

Cardioprotective potential of *Thymus linearis* Benth

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Abstract: In developing countries, myocardial ischemia and the resulting impairments in heart function are the leading cause of illness and mortality. *Thymus linearis* Benth has been used as an antibiotic, antioxidant, and antihypertensive agent for centuries. The goal of this investigation was to see if *Thymus linearis* could protect isoproterenol and doxorubicin-induced myocardial ischemia *in vivo* at doses of 25 mg/kg s.c. and 15 mg/kg i.p., respectively. The level of cardiac enzymes (CK-MB, LDH, and AST) in the serum isolated from the experimental animal's blood was used to determine myocardial ischemia. The anti-ischemic potential was assessed by comparing the levels of the aforementioned cardiac biomarkers in the intoxicated and treated animal groups. The study found substantial increase (p0.0001) in the serum levels of CK-MB, LDH, AST when compared to intoxicated groups, while pretreatment of animals with crude extract of *Thymus linearis* significantly reduced the rise in serum cardiac indicators. The findings of the study indicated that the aqueous methanolic *Thymus linearis* crude extract has cardioprotective potential against Isoproterenol and Doxorubicin-induced cardiac necrosis in rats.

Keywords: Cardioprotective, isoproterenol, doxorubicin, cardiac biomarkers.

INTRODUCTION

Cardiovascular disease is recognized as the leading cause of death worldwide and is expected to continue to be the leading cause of death. (Ramadoss *et al.*, 2012). Despite of all the modern clinical innovations, Myocardial Ischemia is one of the grave and undiagnosed heart related disorder throughout the world, leaving an open window for the investigation of new therapies (Bukhsh *et al.*, 2014). Likewise, in Pakistan, most prevailing cardiovascular diseases are ischemia, myocardial infarction, stable angina, and hypertension. Considering the seriousness of cardiovascular diseases notably myocardial ischemia, the health system of Pakistan does not seem to be promising in terms of managing and treating the most misapprehended and mishandled condition of myocardial ischemia. Furthermore, using medication to treat an ailment comes at a high expense and has long-term adverse effects. The unrelenting nature of disease, horrible morbidity, and ever-increasing medicine costs persuade the thwarted patient to seek treatment from modern medical systems, with a hope that they will raise quality of life (Hilsden *et al.*, 2003). Herbal medicines are gaining popularity day by day because of their easily availability and cheaper to poor person. These

medicines are considered as free from side effects. Many plant foods and extracts reported to have beneficial effects in reducing cardiovascular diseases (Shaito *et al.*, 2020). Continuing research is necessary to elucidate the pharmacological activities of many herbal remedies now being used to treat cardiovascular disease. Abdel Sattar *et al* (2012) precisely endorsed cardio protective activity of methanolic extract of *Stachys schimperi* Vatke against doxorubicin-induced cardiotoxicity. *Thymus linearis* plant grows wildly in India, Afghanistan, Nepal and the Himalaya (ShoaiB *et al.*, 2021) while in Pakistan it is largely found in northern areas such as Chatral, Kashmir and Gilgit-Baltistan (Wazir *et al.*, 2004). Previous studies reported that *Thymus* species owned strong antibacterial, antifungal, antiviral, antiparasitic, antioxidant antihypertensive and antihyperlipidemic agent (Dababneh., 2007). The antiseptic, antioxidant, insecticidal and anesthetic properties of thyme oil are mainly attributed to the presence of thymol, carvacrol, geraniol and other volatile components. No scientific data is existing relating to this plant as anti-ischemic to our utmost information. Hence, the present study was aimed to investigate the *in vivo* anti-ischemic efficacy of *Thymus linearis*.

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MATERIALS AND METHODS

Chemicals

Methanol (RCI Labscan Limited, Bangkok, Thailand), Isoproterenol HCL (Sigma Aldrich Chemie GmbH), Doxorubicin HCL (Zhejiang Hisun Pharmaceutical Co. Ltd, China). Commercial kits of LDH, AST and CKMB (Randox diagnostic kits, Randox laboratories, UK) were used.

Plant material

The aerial parts of *Thymus linearis* were collected in June 2011 in Gilgit District, Shikiyote Village Pakistan, and was identified and authenticated by Dr. Shair Wali Khan, Assistant Professor Botany, Karakorum International University Gilgit Baltistan, Pakistan. Aqueous methanolic (30:70) root extract of *Thymus linearis* was prepared using a cold maceration process. (Alamgeer *et al.*, 2013).

Animals used

Young and healthy Sprague Dawley rats (either sex) were obtained from Islamia University, Bahawalpur (IUB). The animals were kept in specially designed plastic cages with sipper bottles at the Department of Pharmacology, Faculty of Pharmacy, University of Sargodha's animal house, where they were kept at a constant room temperature (25-10oC) and given human care according to National Institute of Health guidelines (NIH). The animals were given one week to acclimate to laboratory settings before being given free access to a conventional laboratory diet with water *ad libitum*. The animal for controls groups used in this article were same with the previously submitted paper in the same journal as main author were same and study was conducted same time. The study was approved by Animal Ethics Committee, University of Sargodha, Sargodha.

Isoproterenol induced myocardial ischemia

The assessment of cardio protective activity of the aqueous methanolic extract of *Thymus linearis* was carried out as described by Rai *et al.* (2011) with slight modifications (Rai *et al.*, 2011). Myocardial ischemia was produced with a subcutaneous injection of ISO hydrochloride diluted in physiological saline at a concentration of 25 mg/kg b.w. The experimental animals were split into four groups (n=5) at random. For 10 days, Group I was given normal saline (0.5 ml/kg b.w. p.o.) as a control. On the 9th and 10th days of the experimental protocol, Group II was ISO Intoxicated and received ISO (25mg/kg b.w s.c). Group III served as Thymus treated group in which animals were pre-treated with AMTL extract (500mg/kg b.w p.o.) for a period of 10 days and were injected ISO (25mg/kg b.w. s.c.) on the 9th and 10th day. Group IV was baseline group in which animals were given only AMTH extract (500mg/kg b.w. p.o.) for a period of 10 days.

Biochemical estimation

On 11th day twelve hours (12 hours) after the 2nd injection of Isoproterenol, the animals were anaesthetized and 5mL of the blood sample was collected and centrifuged (4000 rpm) to separate the serum. The serum was carefully aspirated with a Pasteur pipette into sterilized ependorff tubes for biochemical analysis. CK-MB, LDH and AST levels were measured by using commercially available kits (Randox kits). All measurements were performed according to the manufacturer's instructions. The cardio protective effect of the plant extract was evaluated by comparing the levels of cardiac markers of the treated group with Isoproterenol intoxicated and baseline group.

Doxorubicin induced myocardial ischemia

The assessment of cardio protective activity of the aqueous methanolic extract of *Thymus linearis* was carried out as described by El Sayed *et al.* (2010) with slight modifications (Sayed *et al.*, 2010). Myocardial ischemia was induced in experimental animals by injecting 15mg/3ml/kg body weight of doxorubicin hydrochloride dissolved in physiological saline intraperitoneally. The experimental animals were split into four groups (n=5) at random. For 14 days, Group I was given normal saline (0.5 ml/kg b.w. p.o.) as a control. DOXO was assigned to Group II. On the 12th day of the experimental regimen, the subjects were intoxicated and given doxorubicin hydrochloride (15mg/3ml/kg, i.p.). Group III served as Thymus treated group in which animals were pre-treated with AMTL extract (500 mg/kg b.w p.o.) for a period of 14 days and were injected doxorubicin hydrochloride IP (15 mg/3ml/1000g body weight) on the 12th day. Group IV was baseline group in which animals were given only AMTH extract (500 mg/kg b.w. p.o.) for a period of 14 days.

Biochemical analysis of the serum

On 15th day, seventy two (72) hours after the single injection of doxorubicin hydrochloride and 24 hours after the last dose of aqueous methanolic *Thymus linearis* extract, the rats were anaesthetized and 5ml of the blood sample was collected, centrifuged to separate the serum.

STATISTICAL ANALYSIS

The data was presented as a mean standard deviation. One way analysis of variance (ANOVA) was used to determine the statistical significance of the data. GraphPad Prism software Version 5.00 (GraphPad, San Diego, CA, USA) was used to determine whether there was a significant difference between the groups.

RESULTS

Thymus linearis decreased cardiac marker enzymes in isoproterenol induced myocardial ischemia

The findings of the current study divulged that the experimental animals injected with ISO at a dose of

25mg/kg subcutaneously, resulted in significant increase ($p < 0.0001$) in serum levels of CK-MB, LDH, AST. Whereas, the animals pretreated with *Thymus linearis* extract for 10 days prevented a significant rise ($p < 0.0001$) in the mean serum levels of cardiac biomarker enzymes as compared to ISO intoxicated group (fig. 1).

***Thymus linearis* decreased CK-MB, LDH and AST levels in doxorubicin induced myocardial ischemia**

The results of present study showed that intraperitoneal administration of DOXO at a dose of 15mg/kg significantly raised the cardiac marker enzymes (CK-MB, LDH and AST) in serum of DOXO intoxicated rats. Pretreatment with *Thymus linearis* extract for 14 days has showed a significant prevention of increase ($p < 0.0001$) in mean levels of above-mentioned cardiac enzymes when compared with DOXO-intoxicated group (fig. 2).

DISCUSSION

Nutraceuticals, or herbal medicines and phytonutrients, are becoming increasingly popular around the world, with many individuals resorting to them for treatment of a variety of health conditions in a number of national healthcare contexts (Ekor *et al.*, 2014). There are ongoing efforts underway around the world to discover fresh and curative medications for the treatment of cardiovascular ailments, such as myocardial ischemia, which is yet unidentified and a difficult research field with numerous

clinically relevant issues. The present investigation has endeavored to evaluate the cardio protective effect of traditionally used indigenous medicinal plant *Thymus linearis*. The data obtained have undoubtedly shown that the aqueous-methanolic extract of *Thymus linearis* prevented the rise of cardiac markers indicative of myocardial ischemia.

The result showed that upon the administration of Isoproterenol there was a significant ($p < 0.0001$) increase in the serum level of cardiac enzymes (CK-MB, LDH, AST and ALT) which confirms the induction of myocardial ischemia as they are being the diagnostic indicators of disease. This observation was consistent with previously related studies (Ramadoss *et al.*, 2012). Isoproterenol causes severe stress in the cardiac muscle leading to induction of myocardial ischemia. Stimulation of Beta 2 receptors generates positive inotropic and chronotropic effects on cardiac cells, resulting in myocardial hyperactivity and thus an increase in heart workload. Coronary hypotension is also caused by the activation of Beta 2 receptors. Relative hypoxia is caused by both myocardial hyperactivity and coronary hypotension (Jadeja *et al.*, 2010). Because it is abundant in myocardiocytes but almost non-existent in other tissues, the activity of these enzymes in the serum is useful tool for diagnosing myocardial damage (Abhilash *et al.*, 2011).

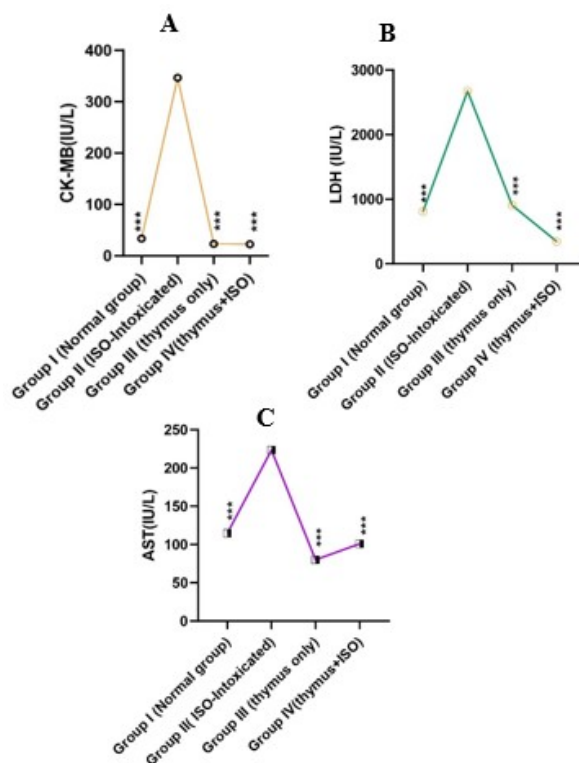


Fig. 1: Effect of *Thymus linearis* crude extract on serum (A) CKMB (B) LDH (C) AST level against ISO induced myocardial ischemia in rats (***) $p < 0.0001$).

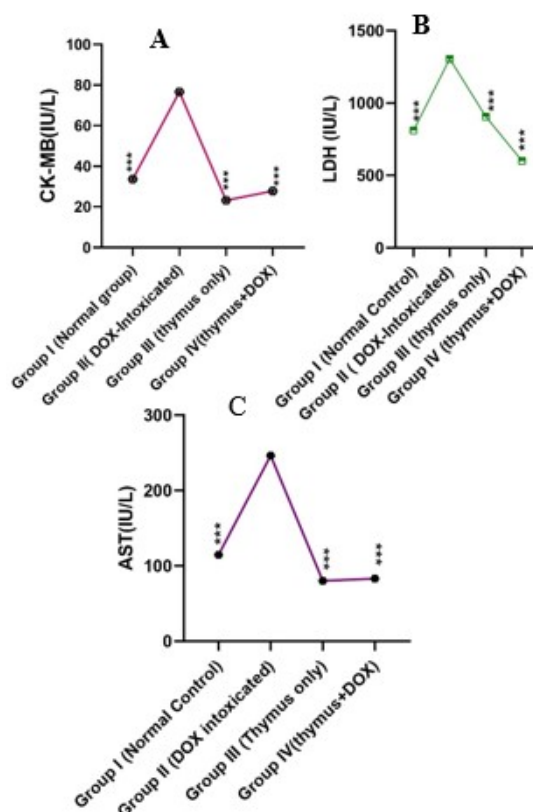


Fig. 2: Effect of *Thymus linearis* crude extract on serum (A) CKMB (B) LDH (C) AST level against DOX induced myocardial ischemia in rats (***)p<0.0001).

As ably delineated by the findings of the current study, pretreatment of animals with aqueous methanolic crude extract of *Thymus linearis* for a period of 10 days, significantly reduced the Isoproterenol-induced leakage of all cardiac diagnostic marker enzymes. The ability of these cardiac indicators to return to normal levels in the pretreated group is a clear indication of the plant's cardioprotective capabilities. *Thymus linearis'* cardioprotective effect can be due to its antioxidant, antihypertensive, and antihyperlipidemic properties as reported by Alamgeer *et al* (2014). Owing to its potent antioxidant activity, the plant might have prevented decomposition of hydro peroxides into free radicals along with the stabilization of the cardiac membrane, resulting in a decrease in these markers' outflow, or the preservation of the myocardium in terms of myocardial ischemia may be due to its negative chronotropic effect, supporting the anti-ischemic potential of the plant. It has previously been observed that hyperlipidemia contributes to doxorubicin-induced cardiotoxicity (El-Shitany *et al.*, 2008).

In the present study, animals of treated group prevented myocardial necrosis against doxorubicin-induced cardiac toxicity, signifying the cardio protective activity of the

plant. The findings of the study suggests that the antioxidant activity of the plant as reported by (Alamgeer *et al.*, 2014) may be one of the several mechanisms which protected these deleterious changes of myocardial membrane. The radical scavenging and antioxidant capabilities of *Thymus linearis* have been reported in the recent literature (Jaric *et al.*, 2015). The antioxidant activity of *Thymus linearis* may be linked to the presence of phenolic compounds (Sultana *et al.*, 2007). All the studied parameters of both models showed that *Thymus linearis* has no harmful effect in rats and the aptness to prevent the rise in serum cardiac marker enzymes rather than decreasing it further authenticates the cardioprotective potential of the plant.

CONCLUSION

According to the findings of the study, an aqueous-methanolic extract of *Thymus linearis* (aerial portions) has a considerable cardioprotective effect and protects the myocardium from Isoproterenol and Doxorubicin-induced cardiac functional and structural injury. To understand the specific mechanism of action of the plant, more isolation, characterization, and purification of the active ingredients, as well as more experiments, would be required.

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