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Original Research Article

Comparison of Cardioprotective Effects of Terminalia Arjuna and Moringa Oleifera in Isoproterenol Induced Myocardial Infarction in Rabbits

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Conflict of interest: Nil

Abstract:

Cardiovascular diseases are major causes of mortality and morbidity in modern world. Herbal drugs have been used traditionally as cardiotonic products since years. This study was conducted to compare the cardioprotective effects of T. arjuna bark extract and M. oleifera Lam. leaf extract on isoproterenol (ISP) induced myocardial infarction by measuring parameters systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), cardiac biomarkers (serum Troponin, CPK-MB, SGOT levels), ECG and histopathology of rabbit hearts. Twenty four albino rabbits were divided in four groups of six each. Group I served as control, group II was injected ISP intraperitonially (i.p.) on 60th day, group III was given T. arjuna bark extract orally for 60 days and injected ISP (i.p.) on 60th day and group IV was given M. oleifera leaves extract orally for 60 days and injected ISP (i.p.) on 60th day. There was highly significant decrease in SBP (123.50±2.56 & 126.83±1.57), DBP (83.75±2.20 & 85.33±1.83) and MBP (97.00±1.71 & 99.16±1.31) among group III & IV as compared to group II SBP (143.83±3.47), DBP (104.58±1.82) & MBP (117.66±1.68). Similarly serum troponin level (0.29±0.03 & 0.38±0.03), SGOT level (17.15±5.60 & 18.8±2.38) and CPK-MB level (109.12±6.31 & 126.59±6.55) among T. arjuna & M. oleifera (respectively) treated rabbits were significantly lesser than ISP treated rabbit troponin (6.53±1.77), SGOT(46.5±9.66) & CPK-MB (367.66±48.82) levels. ECG and histopathology changes too were in accordance with these results which implies the antiatherogenic and cardioprotective effects of T. arjuna and M. oleifera.

Introduction

Coronary artery disease (CAD) has probably affected human beings throughout the history, but it is only in the last century or so that it has emerged as a leading cause of death. World Health Organisation (WHO) recognized that CAD is the leading cause of death globally and has been called the modern "epidemic". [1] Cardiovascular diseases (CVD) are major causes of mortality and morbidity, especially in Indian subcontinent, causing more than 25% of deaths. [2] The projected increase in deaths and disability from CAD is expected to follow closely an explosion in the prevalence of traditional risk factors. Modern day lifestyle's toll on body - a decrease in physical activity and a heightened level of psychosocial stress promote the development of dysglycemia, hypertension and dyslipidemia; all implicated in the genesis of CAD. [3] These are few of the over 200 known risk factors for CAD, resulting into the acute myocardial infarction (AMI). The major identified risk factors for AMI are tobacco use, hypertension, diabetes mellitus, dyslipidemia, obesity, sedentary lifestyle and atherogenic diet. The presence of two or more risk factors is associated with doubling the relative risk of developing atherosclerotic coronary artery disease. [4]

These risk factors can be effectively treated with modern day medicine but there existed traditional medicines, referred to the ancient medical practice in human societies before the application of modern science to health. The WHO estimates that about 80 percent of world population relies on traditional medicine for primary health care.

Although modern medicine is widely spread, traditional medicine still exists in all countries. It is interesting to note that 25 percent of modern medicines are derived from plants that were used traditionally. It is based on the premise that plants contain natural substances that promote health and alleviate illness.

Few herbal plants have generated much recent interest among the scientific and medical communities e.g. Allium *sativa (garlic)*, Eugenia *jambolana (jamun)*, *Ocimum sanctum L. (tulsi), Curcuma longo L. (turmeric)*, *T. arjuna* and *M. oleifera*. Moreover extensive studies on these traditionally used plant extracts can pave the way for further advancement in modern medicine. Keeping it in mind, this study was conducted for studying *Terminalia arjuna* and *Moringa oleifera*.

Terminalia Arjuna

Terminalia arjuna (Roxb) family *combretaceae*, commonly known as 'arjun' tree is found throughout the greater part of the Indian peninsula along rivers and streams in sub Himalayam tract. [5] The bark of this plant is astringent, styptic, cardiotonic and lithotropic. Preliminary pharmacological studies demonstrated cardioprotective, antianginal, antifungal, antibacterial and hepatoprotective activities. [6-9] Bark is rich in saponins, natural antioxidants, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPC), phytosterols, minerals like calcium, magnesium, zinc and copper. [10] Alcoholic decoction of bark was found to be beneficial in stable cases of ischemic heart disease. It has beneficial effects in modifying various known coronary risk factors like obesity, hypertension and hyperglycemia with no significant side effects. [11]

Moringa Oleifera: *Moringa oleifera* (family *Moringaceae*) also known as Drumstick tree or 'sahinjan'. Different parts of this plant contain important minerals and are a good source of protein, vitamins (A&C), β carotenoids, amino acids and phenolics. [12] Almost all the parts of this plant e.g. root, leaves, seeds, flowers etc. have been used in the treatment of various ailments in the indigenous system. The pods are supposed to have preventive role against intestinal worms. The roots possess anti-inflammatory and diuretic effects. They have been found to be useful in anorexia, colic, paralysis, fever, ascites, cardiomyopathy and bronchitis. Young leaves are useful in dog bite, scurvy and catarrhal infection.[6] Bark possesses abortificient, cardiac and circulatory stimulant properties. [13] Alcoholic extract of leaf possesses analgesic activity. [14] The plant is reported to have antispasmodic, hypotensive [15], hypolipidemic [16], diuretic [17], hepato-protective, antiulcer and antibacterial activities [18]. The alcoholic extract of M. Oleifera leaves caused an initial rise in blood pressure in mongrel dogs and cats followed by gradual fall lasting for a considerable duration [19].

There is no dearth of studies regarding the cardioprotective effects of T. Arjuna and M. Oleifera plants. However, no studies are available to show hemodynamic, electrophysiological, biochemical and other cardiovascular effects of T. Arjuna bark and M. *oleifera* leaf alcoholic extract in isoproterenol treated rabbits.

Aim

To compare effects of T. arjuna alcoholic bark extract with M. oleifera alcoholic leaf extract on blood pressure, ECG, serum cardiac markers and histopathology of heart in myocardial infarction induced rabbits.

Results

Both T. arjuna and M. oleifera decreased B.P. (SBP, DBP & Mean) in isoproterenol induced rabbits. But hypotensive effects of T. arjuna are more marked than M. oleifera (Table 1, Fig. 1).

Table 1: Comparative study showing effect of alcoholic extract of T. arjuna (bark) and M. oleifera (leaves) on blood pressure in rabbits (mean ± SD)

Groups	Systolic BP (SBP) mmHg	Diastolic BP (DBP) mmHg	Mean BP mmHg
I (Control)	131.91±1.59	91.83±1.57	105.19±1.13
II (ISP treated)	143.83±3.47*	104.58±1.82*	117.66±1.68*
III (T. arjuna for 2 months + ISP)	123.50±2.56*#	83.75±2.20*#	97.00±1.71*#
IV (M. oleifera for 2 months + ISP)	126.83±1.57*#	85.33±1.83*#	99.16±1.31**#

*p<0.001; **p<0.01; ***p<0.05 when compared with control (Group I)
#p<0.001 when compared with ISP (Group II)

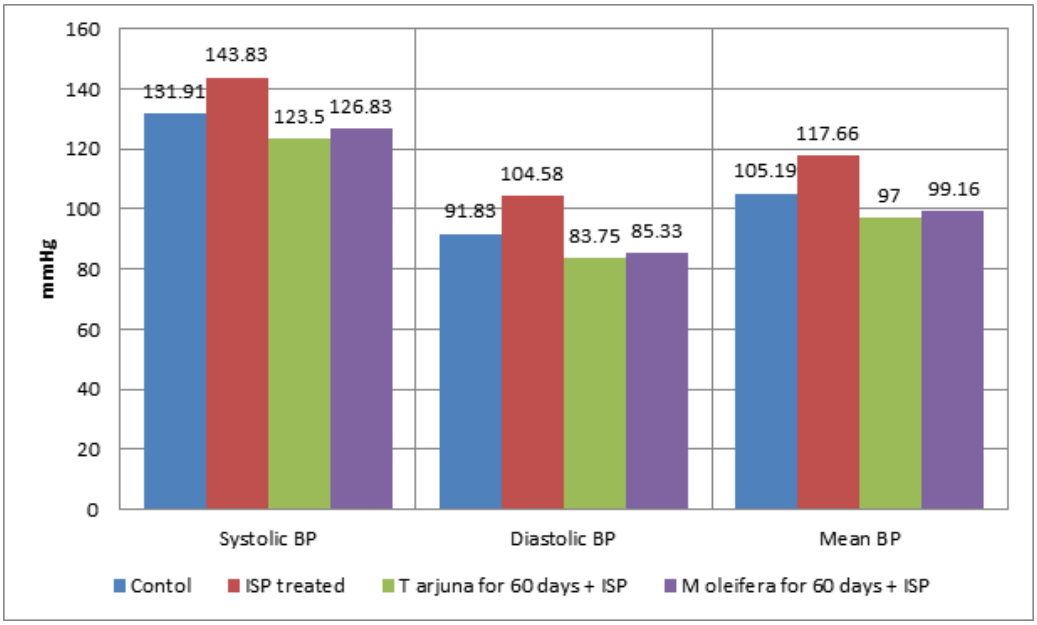

Figure1: Systolic, mean and diastolic blood pressure among control, Isoproterenol, T. arjuna & M. oleifera treated rabbits

T. arjuna as well as M. oleifera both reduced serum cardiac markers in normal as well as isoproterenol induced rabbits when pretreated with T. arjuna and M. oleifera for 2 months. But T. arjuna proved to be more potent than M. oleifera (Table 2; Fig. 2,3 &4).

Table 2: Comparative study showing effect of alcoholic extract of T. arjuna (bark) and M. oleifera (leaves) on serum troponin, SGOT & CPK-MB (cardiac markers) levels in rabbits (mean ± SD)

Groups	S. troponin (ng/ml)	SGOT (IU/L)	CPK-MB (IU/L)
I (Control)	0.43±0.03	24.5±5.20	155.66±5.65
II (ISP treated)	6.53±1.77*	46.5±9.66***	367.66±48.82*
III (T. arjuna for 2 months + ISP)	0.29±0.03*#	17.15±5.60***#	109.12±6.31*#
IV (M. oleifera for 2 months + ISP)	0.38±0.03*#	18.8±2.38***#	126.59±6.55**#

*p<0.001; **p<0.01; ***p<0.05 when compared with control (Group I)
#p<0.001 when compared with ISP (Group II)

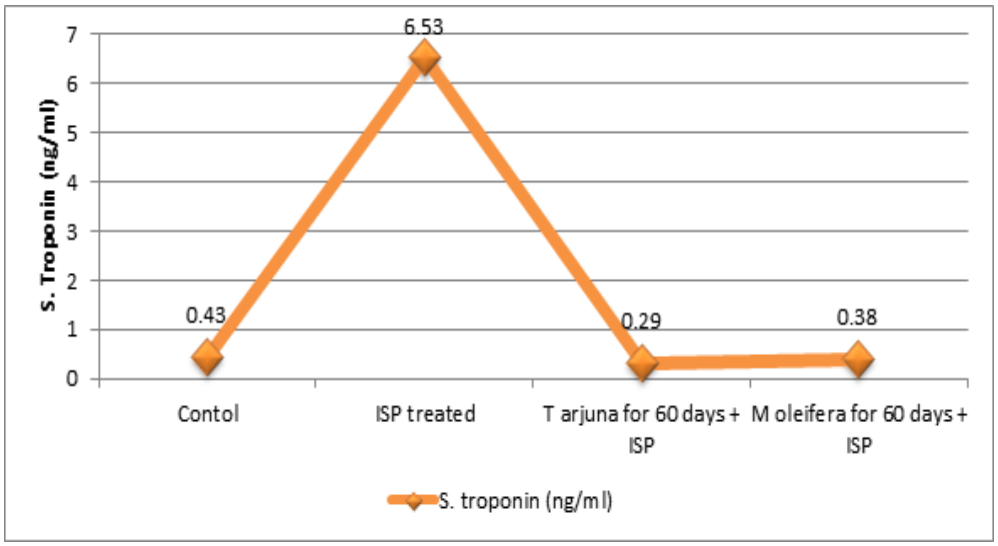

Figure 2: Serum troponin levels among control, Isoproterenol, T. arjuna & M.oleifera treated rabbits

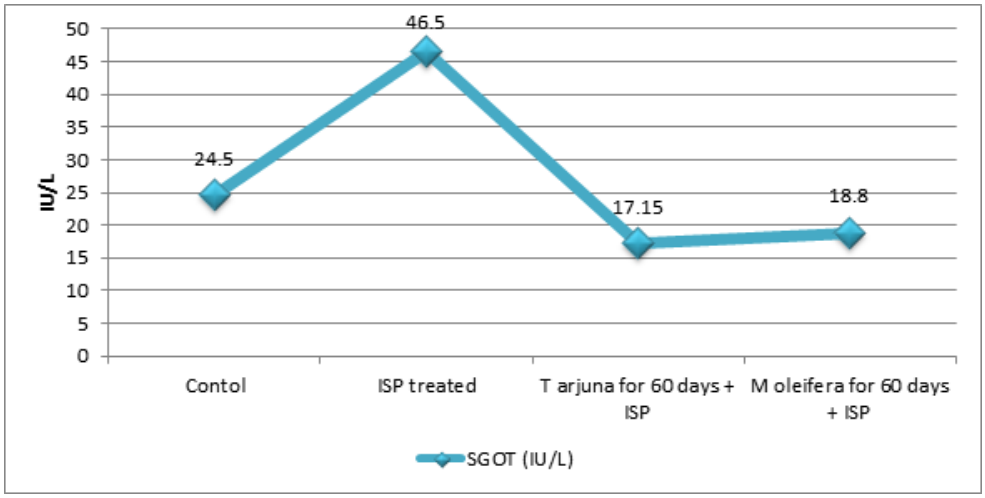

Figure 3: SGOT levels among control, Isoproterenol, T. arjuna & M. oleifera treated rabbits

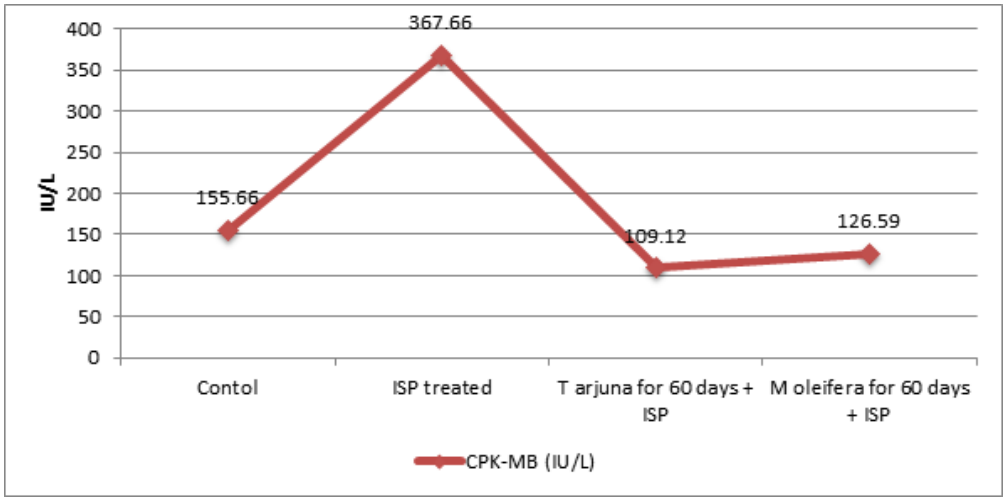

Figure 4: CPK-MB levels among control, Isoproterenol, T. arjuna & M. oleifera treated rabbits

Isoproterenol induced effects like tachycardia, ST segment elevation & T wave inversion were reversed by both T. arjuna & M. oleifera (Fig. 5).

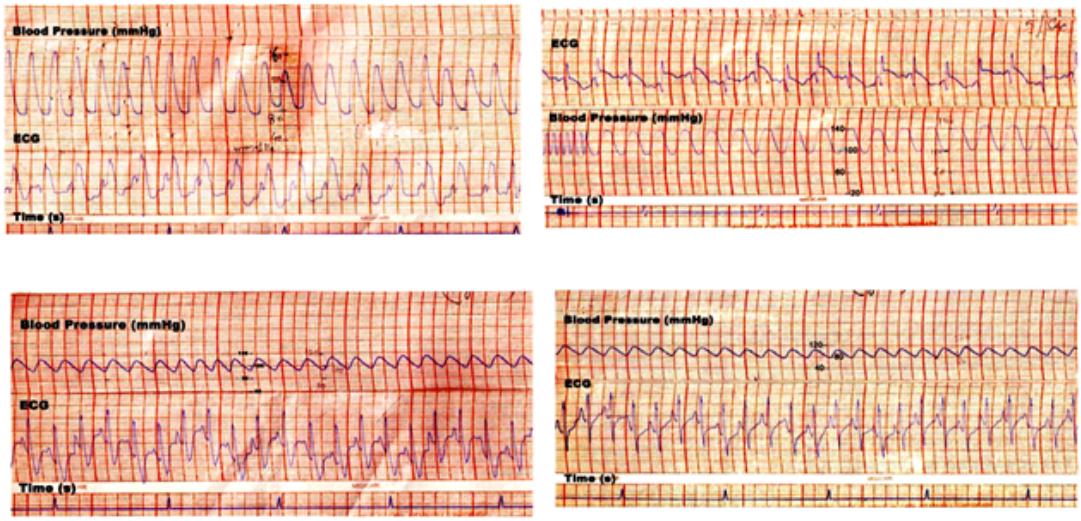

Figure 5: ECG and BP recordings among control, Isoproterenol, T. arjuna & M. oleifera treated rabbits respectively (starting from top left, moving clockwise)

After 24 hours of isoproterenol administration histopathology of heart showed focal vacuolization, pyknotic nuclei, intracellular fibrinoid changes, contraction bands, fatty changes, patches of necrosis and haemorrhage. Sections from T. arjuna pretreated heart showed mild oedema and fatty changes but no necrosis and haemorrhage. However M. oleifera pretreated heart showed few areas of necrosis along with mild inflammatory, oedema and fatty changes (Fig. 6).

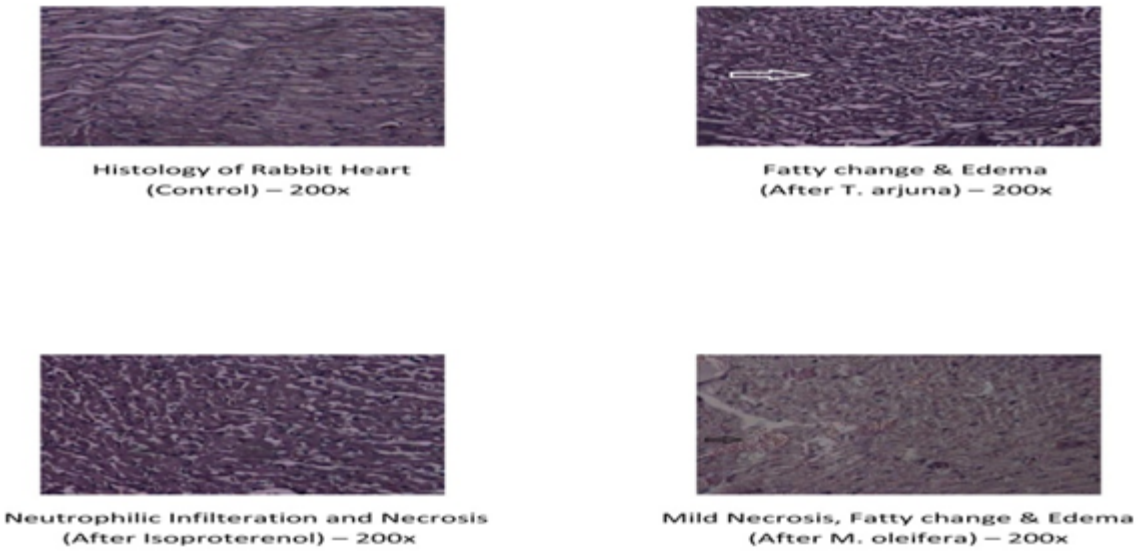

Figure 6: Histology of rabbit hearts

Discussion

Data from the present hemodynamic, biochemical and histological studies revealed marked protective effect of both Terminalia arjuna as well as Moringa *oleifera* alcoholic extract on isoproterenol induced myocardial damage in rabbits.

Increased BP, SBP and DBP in isoproterenol (ISP) treated rabbits are in accordance with the previous studies as by Pinelli et al [20]. ECG tracings (lead II) of rabbits treated with ISP revealed the presence of ST segment elevation with T wave inversion. ISP induced effects are due to direct stimulation of cardiac β receptors and catecholamine release through the involvement of facilitatory presynaptic β2 adrenoreceptor and angiotensin system [21]. Blood pressure response to a β receptor agonist depends on its effect on the heart and vasculature. Isoproterenol activates both β1 and β2 receptors. Activation of β2 receptors leads to vasocdilation in certain vascular beds. The net effect of isoproterenol on BP is slight increase in systolic BP and decrease in diastolic as well as mean BP but in higher doses, there occurs marked tachycardia associated with increased cardiac output, increased systolic, diastolic and mean BP. [22]

The histological sections of the hearts from these rabbits (ISP treated) showed focal vacoulization, pyknotic nuclei, intracellular fibrinoid changes, contraction bands, fatty changes as well as patchy necrosis and haemorrhage (Figure 6). Gunjal et al and Farvin et al also reported the similar histopathological findings. [23, 24] ISP caused Ca2+ channel opening, calcium overload, increased inotropic activity, high O2 demand and myocardial necrosis as probable mechanisms to explain the alterations in ECG and histopathology. [20] The mechanism underlying increase in cardiac markers is the oxidative stress which is usually associated with increased generation of reactive oxygen species (ROS). ISP enhances the susceptibility of myocardial cell membrane to the peroxidative damage, resulting in increased release of cardiac biomarkers (Troponin, SGOT & CPK-MB). ROS modifies membrane phospholipids and proteins leading to lipid peroxidation & oxidation of thiol groups which alters the myocardial membrane permeability and configuration. The depression in Ca^{2+} regulatory mechanisms by ROS ultimately results in intracellular calcium overload and cell death. [25]

Biochemical and ECG findings of the present study showed cardioprotective effect of both T. Arjuna bark extract and M. *oleifera* leaf extract, with former being more potent.

Sumitra et al reported that arjunolic acid derived from T. arjuna prevented decrease in levels of superoxide dismutase (SOD), catalase, glutathione peroxidase, reduced glutathione (GSH) and MPO. [26] The reduced levels of cardiac biomarkers (troponin, SGOT, CPK-MB) in T. arjuna pretreated rabbits were due to the cardioprotective effects conferred against the damage caused by ISP

induced myocardial necrosis. These effects may possibly be mediated either by direct effect of T. arjuna alcoholic extract on coronary blood vessels or via modulating release of catecholaminergic neurotransmission. Similarly hypotension produced by T. arjuna can be explained by the vasodilatory effect on blood vessels. T. arjuna extract decreased blood pressure in rabbit by antagonizing the effect of isoproterenol probably by decreasing Ca^{2+} influx or by blocking calcium channels, thus decreasing calcium overload. Calcium channel blocking property of T. arjuna extract can also relax smooth muscles of blood vessels and ultimately may produce hypotension. A variety of tannins (pyrocatechols, punicallin, pumicalagin, terchebulin, terflavin C, Ca stalaqin, casuanin and casuarinin) have been isolated from the bark of T. arjuna. Tannins are known to enhance synthesis of nitric oxide and relax vascular segments precontracted with norepinephrine to produce hypotension. [27]

Cardioprotective effects of alcoholic leaf extract from M. *oleifera* may be due to well known antioxidant and free radical scavenging activities. M. Oleifera leaf extract possess significant cardioprotective effect, which may be attributed to its antioxidant, antiperoxidative and myocardial preservative properties. Antioxidant effect of M. Oleifera leaves due to presence of ascorbic acid, flavonoids, phenols and carotenoids and modulation of the biochemical enzymes (SOD, CAT, glutathione peroxidase, LDH, CPK-MB) exerted protective effect on myocardial cell membrane which prevented the leakage of cardiac marker enzymes into circulation. Thus, reduction in cardiac markers reflects reduced extent of myocardial damage in rabbits pretreated with M. oleifera leaves.

Isoproterenol administration in rabbits caused marked elevation of lipid peroxidation and suppression in antioxidant defense cascade as evident by significant reduction in superoxide dismutase (SOD) and glutathione (GSH). [28] Nandave et al reported favorable modulation of SOD, catalase, glutathione peroxidase and significant lesser rise in lipid peroxidation in myocardial tissue of M. oleifera fed rats. [29] Isoproterenol leads to destruction of myocardial cells resulting to leakage of cytosolic enzymes into blood stream and thus serving as diagnostic markers (AST, Troponin & CPK-MB). Significant rise in serum levels of AST, Troponin and CPK-MB in isoproterenol induced cardiac necrosis in rabbits indicates the severity of necrotic damage. Thus reduction in cardiac markers in M. *oleifera* leaf extract pretreated rabbits reflects reduced extent of myocardial damage.

Histopathological studies on rabbit hearts further confirm the cardioprotection by T. Arjuna alcoholic bark extract and M. *oleifera* alcoholic leaf extract. Rabbits who received pretreatment with these plants extract demonstrated improvement in structural myocardial morphology in contrast to severe necrosis and haemorrhage in isoproterenol treated rabbits. There was mild fatty change and edema on ISP treatment of T. Arjuna & M. Oleifera fed rabbits hearts. Though there was mild necrosis in M. Oleifera fed rabbits but no necrosis on T. Arjuna fed rabbits. Therefore, these plants extract may have salvaged myocyte and prevented cardiac damage/necrosis induced by isoproterenol in rabbits.

We conclude that alcoholic extract of T. arjuna bark and M. oleifera leaves have been shown to produce hypotensive and negative ionotropic effects. T. arjuna contains glycosides & strong antioxidants which exhibit anti-ischemic and vasodilatory properties. M. oleifera produce calcium antagonistic effects. T. arjuna and M. oleifera exert antiatherogenic and cardioprotective properties; T. arjuna being more potent than M. oleifera. There is still a scope for further research on cardioprotective effect with different doses, duration and other parts of these plants.

Methods

Preparation of alcoholic extracts

Alcoholic extract was prepared using Soxhelt apparatus and ethyl alcohol as solvent. [30, 31]

i. *T. arjuna* – One kg shade dried powdered bark of *T. arjuna* was filled in the inner tube of soxhlet apparatus. When alcohol was boiled, vapours of alcohol were soaked by powdered bark and dissolved material which was alcohol soluble, collected in the flask. Finally obtained solution was evaporated to obtain solid material. The oral dose of T. arjuna bark extract 200 mg/kg was used in the study.[32]

ii. *M. oleifera* – One kg shade dried powdered leaves of *M. oleifera* was filled in the inner tube of soxhlet apparatus. When alcohol was boiled, vapours of alcohol were soaked by powdered leaves and dissolved material which was alcohol soluble, collected in the flask. Finally obtained solution was evaporated to obtain solid material. The oral dose of M. oleifera leaf extract 200 mg/kg was used in the study. [29]

Isoprenaline injections were purchased from Samarth Life Science Pvt Limited, Solan (Himachal Pradesh, India). All the chemicals used in the study were of analytical grade.

Animals and Experimental Design

Albino rabbits of either sex, weighing 2-2.5 Kg were maintained under conditions (ambient temperature 23± 0.5˚C, relative humidity 65±5 %, natural dark: light cycle) with food and water ad libitum. Animals were acclimatized to the laboratory

conditions for at least 7 days prior to experimentation. All experiments were conducted between 9 AM to 4 PM. Care was taken to minimize suffering and pain to animals. All procedures followed the guidelines of the National Institute of Health 1966 (guide for the care and use of laboratory animals). Care was taken to minimize the pain and suffering to the animals. Animals were placed individually in perspex cages during experimentation under standard laboratory conditions. The study was approved from the Institutional Animal Ethical Committee (IAEC).

Rabbits were divided into 4 groups of 6 each. Group I- served control (vehicle). Group II- were given isoproterenol (3mg/kg, i.p., single injection). Group III- received T. arjuna bark extract (200mg/Kg, po) daily for 60 days + isoproterenol 3 mg/kg i.p. single injection on 60th day. Group IV- received M. *oleifera* leaf extract (200mg/kg, po) daily for 60 days + isoproterenol 3 mg/kg i.p. single injection on 60th day.

Induction of Isoproterenol induced myocardial infarction

Myocardial infarction/necrosis was induced in rabbits by injecting Isoproterenol (3mg/kg, intraperitoneally), single injection (Pinelii et al, 2004). [21] Extent of cardiac damage was assessed after 24 hours of isoproterenol administration by recording hemodynamics parameters (BP and ECG), biochemically (Troponin I, AST, CPK-MB and lipid profile) as well as histopathological study of heart.

Recording procedure and setup

Overnight fasting rabbits were anaesthetised with intravenous injection of urethane solution of 20gm %. Urethane solution was administered through marginal vein of pinna in doses of 0.5-1.5gm/kg body weight. Femoral vein was cannulated for injecting i.v. fluids. Blood pressure was recorded by invasive method using 4 channel polyrite. Femoral artery was cannulated by using Stethom's strain gauge pressure transducer loaded previously with heparinized saline. ECG (lead II) was recorded on the same polyrite using needle electrodes. Effects were recorded simultaneously on the moving graph paper at a speed of 5mm/sec and speed was gradually increased to 50mm/sec. Time marker was used to record the signal and to calculate HR, mean BP etc in each group.

Biochemical Parameters

Blood samples were taken from marginal ear vein of rabbits initially, on 60th day and 61st day under aseptic conditions and serum was separated for measurement of serum cardiac markers.

Troponin I and *Aspartate aminotransferase (AST; EC; 2.6.1.1)* – Serum troponin and AST (SGOT) levels were measured in all the groups. [33, 34]

Serum creatinine Phosphokinase MB (CPK-MB; EC; 2.7.3.2) – CPK-MB was estimated in serum (Expert panel of IFCC on enzymes, 1976) of control and other treated groups. [35]

Histopathological examination

At the end of study, rabbits were sacrificed by giving overdose of anaesthesia and immediately hearts were removed for histological study, from each group. Hearts were preserved in 10% buffered formalin before being processed for the histopathological examination. The ventricular mass was sectioned from the apex to the base of the heart in order to obtain 4-5 micron thick transverse slides, which were embedded in paraffin. Paraffin sections were dewaxed in xylene and dehydrated through various grades of ethyl alcohol (from 80% to absolute alcohol). Sections were then stained with Ehrlich's haematoxylin for 8 minutes and with aqueous eosin Y for 2 minutes and again rinsed with water. Sections were dehydrated through different grades of alcohol. Dehydrated sections were passed through two baths of xylene and were dried & mounted with distrene 80 dibutylphthalate xylene (DPX) mountant. Eosin and hematoxylin stained sections were viewed under light microscope at 10x and 40x objectives.

Statistical analysis

Data was reported as mean and their corresponding standard deviation of mean (mean ± SD). Results were statistically analyzed by comparing with control using Student's 't' test (unpaired). Probability (p) value less than 0.05 were considered as significant, $p < 0.01$ highly significant, $p < 0.001$ very highly significant and $p > 0.05$ as not significant.

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