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Protective Effect of *Tecoma stans* Leaves Extract in Isoproterenol Induced Cardiotoxicity

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Abstract: This study was designed to evaluate the protective efficacy of *Tecoma stans* leaves extract in Isoproterenol induced cardiotoxicity in Wistar rat model. Isoproterenol treated control animals exhibited significant elevation in the levels of blood pressure, Heart rate, altered ECG rhythm such as increase in SST segment, prolongation in P waves, QRS complex and RR interval, significant elevation in the serum enzymes and biomarker such as AST, LDH, CK-MB and significant decrease in GSH and increase in MDA content in heart homogenate of experimental animals as compared to normal control group animals. Animals pretreated with different doses of methanolic extract of *Tecoma stans* leaves significantly reduced the elevated levels of blood pressure and Heart rate, exhibited closer to the normal ECG pattern, reduced the elevated levels of AST, LDH and CK-MB, increase in the GSH and decrease in MDA levels as compared to Isoproterenol treated group. In histopathological studies of heart tissue the isoproterenol control animals reflects the increased infraction, congestion, hemorrhage, fibrosis, size of myocytes and inflammation compared to normal control animals. The treatment with standard and different doses of test extract showed dose dependently decreased infarction, congestion, hemorrhage, fibrosis, size of myocytes and inflammation compared to Isoproterenol control animals. The results of our study conclude that the methanolic extract *Tecoma stans* leaves showed protective effect at different dose levels in isoproterenol induced cardiotoxicity could be due to presence of flavonoids and antioxidant enzymes.

Keywords: *Tecoma stans*, Heart rate, Biopac MP 46, ECG, CK-MB, GSH, MDA, Cardiotoxicity

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Introduction

One of the main causes of all deaths from cardiovascular disease is myocardial infarction (MI) (Mnafgui Lopez and Murray, 1998). The prolonged restriction of blood flow through the

coronary artery caused by burst atherosclerotic plaques, myocytes eventually experience oxidative stress and necrosis. MI pathogenesis is still not well understood. However, critical causes of MI include oxidative stress, inflammation, apoptosis, hypoxia, and necrosis (Othman *et al*., 2017).

Isoproterenol (ISO) is a non-selective β-adrenergic agonist and synthetic catecholamine that imposes considerable oxidative stress on cardiomyocytes (after supramaximal dosage) due to auto-oxidation of catecholamine; it results in necrosis, edema and apoptosis, ultimately in myocardial injury and myocardial infarction (Widyaningsi *et al*., 2016). Studies have shown that the pathophysiologies of MI and ISO-induced MI are similar: both trigger various pathological events such as oxidative stress, inflammatory response, necrosis, apoptosis and mitochondrial dysfunction (Goyal *et al*., 2016). Some of the important risk factors of MI are smoking, hypercholesterolemia, high low density lipoprotein, low high density lipoprotein, hyperlipoproteinemia, diabetes, elevated blood pressure, older age and Obesity. Complications of myocardial infarction include Arrhythmias, Congestive Heart Failure, Cardiogenic Shock, Ventricular Aneurysm and Pericarditis (Chawla *et al*., 2011).

Tecoma stans is an erect shrub commonly found in India belonging to the family Bignoniaceae. The shrub has some common names in different native Indian languages. *Tecoma stans* Linn is also known as yellow bells, yellow elder, trumpet flower in English and Piliya in Hindi. *Tecoma stans* is an important medicinal plant. Ginger, Thomas leaves, bark and roots contains biologically active chemicals, and extracts from these tissues are in use as traditional folk medicines (Verma and Singh, 2016).

Tecoma stans is traditionally used in Mexico to control diabetes, hepatic, dysenty, anorexia problems. *Tecoma stans* are having many of the active phytoconstituents like alkaloids, phenols, flavonoids, monoterpenes etc. which leads to the great medicinal value of this plant. The various part of the plants are reported to be having various pharmacological actions like anti-inflammatory, analgesic, anticancer, genotoxic, cytotoxicity, wound healing, anti-hyperglycemic, protect CNS, gastric ulcer healing, antiproliferative, antioxidant, anti-microbial, hemolytic activity, anti-lipoxygenase and acetyl-cholinesterase inhibitory activities (Anand and Basavaraju, 2021).

However**,** upon comprehensive literature survey, no reports found on cardioprotective property of the *Tecoma stans* leaves till date. Hence, the present study was an attempt to explore the cardioprotective property of *Tecoma stans* leaves extract in Isoproterenol induced MI in experimental animals.

Materials and Methods

Collection and preparation of the Tecoma stans leaves extract:

The leaves of *Tecoma stan* were collected from the BLDE Medical College garden after authenticated by Prof. Ramachandra Naik, Department of Botany, SB Arts and KCP Science College, Vijayapur. The fresh leaves of *Tecom stans* were shade dried for 10 days and then powdered. The powdered material was extracted with methanol using Soxhlet apparatus. The solvent was evaporated using rotary flash evaporator. The extract was stored in airtight container in refrigerator below 10 °C for further studies.

Phytochemical investigation on Tecoma stans leaves:

The preliminary phytochemical investigation on methanolic extract of *Tecoma stans* leaves (METSL) was performed (Kokate *et al*., 2015).

Experimental animals:

The Wistar albino rats of 150 - 200 g and Swiss albino mice 20 - 30 g of either sex were used in the experimentation. 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 libitum* under strict hygienic condition. Study

protocol was approved from the Institutional Animal Ethical Committee (IAEC) before initiation of the experiment [Ref. No. bldeacop/IAEC/ 2021/ 01].

Determination acute toxicity study (LD_{50}):

An acute toxicity (OECD Guidelines 423) study of METSL was performed as per the OECD guideline No. 423 on female albino mice weighing 20 - 30 g. The animals were fasted overnight prior to the experimentation. 1/20th, 1/10th and 1/5th (125, 250 and 500 mg) LD_{50} cut of value of the extract were selected as for the cardioprotective activity.

Evaluation of cardioprotective activity (*Mohamed et al., 2021*):

Wistar rats weighing 200 - 250 g was randomly distributed into six groups containing 6 animals each and treated as follow:

Group I: Normal control, received water as vehicle

Group II: Cardiac control, received water + Isoproterenol (ISO) (85 mg/kg) s.c.

Group III: Standard, received ascorbic acid + ISO

Group IV: Received, METSL 125 mg/kg p.o. + ISO

Group V: Received, METSL 250 mg/kg p.o. + ISO

Group VI: Received, METSL 500 mg/kg p.o. + ISO

Group I served as normal control received water as vehicle for 30 days, Group II represents cardiac control, received water as vehicle for 30 days and ISO (85 mg/kg s.c.) on 28th and 29th day to induce MI. Group III was considered as Standard, received ascorbic acid daily for 30 days and ISO on 28th and 29th days. Groups IV, V and VI animals were administered with different doses METSL (125, 250 and 500 mg/kg) orally for 30 days and ISO on 28th and 29th day. At the end of the specified period of treatment following 24 h form the last dose i.e. on 31st day the animals were anaesthetized using ketamine 100 mg/kg and then electrocardiogram (ECG), Blood pressure (BP) and heart rate (HR) of the animals were recorded using Computerized data acquisition system (Biopac MP-46) (USA) instrument. Immediately after measuring all the parameters the blood was collected from the retro orbital plexes to measure the serum enzyme biochemical parameters such as AST, LDH, CK-MD. Later all the animals were scarified by cervical decapitation, the heart was dissected out. Half of the heart was used for estimation of MDA (Wills, 1996) and GSH (Cardoso *et al*., 2017) content and remaining half portion was used for histopathological studies.

Statistical analysis:

The results are expressed in mean ± SEM. All data obtained from the above study were subjected for One way ANOVA followed by Tukey's Krammer Multiple Comparison Test. Data was computed for statistical analysis using Graph Pad Prism 5 Software.

Results

Preliminary phytochemical screening:

The preliminary phytochemical investigation on methanolic extract of *Tecoma stans* leaves exhibited the presence of carbohydrates, flavonoids, alkaloids and tannins.

Acute toxicity study:

METSL was studied for acute toxicity at dose of 2000 mg/kg in female albino mice. The extract did not cause any mortality of the animals at dose of 2000 mg/kg, even at repeated dosing using 3 new mice. Hence, 2500 mg/kg was taken as LD_{50} cut off value as per fixed dose method of OECD guideline number 423.

The screening doses selected for the cardioprotective activity were:

1. 125 mg/kg - 1/20th of 2500 mg/kg b.w. p.o
2. 250 mg/kg - 1/10th of 2500 mg/kg b.w. p.o
3. 500 mg/kg - 1/5th of 2500 mg/kg b.w. p.o

Cardioprotective activity:

Effect of METSL on Blood pressure and heart rate:

It was observed that rats intoxicated with ISO exhibited significant elevation in the levels of blood pressure and heart rate as compared to

Table 1: Effect of METSL on blood pressure and heart rate

Group	Treatment	Blood Pressure (mmHg)		Heart rate/minute
		Systolic	Diastolic	
I	Normal control	100±9	70±9	386±8.0
II	Cardiac control	188±9@	140±11@	515±4.2@
III	Standard	121±9***	90±9***	314±9***
IV	METSL125 mg/kg	145±9*	123±6*	487±7*
V	METSL 250 mg/kg	135±1**	100±11*	436±5***
VI	METSL 500 mg/kg	120±9***	83±7*	301±9***

The values are expressed as Mean ± SEM, (n=6). @p< 0.001 as compared to normal control, *p< 0.05, ** p< 0.01, *** p< 0.001 as compared to cardiac control

Table 2: Effect of METSL on serum enzyme and biomarker (AST, LDH, CK-MB)

Group	Treatment	AST (IU/L)	LDH (IU/L)	CK-MB (IU/L)
I	Normal control	154.2 ± 1.41	109.2±2.93	80.19±2.54
II	Cardiac control	284.2±2.45@	305.5±3.82@	171.2±1.7@
III	Standard	162.6±2.29***	111.69±3.49**	105.0±1.09**
IV	METSL 125 mg/kg	262.1±1.85***	282.3±4.97*	83.74±1.59**
V	METSL 250 mg/kg	205.9±4.45***	196.2±4.18**	70.01±1.20**
VI	METSL 500 mg/kg	174.9±3.54***	91.55±1.41**	58.75±0.62**

The values are expressed as Mean ± SEM, (n=6). @p< 0.001 as compared to normal control, *p< 0.05, ** p< 0.01, *** p< 0.001 as compared to cardiac control

normal group. Animals pretreated with different doses of METSL significantly reduced the elevated levels of blood pressure and heart rate in a dose dependent manner as compared to cardiac control group. The protection offered by test extract was found to be less potent than the reference standard drug (Ascorbic acid). The results are depicted in Table 1.

Effect of METSL on ECG Pattern:

The ECG pattern of normal control, cardiac control, standard and different doses of METSL are represented in Figures 1 - 4. The normal control group animal showed normal ECG rhythm, whereas the animals treated with ISO indicates the increase in SST segment, prolongation in P waves, QRS complex and RR interval. The treatment with ascorbic acid and different doses of METSL exhibited closer to the normal ECG pattern with decreased ST segment, decreased P waves, QRS complex and RR interval when compared to isoproterenol control animals.

Effect of METSL on serum enzymes and biomarker:

The results reflected in Table 2 indicates that the rats intoxicated with ISO control animals exhibited significant elevation in the levels of AST, LDH, CK-MB as compared to normal group animals. The animals pretreated with graded doses of METSL significantly reduced the elevated levels of AST, LDH and CK-MB in a dose dependent manner as compared to ISO treated group. The protection offered by test extract was found to be less potent than the reference standard drug (Ascorbic acid).

Effect of METSL on Tissue GSH and MDA:

The animals administered with ISO exhibits significant decrease in GSH and increase in MDA content in heart homogenate of experimental animals as compared to normal control group animals. The administration of different doses of the METSL and ascorbic acid showed significant increase in the GSH and decrease in MDA levels as compared to cardiac control animals (Table 3).

Histopathological study of heart tissue:

Normal control animals showed normal myocardial structure without any infarction,

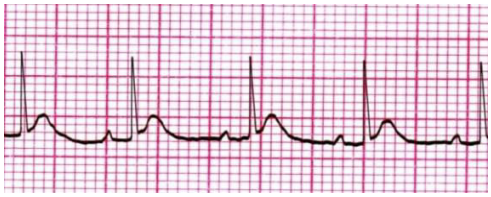

Fig. 1: Normal control ECG (A).

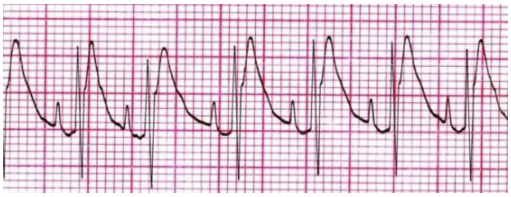

Fig. 2: ISO control ECG (B).

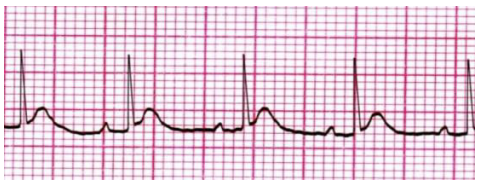

Fig. 3: Standard ECG (C).

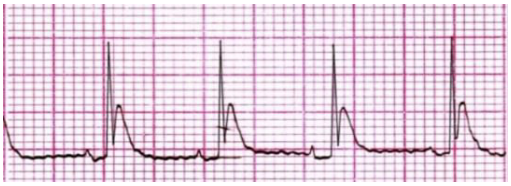

Fig. 4: METSL ECG (D).

Table 3: Effect of METSL on GSH and MDA

Group	Treatment	GSH (U/mg protein)	MDA (U/mg protein)
I	Normal control	95.17±1.561	15.47±1.237
II	Cardiac control	27.13±1.418@	61.09±2.202@
III	Standard	91.04±1.533***	42.36±1.618***
IV	METSL 125 mg/kg	41.09±1.008***	43.38±0.881***
V	METSL 250 mg/kg	64.41±1.228***	2.37±0.649***
VI	METSL 500 mg/kg	88.24±1.118***	25.34±0.9546***

The values are expressed as Mean ± SEM, (n=6). @p< 0.001 as compared to normal control, *p< 0.05, ** p< 0.01, *** p< 0.001 as compared to cardiac control.

congestion, hemorrhage, fibrosis, size of myocytes and inflammation. The ISO control animal reflects the increased infarction, congestion, hemorrhage, fibrosis, size of myocytes and inflammation compared to normal control animals. The treatment with Ascorbic acid and different doses of METSL showed dose dependently decreased infarction, congestion, hemorrhage, fibrosis, size of myocytes and inflammation compared to ISO control animals. The histopathological pictures are represented in Figure 5.

Discussion

The present study was undertaken to evaluate the effect of METSL on ISO induced myocardial infarction. Isoproterenol mediated myocardial toxicity have been well documented in experimental animals as well as in patients (Zhang *et al*., 2008).

The administration of Isoproterenol in experimental animal causes the decrease in systolic and diastolic blood pressure and also heart rate. This is probably due to the effect of isoproterenol on myofibril as a result of which it causes disruption which leads to decrease systolic and diastolic blood pressure and also heart rate (Weinbreg and Signal, 1987). The administration of METSL at different doses restored the blood pressure and heart rate.

The ECG abnormalities are generally used to diagnose the myocardial infarction. The treatment of isoproterenol causes the prolongation of P wave, QRS complex and ST segment (Holland and Brooks, 1977). The prolongation of ECG waves is due to cell membrane damage and also oxidative stress. The treatment with METSL reflected the reduction of P wave, QRS complex and ST

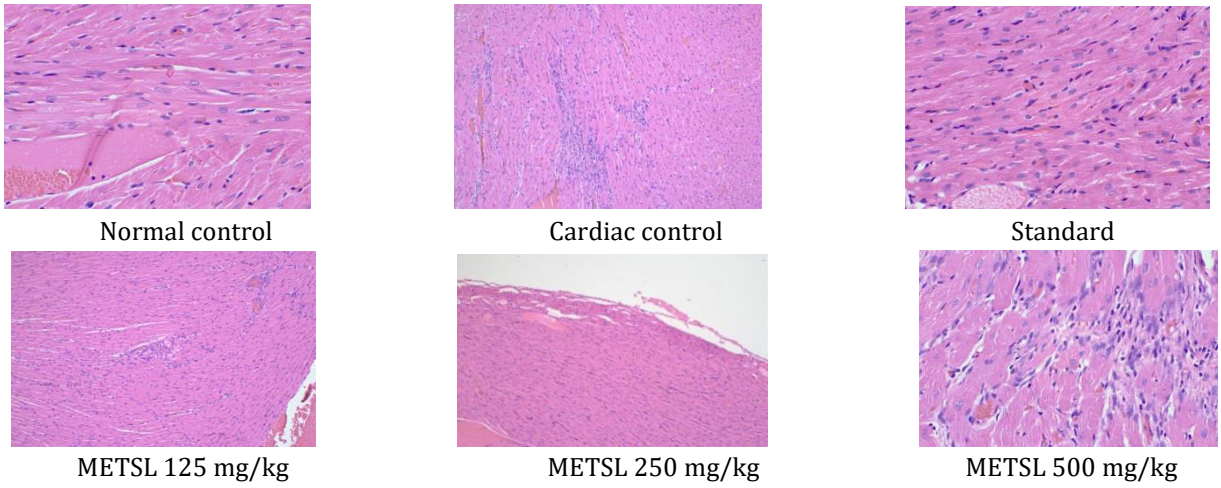

Fig. 5: Histopathological study of heart tissue after METSL.

segment. The protective effect of METSL could be due to cell membrane stabilizing action of myocardium.

The decrease in oxygen supply and glucose may cause the damage to the myocardial cell membrane leading to rapture which causes the leakage of enzymes. These enzymes also called biomarkers and are used to identify myocardial damage. The administration of isoproterenol causes the elevation in the levels of AST, LDH and CK-MB when compared to normal control animals. The treatment with different doses of METSL showed significant decrease in the levels of these enzymes indicating the protective or membrane stabilizing property of test extract on heart.

The oxidative stress and myocardial infarction are meanly associated with heart diseases which results from over production of reactive oxygen species which leads to peroxidation of membrane phospholipids (Weinbreg and Signal, 1987). The treatment of isoproterenol causes the decrease in GSH and increase in lipid peroxidation when compared to normal control. In our study the administration of METSL significantly attenuated the altered levels of MDA and GSH content when compared to cardiac control animals, thus our results demonstrates the protective effect of METSL against oxidative damage caused by isoproterenol.

A histopathological examination of normal control animal heart tissue illustrate the integrity of the myocardial cell membrane whereas the isoproterenol intoxicated rats showed increased infarction, congestion, hemorrhage, fibrosis, size of myocytes and inflammation compared to normal control animals. The treatment with graded doses restored the altered architecture of the myocardium compared to Isoproterenol control animals.

The overall findings obtained in the present study indicates that the administration of METSL significantly protected the myocardial damage caused by isoproterenol. The earlier researcher claimed that medicinal plant extract possessing antioxidant activity has been identified as promising therapeutic approach in disease associated with oxidative stress. Earlier reports reveal that plant extracts having antioxidant activity known to exhibit cardioprotective activity and also the presence of flavonoids and tannin content linked to significant antioxidant activity (Krishna and Verma, 1941). In our study also the phytochemical study revealed the presence of tannins and flavonoids in the test extract and this

could be the reason for protective effect against Isoproterenol induced cardiotoxicity.

Conclusion

It is concluded from the present study that the methanolic extract of *Tecoma stans* leaves showed protective effect at different dose levels (125, 250 and 500 mg/kg) in Isoproterenol induced cardiotoxicity which could be due to the presence of flavonoids and antioxidant enzymes.

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