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EVALUATION OF *MORINGA OLEIFERA* SEEDS FOR THE CARDIO PROTECTIVE EFFICACY

Shivakumar Hugar*, Shivapraksha S. M., Biradar S. S. and Shivakumar B.

BLDEA's SSM College of Pharmacy and Research Centre, BLDE University Campus, Vijayapur-586103, Karnataka, India.

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***Corresponding Author**
Dr. Shivakumar Hugar
BLDEA's SSM College of Pharmacy and Research Centre, BLDE University Campus, Vijayapur-586103, Karnataka, India.

ABSTRACT

Objective: The present study was designed to evaluate cardioprotective activity of seeds of *Moringa oleifera* against isoproterenol induced MI in Wistar albino rats**. Materials and methods:** In this study, at the end of 32nd day, after treatment, fasting blood samples used for the estimation of serum cardiac enzymes namely Lactate dehydrogenase (LDH), Creatine phosphokinase (CPK), Aspartate transaminase (AST), Alanine transaminase (ALT), Creatine phosphokinase-MB (CK-MB) and lipid profile namely Total cholesterol (TC), Triglyceride (TG), HDL and LDL. Further, rats were sacrificed for the removal of heart tissues for the estimation of myocardial marker enzymes (LDH, CPK, AST, ALT and CK-MB) and antioxidant enzymes namely GSH and LPO. **Results:** The results of preliminary phytochemical screening revealed the presence of flavonoids, tannins, glycosides and carbohydrates. Control group (Isoproterenol (85 mg/kg i.p.) animals exhibited significant altered levels of cardiac enzymes in serum and heart tissue and antioxidant enzymes of heart tissue. The extract of *Moringa oleifera* significantly reversed all the parameters of serum and heart tissue cardiac enzymes and antioxidant enzymes level heart tissue. **Conclusion:** The metanolic extract of *Moringa oleifera* seeds exhibited dose dependent significant cardioprotective activity by significantly attenuating the altered parameters of serum as well as heart tissue cardiac enzymes and tissue antioxidant enzymes of heart tissue in experimental animals. Thus the results of the present study suggest that seeds extract of the title plant possesses cardioprotective activity.

KEYWORDS: *Moringa oleifera,* Isoproterenol, cardioprotective.

INTRODUCTION

Cardiovascular diseases such as myocardial infarction (MI) and hypertension are the most important cause of mortality in developing countries due to changing lifestyles.[1] MI is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demands.[2] MI is one of the main causes of death from cardiovascular diseases. Myocardial ischemia occurs while myocardial oxygen demand exceeds oxygen supply causing cell injury known as myocardial infarction, which is one of the most fatal manifestations of cardiovascular diseases.[3]

Medicinal plants are nature's gift to mankind to make disease free healthy life and play a vital role to maintain good health.[4] Present day's research has been focused more on medicinal plants and food products derived from medicinal plants that have been found to have certain preventive measures in the treatment of cardiovascular disease.[5] Use of herbs for the treatment of cardiovascular diseases in Ayurveda, Chinese and Unani system of medicine has given a new lead to understand the pathophysiology of these diseases.

Moringa oleifera Lam (Family-Moringaceae), commonly known as Drum stick tree, grows largely in Asia, tropical Africa and tropical America. Various parts of this tree have been studied for several pharmacological actions. It is reported to be useful as antispasmodic, anti-inflammatory and diuretics.[6] The plant is reported to have antihypertensive, hypocholesterolaemic, anti- ulcer, wound healing, hypolipidemic anti-fungal and anti-tubercular properties.[7-12] Moreover, hydro-alcoholic extract of Drumsticks reported to possess significant antioxidant potential by increasing the activities of catalase, glutathione peroxidase (GPx), glutathione reductase (GR) with decreasing the hepatic MDA level.[13, 14] The cardio-protective activity of *Moringa oleifera* stem bark has been already documented in the literature.[15] *Moringa oleifera* plant is known to contain various phytoconstituents responsible for diver's pharmacological activities.

However, extensive literature review reveals that the cardioprotective efficacy of seeds of the plant has not been scientifically validated so far. In view of this, the present study was under taken for phytochemical analysis and evaluation of *Moringa oleifera* for the cardioprotective efficacy.

MATERIALS AND METHODS

Collection of plant materials

For this study, mature *Moringa oleifera* pods (Drumstick) were collected from Shivananda Nagar, Hagaribommanahalli Taluk, Bellary Dist. Karnataka, after the plant material authenticated by botanist. Drum stick pods were split into two pieces then seeds were separated and shade dried at room temperature for 20 days. The seeds were cleaned and coarse powdered.

Extraction of the plant materials

The shade dried coarse powder was then extracted with pet ether for the removal fatty material followed by methanol using Soxhlet's extraction method. Thereafter, the methanolic extract was concentrated using rotary flash evaporator resulted to yield 8%.

Preliminary phytochemical studies

The reagents required for the preliminary phytochemicals investigation of *Moringa oleifera* seeds extract were freshly prepared using procured chemicals.

The crude extract was subjected to preliminary phytochemical screening following the standard procedures described in the literature.[16]

Procurement of Experimental animals

The Albino mice and Wistar rats were procured from Sri Venkateshwara Enterprises, 4304, 13th main 2nd cross, Subramanyanagar, Bangalore-21 (237/CPCSEA). After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition. All the animals were fed with rodent pellet diet and water *ad libitium* under strict hygienic condition. Study protocol was approved from Institutional Animal Ethics Committee (IAEC) before initiation of the experiment. (Ref.No. BLDEA's COP/IAEC/640/2015-16 dated 24/02/2016).

Determination of Acute toxicity study

An acute toxicity of methanolic extract of *Moringa oleifera* seeds (MEMOS) was determined in female albino mice (20-30 g). The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method was employed for toxicity study. Based on the result of the study, 1/40th, 1/20th and 1/10th of LD_{50} cut off value of the screening doses of extract were selected for the cardio protective activity.[17]

The doses selected for the evaluation of cardioprotective activity of the test extract were.

125 mg/kg - 1/40th dose of LD 50 cut off value, 5000 mg/kg b.w.

250 mg/kg - 1/20th dose of LD 50 cut off value, 5000 mg/kg b.w.

500 mg/kg - 1/10th dose of LD 50 cut off value, 5000 mg/kg b.w.

Evaluation of cardioprotective activity of MEMOS against isoproterenol induced MI in Wistar rats.

Induction of Myocardial Infarction

At the end of treatment period, all the animals, except the normal control rats, were administered isoproterenol (ISO) 85 mg/kg, by intra-peritoneal injection for two consecutive days on the 31 and 32 day at an interval of 24 h. to induce myocardial injury.[18, 19]

Experimental Protocol

The rats were randomly divided into six groups with six rats in each group.

Group I : Normal control animals, Untreated, received vehicle only

Group II: Cardiotoxic control, rats were orally given vehicle 2% W/V aqueous gum acacia 10 ml/kg b. w. once daily for 30 days and in addition received isoproterenol dissolved in normal saline (85 mg/kg b.w., i.p.) on 31 and 32 day at an interval of 24 h.

Group III : Served as standard, pre-treated with Vitamin C dissolved in normal saline (1000 mg/kg body weight, orally) once daily for 30 days in addition, received isoproterenol (85 mg/kg i.p.) on the 31 and 32 day at an interval of 24h.

Group IV : Rats were pre-treated with methanolic extract of *Moringa oleifera* seeds (MEMOS) at 125 mg/kg b.w. for a period of 32 days and in addition, received isoproterenol (85 mg/kg i.p.) on the 31 and 32 day at an interval of 24 h.

Group V : Rats were pre-treated with MEMOS at 250 mg/kg b.w. for a period of 32 days and in addition, received isoproterenol (85 mg/kg i.p.) on the 31 and 32 day at an interval of 24 h.

Group VI: Rats were pre-treated with MEMOS at 500 mg/kg b.w. for a period of 32 days and in addition, received isoproterenol (85 mg/kg i.p.) on the 31 and 32 day at an interval of 24 h.

Collection of Blood and Heart Tissues

At the end of 32nd day, after treatment, fasting blood samples from retro orbital plexus under mild ether anesthesia were collected from all groups into sterilized dry centrifuge tubes, and allowed to coagulate for 30 min. at 37^{o}C. The clear serum obtained after centrifugation was

used for the estimation of serum cardiac enzymes and lipid profile using ready reagent kits by ERBA chem semiautoanalyser.

Estimation of serum enzymes

1. Lactate dehydrogenase (LDH)
2. Creatine phosphokinase (CPK)
3. Aspartate transaminase (AST)
4. Alanine transaminase (ALT)
5. Creatine phosphokinase-MB (CK-MB)

Estimation of Lipid profile

1. Total cholesterol (TC)
2. Triglyceride (TG)
3. HDL and
4. LDL using the respective ready reagent kits.

After collection of blood samples rats were sacrificed for the removal heart tissues and then immersed in physiological saline. The heart tissues were suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and then cut into small pieces and homogenized using a homogenizer (Inco, India). The clear supernatant was used for estimation of cardiac tissue enzymes namely.

1. Lactate dehydrogenase (LDH)
2. Creatine phosphokinase (CPK)
3. Aspartate transaminase (AST)
4. Alanine transaminase (ALT)
5. Creatine phosphokinase-MB (CK-MB), and also antioxidant enzymes namely GSH and LPO.

Statistical analysis

The data obtained from the above findings subjected to statistical analysis using Graph pad prism software by one-way ANOVA followed by Turkey Kramer Multiple Comparison Test to assess the statistical significance of the results.

RESULTS

Preliminary Phytochemical Screening: The results of preliminary phytochemical screening

revealed the presence of flavonoids, tannins, glycosides and carbohydrates in *Moringa oleifera* seeds extract.

Acute toxicity study

In an acute toxicity study, the methanolic extract of *Moringa oleifera* seeds did not cause any mortality of the animals at dose of 2000 mg/kg, even at repeated dosing using new mice. Hence, 5000 mg/kg was taken as LD_{50} cutoff value as per fixed dose method of OECD guideline number 423.

Evaluation of cardioprotective activity against isoproterenol induced MI in Wistar rats.

Effect of MEMOS on serum myocardial marker enzymes

Isoproterenol caused significant elevation of serum myocardial enzymes namely Lactate dehydrogenase (LDH), Creatine phosphokinase (CPK), Aspartate transaminase (AST), Alanine transaminase (ALT), Creatine phosphokinase-MB (CK-MB) when compared to normal control group. Pretreatment of graded doses of MEMOS exhibited dose dependent significant decrease in elevated serum biomarker enzyme levels.

Effect of MEMOS on lipid profile

There was marked increase in the level of lipid parameters such as Total cholesterol (TC), Triglyceride (TG), LDL and decrease in HDL level monitor in isoproterenol injected cardiotoxic control group compared to normal control. These altered lipid profile significantly attenuated by MEMOS treated groups in dose related manner.

Effect of MEMOS on myocardial marker enzymes in heart tissue

Isoproterenol produced significant reduction of myocardial enzymes namely Lactate dehydrogenase (LDH), Creatine phosphokinase (CPK), Aspartate transaminase (AST), Alanine transaminase (ALT), Creatine phosphokinase-MB (CK-MB) in heart tissue when compared to normal control group. Pretreatment of graded doses of MEMOS exhibited significant increase in biomarker enzyme levels in dose dependent fashion.

Effect of MEMOS on GSH and LPO level in heart tissue

Marked elevation of LPO level and significant decrease in GSH content in heart tissue was seen in isoproterenol injected cardiotoxic control group compared to normal control. Pretreatment of MEMOS demonstrated dose dependent reversal of altered LPO and GSH content of the heart tissue.

Table 1: Effect of MEMOS on serum myocardial marker enzymes in ISO- induced myocardial infarction in Wistar rats.

Groups	Treatment	Serum myocardial marker enzymes (IU/L)				
		LDH	CPK	AST	ALT	CK-MB
I	Normal control, untreated	340.59± 8.20	165.47± 4.20	161.35± 3.20	72.35± 2.14	80.45± 3.20
II	Cardiotoxic control (ISO 85 mg/kg b.w.)	509.45± 8.10$^{@}$	345.25± 5.20$^{@}$	310.68± 4.85$^{@}$	142.23± 3.18$^{@}$	196.25± 3.42$^{@}$
III	Standard (Vit. C 1000 mg/kg)	370.45± 7.19***	210.53± 4.95***	170.25± 4.01***	98.47± 3.41***	98.43± 3.43***
IV	MEMOS, 125 mg/kg	440.47± 9.75***	301.58± 4.50***	282.25± 3.85***	120.69± 2.28***	165.24± 3.51***
V	MEMOS, 250 mg/kg	410.81± 6.46***	280.69± 5.47***	261.85± 3.58***	112.47± 2.56***	152.37± 3.01***
VI	MEMOS, 500 mg/kg	390.41± 7.47***	254.53± 5.01***	214.64± 4.10***	103.69± 3.13***	124.95± 3.00***

Values are Mean ± SEM, (n=6), Where ***p<0.001 v/s cardiotoxic control and $^{@}$p<0.001 v/s normal control

Table 2: Effect of MEMOS on lipid profile in ISO - induced myocardial infarction in Wistar rats.

Groups	Treatment	Lipid profile (mg/dl)			
		TC	TG	LDL	HDL
I	Normal control, untreated	86.68±3.20	79.21±2.02	22.45±1.48	39.78±1.15
II	Cardiotoxic control (ISO 85 mg/kg b.w.)	140.24±3.48$^{@}$	132.47±3.08$^{@}$	46.85±2.15$^{@}$	20.87±1.18$^{@}$
III	Standard (Vit. C 1000 mg/kg)	98.45±2.90***	104.42±3.20***	26.61±1.98***	34.47±2.01***
IV	MEMOS, 125 mg/kg	118.21±2.21***	120.50±4.72ns	34.62±2.15**	28.49±1.98*
V	MEMOS, 250 mg/kg	109.47±3.10***	114.41±3.80*	30.80±2.11***	30.65±1.78**
VI	MEMOS, 500 mg/kg	95.86±2.98***	108.47±3.56***	28.57±1.91***	33.51±1.89***

Values are Mean ± SEM, (n=6), Where *p<0.5, **p<0.01, ***p<0.001 v/s cardiotoxic control and $^{@}$p<0.001 v/s normal control

Table 3: Effect of MEMOS on myocardial marker enzymes in heart tissue against ISO-induced myocardial infarction in Wistar rats

Groups	Treatment	Myocardial marker enzymes in heart tissue				
		LDH nM/mg	CPK IU/mg	AST mM/mg	ALT mM/mg	CK-MB IU/mg
I	Normal control, untreated	195.32±2.32	140.29±2.01	260.25±4.01	291.36±3.86	179.35±2.98
II	Cardiotoxic control (ISO 85 mg/kg b.w.)	70.69±2.91$^{@}$	65.39±1.31$^{@}$	140.58±4.13$^{@}$	154.49±4.18$^{@}$	73.25±2.54$^{@}$
III	Standard (Vit. C 1000 mg/kg)	155.23±1.98***	124.29±2.10***	238.56±3.98***	262.54±3.18***	151.21±1.98***
IV	MEMOS, 125 mg/kg	105.32±2.19***	76.29±1.98*	198.25±4.01***	176.47±2.98**	98.26±2.15***
V	MEMOS, 250 mg/kg	117.20±2.52***	89.95±1.56***	209.32±3.54***	198.28±3.48***	115.28±2..13***
VI	MEMOS, 500 mg/kg	124.23±2.29***	101.24±3.10***	218.25±3.19***	223.56±4.19***	128.35±2.17***

Values are Mean ± SEM, (n=6), Where *p<0.5, **p<0.01, ***p<0.001 v/s cardiotoxic control and $^{@}$p<0.001 v/s normal control

Table 4: Effect of MEMOS on GSH and LPO level in heart tissue against ISO- induced myocardial infarction in Wistar rats.

Groups	Treatment	Antioxidant enzymes in heart tissue	
		LPO (nmol of MDA/mg)	GSH (µmol/mg)
I	Normal control, untreated	27.34± 1.20	12.25 ± 1.09
II	Cardiotoxic control (ISO 85 mg/kg b.w.)	61.32± 1.51$^{@}$	04.39± 0.98$^{@}$
III	Standard (Vit. C 1000 mg/kg)	31.27± 1.58***	11.81± 0.90***
IV	MEMOS, 125 mg/kg	49.10± 2.20***	09.01± 0.87*
V	MEMOS, 250 mg/kg	38.23± 1.98***	09.92± 0.92**
VI	MEMOS, 500 mg/kg	30.41± 2.10***	10.86± 0.89***

Values are Mean ± SEM, (n=6), Where *p<0.5, **p<0.01, ***p<0.001 v/s cardiotoxic control and $^{@}$p<0.001 v/s normal control

DISCUSSION

Isoproterenol mediated myocardial infarction is most widely used as a experimental model of screening cardioprotective efficacy of drugs.[20] Isoproterenol is a potent synthetic catecholamine causes subendocardial myocardial ischemia, hypoxia, and finally fibroblastic hyperplasia with decreased myocardial compliance which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction.

The isoproterenol at 85mg/kg is having an ability to destroy myocardial cells. As a result of this, cardiac enzymes such LDH, CK and AST were released into the blood stream and serve as the diagnostic markers of myocardial tissue injury. The amount of these cellular enzymes present in heart reflects the alteration in plasma membrane integrity and/of permeability.[18, 19] Changes in the level of myocardial markers LDH and CK in both serum and heart homogenate in ISO-challenged rats conforms the onset of myocardial necrosis.[20] Oral administration of MEMOS for the period of 32 days demonstrated dose dependent significant changes in the level of cardiac markers (LDH, CK & AST) in both serum and myocardium.

Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the myocardium. High levels of circulating cholesterol along with TG and their accumulation in the heart tissue is usually accompanied by cardiovascular damage.[21] In the present investigation, ISO evidenced its hyperlipidemic effect by elevating serum TC, TG and LDL levels and decreased levels of HDL in comparison with normal control animals. An increase in LDL along with a decrease in HDL was observed in ISO treated rats. LDL is capable of carrying the highest concentration of cholesterol is evidence to increased serum TC.[22] In our study pre-treatment with MEMOS significantly reduced the elevated TC, TG, and LDL levels and increased the level of HDL in dose related manner.

The results of the present study suggest that MEMOS prevented the ISO mediated MI by boosting endogenous antioxidant enzymes. This could be due to antioxidant activity and restoration of altered myocardial markers of serum and heart tissue and lipid profile in the current investigation.

Previous literature reports reveal that the presence of flavonoids in the plant extracts exhibit significant cardioprotective efficacy.[23, 24] In the present study also presence of flavonoids in the *Moringa oleifera* seeds extract which was evident by preliminary phytochemical

screening could be the reason for the observed cardio protective property.

CONCLUSION

It is concluded from the present study by monitoring the restoration of altered cardiac enzymes in serum as well as heart tissue, lipid profile and anti-oxidant enzyme levels in animals pretreated with test extract suggest that MEMOS possesses significant cardioprotective efficacy against isoproterenol induced myocardial infarction in Wistar rats.

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