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Effect of ethanolic extract of *Cyperus rotundus* L. against isoprenaline induced cardiotoxicity

Sadiya Khwaja*[,#], Tarique Mahmood* & Hefazat H Siddiqui

Faculty of Pharmacy, Integral University, Kursi Road, Lucknow-226 026, Uttar Pradesh, India

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Interruption of blood supply to heart results in acute myocardial infarction (AMI) and further leads to damaging of the heart muscles. Available drugs for the treatment MI have one or other side effects, and there is a need for development of better alternative drugs. Here comes the role of herbal sources. In this study, we evaluated cardioprotective effect of *Cyperus rotundus* on isoprenaline-induced myocardial infarction. Thirty five Wistar rats, aged 60-100 days with body wt. 150-200 g, pretreated with ethanolic extract of *Cyperus rotundus* L. (@ 250 and 500 mg/kg body wt.) orally before induction of myocardial necrosis by administrating isoprenaline (85 mg/kg, s.c.) on 19th and 20th day of the pretreatment period. The treated rats were examined for gross functioning of heart, heart weight/body wt. Ratio, and also observed histopathologically. Further, activities of various cardiac enzymes such as aspartate transaminase, alanine transaminase, creatinine kinase-myoglobulin, lactate dehydrogenase, and the gold marker troponin-I were also determined. The levels altered by isoproterenol were found to be restored significantly by the test extracts especially at higher dose. Biochemical observations *viz*., serum ALT (P <0.0001), AST (P <0.0001), creatine kinase-myoglobulin (CK-MB) (P <0.0001), LDH (P <0.0001) demonstrated significant cardioprotective activity of the ethanolic extract of *C. rotundus* (500 mg/kg body wt.), against isoprenaline induced myocardial infarction. These results were also substantiated by physical parameters and histopathological observations. All these results were comparable with that of two standard drugs metoprolol (10 mg/kg/day), ramipril (3 mg/kg/day) as well as polyherbal formulation Abana (50 mg/kg/day).

Keywords: Abana, Ayurveda, Cardiac markers, Cardioprotective, Coco-grass, Herbal drugs, Motha, Myocardial necrosis, Nut grass, Unani

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as heart attack, results from the interruption of blood supply to a part of the heart, causing damage to the heart muscles. Though there are many drugs available for the treatment of MI, the side effects of such drugs have encouraged researchers to look for safe alternative herbal sources. In this context, drugs from natural sources, plants in particular, are considered safer with comparatively lesser or nil adverse effects[1-3]. Considerable research has gone into plants with promising activities from time to time. The coco-grass or nut grass, *Cyperus rotundus* L. is one such plant, well-known in both Unani and Ayurveda for various potential including cardioprotective activity. Locally called Motha, *C. rotundus* is known for its analgesic, antiarthritic, antidiabetic, antidiarrhoeal, anti-inflammatory, antimicrobial, antimutagenic, antioxidant, antipyretic, apoptotic as well as cytoprotective, potential in traditional system of medicine[4]. In Ayurveda, *C. rotundus* is reported for treatment of blood diseases and obesity[3]. The tubers of the plant have reported to possess antioxidant, free radical scavenging, hypolipidemic and hypotensive properties[4,5].

Isoprenaline (ISO), a catecholamine, is a synthetic β-adrenergic agonist that causes severe stress in the myocardium and causes necrosis in the heart muscle[6]. In this study ISO was used to induce myocardial infarction as it serves as a standard model to study the beneficial effect of many drugs on cardiac function[7,8]. ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes[7,8]. Here, we studied the cardioprotective activity of ethanolic extract of rhizomes of *Cyperus rotundus*[7,8] and the comparative evaluation of the rhizomes of the plant extract against some of the clinically established drugs, such as metoprolol, a selective β1 receptor blocker, ramipril, an angiotensin-converting enzyme

*Correspondence:
E-mail: sadiyakhwaja89@gmail.com (SK);
ansaritariq79@yahoo.co.in (TM)
[#]Present add.: Medicinal Chemistry Division, CSIR-CIMAP, Lucknow, Uttar Pradesh, India

(ACE) inhibitor, and abana, a herbal medicine from the Himalaya Herbals.

Materials and Methods

Dried rhizomes of *Cyperus rotundus* were purchased from the local market of Lucknow and was authenticated (Ref No.NBRI/CIF/358/2013, Specification-NBRI-SOP-202) by a botanist of CSIR-National Botanical Research Institute, Lucknow (UP). A specimen sample of the same was preserved in the section of the Faculty of Pharmacy, Integral University, Lucknow, with the voucher No. NBRI-SOP-202, for future reference. Metoprolol tartrate was purchased from Sigma-Aldrich; Ramipril, from Sanofi India Limited; and Abana from The Himalaya Drug Company.

Preparation of ethanolic extract of *Cyperus rotundus*

The dried rhizomes of *C. rotundus* were chopped into small pieces and extracted with 90% ethanol at room temperature (24-30°C) for 48 h by soxhlet method. The extract was pooled, filtered and concentrated by evaporation to yield ethanolic extract of rhizomes. The concentrated extract was weighed according to the body weight, and was accurately dissolved in 0.9% normal saline for dose treatment daily.

Phytochemical studies

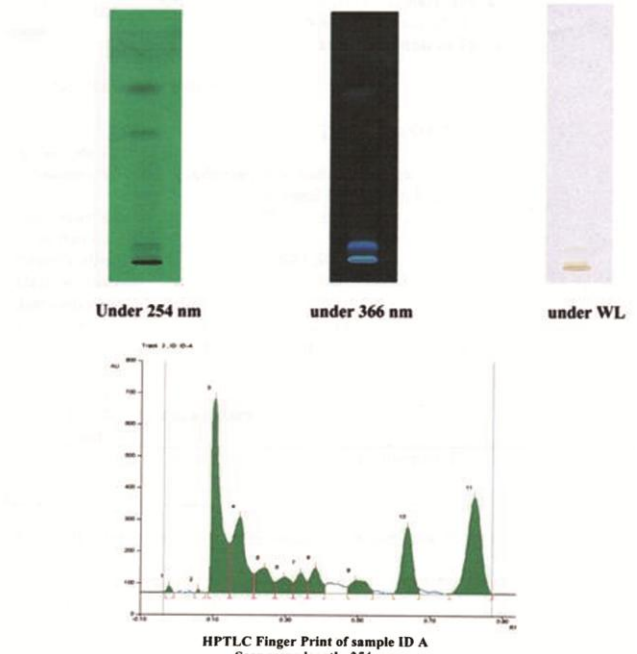
For phytochemical studies, *C. rotundus* rhizomes were extracted with 90% ethanol (v/v) and processed for HPTLC analysis at CSIR-NBRI, Lucknow. The application was done on Linomat 5 applicator (CAMAG) at volume 10 µL using the solvent system toluene:ethyl acetate (5:1) at scan wavelength-254 nm. The TLC plate was pre-saturated in CAMAG Twin Trough Chamber. The qualitative HPTLC analysis showed presence of 11 components in the form of 11 peaks with different R_f values (Fig. 1).

Experimental animals

Thirty five male Wistar rats (l50-200 g) aged 60-100 days were procured from Central Animal House, CDRI, Lucknow and housed in seven groups (2 test groups + 3 test drugs + 1 control+ 1 isoprenaline) of five rats in each in sanitized polypropylene cages containing paddy husk as bedding under standard laboratory conditions at room temperature (23±2°C) and relative humidity 45-55% with 12 h light/dark cycles after initial acclimatization for about a week. They had free access to standard rodent pellet diet and water *ad libitum*. The experimental study was conducted in accordance with the Institutional Animal Ethics Committee (Proposal No. IU/Pharm/MPharm/CPCSEA/13/02 dated 21 January 2013). The animals were grouped into seven groups of five animals each experimental and control groups and were treated as follows for 20 days[7].

Experimental protocol

Rats were pretreated with two different doses of 90% ethanolic extract of *C. rotundus* rhizomes (250 and 500 mg/kg/day), metoprolol (10 mg/kg/day; p.o.), ramipril (3 mg/kg/day; p.o.) and Abana (50 mg/kg/day; p.o.) using an oral feeding tube daily for 20 days[8]. At the end of 19 days of pretreatment with test drugs rats were weighed and isoprenaline HCl injection was administered at the dose of 85 mg/kg/day, s.c. twice at the interval of 24 h (i.e. 19th & 20th day of extract pretreatment)[9]. Rats were then sacrificed on 21st day, blood collected by tail vein under light ether anaesthesia and was allowed to clot for 30 min at room



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	5.5 AU	-0.02 Rf	20.9 AU	1.21 %	-0.01 Rf	0.3 AU	230.3 AU	0.48 %	unknown 1
2	0.05 Rf	0.8 AU	0.06 Rf	11.8 AU	0.69 %	0.08 Rf	0.4 AU	90.2 AU	0.19 %	unknown 2
3	0.09 Rf	1.7 AU	0.10 Rf	614.4 AU	35.71 %	0.14 Rf	35.9 AU	13867.4 AU	28.91 %	unknown 3
4	0.14 Rf	156.7 AU	0.17 Rf	240.1 AU	13.96 %	0.21 Rf	31.0 AU	7860.7 AU	16.38 %	unknown 4
5	0.21 Rf	81.6 AU	0.24 Rf	80.3 AU	4.67 %	0.27 Rf	34.7 AU	2834.7 AU	5.91 %	unknown 5
6	0.27 Rf	34.7 AU	0.29 Rf	51.1 AU	2.97 %	0.32 Rf	33.2 AU	1700.3 AU	3.55 %	unknown 6
7	0.32 Rf	34.0 AU	0.34 Rf	64.8 AU	3.77 %	0.36 Rf	37.5 AU	1516.5 AU	3.16 %	unknown 7
8	0.36 Rf	38.0 AU	0.38 Rf	80.9 AU	4.70 %	0.41 Rf	27.8 AU	2063.8 AU	4.30 %	unknown 8
9	0.47 Rf	21.9 AU	0.49 Rf	41.9 AU	2.44 %	0.54 Rf	8.3 AU	1775.4 AU	3.70 %	unknown 9
10	0.60 Rf	1.2 AU	0.64 Rf	210.7 AU	12.25 %	0.67 Rf	17.8 AU	5376.3 AU	11.21 %	unknown 10
11	0.75 Rf	1.3 AU	0.82 Rf	303.6 AU	17.64 %	0.87 Rf	1.4 AU	10647.9 AU	22.20 %	unknown 11

Fig. 1—HPTLC fingerprint profile (Qualitative analysis)

temperature[10].The serum was then separated by centrifugation at 3000 rpm at 30°C for 15 min and used for estimation of marker enzymes. The rats were sacrificed under high dose of ether and heart was removed immediately and washed with ice cold saline. Blood was collected from retro-orbital plexus and cardiac puncture both[5] by tapping the heart on a Kim wipe (absorbent pad) or surgical compress. This process was repeated until the heart was totally dry. The dried hearts were weighed[11], and kept in 10% formaline solution. All the histopathological observations were done in RS Diagnostic Pvt. Ltd., Lucknow. Further, the dried hearts were visually examined for inflammation, redness, capillary dilatation, scar formation and colour in all parts of heart and were graded[12].

Estimation of cardiac marker enzymes

Serum aspartate aminotransferase (AST), alanine transaminase (ALT), creatine kinase- myoglobulin (CK-MB) and lactate dehydrogenase (LDH) were determined using standard kits from Merck Specialties Pvt. Ltd. Troponin was estimated 4-6 h after giving isoprenaline using standard kits from Reckon Diagnostic Pvt. Ltd.

Statistical analysis

Values of heart weight and body weight ratio, AST, ALT, CK-MB and LDH were expressed as mean±SEM of five animals in each group. Isoprenaline group was compared with normal group, while plant extract groups were compared with isoprenaline group and these were calculated by one way ANOVA followed by Tukey's test.

Results

Gross examination of heart

In visual examination, the heart of isoprenaline (ISO) group showed marked inflammation, scar formation, and diffused compared to the normal control (NC) group. The grading shifted from grade 0 to 4. The standard (STD) group showed reduction in edema, capillary dilation, and scar formation, with little redness compared to the ISO group. Rats given *Cyperus rotundus* extract @ 500 mg/kg showed remarkable decrease in inflammation, redness, capillary dilation and scar formation as compared to the ISO damaged group. Its grading shifted from grade 4 to 2 (Table 1 and Fig. 2).

Heart weight:body weight ratio

The heart weight and heart weight/body weight ratio were analyzed in various treatment groups. The isoprenaline (ISO) group showed marked increase in heart weight due to hypertrophy as compared to the normal control (NC) group. Whereas, the standard (STD) group showed reduction in heart weight and heart/body weight ratio as compared to the ISO group. Similarly, the *Cyperus rotundus* extract (500 mg/kg) treated group showed remarkable decrease (P <0.001) in heart weight and heart/body weight ratio compared to the ISO group (Table 2).

Table 1—Grading of heart on basis of cardiac damage

Groups	Grading of cardiac damage
Normal control	Grade 0
Isoprenaline (85 mg/kg)	Grade 4
Metoprolol (10 mg/kg)	Grade 1
Ramipril (3 mg/kg)	Grade 2
Abana (50 mg/kg)	Grade 2
Cyperus rotundus (250 mg/kg)	Grade 3
Cyperus rotundus (500 mg/kg)	Grade 2

Table 2—Heart weight:body weight ratio ($\times 10^3$)

Groups	Value (Mean±SEM)
Normal Control	3.8±0.2
Isoprenaline (85 mg/kg)	5.4±0.1
Metoprolol (10 mg/kg)	4.0±0.34
Ramipril (3 mg/kg)	4.2±0.37
Abana (50 mg/kg)	4.6±0.38
Cyperus rotundus (250 mg/kg)	5.1±0.85
Cyperus rotundus (500 mg/kg)	4.2±0.24
Values are expressed as N=5±SEM	

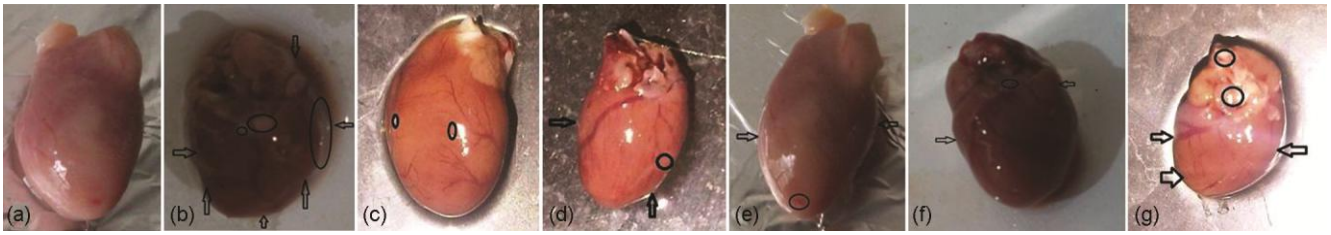


Fig. 2—Assessement and Grading of Heart of different groups. (a) Normal control; (b) Isoprenaline; (c) Metoprolol; (d) Ramipril (e) Abana; (f) *Cyperus rotundus* (500 mg/kg); and (g) *C. rotundus* (250 mg/kg)

Biochemical estimations

The effects of oral administration of ethanolic extract of *C. rotundus* on serum marker enzymes AST, ALT, LDH and CK-MB after 21 days are outlined in Fig. 3. Rats treated with isoprenaline (ISO) showed a highly significant increase (P <0.001) in activities of serum marker enzymes compared with that of normal rat (NC) group. Rats pretreated with metoprolol (STD group) compared to the ISO group showed a significant (P <0.001) reduction in cardiac marker enzyme. Pretreatment with high dose of *Cyperus rotundus* extract (500 mg/kg) to rats for 20 days, followed by ISO subcutaneous injection on the 19 and 20th days, elicited a significant (P <0.001) reduction in the ISO induced increased activities of AST, ALT, LDH, and CK-MB. The release of troponin-I was estimated by Rapid test kit after 4 h of infarction. ISO group showed the presence of troponin in serum. However, more than half of the animals in the standard group showed absence of troponin in their serum. *Cyperus rotundus* extract (500 mg/kg) remarkably decreased the release of troponin (Table 3).

Histopathological examination

Photomicrograph of rat heart of the normal control group (Fig. 4a) has shown the endocardium, myocardium and epicardium as well as papillary muscles and vasculature to be normal and healthy. There was no muscular hypertrophy or evidences of myositis (necrosis and/or round cell infiltrates), clearly visible in 10X (prominently). However, the ISO treated group exhibited focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium, vacuolar changes and prominent oedema along with chronic inflammatory cells were clearly visible in 10X (prominently) (Fig. 4b). Rat heart pretreated with the standard (STD) metoprolol (10 mg/kg) showed lesser degree of myonecrosis, myophagocytosis and lymphocytic infiltration, oedema and insignificant infiltration of inflammatory cells clearly visible in 10X (prominently) (Fig. 4c). Pretreated rat heart with *Cyperus rotundus* (500 mg/kg) showed little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagophytosis and subendocardium vacuolar changes clearly visible in 10X (prominently) (Fig. 4f). Similarly, pretreatments with ramipril, abana and *Cyperus rotundus* extract 250 mg/kg also exerted a protective effect as evident from

Table 3—Presence/absence of Troponin-I in different experimental groups on basis of cardioprotective activity

Groups	Troponin-I	
	+ve	–ve
Normal Control	-	5
Isoprenaline (85 mg/kg)	5	-
Metoprolol (10 mg/kg)	1	4
Ramipril (3 mg/kg)	2	3
Abana (50 mg/kg)	2	3
Cyperus rotundus (250 mg/kg)	3	2
Cyperus rotundus (500 mg/kg)	2	3

+ve: presence of enzyme; and –ve: absence of enzyme

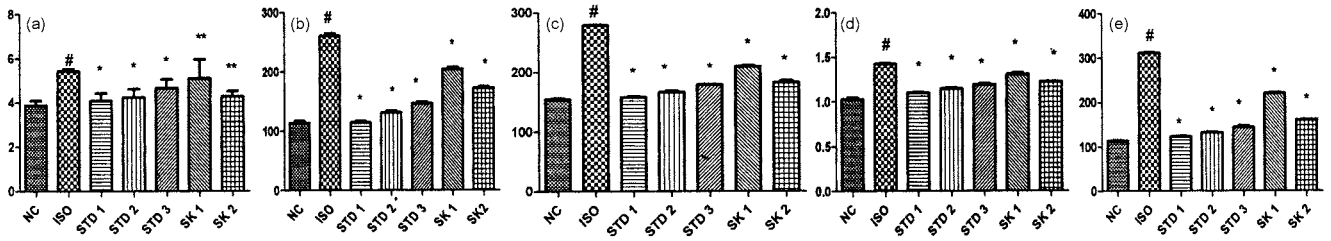


Fig. 3—Graphical representation of results of cardiac marker enzymes. (a) Heart wt.:body wt. ratio; Effect of *Cyperus rotundus* pretreatment on serum enzymes (b) ALT; (c) AST; (d) CK; and (e) LDH. [NC, normal control; ISO, isoprenaline control; STD 1, metoprolol; STD 2, ramipril; STD 3, Abana; SK 1, *Cyperus rotundus* low dose (250 mg/kg/day); and SK 2, *C. rotundus* high dose (500 mg/ kg/ day)]

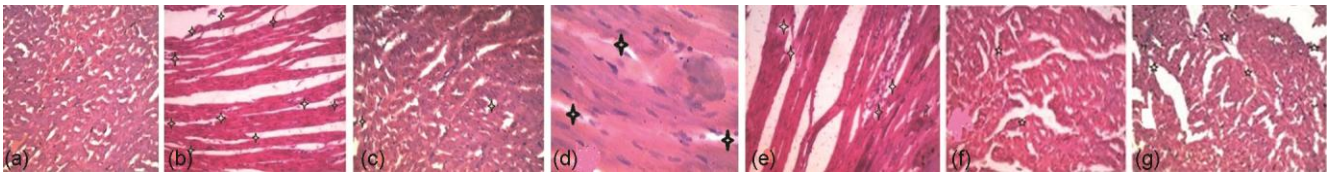


Fig. 4—Photomicrographs of histopathological studies. (a) normal control; (b) isoprenaline group; (c) metoprolol; (d) ramipril; (e) abana; (f) *Cyperus rotundus* (500 mg/kg); and (g) *C. rotundus* (250 mg/kg).

the normal myofibrillar structures with striations (Fig. 4 d, e and g). The cardioprotective effect of *Cyperus rotundus* extract 500 mg/kg was comparable to the test drugs as shown in most of experimental parameters, while in 250 mg/kg group effect was less.

Discussion

Myocardial infarction remains a leading cause of morbidity and mortality worldwide[11,13,14]. Heart attack demands prompt treatment to prevent permanent damage and to save lives. Plants *viz*., *Terminalia arjuna* (Roxb.) Wight & Arn.[13], *Marrubium vulgare* L.[14], *Punica granatum*[15], *Azadirachta indica*[16] and *Picrorhiza kurroa* (Royle Ex Benth)[17] are known to possess cardioprotective activity. The Nut grass, *Cyperus rotundus* L. too is no less[4,18]. Isoproterenol (ISO) causes significant damage to myocardium and endocardium and a significant increase in the levels of serum marker enzymes such as AST, ALT, CK-MB, LDH, and troponin-I. This might be due to the damage to the heart muscle, rendering the leakage of enzymes into the serum. The biochemical markers that are used widely in detection of myocardial necrosis, are CK-MB, LDH, and transaminases[7]. CK-MB has greater than 95% sensitivity and specificity for myocardial injury when measured between 24-36 h. Estimation of elevated serum enzymes is a useful guide for necrosis of myocardium[7].

Cyperus rotundus extract (250 mg/kg) didn't show significant protective effect but the same at 500 mg/kg showed significant cardioprotection against Isoprenaline induced damage ALT (P <0.0001), AST (P <0.0001), CK-MB (P <0.0001) and LDH (P <0.0001) (Fig. 3). Troponin test showed negative result (absence of troponin) in *Cyperus rotundus* (500 mg/kg) group compared to the isoprenaline group that showed positive result (Table 3). All these results were compared and found similar against two clinically established standard drugs metoprolol (10 mg/kg) and ramipril (3 mg/kg) as well as the polyherbal formulation Abana (50 mg/kg). These results were also confirmed by the histopathological observations which showed that normal architecture of the myocardium with no evidence of changes in the normal control group (Fig. 4a). Whereas, the cardiac sections of the ISO treated animals revealed degenerative changes in the muscle fiber, showing a coagulative necrosis (Fig. 4b). Pretreatment with, metoprolol, ramipril, abana and *Cyperus rotundus* extract exerted a protective effect as evident from the normal myofibrillar structures with striations (Fig. 4 c-g).

The cardioprotective effect of *C. rotundus* could be attributed to the presence of flavonoid content which has antioxidant potential[13]. Abi *et al*.[19] who studied the methanolic extract of *Croton sparciflorus* for its cardioprotective activity against isoproterenol induced myocardial infarction also observed low serum levels of various biochemical parameters such CK-MB, LDH and transaminases as observed in the present study.

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