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Original Research Article

Evaluation of cardioprotective potential of hydroalcohol peel extract of *Citrullus colocynthis* Linn. (Cucurbitaceae)

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Abstract

Purpose: *To investigate the cardioprotective potential of hydroalcoholic peel extract of C. colocynthis against adrenaline-induced myocardial ischemia in rabbits.*
Methods: *24 healthy male rabbits were split into 4 groups. Group-I rabbits were administered with subcutaneous injection of adrenaline for two days consecutively. Group-II, Group-III and Group- IV rabbits were given 100, 200 and 300mg/kg of peel extract of C. colocynthis for 14 days orally and the adrenaline was injected on the 14th and 15th day. Histopathology was done to the necrosis level.*
Results: *Adrenaline-induced group significantly ($p< 0.001$) elevated the levels of C-reactive protein (CRP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine-kinase (CK-MB) and troponin-I. Groups treated with plant extract showed cardioprotective effects by causing a significant decrease ($p < 0.001$) of all the above-mentioned biomarkers in comparison with the adrenaline treated group. The cardiac tissues of the adrenaline-treated group showed more necrosis, and mild disintegration of cardiac tissues was observed in groups that had been pre-treated with extract.*
Conclusion: *Pretreatment of rabbits with peel extract produces a cardioprotective effect against adrenaline-induced myocardial damage. Thus, the extract can potentially be utilized as a therapeutic agent for the treatment of cardiovascular disorders.*

Keywords: *Adrenaline, Citrullus colocynthis, Cardioprotective, Biomarkers, C-reactive protein*

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INTRODUCTION

In several developed countries, heart attack is a major cause of morbidity in men as well as in women. Because of changing lifestyle patterns, many developing countries, including Bangladesh, Afghanistan, Pakistan, and India, are catching up with this epidemic at an alarming rate [1]. Though synthetic medicaments are very effective in the treatment of cardiovascular disorders, but the associated side effects restricted their use [1]. Myocardial infarction (MI)

is a profound ischemic condition in which myocardial tissues undergo extreme necrosis as a result of an imbalance between blood supply and the oxygen demand of the myocardium [2].

Adrenaline is a catecholamine synthesized by the adrenal gland. Adrenaline binds to all adrenergic receptors and acts as a non-selective agonist consisting of beta-1, alpha-1, beta-2, alpha-2, beta-3 receptor and various others. It causes myocardial infarction at a higher dose [3]. Lipid peroxidation (LPO) leads to the depletion of cellular antioxidants and overproduction of nitrosative derivatives which results in MI [4]. Oxidative stress occurs due to the overproduction of reactive oxygen species (ROS) [3]. Adrenaline induced MI model is used to explore the cardioprotective effect of plant extract in the experimental animals.

The importance of Phytomedicines in the cure of various disorders is indispensable globally [5]. Plants are less toxic than synthetic medicines, they continue to provide important therapeutic agents not only in traditional but also in modern medicine [6]. *Citrullus colocynthis* Linn. (Cucurbitaceae) is a well-known medicinal plant. In Arab countries, it was commonly used to treat skin eruption in camels and insects bite [7]. It is traditionally used to treat asthma, cough, oedema, and constipation [8]. Its pulp and seeds possess anti-inflammatory properties [9]. Currently there is no experimental evidence to support its use as a cardioprotective agent. The current research was performed to evaluate the cardioprotective potential of the hydroalcoholic peel extract of *C. colocynthis*.

EXPERIMENTAL

Plant material and extract preparation

Fresh fruits of *C. colocynthis* plant were bought from a nearby Multan market. It was authenticated by taxonomists at the Department of Botany, Bahauddin Zakariya University, Multan. A voucher specimen (R.R. Stewart F.W. Pak.702/10) was deposited for further reference. The peel part of the fruit was subjected to shade drying. Foreign adulterants and vegetative waste were removed manually before grinding the dried peel into a coarse powder with the help of an herbal grinder and was stored in air-tight jars. The powder obtained from only one batch was used in the experiment. A 250 g *C. colocynthis* peel powder was soaked in a hydroalcohol solvent (70:30 v/v) for 9 days in 2.5L amber colored air-tight glass jars. Then it was filtered and the filtrate was evaporated at 37 degrees celsius under reduced pressure using a rotatory evaporator to obtain a thick paste-like consistency [10]. The extract of the peel of *C. colocynthis* was utilized for the evaluation of adrenaline-induced cardioprotective effect.

Animals

Male rabbits weighing 1.5 kg had been obtained from the nearby market of Multan. They were feed on standard, commercially available food and tap water *ad libitum.* The rabbits were kept at the Department of Biochemistry, Muhammad Institute of Medical and Allied Sciences, Multan. The temperature was maintained at 25 ^{o}C. The experiments were performed according to National Research Council guidelines [11] and were authorized by the Ethical Committee of Muhammad Institute of Medical and Allied Science Multan, Pakistan (approval no. MIMAS/03/Biochem/315/20).

Chemicals

Adrenaline, ethanol, and distilled water were purchased from M/s Sigma Chemical Company, St. Louis. MO. All the other chemicals used in the study were of analytical grade. AST, ALP, ALT, CRP, LDH kits were purchased from Javeed Pharmacy Lahore.

Acute oral toxicity dose test

Acute oral toxicity of the hydroalcohol peel extract of *C. colocynthis* was assessed in 12 rabbits. They were divided into 3 groups, each group contained 4 rabbits. Animals were kept on fasting for 24 h and dosed with the extract 350, 550 and 2000mg/kg body weight respectively. The rabbits were monitored for 14 days after dosing for signs of judder, lethargy, and death [5].

Study design

The rabbits were divided into 4-groups, each group contained 6 rabbits. Group-1 rabbits were administered 2mg/kg of adrenaline subcutaneously in a 24 h period for two successive days. Group-2 rabbits were pre-treated orally with 100mg/kg of extract for 14 successive days and 2 mg/kg of adrenaline was administered on the 14th and 15th day with 24 h gap. The rabbits in Group-3 were pre-treated orally with 200mg/kg of extract for 14 days consecutively and on the 14th and 15th day, 2mg/kg of adrenaline was administered with a gap of 24 h. Group-4 rabbits were pre-treated with 300mg/kg of extract for 14 days successively and on the 14th and 15th-day,

2mg/kg of adrenaline was administered at an interval of 24 h [1,12].

Then, 24 h after administering the final dose of adrenaline, the rabbits were anesthetized and blood samples were collected from each group to screen the cardiac biomarkers; ALP, CK-MB, ALT, troponin-I, CRP, AST, LDH levels [1].

Histopathological examination

Rabbits were killed under anesthesia, and the heart was dissected for histological examination. One cm ventricular portion of the heart was carefully transferred into a 10 % formalin solution. After that tissue had been submerged in the paraffin, a 5 µm thick segment was cut and stained with a hematoxylin-eosin dye and mounted in the diphenyl xyline. The histopathology of the cardiac muscle was determined with the help of a compound microscope attached to the camera, and micro-images were captured [13].

Statistical analysis

The data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Dunnett's multiple comparison test were carried out on the data using SPSS software. $P < 0.005$ and $p < 0.001$ were considered statistically significant as appropriate.

RESULTS

Acute oral toxicity

No mortality and morbidity were observed up to the 2,000 mg/kg dose level. This showed that LD_{50} of hydroalcoholic peel extract of *C. colocynthis* is considerably more than 2,000 mg/kg.

Biochemical parameters

The level of biochemical parameters ALP, ALT, AST, CKMB, CRP, troponin-I, LDH were found significantly high in adrenaline treated group-1 in comparison with extract-treated Group-2, Group-3 and Group-4. The levels of the different biochemical parameters in the heart tissues of adrenaline-induced and extract-treated groups were framed in Table 1.

Highly significant ($p < 0.001$) elevated levels of biochemical parameters CRP, AST, ALT, ALP, CKMB, LDH, and troponin-I were found in adrenaline treated Group-1. Group-2 rabbits pre-treated with the 100 mg/kg extract for 14 successive days presented a mild decrease in the levels of biochemical parameters. Group-3 rabbits pretreated with 200 mg/kg extract for 14 consecutive days exhibited a mild to moderate decrease in the biochemical parameters. Group-4 rabbits pre-treated with 300 mg/kg extract for 14 successive days considerably ($p < 0.005$) resisted the effects of adrenaline and maintained the biochemical parameters at a normal level as shown in Figure 1.

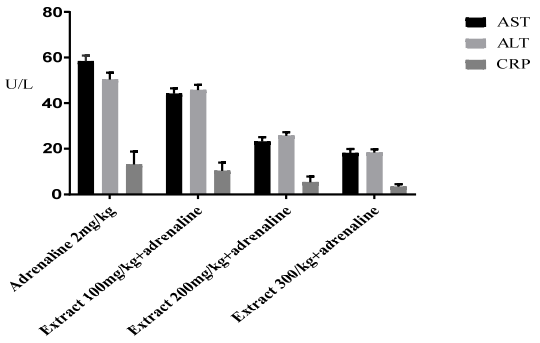

Figure 1: Cardioprotective effect of hydroalcohol peel extract of *C. colocynthis* LDH, troponin, CK-MB, ALP (a) and AST, ALT and CRP (b)

Table 1: Effect of hydroalcohol peel extract of *C. colocynthis* on biochemical parameters

Group	Dose	AST (U/L)	ALT (U/L)	ALP (U/L)	CKMB (U/L)	LDH (U/L)	Troponin-(I Ng/L)	CRP (mg/L)
Adrenaline	2mg/kg	58.4±2.5	50.4±3.0	487.1± 18.6	327.5±14.2	462.4±12.0	267.0±14.8	13.10±5.7
Extract+ adrenaline	100mg/kg+ 2mg/kg	44.1±2.3	45.7±2.3	349.9± 15.9	203.3±10.5	380.2±10.9	200.2±12.1	10.30±3.6
Extract + adrenaline	200mg/kg+ 2mg/kg	23.1±2.0	25.7±1.6	240.1±12.6	150.2±7.6	304.3±7.0	120.4±10.2	5.33±2.5
Extract + adrenaline	300mg/kg+ 2mg/kg	18.1±1.8	18.3±1.5	150.2±10.4	80.0±5.7	200.2±6.5	85.5±8.6	3.33±1.1

Data are showed as Mean ± S.D, $p < 0.001$

Histopathological features of heart tissues

Microscopic analysis of the heart tissue in the adrenaline intoxicated group showed a remarkable change in cardiac cell structure; hemorrhage, mononucleate cellular infiltration, muscular fibers tear, interstitial edema, disintegration, distention of capillaries, mottled staining, vascular disintegration, and obstruction of the myocardium were observed. The cardiac tissues of the adrenaline treated group showed more necrosis when compared with groups treated with the extract showed a slight deterioration in the cardiac structure as shown in Figure 2.

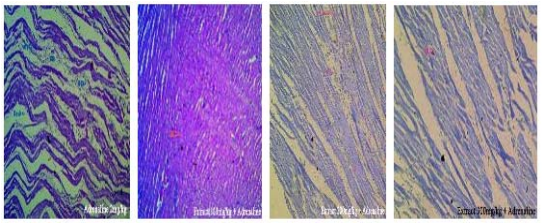

Figure 2: Photomicrographs of the ventricular portion of the heart section of adrenaline treated group (a), 100 mg/kg extract + adrenaline treated group (b), 200 mg/kg extract + adrenaline treated group (c) and 300 mg/kg extract + adrenaline treated group (d)

DISCUSSION

MI is a serious ischemic condition, in which sever necrosis of myocardial tissue takes place [14]. MI occurs due to exaggerated LPO that leads to the destruction of cellular antioxidants, which are mandatory for normal functioning [4]. The over production of ROS causes oxidative stress within the myocardium [3].

Myocardium has a variety of diagnostic biomarkers, such as troponin-I, LDH, CK-MB and CRP and transaminases such as AST, ALP, and ALT. In a case of metabolic injury to the heart, it transfers its material into ECF (Extra Cellular Fluid) [1]. In this study, adrenaline induced necrosis in the myocardium of experimental animals by increasing the workload/stimulation of adrenoreceptors, which increased the levels of the enzymes present in serum, resulting in the reduction of myocardial homogenate. This exaggerated lipid peroxidation and myocardial injury elevated the level of group-1 treated animals in comparison to all 3 groups (2,3,4) treated with *C. colocynthis* [Figure 1]. Adrenaline at a dose of 2mg/kg body weight is well documented [15-16]. The generation of free radicals and stimulation of lipid peroxidation is responsible for the permanent damage of the myocardium. Histological variation (intense myocardial necrosis) complemented the biochemical investigation.

All the 3 doses of *C. colocynthis* utilized in the current experiment showed significant resistance against adrenaline induced MI in a dose – dependent fashion. Phytochemical screening of the plant displayed the presence of phenolics, flavonoids, glycosides, and alkaloids [17]. Phenols and flavonoids were reported for the cardioprotection in many previous studies [18]. It is logical to believe that the cardioprotective activity of *C. colocynthis* may also be due to the presence of phenols and flavonoids. Previous studies have documented its hepatoprotective [19], antidiabetic and hypolipidemic potential [20]. The actual mechanism which is responsible for its cardioprotective effect though is still unclear. It is possible that it provides a cardioprotective activity by other mechanisms such as the stabilization of membranes and a reduction in the production of free radicals, by the inhibition of alpha-glucosidase, and could also lower the level of cholesterol and triglycerides in the body [21].

In addition, it could reduce the damage to vascular smooth muscle cells, monocytes, macrophages, endothelial cells and myocardium due to its strong antioxidant potential [22]. It may reduce the risk of hypertrophic cardiomyopathy by the opening of the K_{ATP} channel, and enhance the production of atrial natriuretic factor, or it may provide cardioprotection by enhancing the function of the mitochondria through the regulation of the level of calcium in the blood and the role of different proteins e.g., glycoproteins, structural and contractile proteins in the body [1]. The outcomes of this research shows that adrenaline administration caused myocardial damages illustrated by increasing levels of biochemical markers and histomorphological variations in the cardiac tissues, which were linked with secretion of ROS as shown in myocardial tissues. *C. colocynthis* offered cardioprotection by decreasing the oxidative stress induced in experimental animals by preventing the ROS mediated damage of catecholamine assault.

CONCLUSION

The hydroalcohol peel extract of *Citrullus colocynthis* exerts cardioprotective effect by down-regulating the oxidative stress caused by adrenaline. Less cardiac cell deterioration is observed in all three groups pre-treated with *C. colocynthis.* Thus, the plant extract possesses cardioprotective properties.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Imran Ahmad Khan and Shaukat Hussain Munawar conceived and designed the study, Muhammad Omer Iqbal and Perwasha Perwasha collected and analysed the data, Ashira Manzoor acquisition of experiment, Saba Kousar supervised the study, Zahid Manzoor wrote the manuscript.

REFERENCES

1. *Khan IA, Hussain M, Munawar SH, Iqbal MO, Arshad S, Manzoor A, et al. Jasminum sambac: A Potential Candidate for Drug Development to Cure Cardiovascular Ailments. Molecules. 2021;26(18): 5664.*
2. *Mundugaru R, Udaykumar P, Senthilkumar S, Bhat S. Cardioprotective activity of fruit of garcinia pedunculata on isoprenaline-induced myocardial infarction in rat. Bangladesh J Pharmacol 2016; 11(1): 231-235.*
3. *Mishra S, Naaz S, Ghosh AK, Paul S, Ghosal N, Dutta M, et al. Orally administered aqueous bark extract of Terminaliaarjuna protects against adrenaline-induced myocardial injury in rat heart through antioxidant mechanisms: an in vivo and an in vitro study. J Pharm Res 2016; 10(6): 454-478.*
4. *El-marasy SA, El Awdan SA, Hassan A, Abdallah HM. Cardioprotective effect of thymol against adrenaline-induced myocardial injury in rats. Heliyon 2020; 6(7): 04431.*
5. *Khan IA, Lodhi H, Munawar SH, Manzoor A, Manzoor Z, Raza MA, Iqbal O. Assessment of ameliorative effect of Aab-e-Shifa polyherbal formulation in experimentally-induced wound in rabbits. Trop J Pharm Res 2020, 19, 2357–2362.*
6. *Khan IA, Lodhi AH, Munawar SH, Manzoor A, Manzoor Z, Raza MA. Formulation and evaluation of allicin and curcumin gel improves normal and diabetic ulcers in rabbits. Lat Am J Pharm 2018, 37, 1602–1607.*
7. *Thangavel P, Ramasamy RK. Phytochemical screening and antibacterial and antifungal activity of the stem, leaf and fruit extracts using different solvent of Citrullus colocynthis (L.) Schrad. Res Rev J Pharmacogn Phytochem 2019; 8(1): 352-355.*
8. *Delazar A, Gibbons S, Kosari AR, Nazemiyeh H, Modarresi M, Nahar L, et al. Flavone C-glycosides and cucurbitacin glycosides from Citrullus colocynthis. DARU 2006; 14(3): 109-14.*
9. *Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. Screening of analgesic and anti-inflammatory activities of Citrullus colocynthis from southern Tunisia. J Ethnopharmacol 2010; 128(1): 15-19.*
10. *Bashyal S, GUHA A. Evaluation of Trachyspermum ammi seeds for antimicrobial activity and phytochemical analysis. Evaluation 2018; 11(5): 148-222.*
11. *Touitou Y, Portaluppi F, Smolensky MH, Rensing L. Ethical principles and standards for the conduct of human and animal biological rhythm research. Chronobiol Int 2004; 21(1): 161-170.*
12. *Begum S, Akhter N. Cardioprotective effect of amlodipine in oxidative stress induced by experimental myocardial infarction in rats. Bangladesh J Pharmacol 2007; 2(2): 55-60.*
13. *Abi Beaulah G, Mohamed Sadiq A, Sivakumar V, Jaya Santhi R. Cardioprotective activity of methanolic extract of Croton sparciflorus on isoproterenol induced myocardial infarcted wistar albino rats. J Med Plants Res 2014; 2(6): 1-8.*
14. *Khan IA. Pharmacotherapeutic modifications in cardiopulmonary patients during COVID-19 outbreak. J Coll Phy Surg Pak 2020, 30, 15–17.*
15. *Nmila R, Gross R, Rchid H, Roye M, Manteghetti M, Petit P, et al. Insulinotropic effect of Citrullus colocynthis fruit extracts. Planta Medica 2000; 66(05): 418-423.*
16. *Shah SMA, Akram M, Riaz M, Munir N, Rasool G. Cardioprotective potential of plant-derived molecules: a scientific and medicinal approach. Dose-response 2019; 17(2): 15-22.*
17. *Dar AI, Sharma V, Saxena R, Bansal S. Hepatoprotective Activity of Citrullus colocynthis Linn. J Ethnopharmacol 2011; 7(3): 44-60.*
18. *Kumar S, Kumar D, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of Citrullus colocynthis (L.) Schrad. methanolic fruit extract. Acta Pharmaceutica 2008; 58(2): 215-220.*
19. *Vakiloddin S, Fuloria N, Fuloria S, Dhanaraj SA, Balaji K, Karupiah S. Evidences of hepatoprotective and antioxidant effect of Citrullus colocynthis fruits in*

paracetamol induced hepatotoxicity. Pak J Pharm Sci 2015; 28(3): 111-222.

20. *Gurudeeban S, Ramanathan T. Antidiabetic effect of Citrullus colocynthis in alloxon-induced diabetic rats. Inventi Rapid: Ethno pharmacol 2010; 1(6): 112.*
21. *Saiprasanna B, Babu M, Ramani R, Kumar P, Rajeshree P. Cardioprotective effect of Pongamia pinnata hydro-alcoholic leaf extract against Isoproterenol induced myocardial infarction in wistar rats. Int J Med Pharmaceut Sci 2012; 2(3): 1-15*
22. *Iqbal MO, Khan IA, Manzoor A, Arshad S, Sial AS, Dar E, et al. Cardioprotective effect of hydroalcoholic leaf extract of Jatropha mollissima on isoproterenol-induced myocardial infarction in rats. Phcog Mag 2021; 17: 251-256.*