaf extract of Acalypha indica (AIE).

METHODS: An animal model of myocardial ischemic injury was induced by isoproterenol (ISO) in adult rats. Albino Wist rats were pretreated with the AIE (100 and 200 mg/kg b.w, orally) for 30 days followed by ISO (85 mg/kg s.c) at an interval of 24 h for two days. At the end of the experimental period (12 h after the second dose of ISO injection) rats were sacrificed by anaesthetization with an intramuscular injection of ketamine hydrochloride (50 mg/kg b.w). To ensure antiischemic potential of AIE, the plasma lipids - total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), free fatty acids (FFA), phospholipids (PL), myocardial lipids and hepatic lipids (TC, TG, FFA, PL) were estimated. Histopathology of heart was also done to assess the protective effect.

RESULTS: In the experiment, ISO injected rats were treated to test the antiischemic effect of AIE. Our results suggest that prior administration of AIE maintained the levels of the plasma lipids- myocardial and hepatic lipids (TC, TG, FFA, PL) in all the treatment groups (100 and 200 mg/kg) more protective with higher dose when compared to ISO-injected rats. Significant alterations occurred in histopathological examination of heart of ISO administered rat revealed myofiber loss, extensive subendocardial necrosis, infiltration of inflammatory cells, marked myocellular edema plus vacuolar degeneration. However, pretreatment with AIE at 200 mg/kg dose showed predominantly normal myocardium structure with myofibers appeared and no inflammatory cell infiltration, edema and necrosis also supported by computed ischemic markers atherogenic index of plasma (AIP) and non-HDL-C.

CONCLUSION: The biochemical and histological evidence from this study shows that AIE is protective against myocardial ischemia.
KEYWORDS: Acalypha indica-flavonoid-hepatic lipids-histopathology-myocardial ischemia-plasma lipids.

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in a worldwide epidemic. Myocardial ischemia is characterized by an imbalance between myocardial oxygen supply and demand, causing cardiac dysfunction, arrhythmias, myocardial infarction, and sudden death\(^1\). It is the most dreaded sequel among IHDs invariably followed by biochemical alteration such as hyperlipidemia leading to qualitative and quantitative alteration of myocardium\(^2\). Hypercholesterolemia is considered a major risk factor in the progression of coronary atherosclerosis and associated with an increase in the incidence of myocardial ischemia and cardiac events\(^3\).

The model of isoproterenol (ISO)-induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function\(^4\). ISO is a synthetic β-adrenergic agonist that induces myocardial ischemia leading to severe increase in levels of serum and myocardial lipids\(^5\). It would be ideal to remove excess lipids which may be a causative factor for damage to the myocardial membrane to such an amount consistent with maintenance of the normal body function.

Plants are appreciated as the major resource for molecules and medicines and a growing body of medical literature supports the clinical efficacy. Recently, there has been a growing interest in establishing the therapeutic potential of medicinal plants against various diseases. The use of plant extracts for medicinal purposes seems to be more natural, less expensive and with no apparent side effects\(^6\).

Hyperlipidemia and hypercholesterolemia has also been implicated in the pathogenesis of myocardial ischemia. Therefore therapeutic interventions having hypolipidemic and hypcholesterolemic activity may exert beneficial effects against IHDs\(^7\).

Acalypha indica L. is an annual erect herb found throughout various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa. The plant has wide uses in the traditional medicines of various countries and reportedly possesses diuretic, purgative and antihelmintic properties, besides being also used for bronchitis, asthma, pneumonia, scabies and other cutaneous diseases. A drug used for prevention and reversal of atherosclerotic disease process in the Sidha system of Indian medicine, anna payava siribharam, contains the leaves of this plant as one of the ingredients\(^8\). Chemical constituents reported from this plant include acalyphamide (as acetate), aurantiamide and its acetate, succinimide acalypho-lactate, 2-methyl anthraquinone, tri-O-methyl ether acid, β-sitosterol and its β-D-glucoside (leaves); a cyanogenetic glucoside, acalyphine, two alkaloids, acalyphine and triacetonamine, an essential oil n-octacosanol, kaempferol, quebrachitol, β-sitosterol acetate and tannin (whole plant); stigmasteryl (root)\(^9\). Recently, four kaempferol glycosides, mauritianin, clitorin, acalyphorin and biorobin have been isolated from the flowers and leaves of this plant\(^6\). The active ingredients behind the therapeutic effects have been identified are due to the presence of various biologically active compounds like alkaloids, polyphenols and flavonoids\(^6\). The medicinal properties of A.indica are not exploited properly.

We were interested in testing the flavonoids of A. indica as a potential agent improving the lipid metabolism in the ISO-induced myocardial ischemia in experimental rats. Ethnomedicinal information from the indigenous people of the tribes of Chittoor district, Andhra Pradesh, India revealed that the herb is used for the treatment of heart related diseases. However, there is no scientific confirmation of this
claim. The present study highlights the possible ability of methanolic extract of *A. indica* against ISO-induced plasma, myocardial, hepatic lipid and histological alterations.

1 Materials and methods

1.1 Collection and preparation of flavonoid rich plant extract Fresh mature leaves of *A. indica* were collected from Tirumala hills (Chittoor district, Andhra Pradesh, India). The plant was authenticated by Dr. K. Madhava Chetty, taxonomist (Member IAAT-No.357) from Department of Botany of Sri Venkateswara University, Tirupati, Andhra Pradesh, India and a voucher specimen (No.SVUBH/1012) was deposited in the herbarium for future reference. After collection, plant was washed thoroughly with tap water. The leaves were separated manually, shaded dried and grounded into fine powder. The leaf powder was extracted with methanol (5:1, volume/weight) at room temperature (30±1 °C) under stirring for 8 h and the extraction process was repeated till the solvent became colorless. Extract was then filtered through Whatman No. 1 filter paper followed by evaporation under reduced pressure.

The concentrated extract was again exhaustively defatted by refluxing with n-hexane and benzene (15 h twice). The two fractions were negative for polyphenols. Then the defatted bulk residue was successively extracted by refluxing with ethyl acetate (15 h twice). Total flavonoids were spectrophotometrically estimated in the ethyl acetate fraction which was found to contain the bulk of flavonoids (total flavonoids content 2.89 mg quercetin equivalent (Qb/g dry weight) and this fraction was evaporated in a rotary evaporator under reduced pressure, freeze-dried and used for the study.

1.2 Experimental animals and housing conditions Male Wistar rats, 10 to 12 weeks old, weighing 150 to 175 g, were used in the study. Animals were obtained from the Sri Venkateswara Animal Agency, Bangalore, Karnataka, India. They were kept in the departmental animal house under controlled conditions of temperature at (25±2) °C, relative humidity of 60±5% and a light-dark cycle of 12 h each. The animals were fed with show pellets (Sri Sai Durga Animal Feeds, Bangalore, Karnataka, India) and allowed water *ad libitum*. Animals were maintained in polypropylene cages, each cage containing a maximum of four animals. The study was conducted in accordance with the protocol approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC/SVU/TVM-KCM/06(i)/a).

1.3 Induction of myocardial ischemia Myocardial ischemia was induced by subcutaneous injection of ISO (85 mg/kg body weight), dissolved in physiological saline, for two consecutive days.

1.4 Experimental procedure Twenty-four male rats were completely randomized into four groups of six animals. Group 1 was normal control (distilled water per oral). Group 2 was ISO-treated (85 mg/kg, subcutaneously) at an interval of 24 h for 2 d. Groups 3 and 4 were administered with 100 and 200 mg/kg body weight/day p.o. of flavonoid-rich methanolic *A. indica* leaf extract (AIE), respectively for 30 d followed by ISO (85 mg/kg subcutaneously) at an interval of 24 h for 2 d. AIE did not produce any signs of toxicity up to a dose of 200 mg/kg. Hence, the doses (100 and 200 mg/kg body weight) were selected. Moreover, previous experimental studies also reported that the doses below 200 mg/kg of AIE per day are more efficacious and without any recognized side effects.
At the end of the experimental period (12 h after the second dose of ISO injection), rats were sacrificed by anesthesia with an intramuscular injection of ketamine hydrochloride (24 mg/kg body weight). Blood samples were collected via cardiac puncture in a heparinized tube, centrifuged at 900xg for 10 min. The separated plasma was used for lipid estimations. After the heart and liver were excised, washed in ice-cold isotonic saline and blotted with a filter paper. A portion of the tissue was weighed, homogenized in 0.1 mol/L Tris-HCl buffer (pH 7.4) and the homogenate was used for tissue lipid estimations.

1.5 Biochemical determinations in the plasma

Following the procedure described in the Kamineni Life Science Pvt Ltd. assay kits, the concentrations of total cholesterol, triglycerides, high density lipoprotein cholesterol were determined in the plasma. Phospholipids and free fatty acids content were determined by the method of Zilversmit et al. and Falholt et al. Low density lipoprotein cholesterol and very high density lipoprotein cholesterol in the plasma were calculated by the Friedewald et al formula. LDL-C and VLDL-C fractions were calculated as VLDL-C=TC-HDL-C and LDL-C=TC-(HDL-C+VLDL-C) respectively. Non-HDL-C was calculated as TC-HDL-C. Atherogenic index of plasma log(TG/HDL-C) was also calculated.

1.6 Biochemical determinations in the myocardial and hepatic tissues

Lipids were extracted from tissues by the method of Falcetti et al. using chloroform-methanol mixture (CHCl3:MeOH = 2:1 volume ratio). The TC was estimated by the method of Zlatkis et al. To 0.1 mL of the lipid extract, 0.9 mL of ferric chloride-acetic acid reagent was added and allowed to stand for 15 min and then centrifuged. To 5 mL of the supernatant, add 3 mL of concentrated sulfuric acid. The colour developed was read after 20 min at 560 nm against a reagent blank. Values were expressed as milligram per gram wet tissue.

TG was estimated by the method of Rogen and Dunn. To an aliquot of lipid extract, evaporated to dryness. 0.1 mL of methanol was added followed by 4 mL of isopropanol. Then 0.4 mL of ammonia was added to all the tubes, shaken well for 15 min, centrifuged and then 2 mL of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65 °C for 15 min for saponification after adding 0.6 mL of the saponification reagent (5.0 g of potassium hydroxide in 60 mL of distilled water and add 40 mL of isopropanol) followed by 0.5 mL of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65 °C for 1 h, the contents were cooled and absorbance was read at 420 nm. The TG content was expressed as milligram per gram wet tissue.

PE content was determined by the method of Zilversmit et al. To 0.1 mL of lipid extract, 1 mL of 5 mol/L H2SO4 and 1 mL of concentrated nitric acid were added and digested to a colourless solution. The phosphorus content in the extract was determined by the method of Fiske and Subbarow. The values were expressed as milligram per gram wet tissue.

PAs were estimated by the method of Falholt et al. To 50 μL lipid extract, 1 mL phosphate buffer (pH 6.4) and 6 mL chloroform:heptane:methanol solution (49:49:2 volume ratio) were added in a test tube with polyethylene cap. This was shaken for 10 min and centrifuged (2860xg for 10 min). The buffer is removed carefully by suction and 5 mL of the organic phase was shaken with 2 mL of a solution containing 0.05 mol/L copper nitrate and 0.1 mol/L triethanolamine, pH 8.1 for 5 min. After centrifugation (2860xg for 5 min), 3 mL of the upper phase was transferred to a test tube containing 0.5 mL of a solution prepared from 10 mL of 4 g/L 1,5-diphenylcarbazide in ethanol and 0.1 mL of 1 mol/L triethanolamine and mixed.
carefully. Colorimetric determination was made after 15 min at 550 nm. For the calculation a standard curve is prepared (0.2, 0.4, 0.8 and 1.0 mmol/L palmitic acid). The amount of FFAs was expressed as milligram per gram wet tissue.

1.7 Histopathological analysis of heart  The heart and liver tissues were rapidly dissected out and fixed by immersion at room temperature in 10% formalin solution. For the histological examinations, paraffin embedded tissue sections of heart (5 μm) were stained with hematoxylin-eosin (HE). The tissue samples were then examined and photographed under a light microscope (Olympus CX31, Olympus, Japan), for observation of structural abnormality.

1.8 Statistical analysis  The SPSS statistical program was employed for statistical evaluation. The results were evaluated using analysis of variance (ANOVA) utilizing the F-test. The results were presented as the mean ± standard deviation. Differences among the means for the groups were assessed using the Duncan’s Multiple Range Test (DMRT) to determine which values were significantly different at *P* < 0.05.

2 Results

2.1 Effects of AIE on plasma lipids and ischemic markers  Table 1 represents the plasma lipid profile of control and experimental rats. There were alterations in plasma lipid profile of the rats administered with ISO when compared with the control. ISO raised the plasma levels of TC, LDL-C, VLDL-C, TG, FFA, and PL significantly (*P* < 0.05) with a decrease in HDL-C (*P* < 0.05). On pretreatment with the plant extract for 30 d restored the lipid profile to near normal and improved myocardial ischemic risk markers (Table 1).

2.2 Effects of AIE on myocardial and hepatic lipids  As reported in Tables 2 there was significant (*P* < 0.05) increase in the levels of cholesterol, TG and FFA with a significant (*P* < 0.05) decrease in PL in the heart and liver of ISO-treated rats. On pretreatment with AIE, the levels of all the above parameters brought to normal level. Generally, the effect of the extract was more prominent at the higher dose (200 mg/kg bw).

2.3 Effects of AIE on histopathological alterations  Figure 1 shows the normal histology of the myocardium in control animals (A). ISO treated animals (Group 2) shows cardiac muscle separation, myofiber loss, extensive subendocardial necrosis and inflammatory infiltrate into the myocardium (B). Group 3 rats treated with 100 mg/kg showed mild muscle separation and few inflammatory cells (C). AIE treatment (Group 4-200 mg/kg) showed no changes (D).

3 Discussion

The present investigation was carried out to evaluate the lipid lowering effects of *A. indica* in rat model of experimentally induced myocardial ischemia. To the best of our knowledge, the study provides first evidence of *A. indica* in experimental ischemic animals. The results obtained provide substantive evidence in favor of antiischemic potential of *A. indica*. Out of the two doses studied (100 and 200 mg/kg), the higher dose was found to be more protective.

ISO-induced myocardial ischemia model was used in the present study due to its technical simplicity, excellent reproducibility and an acceptable low mortality. In accordance with the previous studies ISO at 85 mg/kg, s.c., induces myocardial ischemia[12]. This is, in part, due to its agonistic effect on beta-adrenergic receptors. Lipid metabolism plays an important role in myocardial ischemia produced by ISO.
ISO causes hyperlipidemia and it increases the LDL-C in the blood, which in turn leads to the accumulation of circulating cholesterol at high level in the heart tissue is usually accompanied by cardiovascular damage. Hypercholesterolemia and hypertriglyceridemia were seen in ISO-treated rats which might be due to increased mobilization of lipids from adipose tissue. Free radical mediated peroxidation of membrane phospholipids and consequent changes in membrane permeability is the primary target responsible for ischemia induced by ISO. The free radical generated due the auto oxidation of ISO resulted in severe changes in the biochemical status of the myocardium. The deleterious effect of these free radicals is mainly on phospholipid membrane causing its degradation.

In the present study, aqueous extract of A. indica restored TC level to near normal levels, thereby reducing the risk of cardiovascular disease. This observation is in agreement with the findings of Raju et al. who reported a positive correlation between Momordica cymbalaria extract and cholesterol level. ISO administration caused a significant raise in the plasma lipids. Rats treated with AIE showed decreased concentration of the plasma levels of TC, TG, LDL-C, VLDL-C, FFA and PL indicates the beneficial effects of AIE in reducing hyperlipidemia caused by ISO administration. LDL-C formation occurs primarily in the cytoplasm of VLDL-C. LDL-C is capable of carrying the highest concentration of TG and PL. It has also been suggested that oxidatively modified LDL-C rather than unmodified LDL-C is responsible for atherogenesis. Oxidized LDL-C promotes the production of several cytokines, immune cell chemoattractant proteins, and growth factors. In addition, they increase platelet aggregation, which aggravates the lesion and causes arterial wall thickening. An increased level of LDL shows a positive correlation with coronary artery disease, whereas HDL-C shows a negative correlation. HDL-C inhibits the uptake of LDL-C from arterial wall and facilitates the transport of cholesterol from peripheral tissues to the liver, where it is catabolized and excreted from the body.

Pretreatment with naringin to ISO-induced rats minimized the alterations in serum lipoprotein levels by increasing HDL-C and decreasing LDL and VLDL-C levels. The above result is an indication that the myocardial membrane is intact and not damaged. Our results are in agreement with previous report.

TGs are elevated markedly in myocardial diseases with reduced HDL-C levels. The authors attributed this to the metabolism of triglyceride-rich lipoproteins present in HDL-C, particularly the sub-fraction HDL2 which has a negative association with myocardial damage. Therefore, the increased plasma TGs coupled with decreased HDL-C levels in ISO-treated rats as observed in this work, suggests predisposition to cardiac ischemic risk. Further our study also demonstrates that the extract increased the plasma levels of HDL-C in the treated groups (group III, group IV) as compared to isogenic group (group II). Thus cholesterol and TG lowering properties of AIE could be attributed to hypocholesteremic compounds that may act as inhibitors or activators for some enzymes which participate in cholesterol metabolism or due its ability to suppress lipid peroxidation and may be partially attributed the antiradical activities of the flavonoid components known to act by free radical scavenging or chain-breaking mechanisms. Further, Guo et al. reported the hypolipidemic and hypocholesteremic effects of flavonoids. The elevated plasma PL and FFA in ISO-treated rats may be due to increased peroxidation of membrane lipids via phospholipaseA2 also may be due to increased lipolysis in adipose tissue. Treatment with AIE showed decreased plasma PL and FFA which might be due to the free radical scavenging property and through its antilipolytic activity of AIE.
Atherogenic lipoprotein profile is an important risk factor for ischemia. It is characterized by high ratio of LDL-C to HDL-C and increased level of TGs. Predominance in plasma of small dense LDL-C and small HDL-C particles is associated with an increased risk of ischemia while large HDL-C particles are associated with decreased risk. TGs were of prognostic significance and are known to contribute to ischemic risk with high LDL-C/HDL-C ratio, when used in a ratio with HDL-C become an excellent predictor of likelihood of future myocardial ischemia. AIP has a place in the assessment of lipid-related risk for ischemia as it is derived from more precise measurements of atherogenic lipoprotein profile [34].

Ischemia and related conditions (metabolic syndrome, obesity, and diabetes) often have elevated triglycerides, low HDL-C, and relatively normal calculated LDL-C values. Relying on LDL-C targets alone can be misleading in such cases, since they produce highly atherogenic VLDL-C and intermediate-density lipoproteins (IDLs), as well as small dense atherogenic LDL-C particles, in spite of the normal LDL-C values. LDL measurement can be very misleading when the TGs are in the (200-500 mg/dl) range. Sustained hyperglyceridemia evenuates in elevated levels of VLDL-C, IDL, and abnormal highly atherogenic LDL-C particles. Stated otherwise, non-HDL cholesterol may be a stronger predictor of coronary risk than LDL-C or TGs in certain populations, since it reflects the sum of serum cholesterol carried by all of the potentially atherogenic lipoproteins LDL-C, VLDL-C, IDL, and other remnant lipoproteins. Moreover, since it is calculated from total cholesterol and HDL cholesterol, both of which are measured directly, it is not affected by the TG level and does not require a fasting specimen. Non-HDL cholesterol provides a single index of all these apolipoprotein B-containing lipoproteins, essentially acting as a surrogate for direct apo B determinations. The bottom line is that the measurement of LDL-C alone is not an adequate measure of atherogenic risk in ischemia [35]. Therefore, the reduction in the computed AIP and non-HDL cholesterol the secondary lipid lowering targets, further corroborated the school of thought that the extract has antiischemic effect.

An ISO-treated rat showing altered lipid profiles in the heart agrees well with a previous report [12]. A significant rise in cholesterol content suggested that the redistribution of cholesterol in ischemic cells [36]. The elevated level of cholesterol in heart is well associated with myocardial ischemia. The significant increase in heart TG in ISO-induced rats might be due to a decrease in the activity of lipoprotein lipase, resulting in decreased uptake of TGs from the circulation [37]. In aerobic conditions, cardiomyocytes prefer free fatty acids for energy. Ischemic myocardium is not in a position to oxidize the available fatty acids and results in the accumulation of fatty acyl CoA derivatives. The significant increase observed in the lipid profiles except PLs in the rat treated with ISO alone could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate (cAMP) [38]. A significant decrease in phospholipid content could be due to an accelerated degradation of membrane phospholipids by phospholipases. Cell membranes are rich sources of PL and degradation of PL results in membrane dysfunction, which leads to cell injury. The increased peroxidation of membrane PL releases FFA by the action of phospholipase A2 [21]. Ca2+ have been reported to be one of the inducers of phospholipase A2. Therefore, increased levels of FFA in ISO-induced rats might be due to the indirect effect of Ca2+. AIE pretreatment significantly altered the levels of myocardial lipids in ISO-induced rats.

ISO-treated rats showed increased levels of hepatic TC, TG and FFA. An additional feature of the disordered lipid metabolism in hepatic ischemia is that the changes in
PL content are not accompanied by corresponding changes in the quantities of other lipids. AIE treatment significantly decreased the TC and TG content in the rat liver. These effects could be due to inhibition of intestinal absorption of cholesterol, enhancement of cholesterol excretion, alteration of endogenous cholesterol metabolism[39]. Several studies have reported that liver TG content was significantly decreased by consumption of dietary flavonoids, such as isorhamnetin and rhamnetin[40]. Bok et al[41] reported the cholesterol and TG lowering effect of hesperidin and naringin.

FFAs appear to be important mediators of lipotoxicity, both as potential cellular toxins and inducing lipid over accumulation through insulin resistance[42]. Furthermore, FFAs not only promote hepatic lipotoxicity by stimulating TNFα expression via a lysosomal pathway[43], but also are the most important source of reactive oxygen species. In this study, reduced liver FFAs content by AIE treatment in ischemic rats might be due to enhanced clearance and also decreased production of the major transporters of endogenously synthesized cholesterol and TGs; the result indicate that AIE may be a potential candidate to treat ischemia. PL content found significantly reversed in pretreated ISO induced ischemic rats. These results with lipids are, on one hand, consistent with that of study by Nassar et al[44] showed citrus flavonoids hesperetin and naringenin, which were shown to have little inhibitory effect and, in some cases, a stimulatory effect on phospholipid synthesis in HepG2 cells. On the other hand, our results are in contrast with the silybin and silymarin decreased hepatic synthesis PLs[45]. The discrepancy in results may stem from structural differences in the flavonoid molecules. The flavonoid component of AIE might have stimulated malonyl-CoA formation, fatty acids and, consequently, triacylglycerol synthesis. Hepatic lipase (HL) influences metabolism of HDL-C, LDL-C, and IDL risk factors for myocardial ischemia. In addition to converting large HDL2 particles to small dense HDL3 particles and LDL particles, IDLs to small dense LDL particles, hepatic lipase may facilitate hepatic uptake of lipoproteins.

Inhibition of hepatic lipase found primarily on the endothelium of the hepatic sinusoids would be expected to increase HDL and apo A, transferred with phospholipid into HDL-C during lipolysis. There is overwhelming evidence relating independent negative association between HDL-C and ischemia[46]. This factor also accounts for the pronounced synthesis of PLs.

The histopathological examination of heart sections from control rats showed a homogeneous myocardium without necrosis, edema and inflammation. The ISO-treated rat hearts revealed significant myofiber loss, extensive subendocardial necrosis, infiltration of inflammatory cells, marked myocellular edema plus vacuolar degeneration. AIE treatment at 100 mg/kg dose showed no sign of inflammation, but at the same time mild necrosis and edema were observed. At the next higher dose of 200 mg/kg, the myocardium appeared absolutely normal showing no inflammatory cell infiltration, edema and necrosis. This shows the protective effects of AIE on heart against ISO induced ischemia.

In conclusion, the data obtained from the present study indicates that the flavonoid-rich fraction of AIE contains bioactive molecules such as various flavonoid derivatives and their glycosides which may have beneficial effects as lipid lowering agents. To the best of our knowledge, our finding provides the first ever evidence on the role of flavonoid rich AIE in ischemia. It sounds necessary to identify and isolate the particular compound showing the desired effect from the AIE so as to understand its exact mechanism of action. However, the present study gives a preliminary indication that the flavonoid-rich extract has potential to act at multiple sites of
regulatory pathways. Considering all these facts, it is reasonable to undertake further studies on possible usefulness of flavonoid-rich AIE in treating biochemical and histopathological abnormalities of myocardial ischemia.

4 Acknowledgement

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5 Competing interests

The authors declare that they have no competing interests.

REFERENCES


Figure 1 Photomicrographs showing histopathology of heart. Photomicrographs showing: (A) normal architecture of rat myocardium in control group (H&E 40×); (B) extensive myocardial necrosis and inflammatory cell infiltration (→) in isoproterenol group (H&E 40×); (C) normal myocardium with the presence of sparse inflammatory cells and focal edema (→) in AIE 100mg/kg group (H&E 40×). (D) normal myocardium in AIE 200mg/kg group (H&E 40×); Figures are representative of at least four independent experiments.
Table 1 Effect of flavonoid rich methanolic leaf extract of *Acalypha indica* on the levels of plasma lipids and ischemic markers in isoproterenol-induced rats.

| Groups                        | TC (mg/dL) | TG (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) | FFA (mg/dL) | PL (mg/dL) | AIP (mg/dL) | non-HDL (mg/dL) |
|-------------------------------|------------|------------|-------------|-------------|--------------|-------------|------------|------------|----------------|----------------|
| Control                       | 74.7±6.14  | 52.7±3.27  | 42.7±3.24   | 21.4±8.31   | 10.5±0.65    | 50.18±3.57  | 80.35±6.22 | -0.26±0.03 | 31.96±8.54    |
| Isoproterenol [85 mg/kg bw]   | 107.5±49.19| 72.9±5.77  | 30.7±2.26   | 14.5±1.15   | 68.1±6.59    | 119.6±10.68 | 0.01±0.04  | 76.8±7.68   |
| Isoproterenol+ *A.indica* extract [100 g/kg bw] | 92.5±6.46  | 59.5±3.23  | 45.1±3.03   | 11.9±1.54   | 59.13±3.91   | 98.86±7.87  | -0.239±0.02 | 47.4±5.07   |
| Isoproterenol+ *A.indica* extract [200 g/kg bw] | 85.7±5.85  | 53.8±2.91  | 43.4±2.53   | 10.7±0.58   | 53.48±3.44   | 74.68±5.25  | -0.218±0.002 | 42.2±6.26   |

Values are given as means±S.D, n=6. Values not sharing a common super script differ significantly at *P* < 0.05, *P* < 0.05, *P* < 0.05 vs control (DMRT).
Table 2 Effect of flavonoid rich methanolic leaf extract of *Acalypha indica* on the levels of myocardial and hepatic lipids in isoproterenol-induced rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>myocardial lipids (mg/g wet tissue)</th>
<th>hepatic lipids (mg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>TG</td>
</tr>
<tr>
<td>Control</td>
<td>3.5±0.12</td>
<td>3.7±0.11</td>
</tr>
<tr>
<td>Isoproterenol [85 mg/kg bw]</td>
<td>4.94±0.10</td>
<td>5.49±0.18</td>
</tr>
<tr>
<td>Isoproterenol+<em>A.indica</em> extract [100 mg/kg bw]</td>
<td>4.21±0.15</td>
<td>4.61±0.16</td>
</tr>
<tr>
<td>Isoproterenol+<em>A.indica</em> extract [200 mg/kg bw]</td>
<td>3.82±0.11</td>
<td>4.36±0.12</td>
</tr>
</tbody>
</table>

Values are given as means±S.D, n=6. Values not sharing a common super script differ significantly at *P* < 0.05, *P* < 0.05, *P* < 0.05 vs control (DMRT).