Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

An Experimental Study to Assess the Effect of *Dadima* as *Hrudya* on *Hrudaya* by Inducing Cardio-Toxicity in Wistar Strain Albino Rats

Author: Arfa Mohamed Akif[1]

Co Authors: Shrikanth P H[2], Arhanth Kumar A[3] and Sudhakar Bhat[4]

[1-3]Department of Samhita & Siddhanta, Shri Dharmasthala Manjunatheshwara Ayurveda Medical College & Hospital, Udupi, Karnataka India
[4]Department of Pharmacology & Toxicology, Shri Dharmasthala Manjunatheshwara Research Centre in Ayurveda & Allied Sciences, Udupi, Karnataka, India

ABSTRACT

Background: *Dadima* is a nutrient packed fruit, rich in phytochemical compounds like punicalagins which is a potent anti-oxidant responsible for free radical scavenging activity against oxidative stress. It has emerged to be the best *Hrudya Dravya* and a *Nitya Sevaniya Rasayana*, often referred as *Pathyakari* and *Sada-hita*. *Hrudya* is a unique phenomenon which is pondered as pleasing and congenial to the *Hrudaya*. *Amla Rasa* is considered to be the best among the *Hrudya Dravyas*. Its cardioprotective, anxiolytic, anti-inflammatory, anti-apoptotic, anti-depressant and CNS stimulant activity has shown to prevent and reverse cardiovascular diseases. **Objective:** To assess the *Hrudya* action of *Dadima*, one of *Charakokta Hrudya Dashemani*. **Methodology:** A pharmacological study was carried out to ascertain the cardioprotective activity of *Dadima* on Isoprenaline (ISO) induced Myocardial infarction (MI) in wistar strain albino rats. Fresh fruit juice was administered to the rats for a stipulated period of time and the study was conducted through different experimental parameters. **Observation & Results:** The bodily weight changes, bio-chemical investigations, anti-oxidant and histopathological study on heart against ISO induced MI, showed statistically significant results establishing its anti-oxidant potential, proving the efficacy of the drug *Dadima* as *Hrudya*.

Key Words: *Dadima, Hrudya, Hrudaya, Myocardial infarction, Anti-oxidant, Oxidative stress*

INTRODUCTION

Cardiovascular diseases are rising at an exponential scale for a couple of decades now. An estimated 17.9 million people have died from cardiovascular diseases annually, representing 31% of all global deaths emerging as a state of epidemic. As the magnitude of cardiovascular diseases continue to accelerate worldwide, the pressing need for increased awareness and more focused preventive response is the need of the hour for root level rectification of the cause and to bring about the quality of life.

Among the *Hrudya Mahakashaya*[1] mentioned in *Charaka Samhita*, *Dadima* is taken up for the study. It possesses *Amla*, *Madhura* and *Kashaya Rasa*, *Madhura Vipaka*, *Anushna Veerya* and

Snighdha Guna[2]. *"Hrudyaani hrudayaya hitaani amlatvaat" Hrudya* is *Hrudaya Hita* because of the presence of *Amla Rasa* in it[3], which helps in breaking down the *Hrudroga Samprapti* effectively. *Amla Rasa* and *Rakta Dhatu* are closely related to each other in terms of *Panchabhautikatva, Guna*, influence on *Dosha* and *Hrudaya, Nidana, Roga* and *Chikitsa* thereby contributing their combined effect in understanding the mechanism of *Hrudya Karma*. *Dadima*, the cheapest and easily available dietary fruit can play a major role in the treatment of *Hrudroga* in a preventive way, as oxidative stress forms the main reason for CVD.

Objective:

To assess the *Hrudya* action of *Dadima*, one of *Charakokta Hrudya Dashemani.*

METHODOLOGY

To assess the effect of *Dadima* on Isoprenaline (ISO) induced Myocardial infarction (MI) in Wistar strain albino rats.

- Weight of the heart. body weight changes and clotting time.
- Anti-oxidant property of heart– catalase, lipid peroxidase and protein.
- Cardiac cyto-architecture (histopathology).
- Serum biochemical parameters - SGOT, SGPT, serum total cholesterol, serum triglycerides, serum creatinine, serum glucose, LDH activity, Blood urea and CK-MB.

Plant material and extract preparation:

Fully matured seasonal fruits of *Dadima* (pomegranate) were collected from nearby market in Udupi. Fresh fruits were peeled and seeds were collected in a soft white cotton cloth and squeezed to get the juice extract, measured and used.

Experimental animals:

Wistar strain albino rats of either sex, weighing between 200-250g were obtained from the animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi. They were housed in standard transparent polypropylene cages with wheat husk bedding, renewed every 24hours. They were kept under controlled room temperature at around 25±3̊c with relative humidity of 40-60%, on a 12hour natural day and night cycle. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. They were fed with standard rat pellet diet and tap water *ad libitum.* IAEC had approved the experimental protocol (IAEC No-SDMCRA/IAEC/SS-03) and the care of animals was undertaken as per the CPCSEA guidelines.

Chemicals:

Chemical to induce MI – Isoprenaline hydrochloride (ISO) 15627-5G, source # BCBW7995, batch no. # 0000069674 by SIGMA – ALDRICH.

Other chemicals - All other chemicals and reagents used in the study were procured from standard and reputed firms.

Experimental design:

Rats were randomly divided into 3 groups of 6 rats each as mentioned in table no.1. Group 1 received standard rat pellet diet and tap water *ad libitum* for 21 consecutive days and served as normal control group. Group 2 rats received the same as group 1

Table 1 Experimental design & Animal grouping

Day	Control group (G1)	ISO control (G2)	Test group (G3)
1st-20th day	Normal diet	Normal diet	Test drug
20th day	Normal diet	ISO (1st dose)	Test drug + ISO(1st dose)
21st day	Normal diet	ISO (2nd dose)	Test drug + ISO(2nd dose)

in addition, it received isoprenaline 80mg/kg body wt. subcutaneously on 20th and 21st day at an interval of 24 hours and served as ISO control group. Group 3 rats were pre-treated with 4.5ml/kg body wt. fruit juice extract of *Dadima* for a period of 21 consecutive days, in addition it received isoprenaline 80mg/kg body wt. subcutaneously on 20th and 21st day at an interval of 24 hours and served as test group. Rats were weighed regularly every 4th day.

On 22nd day, 24 hours after the last dose of isoprenaline, rats were anesthetised and blood samples were collected from retro-orbital puncture for the estimation of clotting time and biochemical parameters such as CK-MB, LDH activity, SGOT, SGPT, total cholesterol, triglycerides, serum creatinine, glucose and blood urea.

The rats were weighed and sacrificed by overdose of diethyl ether anesthesia. The abdomen was opened through midline incision and then heart was dissected out along with aorta, weighed, and transferred to normal saline for assessment of anti-oxidant property of heart by following the standard operating procedures(SOP) for catalase activity(Sinha1972), lipid peroxidase activity(Ohkawaet al.,1979) and protein estimation (Lowry's method). The specimen meant for the cardiac cyto-architecture (histopathology)was transferred into 10% formalin solution. The tissue was embedded in paraffin. The section was cut into 5-6 µm thickness and stained with hematoxylin-eosin stain and mounted in diphenyl phthalein xylene. The histopathological changes of heart tissue were observed under compound microscope and microphotographs were taken.

Statistical analysis:

The experimental data was expressed as mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by Dunnett's multiple comparison t-test with post HOC test.A level for $p<0.05$ was considered statistically significant, $p<0.01$ was considered statistically very significant and $p<0.0001$ was considered statistically extremely significant.

Table 2 Effect of fruit juice extract of *Dadima* on cardio-toxicity induced experiment

	Normal control	ISO control @	Test drug + ISO #
Body weight (g)	9.65 ± 0.525	32.47 ± 3.118**	54.40 ± 3.221**
Weight of heart(g)	0.81 ± 0.010	0.96 ± 0.056*	1.01 ± 0.024
Clotting time	1.5 ± 0.223	1.5 ± 0.223	2 ± 0.2582

Data expressed in mean ± SEM, *p<0.05, **p<0.01, @ - compared with normal control, # - compared with ISO control

OBSERVATION & RESULTS

In table no.2, both the test group and ISO control group resulted in statistically very significant increase in bodyweight in comparison to the normal control group. The ISO control group showed a statistically significant increase in weight of heart when compared with the normal

control group and the test group showed statistically non-significant increase in weight of heart in comparison to ISO control group. In ISO control group, statistically no changes were observed with clotting time in comparison to the normal control group and the test group showed statistically non-significant increase in clotting time when compared with the ISO control group.

In table no.3, significant increase in the LDH, SGPT and serum creatinine activity were observed, non-significant increase was noted in CK-MB, SGOT and triglycerides and non-significant decreased activity was seen in total cholesterol and blood urea values in ISO treated myocardial infarcted rats as compared to the control group.

Table 3 Effect of fruit juice extract of *Dadima* on biochemical parameters

	Normal control	ISO control @	Test drug + ISO #
CK-MB (IU/L)	127.6 ± 23.224	208.54 ± 30.536	143.82 ± 17.427
LDH activity (IU/L)	210.66 ± 34.216	616.7 ± 55.469**	550.93 ± 123.36
SGOT activity (IU/L)	116.83 ± 5.016	130 ± 11.520	127.16 ± 8.998
SGPT activity (IU/L)	55.5 ± 4.039	79.16 ± 5.828**	69 ± 3.033
Total cholesterol (mg/dl)	70.33 ± 4.731	58.5 ± 6.276	74.75 ± 9.349
Triglycerides (mg/dl)	89.66 ± 5.220	118.33 ± 10.704	101.5 ± 8.671
Serum creatinine (mg/dl)	0.36 ± 0.021	0.5 ± 0.036**	0.53 ± 0.021
Glucose (mg/dl)	93 ± 2.033	99.16 ± 5.263	151.83 ± 5.576**
Blood urea (mg/dl)	51 ± 2.817	45.16 ± 4.743	44 ± 2.408

Data expressed in mean ± SEM, *p<0.05, **p<0.01, @ - compared with normal control, # - compared with ISO control

Extract pre-treated test group non-significantly reduced the activity of serum biomarkers such as LDH, SGPT, SGOT, CK-MB, triglycerides and blood urea levels and non-significant increase was noted in total cholesterol and serum creatinine levels as compared to ISO control group.

Table 4 Effect of fruit juice extract of *Dadima* on anti-oxidant activity of heart:

	Normal control	ISO control @	Test drug + ISO #
Catalase activity	0.928 ± 0.071	1.493 ± 0.204	1.416 ± 0.2590
Lipid peroxidase	1.473 ± 0.0871	1.603 ± 0.5010	1.018 ± 0.0219
Protein estimation	0.054 ± 0.0053	0.04 ± 0.0026	0.065 ± 0.014

Data expressed in mean ± SEM, *p<0.05, **p<0.01, @ - compared with normal control, # - compared with ISO control

In table no.4, non-significant elevation in the catalase and lipid peroxidase activity and non-significant reduction in the protein estimation was observed in ISO treated group as compared to the normal control.

Whereas, non-significant decrease values of catalase and lipid peroxidase activity and non-significant increased values in protein estimation were obtained in the test group as compared to ISO control.

Histopathological examination of heart tissue:

Normal cyto-architecture was observed with no specific changes noted in normal control rats. The tissue sections showed vacuolation of muscle fibres with inflammatory cells. Many degenerated muscle fibres were also seen. There were many areas of necrosis also. Severe toxic changes were noted ISO control. The changes observed in test group compared with ISO control there was only slight reduction in inflammation, degeneration and necrosis. Moderate toxic changes were noted. Mild protection was seen.

DISCUSSION

The pathophysiological and morphological aberrations produced in the heart of a myocardial necrotic rat model are comparable to those taking place in human MI. The low mortality, high reproducibility and validity compared with other animal models make it more suitable for the evaluation of cardioprotective agents.

ISO induced myocardial damage in the rat model:

Isoprenaline hydrochloride(ISO)[4] a synthetic catechol compound and a potent β-adrenergic agonist with peripheral vasodilator, bronchodilator and cardiac stimulant properties was used.

ISO exerts its effect on the β-1 adrenergic receptors in the myocardium, thereby increasing heart rate and cardiac output. In addition, it acts on β-2 adrenergic receptors in bronchiolar and vascular smooth muscle, thereby causing muscle relaxation.

It is found to cause severe oxidative stress in the myocardium resulting in the infarct like necrosis of heart muscle. Prolonged ISO exposure causes myocardial hypertrophy in animals. Thus, ISO is employed at submaximal dose as a non-invasive method to induce myocardial lesions in rats.

ISO induces cardiac necrosis by several mechanisms which include increased oxygen consumption, poor oxygen utilization, increased calcium overload and accumulation, altered myocardial cell metabolism, deranged electrolytes, altered membrane permeability, intracellular acidosis, oxidation of catecholamines, excessive formation of free radicals and increased levels of lipid peroxidases.

Mechanism of *Dadima* as cardioprotective in ISO induced MI:

Anti-oxidants are compounds that inhibit oxidation. Anti-oxidants are bodyguards for the heart vessels which prevent them from clogging. The anti-oxidants present in *Dadima* such as punicalagins, ellagitannins, flavonoids, β-carotene, vitamin-c terminate these chain reactions by neutralizing the free radicals by preventing them from damaging the body. They often act by donating electrons to the free radicals making them more stable.

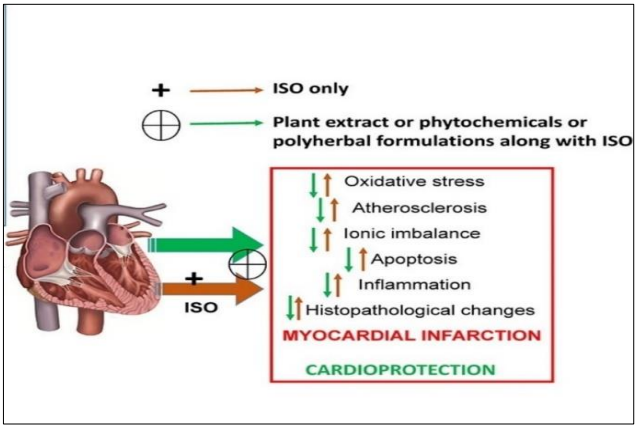

Figure 1 Physiological effects of ISO with the test drug in the myocardium

The fruit juice extract of *Dadima*, has shown to protect against ISO induced cardiac damage[5] through attenuation of oxidative stress(fig.1) by increased serum anti-oxidant capacity, reduction of plasma lipids and LPO, decreased oxidised LDL uptake by macrophages, decreased intima media thickness, decreased atherosclerotic lesion areas, enhanced biological actions of nitric oxide, decreased inflammation leads to scavenging of free radicals and ROS and myocardial tissue necrosis. This would then protect and maintain the

natural permeability and structural integrity of the heart, preventing the leakage of cardiac diagnostic marker enzymes including CK-MB , LDH, AST, SGOT and membrane disruption.

Effect on anti-oxidants of heart:

Oxidative stress[6] is defined as an imbalance between the production of reactive oxygen species (ROS) and reactive metabolites, and their elimination by protective mechanisms comprising an enzymatic defence system which include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Catalase[7] is an enzymatic anti-oxidant present in the peroxisome of aerobic cells and very efficient in regulating the production of ROS through catalytic removal and conversion of superoxide radicals to hydrogen peroxide, and hydrogen peroxide to water and molecular oxygen thereby neutralising the chain reaction initiated by the free radicals. The catalase activity was found to be non-significantly decreased in all the treated groups in comparison to the ISO control group statistically. Wherein statistically non-significant increase of CAT was noted in ISO control group when compared with normal control. Hence, only moderate effect of *Dadima* on CAT activity was noted against the ISO induced cardiac damage.

The free radicals attack all major classes of biomolecules, mainly the polyunsaturated fatty acids (PUFA) of cell membranes. The oxidative damage of PUFA, known as lipid peroxidation[8] is particularly destructive because it proceeds as a self-perpetuating chain reaction forming peroxyl radicals, which are the carriers of the chain reactions. LPO has been widely associated with the tissue injuries and diseases. The tissue LPO activity was determined by measuring the content of Thiobarbituric acid reactive substances (TBARs). The LPO activity in the ISO control group showed non-significant increase in comparison to normal control. Whereas, in the test group non-significant decrease was observed in LPO activity when compared to ISO control group. Therefore, it can be inferred that the test drug *Dadima*, plays a significant role in reducing the ISO induced cardiac injury.

There is increasing evidence about the significant anti-oxidant activity in proteins[9] such as albumin. They inhibit lipid oxidation through multiple pathways including inactivation of reactive oxygen species (ROS), scavenging free radicals, chelation of pro-oxidative transition metals and reduction of hydroperoxides. Statistically, non-significant increase was observed in the protein activity when compared with the ISO control group. The non-significant decrease of protein activity in ISO control have provided a better evidence proving the efficacy of *Dadima* as cardioprotective against oxidative damage caused by ISO.

Oxidative stress and the free radicals can be understood by relating it to the concept of *Aama* in Ayurveda. *Aama Samdharana* resulted from vitiation of *Tridoshas* and *Srotorodha*, hampers the *Agni* at the level of *Jataragni* and *Rasagni* leading to *Rasa Dushti*, can also be interpreted as the state of oxidative stress causing *Hrudroga*. This *Dushita Rasa-Rakta* complex along with

Avalambaka Kapha hinders the normal functioning of *Hrudaya*, creating obstruction in *Rasavaha Srotas* and leading to aggravation of *Vata*.

Dadima brings about the qualities of *Amla Rasa*[10] like *Vatanulomana, Agni Deepana, Aama Pachana* does the *Sroto Shodhana* by virtue of its *Agneya* nature which activates the microcirculation. Thus, *Dadima* acts at the level of *Rasa* which in turn facilitates the synthesis and nourishment of successive *Dhatus* resulting in *Deha Brmhana, Hrudaya Tarpana* and *Preenana* thereby increasing *Bala* and *Urja*.

The effect of *Rasayana* can be attained by the mere intake of *Nitya Sevaniya Dravyas*[11] like *Dadima*, this being *Amla Rasa Pradhana* produces the effect of *Brumhana* and *Balakara*. As *Amla Rasa* is having *Prithvi Mahabhoota* along with *Agni*[12], it does the *Brumhana* of *Shareera*. Thus, a strong relationship can be established between *Rasayana* and its anti-oxidant potential, proving the efficacy of the drug *Dadima* as *Rasayana.*

No specific changes were observed in this experiment to check the efficacy of *Dadima* on clotting time factor. However, the haemostatic quality of *Dadima* can be inferred from the references available in our classics where the *Dadima Rasa* was used as *Pana* in the treatment of *Rakta-atisara*[13] and *Dadima Pushpa Toya* was used as *Nasya* in *Granapravrutta Raktapitta Chikitsa*[14]. This may be due to *Grahi* property of *Dadima.*

CONCLUSION

The study was mainly aimed at protective and preventive care towards cardiac ailments caused due to oxidative stress with the help of *Nityasevaniya Aahara Dravyas* like *Dadima.* The juice extract of *Dadima* was proved to possess anti-oxidant, anti-inflammatory, anti-apoptotic, anti-atherosclerotic and haemodynamic properties acting as a free radical scavenger in oxidative stress showing prevention and reversal of CVD. Thus, Dadima was proved to protect the heart against ISO induced myocardial damage in wistar strain rats.

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