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EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF ABROMA AUGUSTA ON ISOPRENALIN INDUCED MYOCARDIAL NECROSIS IN RATS

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Medicinal herbs, *Abroma augusta* , isoprenaline, antioxidants, myocardial infarction.

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Abstract

Myocardial infarction (MI) means that part of the heart muscle suddenly loses its blood supply. Without prompt treatment, this can lead to damage to the affected part of the heart. The present study was undertaken to study the cardioprotective effect of methanol extract of *Abroma augusta* leaves. The powdered crude leaves were extracted in a Soxhlet apparatus with methanol. Extract was dried at 40°C under pressure and stored at 4°C until use. Phytochemical screening of extract was done by standard methods. Methanol extract of *Abroma augusta* was studied for acute oral toxicity according OECD guidelines No. 423 on Wistar rats. Isoprenalin induced myocardial necrosis in rats model was used to study cardioprotective effect. After 10 days blood samples from animals were collected. Serum samples were analyzed for lactate dehydrogenase (LDH) and aspartate transaminase (AST) by using standard kits. The treatment with methanolic extract of *Abroma augusta* significantly decreased the elevated levels of LDH, and AST as compared to control group. The present results indicated that the rats pretreated with *Abroma augusta* were significantly protected from myocardial damage caused by ISO may be due to the presence of flavonoids, polyphenols, alkaloids found in extract

Introduction

Cardiovascular diseases are the major health problem of advanced as well as developing countries of the world. Hypertension is the common cardiac disease followed by ischemic heart disease (IHD) (1). Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardium demand (2). Myocardial infarction is the rapid development of myocardial necrosis. Oxidative stress resulting from increased production of free radicals associated with decreased levels of antioxidants in the myocardium plays a major role in cardiovascular diseases. (3)

Myocardial infarction (MI) means that part of the heart muscle suddenly loses its blood supply. Without prompt treatment, this can lead to damage to the affected part of the heart. An MI is called a heart attack or a coronary thrombosis (4). There are different types of MI. The two main types are called ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI) (5). In a STEMI, the artery supplying an area of the heart muscle is completely blocked. In a NSTEMI, the artery is only partly blocked (6). The common cause of an MI is a blood clot (thrombosis) that forms inside a coronary artery, or one of its branches. This blocks the blood flow to a part of the heart (4).

Isoprenaline (ISO), a catecholamine, was administered in the present study, as it serves as a standard model to study the beneficial effect of many drugs on cardiac function. ISO is a synthetic β-adrenergic agonist that causes severe stress in the myocardium and causes necrosis in the heart muscle. ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes

(3). On auto-oxidation, ISO generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phos-pholipids and cause severe damage to the myocardial membrane (7). Several medicinal plants have been found to possess antioxidant properties and have beneficial effects in pathological conditions like cancer, liver diseases, cataract and myocardial ischaemia (8, 9).

Recently, attention has been focused on non- nutrient phytochemicals and polyphenols such as the flavonoids, alkaloids and xanthones derived from different plant species as potential therapeutic agents in the prevention and management of cardiovascular diseases due to their antioxidant nature (10). With technological advancement of science, the isolation, identification and elucidation of the chemical principles from medicinal plants have become much simpler and have contributed significantly to the development of new drugs for almost all type of diseases including cardiovascular, cancer and hepatic diseases (11).

Materials and methods

Plant material

The leaves of *Abroma augusta* (Ulatkambal) were collected in the month of January from Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur (M.P.). The plant was identify and authenticated by Dr. A.B. Tiwari, Sr. Scientist, Department of Crop & Herbal Physiology, Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur (M.P.). Leaves of *Abroma augusta* were dried in shade, coarsely powdered and used for the preparation of extracts. The powdered crude drug was extracted in a Soxhlet apparatus with methanol. Finally extract was dried at 40°C under pressure and stored at 4°C until use. Phytochemical screening of extract was done by standard methods. (12)

Drugs and Chemicals

Gliclazide, Isoprenalin (Microlabs, India) were used in the study. All other reagents used in this study were of analytical grade.

Animals

Healthy adult male Wistar albino rats between 6 and 8 months of age and weighing about 150-250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 30 C; 35–60% humidity). All the experimental protocols were approved by Institutional Animal Ethics Committee.

Acute Toxicity Studies

Methanol extract of *Abroma augusta* was studied for acute oral toxicity as per revised OECD guidelines No. 423 (13) The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence 200 and 400 mg/kg doses of extract were used for the study.

Cardioprotective activity

Isoprenalin induced myocardial necrosis in rats

Animal grouping

The experimental animals were divided into five groups, six animals in each group

- Group 1: Control group, animals received distilled water as a vehicle for 8 days and normal saline on day 9 and 10.
- Groups 2 animals received ISO (5.25 mg/kg, i.p. on day 9 and 8.5 mg/kg on day 10 in normal saline)
- Groups 3: animals received Gliclazide 25 mg/kg +ISO (5.25 mg/kg, i.p. on day 9 and 8.5 mg/kg on day 10 in normal saline)
- Groups 4: Methanolic Extract (200 mg/kg, orally) in distilled water for 8 days and then ISO (5.25 mg/kg, i.p.) on day 9 and 8.5 mg/kg on day 10 in normal saline).
- Groups 5: Methanolic Extract (400 mg/kg, orally) in distilled water for 8 days and then ISO (5.25 mg/kg, i.p.) on day 9 and 8.5 mg/kg on day 10 in normal saline). **(**14)

Serum parameters

After 24 hr of the last dose of ISO, blood was collected from the retro-orbital plexus of each rat under mild ether anaesthesia (TKM Pharma, India) for determination of biochemical parameters. Serum was separated in cryocentrifuge (Eppendroff, India) at 4 ˚C at 6 000 rpm. for 15 min and lactate dehydrogenase (LDH) and aspartate transaminase (AST) were measured by using standard kits according to the manufacturer's instruction manual (Merck Specialities Pvt. Ltd. India) using an autoanalyser (Nihon Kohden, Japan).

Histopathological studies

At the end of the experiment, myocardial tissues from all the groups were subjected to histopathological studies (15).The tissues were fixed in formalin (10%), routinely processed and embedded in paraffin wax. Paraffin section (5μm) were cut on glass slides and stained with hematoxylin and eosin (H&E) after dewaxing, and examined under a light microscope.

Statistical analysis

Experimental results were mean±SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's test. Data were considered statistically significant only when p value < 0.05, $p< 0.01$.

Results and discussion

The effects of *Abroma augusta* on serum marker enzymes LDH, and AST are shown in Table 1. Serum LDH and AST levels were significantly increased in group II when compared with control group I, while methanolic extract *Abroma augusta* group IV and group V significantly decreased the elevated levels of LDH, and AST though not to control value and Gliclazide treated group III significantly decreased the elevated levels of LDH, and AST as compared to control group.

Histopathological studies of Heart – Photomicrograph of vehicle control group revealed a normal architecture with regular morphology of myocardial cell membrane. Photomicrograph of ISO treated group showed inflammation infiltrate. Gliclazide treated group showed normal morphology with absence of inflammation and sign of muscle necrosis. In animal treated with methanolic extract *Abroma augusta* pretreatment, the morphology of myocardium was essentially within normal limits. No area of necrosis and cellular infiltration was seen indicating that methanolic extract of *Abroma augusta* has significant cardioprotective effect and it also, maintained myocardial membrane integrity.

The methanolic extract of *Abroma augusta* showed strongest antioxidant activity due to the presence of flavonoids (16). Methanolic extract of *Abroma augusta* has significant cardioprotective effect and it also, maintained myocardial membrane integrity. *Abroma augusta* might reduce or prevent excessive production of free radicals, exhibiting its cardioprotective effect due to the presence of flavonoids, polyphenols, alkaloids found in extract.

Furthermore, histopathological observation revealed that *Abroma augusta* prevented the degeneration of myofibriller tissue and leucocytic infilteration in myocardial infaraction. In conclusion, the present results indicated that the rats pretreated with *Abroma augusta* were significantly protected from myocardial damage caused by ISO. Pharmacological augmentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in disease associated with increased oxidative stress . The finding of current study proves that the methanol extract of *Abroma augusta* effectively treated hyperlipidemia in murine model. This activity may be due to presence of flavonoids, polyphenols, alkaloids found in extract. However isolation and characterization of active constituent responsible for this activity is necessary.

References

1 Begum S, Akhtar N. Cardioprotective effect of amlodipine in oxidative stress induced by experimental myocardial infraction in rats. Banjladesh J Pharmacol 2007; 2: 55-60.
2 Prabhu S, Mallika J, Sabitha KE, Shyamaladevi CS. Cardioprotective effect of mangiferin on isoproterenol inducednmyocardial infarction in rats. Indian J Exp Biol 2006; 44:209-15.
3 Siddiqui HH. 1962. Pharmacology of an alcoholic extract of *Bombyx mori* cocoons. Indian J Pharm, 24:183
4 Thygesen K, Alpert JS and White HD (2007). "Universal definition of myocardial infarction". *European Heart Journal*, 28 (20) 2525–38.
5 Montalescot G, Dallongeville J and Van Belle E (2007). STEMI and NSTEMI: are they so different? 1 year outcomes in acute myocardial *European Heart Journal*, 12 1409-17.
6 Lamas GA, Escolar E and Faxon DP (2010). Examining treatment of ST-elevation myocardial infarction: the importance of *Journal of Cardiovascular Pharmacology & Therapeutics*, 1 6-16.
7 Huh H, Staba EJ. Botany and chemistry of *Ginkgo biloba* L. *J Herbs Spices Med Plants* 1992;1:92-124.
8 Hertog MGL., Feskens EJM, Hollam PCH, Katan MB, Kromhout D. 1993. Dietary antioxidant flavonoids and risk of coronary heart diseases, The Zutphen elderly Study. Lancet 342: 1007-1020.
9 Shalini VK, Srinivas L. 1987. Lipid peroxide induced DNA damage: protection by turmeric (*Curcuma longa*). Mol Cell Biochem 77: 3-10.
10 Pauletti P M, Castro-Gamboa I, Siqueira Silva D H, Young M C, Tomazela D M, Eberlin M N & da Silva Bolzani V, New antioxidant C-glucosylxanthones from the stems of Arrabidaea samydoides, J Nat Prod, 10 (2003) 1284.
11 Sato T, Kawamoto A, Tamura A, Tatsumi Y & Fujii T, Mechanism of antioxidant action of pueraria glycoside (PG)- 1 (an isoflavonoid) and mangiferin (a xanthonoid), Chem Pharm Bull (Tokyo), 40 (1992) 721.
12 Harborne JB. Phytochemical Method: A guide to modern techniques of plants analysis. New York: Chapman and Hall; 1983.
13 Organization for Economic Cooperation and development (OECD). Guideline 423 for testing chemicals: Paris; 2001;1-14 .
14 Mali VR., Bodhankar SL., 2010. Cardioprotective eff ect of *Lagenaria siceraria* (LS) fruit powder in isoprenalin-induced cardiotoxicity in rats. *Eur J Integrative Med* **2**: 143–149
15 Luna L C.,1960. Mannual of histological screening methods of armed forces institute of pathology ,Mc. Graw Hill book Co. New York),125.
16 Nahar, L., Ripa, F., Rokonuzzaman., 2009. Investigation on Antioxidant Activities of Six Indigenous Plants of Bangladesh . *Journal of Applied Sciences Research*, 5(12): 2285-2288

Table.1 Effect of Abroma augusta on serum parameters in rats after isoprenalin induced myocardial infarction.

Parameters	Control	ISO group	Standard (Gliclazide)	Extract(200mg/kg)	Extract(400mg/kg)
AST IU/L	194.33 ±2.58	325.33±2.73	205.83±2.13*	245.83±2.56* ψ	225±3.74* ψ
LDH IU/L	193.33±1.75	425.33±3.26	256.33±2.06*	366.5±1.87* ψ	265±2.36 * ψ

Values are expressed as Mean ± SEM (n=6) Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to ISO control group ($p< 0.01$). ψ Significantly compared to Standard group ($p< 0.01$)

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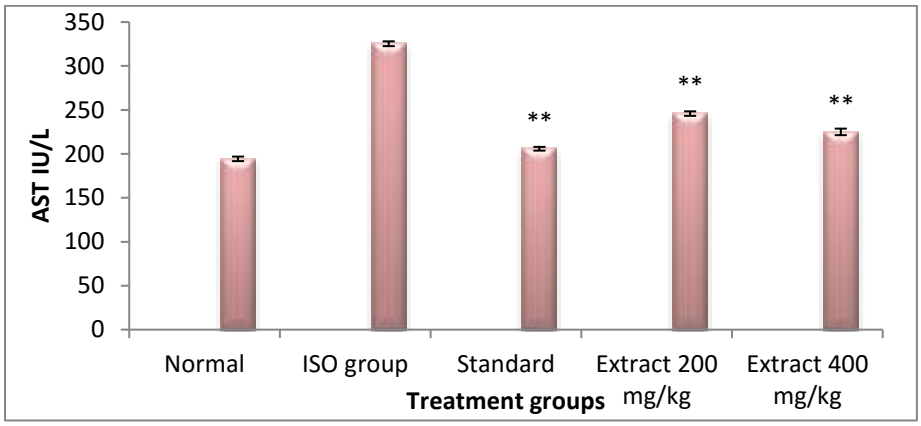

Fig 1 Effect of Abroma augusta on AST in rats after isoprenalin induced myocardial infarction

Values are expressed as Mean ± SEM. Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to ISO control group ($p< 0.01$).

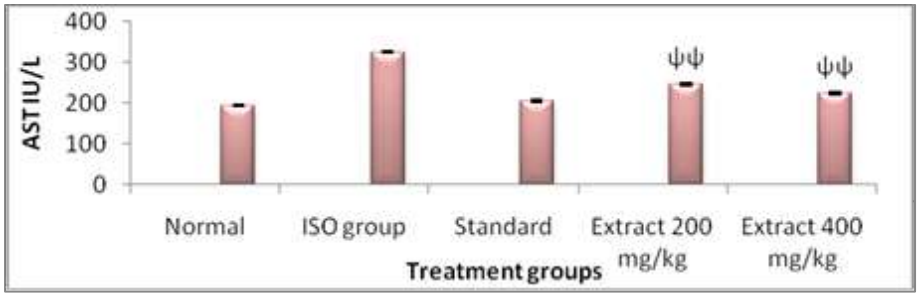

Fig 2 Effect of Abroma augusta on AST in rats after Isoprenalin induced myocardial infarction

Values are expressed as Mean ± SEM. Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p< 0.01$).

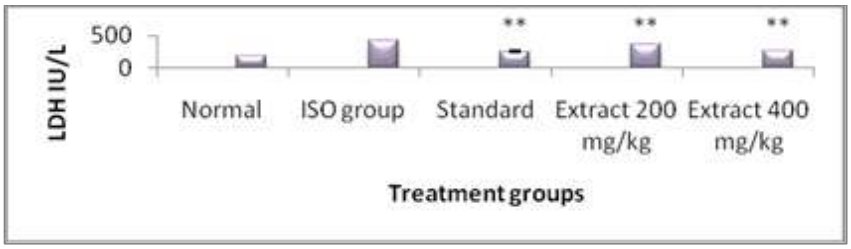

Fig 3 Effect of Abroma augusta on LDH in rats after Isoprenalin induced myocardial infarction.

Values are expressed as Mean ± SEM. Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to ISO control group ($p< 0$).

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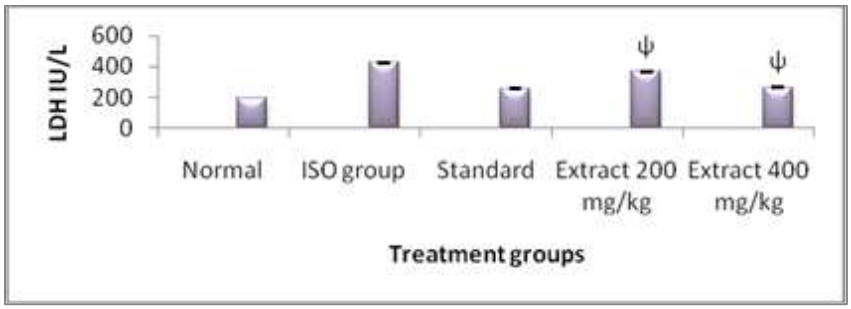
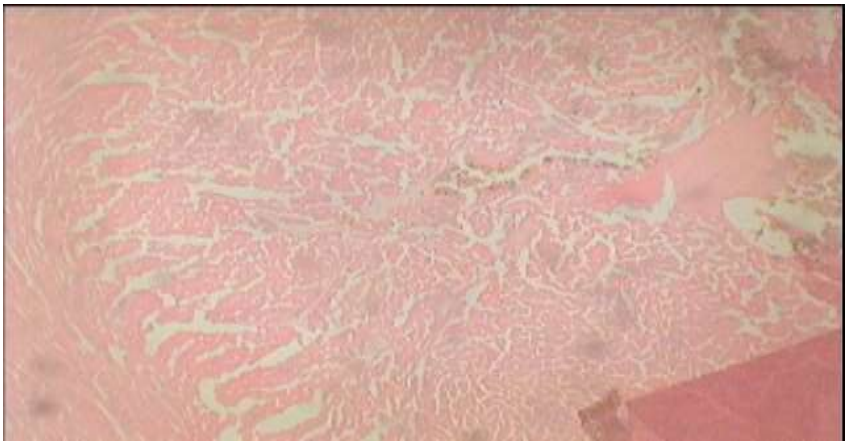


Fig 4 Effect of Abroma augusta on LDH in rats after Isoprenalin induced myocardial infarction.

Values are expressed as Mean ± SEM. Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p < 0.01$).



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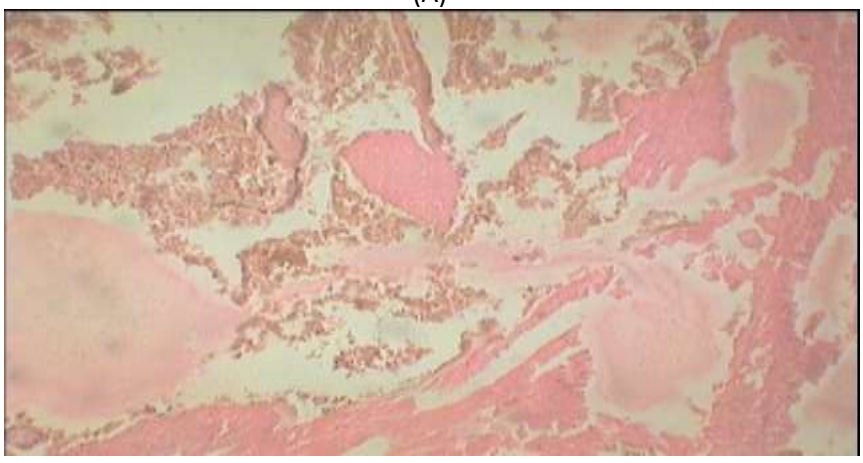

(B)

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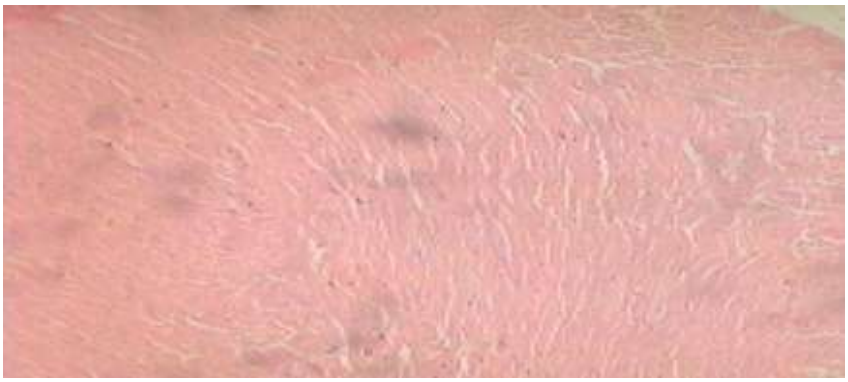
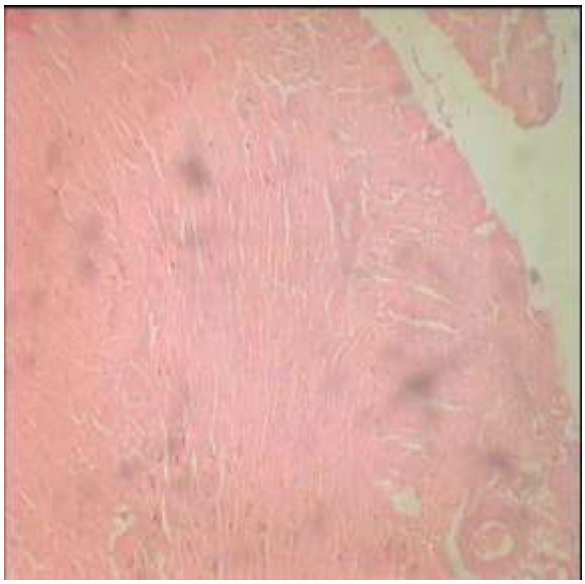


(C)



(D)

(E)

Fig 5 Histological examination of heart in experimental animals. (A) normal group (group I) rat showing normal architecture of heart with regular morphology of myocardial cell membrane .(B) ISO group rat showing inflammation and sign of muscle necrosis in heart section (C) standard group (group III) rat showing normal myocardial fibres.(D) Extract 200 mg/kg group (group IV) rat showing absence of inflammation and sign of muscle necrosis in heart section .(E) Extract 400 mg/kg group (group V) rat showing absence of inflammation and sign of muscle necrosis in heart section.