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Research Article

Comparative Cardioprotective Effect of Herbs of *Hridya dashemani* (*Karamarda- Carissa carandas* L., *Badara- Ziziphus jujuba* Lam.) versus *Arjuna-Terminalia arjuna* (Roxb.ex.DC.) Wight & Arn. against Isoprenaline induced Myocardial Infarction in Rats

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ABSTRACT

Hridya dashemani (ten cardioprotective herbs) includes fruits of Amra, Amrataka, Lakucha, Karamarda, Vrukshamla, Amlavetasa, Kuvala, Badara, Dadima and Matulunga. In Ayurveda, these herbs have been known to be conducive to heart. The present study was designed to evaluate the cardioprotective action of two herbs of *Hridya dashemani* fruits of *Karamarda* (*Carissa carandas* L.) and fruits of *Badara* (*Ziziphus jujuba* Lam.)] on the basis of biochemical & histopathological parameters in isoprenaline (ISO) induced myocardial infarction (MI) in experimental rats and to compare with stem bark of *Arjuna* (*Terminalia arjuna* (Roxb.ex.DC.) Wight & Arn.), a well-known cardioprotective herb. Total 36 male wistar albino rats were randomly divided into six groups. Group I- normal control (NC), group II- ISO induced MI, group III- positive control with hesperidin (100 mg/kg b.w), group IV- *Badara* (*Z. jujuba* – 450 mg/kg b.w), group V- *Karamarda* (*C. carandus* – 450 mg/kg b.w), group VI- *Arjuna* (*T. arjuna* – 450 mg/kg b.w). After 21 days of pre-treatment, experimental MI was induced in all groups except NC by injecting ISO subcutaneously (85 mg/kg) on 19th & 20th day at an interval of 24 hours. Serum biochemical parameters, including cardiac biomarkers and histopathological examination of heart tissues were evaluated. ISO treated rats had a significant ($p < 0.05$) elevation in serum levels of diagnostic marker enzymes (AST, ALT, CK-MB, LDH, ALP) when compared to NC. All the pre-treated groups had significantly ($p < 0.05$) reduced marker enzyme serum levels compared to the ISO treated control. The protective role of these herbs was further confirmed by histopathological examination. The comparison revealed that *Karamarda* (*C. carandus*) pre-treated had similar protective effect as *Arjuna* (*T. arjuna*) in various biochemical parameters (AST, ALT, ALP, LDH, CK-MB, HDL, cholesterol, creatinine). It may be concluded from the present study that 450 mg/kg b.w of water extract of the above herbs have cardioprotective action against isoprenaline induced MI, with *Karamarda* (*C. carandus*), *Arjuna* (*T. arjuna*) having almost similar effects. The study paves way to further evaluate these herbs as preventive and curative in cardiovascular disease (CVD) clinical management.

Introduction

Globally an estimated 17.9 million deaths were reported from cardiovascular disease (CVD) in 2019, representing 32% of all deaths and of these, 85% deaths were due to heart attack and stroke.[1] It is estimated that non-communicable diseases account for 63% of all deaths in India with 27% due to CVD.[2] This is a major challenge to the healthcare system and requires a safe, cost-effective and efficacious treatment. The therapeutic potential of herbs in healthcare, either for prevention or cure is

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well known in Indian system of medicine, *Ayurveda*. *Hridyadashemani*, is a group of ten fruits comprising *Amra (Mangifera indica* L.*)*, *Amrataka (Spondias pinnata* (L. f.) Kurz), *Lakucha (Artocarpus lakoocha* Roxb.*)*, *Karamarda (Carissa carandas* L.*)*, *Vrukshamla (Garcinia indica* Chois.*)*, *Amlavetasa (Garcinia pedunculata* Roxb.ex Buch.-Ham*)*, *Kuvala (Ziziphus sativa* Gaertn), *Badara (Ziziphus jujuba* Lam.), *Dadima (Punica granatum* L.), *Matulunga (Citrus medica* L.) which are stated to be conducive to the heart.[3] Based on previous phytochemical and related experimental studies,[4-7] it is hypothesized that *Hridyadashemani* could be useful in CVD but not investigated for its cardioprotective action. Hence the primary objective of this study was to evaluate the cardioprotective action of two herbs of *Hridyadashemani*, i.e., *Karamarda* & *Badara*. The bark of *Arjuna* (Terminalia arjuna (Roxb.ex.DC.) Wight & Arn.) is utilised in *Hrdroga* (diseases of the heart)[8,9] and have been extensively investigated through *in-vitro*, *in-vivo* and clinical studies for its efficacy in the management of CVD.[10,11] Previous animal experimental studies have shown that 250 mg/kg b.w aqueous and ethanolic extracts of *T. arjuna* bark have cardioprotective activity in ISO induced MI in rats.[12] *T. arjuna* stem bark powder, given 500 mg 8 hourly in patients of MI with angina and/or ischaemic cardiomyopathy, have shown to improve left ventricular ejection fraction and reduce left ventricular mass.[13] In another clinical study, *T. arjuna* bark extract was administered as adjuvant therapy in patients with refractory heart failure and found that the extract caused long lasting improvement in symptoms and signs of heart failure.[14] Thus, various evidences reflect the potential of *T. arjuna* in CVD and hence the secondary objective of the study was to compare the *in-vivo* cardioprotective efficacy of *Karamarda* (*Carissa carandas* L.), *Badara* (*Ziziphus jujuba* Lam.) with stem bark of *Arjuna* (Terminalia arjuna (Roxb.ex.DC.) Wight & Arn.) against isoprenaline induced myocardial infarction (MI) in rats. As the herbs investigated in the study are wild fruits and abundantly available, they have the potential to be suitably modified for the production of value-added products in the prevention and adjuvant therapy of cardiovascular diseases.

Material and Methods

Plant Material & Extract preparation

The fruits of *Karamarda* (*Carissa carandas* L.), *Badara* (*Ziziphus jujuba* Lam.) and stem bark of *Arjuna* (*Terminalia arjuna* (Roxb.ex.DC.) Wight & Arn.) were collected from their natural habitat (Fig. 1) The collected samples were authenticated by scientists at ICMR- National Institute of Traditional Medicine and herbarium of these plants maintained in the laboratory. (Voucher specimen No-RMRC- 1622,1624,1226). The collected herbs were cleaned, chopped into smaller pieces, and dried under shade. (Fig. 1) A portion of dried herbs were powdered and stored in containers for further study. The dried powder of the herbs was soaked in water for 24 hours and the juice extracted, to be administered to animals. Based on the recommended human dose,[15-17] stock solution of the herbs was prepared in such a concentration that the requisite dose (450 mg/kg body weight) could be obtained by administration of stock solution.

Chemicals and Reagents

Hesperidin & Isoproterenol hydrochloride, were purchased from 15627-5G, CAS: 51-30-9, SIGMA-ALDRICH, USA. All other chemicals and reagents used in the study were procured from standard firms and were of analytical grade.

Experimental Animals

Albino rats of wistar strains of either sex weighing 150 to 250 g were obtained from the animal house (Registration No.548/2002/CPCSEA) attached to SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved and performed in compliance with the guidelines from the Institutional Animal Ethics Committee under reference no. SDMCRA/IAEC/H-D-01 dated 7/09/2019. All experimental procedures were strictly carried out following ethical guidelines.

Six animals were housed in a poly-propylene cage with stainless steel top grill. The dry wheat husk (post hulled) was used as bedding material and was changed every 3rd day. The animals were fed with normal rat diet- Laboratory Animal feed pellets supplied by VRK Nutritional Solutions and tap water ad libitum throughout the study. They were acclimatized in the laboratory condition for 7 days prior to the experiment in standard laboratory conditions, 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and relative humidity of approximately 50%.

Induction of Myocardial Infarction

Isoprenaline was dissolved in normal saline and injected subcutaneously at a dose of 85 mg/kg body weight once daily for 2 consecutive days.

Experimental Method

The *in-vivo* cardioprotection was evaluated against Isoprenaline induced myocardial infarction (MI) in wistar albino rats.[18] Thirty-six male wistar albino rats weighing 150 to 250 g were randomly divided into six groups (n=6) as follows:

Group I-Normal control (NC); received tap water orally for 21 consecutive days and served as normal control group. Group II-ISO induced MI control (ISO); rats received tap water orally for 21 consecutive days and in addition received isoprenaline 85 mg/kg, subcutaneously on 19th and 20th day at an interval of 24 hours. Group III-Standard (SH); rats received 100 mg/kg b.w of hesperidin orally for 21 consecutive days. Group IV-ZJ; rats were pre-treated

Table 1: Showing the grouping of animals for the study

Group No and Code	*Drug*	*Dose*
I- NC	Normal control	Tap water
II- ISO	Isoprenaline induced MI Control	85 mg/kg b.w
III- SH	Standard - Hesperidin	100 mg /kg b.w
IV- ZJ	*Badara* (*Z. jujuba*)	450 mg/kg b.w
V- CC	*Karamarda* (*C. carandus*)	450 mg/kg b.w
VI-TA	Arjuna (*T.arjuna*)	450 mg/kg b.w

orally with 450mg/kg b.w of water extract of *Badara* (*Z. jujuba*) for 21 consecutive days. Group V - CC; rats were pre-treated orally with 450 mg/kg b.w of water extract of *Karamarda* (*C. carandus*) for 21 consecutive days. Group VI-TA; rats were pre-treated orally with 450 mg/kg b.w of water extract of Arjuna (*T. arjuna*) for 21 consecutive days. In addition to the respective extracts, group III, IV, V and VI received isoprenaline 85 mg/kg b.w, subcutaneously on 19^{th} and 20^{th} day at an interval of 24 hours.

The initial weight of all animals was recorded prior to the administration of drugs and dose of medicine was calculated accordingly. Test drug and standard drug were administered to respective groups at morning hours and continued for 21 days. (Table 1) On 19^{th} day, body weight was again recorded, accordingly, the Isoprenaline dose was calculated and 1^{st} dose of Isoprenaline injection(s/c) was given. On 20^{th} day, i.e., after 24 hours of 1^{st} dose, 2^{nd} dose of Isoprenaline injection was given (s/c). On 21^{st} day, blood samples were collected through supraorbital puncture by capillary for estimation of serum biochemical parameters like CK-MB, aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, albumin, cholesterol, triglycerides, HDL (High-density lipoprotein) as per standard methods. Rats were then sacrificed by overdose of diethyl ether anesthesia. The abdomen was opened by midline incision. Then the heart was dissected with aorta and transferred to normal saline for histopathology in 10% formalin solution. The section was cut at 5 µm thickness, stained with hematoxyline–eosin stain, and mounted in DPX (Diphenyl xyline). The histopathological changes of heart tissue were observed under a compound microscope and their micro-photographs were taken.

Statistical Analysis

The experimental data were expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple t-test for comparison with positive control and Tukey test for inter-group comparisons using statistical software. A level for $p < 0.05$ was considered to be statistically significant.

Results

Table 2 represents the effect of the herbs on AST, ALT, CK-MB, LDH, ALP in normal and ISO- induced animals. A significant ($p < 0.05$) elevation was observed in the serum levels of diagnostic marker enzymes (AST, ALT, CK-MB, LDH, ALP) in ISO group when compared to NC. The other pre-treated groups (SH, ZJ, CC and TA) significantly reduced serum levels of marker enzymes AST, ALT, CK-MB, LDH, ALP when compared with ISO, except CC whose decreased levels in LDH was not statistically significant.

The effect of herbs on other biochemical parameters like Total cholesterol, triglycerides, high-density lipoprotein, creatinine, and albumin in normal and ISO induced animals are represented in Table 3. In ISO group, there was increase in triglycerides, albumin but the increase was not statistically significant, while there was a significant increase in creatinine (0.73 ± 0.07). There was decrease in total cholesterol and HDL in ISO group but the decrease was not statistically significant. Among the pre-treated groups, there was a significant increase in HDL in SH, ZJ group and a significant decrease in creatinine and albumin in all the groups. Concerning the ponderal changes, there was a significant increase in the weight of the heart in ISO group and a significant decrease in the TA group. (Table 4)

Histopathology

Examination of the sections of the heart from the ISO group revealed extensive changes in cytoarchitecture. There was marked degenerative changes in the form of pale vacuolated muscle fibres, areas of necrosis and inflammatory infiltration. All the rats in this group exhibited chronic lymphocytic infiltration in all areas of the tissue. (Fig. 2) Among the pre-treated groups, ZJ showed areas of necrosis, and inflammatory infiltration and there was no reduction in the degenerative changes compared to ISO. In CC group few animals exhibited small areas of

Table 2: Effect of the herbs on the activities of cardiac marker enzymes in serum of normal and ISO-induced myocardial infarcted rats.

Group	AST	ALT	CK-MB	LDH	ALP
Group I-NC	87.5 ± 2.09	47.17 ± 1.85	80.17 ± 3.07	210.67 ± 34.22	359.5 ± 49.93
Group II- ISO	$164.83 \pm 9.76^{@**}$	$93.83 \pm 6.71^{@**}$	$174.27 \pm 20.20^{@**}$	$616.7 \pm 55.47^{@**}$	$828.33 \pm 49.98^{@**}$
Group III- SH	$116.17 \pm 3.91^{\#**}$	$42.17 \pm 1.35^{\#**}$	$84.82 \pm 4.08^{\#**}$	$311.15 \pm 24.67^{\#**}$	$417 \pm 76.01^{\#**}$
Group IV- ZJ	$114.67 \pm 3.38^{\#**}$†	$72 \pm 2.82^{\#**}$†	$100.67 \pm 4.45^{\#**}$	$284.7 \pm 25.56^{\#**}$†	$599.33 \pm 71.71^{\#**}$†
Group V- CC	$81.67 \pm 5.07^{\#**}$	$46.33 \pm 6.37^{\#**}$	$82.17 \pm 3.74^{\#**}$	$504.83 \pm 18.17^{\#}$	$242.17 \pm 48.31^{\#**}$
Group VI-TA	$85.5 \pm 4.52^{\#**}$	$43.33 \pm 1.71^{\#**}$	$81.67 \pm 1.67^{\#**}$	$461.33 \pm 34.21^{\#**}$	$315 \pm 47.32^{\#**}$

$^{@}$ ISO compared with NC; # Other groups compared with ISO; ** $p<0.05$; † significant when compared with Group 6- TA

Table 3: Effect of the herbs on biochemical parameters in serum of normal and ISO-induced myocardial infarcted rats

Group	*Total Cholesterol*	*Triglycerides*	*High-density lipoprotein*	*Creatinine*	*Albumin*
Group I-NC	70.33 ± 4.73	89.67 ± 5.22	19.33 ± 1.45	0.37 ± 0.02	4.07 ± 0.08
Group II- ISO	58.5 ± 6.28$^{@}$	118.33 ± 10.70$^{@}$	12.38 ± 0.42$^{@}$	0.73 ± 0.07$^{@}$**	4.17 ± 0.07$^{@}$
Group III- SH	94.17 ± 9.19$^{\#}$**	76.83 ± 11.61$^{\#}$**	34 ± 3.42$^{\#}$**	0.5 ± 0.03$^{\#}$**	3.63 ± 0.04$^{\#}$**
Group IV- ZJ	79.17 ± 8.41$^{\#}$	99 ± 8.79$^{\#}$	46.17 ± 3.21$^{\#}$**†	0.47 ± 0.03$^{\#}$**	3.68 ± 0.09$^{\#}$**
Group V- CC	61.67 ± 6.34$^{\#}$	76.17 ± 6.93$^{\#}$**	18.52 ± 2.31$^{\#}$	0.45 ± 0.04$^{\#}$**	2.95 ± 0.12$^{\#}$**
Group VI-TA	91.83 ± 12.23$^{\#}$**	82.83 ± 4.72$^{\#}$**	21.07 ± 2.38$^{\#}$	0.4 ± 0.03$^{\#}$**	3.5 ± 0.13$^{\#}$**

$^{@}$ ISO compared with NC; # Other groups compared with ISO; ** $p < 0.05$; † significant when compared with Group 6- TA

Table 4: Effect of the herbs on ponderal parameters in normal and ISO-induced myocardial infarcted rats

Group	*Weight of the heart*	*% change in body weight*
Group I-NC	0.79 ± 0.01	21.44 ± 1.84
Group II- ISO	0.98 ± 0.05$^{@}$**	2.99 ± 0.53$^{@}$**
Group III- SH	0.85 ± 0.07$^{\#}$	11.24 ± 1.04$^{\#}$**
Group IV- ZJ	0.96 ± 0.03$^{\#}$	5.99 ± 0.94$^{\#}$
Group V- CC	0.84 ± 0.03$^{\#}$	3.05 ± 0.18$^{\#}$
Group VI-TA	0.77 ± 0.04$^{\#}$**	5.16 ± 0.43$^{\#}$

$^{@}$ ISO compared with NC; # Other groups compared with ISO; ** $p<0.05$

Fig. 1a: Fresh fruits of Badara (*Z. jujuba* Lam.)

Fig. 1b: Dried de-seeded fruits of Badara (*Z. jujuba* Lam.)

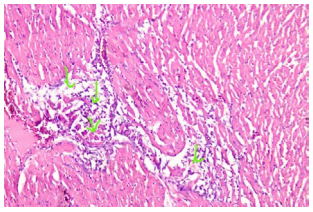
Fig. 1c: Fresh fruits of *Karamarda* (*C. carandas* L.)

Fig. 1d: Dried fruits of *Karamarda* (*C. carandas* L.)

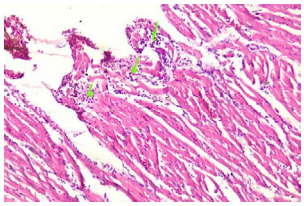
Fig. 1e: Fresh stem bark of *Arjuna* (*T. arjuna* (Roxb. ex. DC.) Wight & Arn.)

Fig. 1f: Dried stem bark of *Arjuna* (*T. arjuna* (Roxb. ex. DC.) Wight & Arn.)

Fig. 1: Fresh & dried herbs used for the study

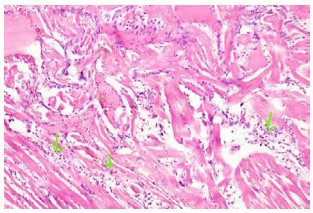


Necrosis

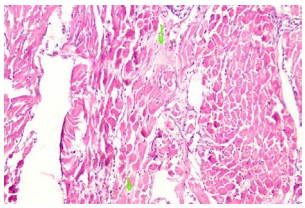


Inflammatory infiltration

Fig. 2a: Histopathology of heart tissue- Positive Control (PC)

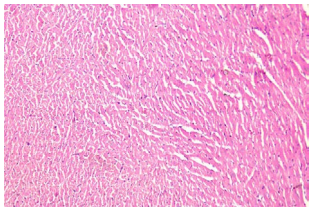


Inflammatory infiltration

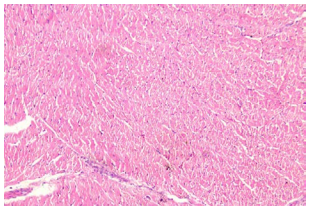


Necrosis areas

Fig. 2b: Histopathology of heart tissue- Group IV-1 Test drug- *Badara* (*Z. jujuba*)

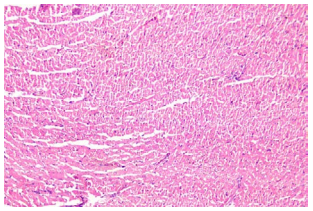


No histological changes

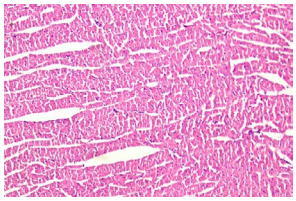


Reduced histological changes

Fig. 2c: Histopathology of heart tissue- Group V-2 Test drug- *Karamarda* (*C. carandus*)



Minor histological changes



No histological changes

Fig. 2d: Histopathology of heart tissue- Group VI- Arjuna (*T. arjuna*)

Fig. 2: Photomicrographs of representative heart section of different groups

necrosis and mild inflammatory infiltration, while in TA pre-treated group, there was mild degenerative changes and two animals showed no necrosis in the tissue. Thus CC & TA showed mild degenerative changes when compared to the isoprenaline control group.

Comparison of cardioprotective effect of two herbs of *Hridya dashemani* (*C. carandas* L., *Z. jujuba* Lam.) with *arjuna* (*T. arjuna* (Roxb.ex.DC.) Wight & Arn) against isoprenaline induced myocardial infarction in rats

One of the objectives of the current study was to compare the cardioprotective action of two herbs of *Hridya dashemani*, (*Karamarda*, *C. carandas* L. and *Badara*, *Z. jujuba* Lam.) with *Arjuna*. (Table 2,3 and 4) Comparing the diagnostic marker enzymes, like AST, ALT, CK-MB, LDH, ALP revealed that TA had a statistically significant decrease in AST, ALT, ALP levels when compared to ZJ while CC has shown almost similar effects as TA group. With respect to CK-MB, though *Arjuna* has the least levels, it is not statistically significant compared to other pre-treated groups. ZJ had significant increase in HDL & decrease in LDH compared to TA & CC groups. There was no significant difference in creatinine, albumin, cholesterol, or triglyceride levels among the pre-treated groups (ZJ, CC) compared to TA. With respect to ponderal changes, there was a significant decrease in weight of the heart in TA when compared to other pre-treated groups, almost near the normal group's levels. The study shows that the cardioprotective action of CC is comparable to TA as revealed by diagnostic marker enzymes.

Discussion

Hridya dashemani includes ten fruits that are conducive to the heart.[3] Of these ten, two herbs were chosen for the present study as these are easily available sour fruits and edible. In Ayurveda, *Arjuna* (TA) bark is recommended in heart disease[8,9] and in recent years it has gained importance in the treatment of cardiac disorders.[19] Previous experimental and clinical studies suggested that TA possesses anti-ischemic, antioxidant, hypolipidemic, and antiatherogenic activities.[20] An earlier clinical study has implicated that TA is effective in patients with symptoms of stable angina pectoris.[21] Hence TA was included in the present study as a positive standard to compare with the test herbs (ZJ, CC).

In the study, hesperidin was used as a standard drug, a naturally occurring flavonoid found to possess anti-inflammatory, antioxidant and cardiovascular effects.[22] Studies have proven hesperidin to possess cardioprotective effect against isoproterenol-induced myocardial ischemia.[23,24]

Isoprenaline is a synthetic catecholamine and a powerful nonselective β-agonist which is reported to cause oxidative stress leading to impairment in cardiac function and deleterious effects on myocardium similar to MI in humans. Hence, it serves as a simple, non-invasive, economical and well-standardized model to study the cardioprotective effects of many drugs.[25] The significant ($p<0.05$) increase observed in the levels of serum diagnostic marker enzymes (AST, ALT, CK-MB, LDH, ALP) in ISO group as compared to NC is evidence to damage caused by isoprenaline. Cystolic enzymes like LDH, CK-MB are the diagnostic markers of myocardial tissue damage as they leak out from damaged tissues into blood when the cell membranes become permeable or ruptured.[26] Thus the elevated levels of these markers suggest necrotic damage in the myocardial membrane of ISO treated rats. This observation agrees with earlier studies[27] which show elevated plasma marker enzyme levels in plasma as indicative of necrotic lesions in the myocardial membrane. An increase in heart weight and marked degenerative changes in the cytoarchitecture of ISO group in the current study are also suggestive of myocardial damage.

Pre-treatments with 450 mg/kg b.w of *Badara* (*Z. jujuba*), *Karamarda* (*C. carandus*) & *Arjuna* (*T. arjuna*) for a period of 21 days individually has shown a significant decrease in the serum marker enzymes (ALT, AST, CK-MB, LDH, ALP), which shows its cardioprotective action. Previous studies provide evidence for the relation of aminotransferases (ALT & AST) with cardiovascular disease (CVD),[28] and elevated levels have been used as a risk factor for CVD along with other biomarkers.[29] Creatine kinase is an enzyme found in three isoenzymes, of which CK-MB is useful for the diagnosis of MI. Thus CK-MB is used as a criterion for myocardial injury along with other biomarkers.[30] In the present study, the pre-treated groups have decreased levels of serum CK-MB compared to the ISO group, which shows the cardioprotective action of all the herbs as previous studies have shown that serum levels of CK-MB are proportional to myocardial damage.[30-32] An increase in lactate dehydrogenase (LDH) activity is observed 6 to 12 hours following MI.[30] An analysis of LDH and its isoenzymes between 24 to 48 hours after the onset of chest pain is one of the confirmatory tests for acute MI and other cardiac markers.[33] In the present study, all the pre-treated groups have decreased serum levels of LDH when compared with ISO. Previous research work suggests a positive association of ALP with CVD events & deaths.[34] An elevated serum ALP level may promote inflammation, vascular calcification, which lead to damage in vascular integrity and promote atherosclerosis, thus increasing risk to CVD.[35] In the present study, all the pre-treated groups have significantly decreased serum levels of ALP when compared with ISO, showing its cardio-protective action. Lipids play a major role in cardiovascular disease and triglyceride concentration is the measure that is positively associated with the incidence of CVD.[36] Also, many studies have seen an inverse relationship of HDL to atherosclerotic CVD.[37] In the present study, a significant increase in HDL in the pre-treated groups reflect protection from atherosclerotic CVD. Impairment of renal function is a potential marker for CVD risk; creatinine is particularly an important indicator.[38] In the present study, the significant

decrease in creatinine levels in the pre-treated groups shows its nephroprotective action.

Concerning the ponderal changes in heart weight and body weight, the study revealed that the pre-treated groups could protect the heart from damage, especially the TA group with heart weight nearing to NC group. Histopathological studies reveal that CC & TA showed mild degenerative changes compared to the isoprenaline control group, suggesting that CC & TA could adequately prevent isoproterenol-induced myocardial damage. Thus, the effect of 450 mg/kg b.w of ZJ, CC & TA on various biochemical markers and histology reveals that the herbs provide cardioprotection comparable to the standard. Often neglected, nutraceuticals and dietary interventions could play a key role in preventing and curing non-communicable disease.[39] The fruits explored in the present study (Z. *jujuba* and *C. carandus*) are sour and edible; thus, their inclusion in diet might have greater significance in preventing cardiovascular disease. In addition, suitable pharmaceutical modifications of these fruits could lead to products that may be included as adjuvant therapy in clinical management.

Among the three herbs, TA showed a significant decrease in the majority of the biomarkers compared to other groups. This finding is similar to a previous study[12] which reports the protective action of aqueous & ethanolic extracts of *T. arjuna* against isoprenaline induced increase in liver enzymes. Gallic acid, an important flavonoid of *T. arjuna*[40] has been reported to have a protective effect on the myocardium[41] and may be responsible for cardioprotection.

ZJ treated group had significantly lower levels of LDH when compared to other groups. The fruits of *Z. jujuba* Lam are a rich source of flavonoids[42] and the cardioprotective & antioxidant effect of flavonoids are well documented.[43] The cardioprotective action observed in the current study, could be due to the presence of flavonoids in the fruits of *Z. jujuba*. A previous study has shown that pre-treatment with jujube polyphenols extracted from the peel of the fruits prevented myocardial ischemia induced by isoprenaline in rats.[44]

Fruit juice of CC contains major phenolic compounds such as cyanidin-3-glucoside, ferulic acid, rutin, flavonoid, quercetin and anthocyanin compounds, which may be responsible for its anti-inflammatory, hepatoprotective, antihyperlipidemic and antioxidant activity.[45] In the current study, CC pre-treated group has shown effects comparable to TA group on various biochemical parameters (AST, ALT, ALP, LDH, CK-MB, HDL, cholesterol, and creatinine).

Thus, from the present study, it may be concluded that all three herbs (450 mg/kg b.w of ZJ, CC & TA) have cardioprotective action against isoprenaline induced MI, with CC & TA having almost similar effects while ZJ showing better effect in reducing LDH levels and increasing HDL levels. Further evidence-based research on the dietary inclusion of these fruits to evaluate its cardioprotective role will be of great value in the prevention of CVD.

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