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Effect of *Piper longum* linn on histopathological and biochemical changes in isoproterenol induced myocardial infarction in rats.

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ABSTRACT

In this study, cardioprotective effect of methanolic extract of *Piper longum* (MePl) was evaluated in a rat model having acute myocardial infarction, induced by Isoproterenol (ISO) (85 mg/kg,sc, for two consecutive days). MePl (250 mg/kg and 500 mg/kg) pretreatment orally for 28 days significantly prevents the damage induced by isopreterenol, is supported in histopathological examination evinced by decresed vacular and fatty degeneration, granular disintegration and hyaline necrosis of muscle fibers.. The results of histopathological examination of rat's heart sections was confirming the myocardial injury which was further supplemented by biochemical observations like decreased levels of Creatine Kinase- MB (CKMB) and Lactate dehydrogenase (LDH) as serum myocardial markers. The results were comparable to that of ascorbic acid treated group. The present results provide evidence for the first time, that MePl pretreatment prevents myocardial injury against ISO-induced myocardial infarction in rats.

Key words: Creatine Kinase-MB, Isoproterenol, Lactate dehydrogenase, Myocardial Infarction, *Piper longum*.

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INTRODUCTION

Globally, myocardial infarction (MI) is one of the leading causes of death for both men and women [1] Due to changing lifestyles in developing countries, such as India, and particularly in urban areas, MI is making an increasingly important contribution to mortality statistics [2].

MI is a complex phenomenon affecting the mechanical, electrical, structural, and biochemical properties of the heart [3] Several methods have been used to study the beneficial effects of many drugs on cardiac function [4] The administration of isoproterenol, a β-adrenergic agonist, has been found to cause severe stress in the myocardium, resulting in the infarct-like necrosis of the heart muscle [5].

Despite considerable progress in the management of MI by synthetic drugs, the search for indigenous cardio protective agents still continue. Some plant products have also been demonstrated to cause augmentation of myocardial antioxidants [6,7]. Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life [8].

Piper longum Linn. is the drug belonging to the family Piperaceae contains alkaloid piperine, piperlongumine, piperlonguminine. It is a common Indian dietary spice which has been shown to possess a wide range of therapeutic utilities in the traditional Indian medicines. It has been reported to possess immunomodulatory, antiasthamatic, hepatoprotective, hypocholestremic and antiinflammmatory activities. [9] It has also TXA2 receptor antagonistic action thereby having thrombolytic activity. [10] Further more it possess negative chronotropic and negative inotropic activities. [11] It has been found to possess antioxidant activity which neutralizes harmful effects of excessive free radicals produced in the body [12].

In light of antioxidant activity of MePl, the present investigation was carried out to observe the prevention of various histopathological and biochemical changes in MI induced by ISO in rats.

MATERIALS

Identification and collection of Plant material

Dried fruits of *Piper longum* Linn. were obtained from a commercial supplier of Ahmedabad. Drug was identified and authenticated by Dr. Geetha K. A., Senior Scientist (Plant Breeding) at National Research Center for Medicinal and Aromatic Plants, Boriavi, Anand, Gujarat.

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Extraction

Dried fruits of *Piper longum* Linn. were finely powdered and passed through 60 mesh size sieve. 50 gm of fine powder was extracted with diethyl ether in a continuous soxhlet apparatus for 3 hours to remove the fatty materials. Then, the defatted powder was extracted with 300 ml methanol for 7-8 hours. The filtrate was concentrated and the methanolic extract of *Piper longum* Linn. (extractive value 18.5 %w/v) was collected and stored in a cool dry place until further use [9].

Chemicals and drugs

Isoprenaline (isoproterenol) hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO, USA). All the other chemicals used in the study were of analytical grade.

Animals

Healthy male Wistar Albino rats (250-300gm, 12-14 weeks age) were housed in cages with free access to standard rat chow (diet) and water *ad libitum* and acclimatized to the surroundings for one week prior to the experiment. Animals were harbored on a 12hr light/dark cycle at a constant temperature (22º±1ºC) and humidity (55±1%).The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. 8009 dated 22th Dec 2008).

METHODS

Isoproterenol induced Myocardial Infarction

Male Wistar Albino rats (250-300gm, 12-14 weeks age) were randomly allocated to 5 groups containing eight animals each. Group I (Normal Control) and Group II (Model Control) animals received distilled water throughout the study period. Group III, Group IV and Group V received Ascorbic acid (250mg/kg), MePl 250mg/kg and MePl 500 mg/kg respectively once a day per oral for 28 days. On the 27th day MI was induced in animals of Group II to Group V by administration of the first dose of ISO (85 mg/kg, S.C) followed by second dose of ISO after 24 hours. 12 hours after the second injection of ISO, rats were anesthetized by chloroform and decapitated. Blood was collected from each animal and was stored for 1 to 2 hours at room temperature. Serum was obtained by centrifugation at 4000 rpm for 15 min and at the same time heart was rinsed with ice-chilled physiologic saline. It was then used for estimation of biochemical parameters like CKMB and LDH. [13].

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Histopathology of heart

Heart (finely sliced) was fixed in 10% formalin for 24 hrs and washed in running water for 24hrs. it was processed with an automate tissue processor (Shandon Citadel Model 2000). Samples were dehydrated with Alcohol in an autotechnican.& then cleared in Benzene to remove absolute alcohol Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C & then in a cubical block of paraffin made by the "C" moulds following dehydration & embedding. Section were cut at 3µm with a rotary microtone, stained with hematoxylin & eosin & examined microscopically, by a pathologist Dr. Brahmbhatt (M.D. Pathologist) of the experiments being performed. Microscopically the lesions of the heart were graded as follows [15]:

Grade-0: No lesion.
Grade-1: Fibroblastic swelling or proliferation and accumulation of histiocyte.
Grade-2: Edema mottled staining, fragmentation and segmentation of muscle fiber.
Grade-3: Vacular and fatty degeneration, granular disintegration and hyaline necrosis of muscle fibers. Marked capillary dilatation with hemorrhage, extensive edema occasionally with a mucoid component caused sequestration of muscle fibers.
Grade-4: Confluent lesion throughout the heart. Lesions were similar in characteristics to those in Grade- 3.

Estimation of serum enzyme levels in rats

Activities of CK-MB and LDH were measured in serum using commercial kits purchased from Vallabha Enterprise (Ahmedabad, India).

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Data's were analyzed as a completely randomized design using One Way Analysis of Variance (ANOVA). Any significant difference between means was assessed by the Dunnett's Post Hoc Test. 95% level of significance ($p<0.05$) was used for the statistical analysis. Statistical analysis was performed using primer statistical software.

RESULTS

Effect of MePl on histopathology of heart

Microscopic examination of heart sections of rats treated with isoproterenol revealed Vacular and fatty degeneration, granular disintegration and hyaline necrosis of muscle fibers. Marked capillary dilatation with hemorrhage, extensive edema occasionally with a mucoid component caused sequestration of muscle fibers. (Grade 3). Rats pretreated with MePl extract at doses of 250 mg/kg shows edema mottled staining, fragmentation and segmentation of muscle fiber. (Grade 2) while MePl 500 mg/kg and ascorbic acid shows fibroblastic swelling or proliferation

and accumulation of histiocytes (Grade 1). It further showed dose-dependent protection characterized by less leucocytic infiltration and degeneration of myofibrillar tissues.

Effect of MePl on Serum levels of CK-MB and LDH

ISO model control animals showed significant increased in CKMB level (866.30±8.95; P<0.001) as compared to normal control (21.081±1.23). Ascorbic acid (250 mg/kg, p.o. for 28 days) treatment significantly decreased the CKMB level (215.18±26.072; P<0.001) as compared to ISO Control animals. MePl (250 mg/kg and 500 mg/kg, p.o. for 28 days) treatment significantly decreased the CKMB level (517.03±6.64, 495.18±16.32 ; P<0.001 respectively) as compared to ISO Control animals (Table 1).

In similar way LDH level was significantly increased (1148.55±169.41; P<0.001) as compared to normal control (438.65±36.26). Ascorbic acid (250 mg/kg, p.o. for 28 days) treatment significantly decreased the LDH level (480.34±27.18; P<0.001) as compared to ISO Control animals. MePl (250 mg/kg and 500 mg/kg, p.o. for 28 days) treatment significantly decreased the LDH level (567.16±38.19, 353.29±11.26; P<0.001 respectively) as compared to ISO Control animals (Table 1)

DISCUSSION

The present study demonstrated that the MePl has efficiently protected the myocardium against isoproterenol-induced MI. Karthick et al., 2006 and zhou et al., 2008 have reported that supramaximal doses of isoproterenol induce subendocardial myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction.[15,16] Thus, it is widely used as a model of evaluating cardioprotective drugs and studying myocardial consequences of ischemic disorders [14]

In line with above notion, Isoprenaline (85 mg/kg, S.C) was used in present study, for induction of MI in experimental animals. Subcutaneous administration of isopreanline, a synthetic catecholamine and β-adrenergic agonist, into adult rats leads to biochemical and morphological alterations in the heart tissue of experimental animals similar to those observed in human MI [17]. It causes fatty changes in the myocardium, together with disorganization of nucleoli, the appearance of abnormally increased smooth endoplasmic reticulum and atypical dense bodies, and distorted shape changes in mitochondria. [18].

Significant changes in the normal architecture of myocardium was observed on induction of MI by Isoprenaline in experimental animals like vacular and fatty degeneration, granular disintegration and hyaline necrosis of muscle fibers along with marked capillary dilatation with hemorrhage, extensive edema occasionally with a mucoid component caused sequestration of muscle fibers were observed in histopathological evaluation of model

control heart muscle fibers. Treatment with MePl (250 mg/kg and 500 mg/kg) produced significant prevention of destruction of normal myocardial architecture induced by ISO as revealed by evidences of histopathological study. [14] These findings suggest that MePl has protective action against ISO induced MI.

It has been reported that myocardial necrosis show membrane permeability alterations which bring about the loss of function and integrity of myocardial membranes. Of all the macromolecules to leak from damaged tissues, enzymes, because of their tissue specificity and catalytic activity, are the best markers of tissue damage. Increased activities of these marker enzymes in the serum are indicative of cellular damage, severity of necrotic damage and loss of functional integrity of cell membrane. [19, 20]. The levels of these enzymes present in plasma are reported to be directly proportional to the number of necrotic cells present in the cardiac tissue [21]. In context it has been reported that CKMB is present in the higher proportion and concentration in the myocardium and also is referenced as early marker of MI [22]. Moreover LDH levels get elevated when there is tissue inflammation and necrosis. Numerous researchers have also reported elevated levels of CKMB and LDH in MI induced by ISO. Finding of the present study were in accordance with the above experimental evidences whereby treatment with MePl significantly decreased level of CKMB and LDH as compared to ISO model control animals, suggesting improvement in necrotic damage induced by ISO.

The MePl extract used in the present investigation was in crude form and likely to contain compounds such as alkaloids and amides, lignans, esters and volatile oil. It is not possible to determine which of these compounds are responsible for protective action in the heart but this action might be due to one of the above mentioned compounds. The study needs further investigation in order to know the exact mechanism behind the cardioprotective effect of MePl.

CONCLUSION

To conclude, MePl significantly prevents the damage induced by Isoproterenol on histopathological and biochemical changes in rat model of Myocardial Infarction.

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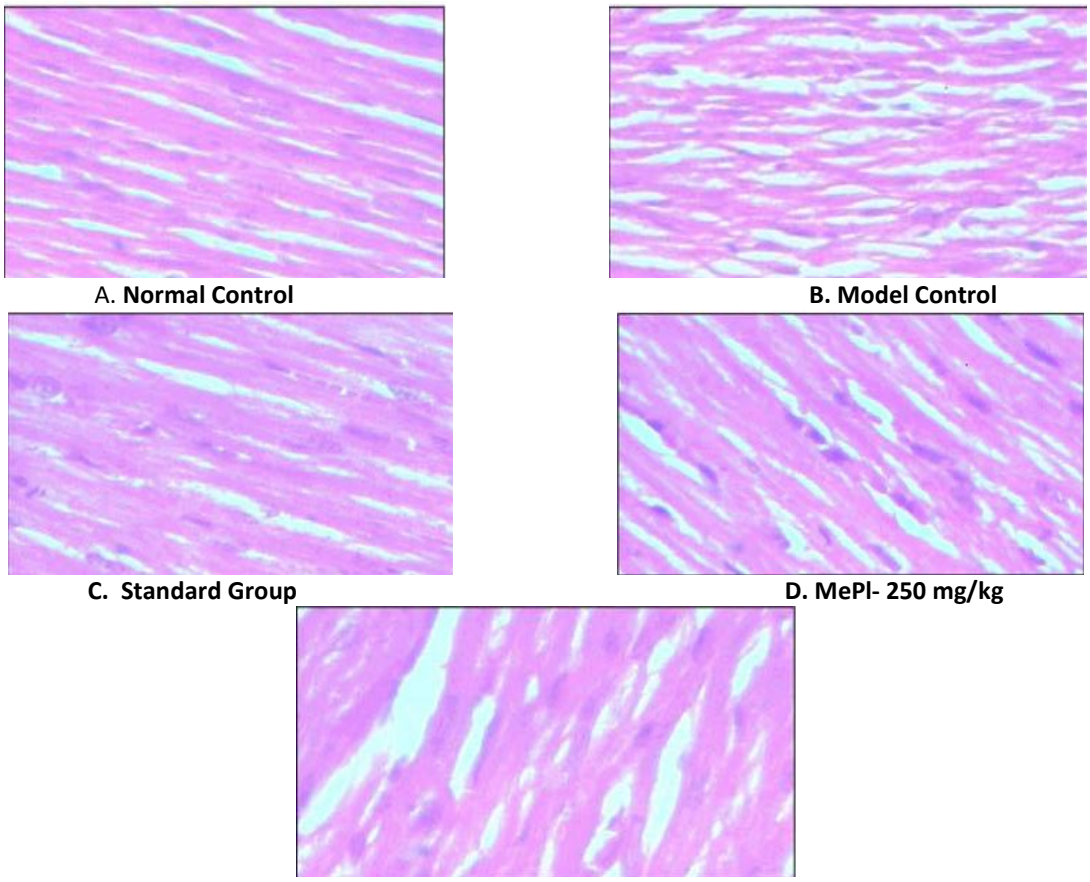

A. **Normal Control**

B. Model Control

C. Standard Group

D. MePl- 250 mg/kg

E. MePl-500 mg/kg

Figure 1: Photographs of hematoxyline & eosin stained paraffin sections of rat hearts. a) Normal control, b) Model control c) Ascorbic acid 250 mg/kg, p.o for 28 days, d)MePl-250 mg/kg, p.o for 28 days & e) MePl-500 mg/kg, p.o for 28 days respectively, attenuated the extent & severity of the histological signs of cell damage. Magnification 100 times.

Table 1: Effect of MePl on the levels of CKMB & LDH in serum of normal and ISO induced MI.

Groups	CKMB level in U/ml	LDH level in U/ml
Normal	21.081±1.23	438.65±36.26
Model	866.30±8.95#	1148.55±169.41#
Standard	215.18±26.072*	480.34±27.18*
MePl-250 mg/kg	517.03±6.64*	567.16±38.19*
MePl-500 mg/kg	495.18±16.32*	353.29±11.26*

The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnet's Post Hoc test. P values <0.05 were considered significant.

* Significantly different from Normal control group at $P < 0.05$.

\# Significantly different from Model control group at $P < 0.05$.

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