INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

ISSN: 2395-6429, Impact Factor: SJIF: 4.656
Available Online at www.journalcmpr.com
Volume 4; Issue 2(B); February 2018; Page No. 3045-3047
DOI: http://dx.doi.org/10.24327/23956429.ijcmpr20180395

Research Article

EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF TERMINALIA CATAPPA LEAVES AGAINST DOXORUBICIN INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS

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ARTICLE INFO

Article History:
Received 6th November, 2017
Received in revised form 21st December, 2017
Accepted 18th January, 2018
Published online 28th February, 2018

Key words:
Terminalia catappa leaves, Cardiotoxicity, Doxorubicin, Methanolic extract.

ABSTRACT

Objective: The present study was intended to evaluate the cardioprotective activity of (METCL) methanolic extract of Terminalia catappa leaves.

Method: The methanolic extract of Terminalia catappa leaves at the dose of 100, 250 and 500 mg/kg was administered orally to Wistar albino rats with Doxorubicin (2.5mg/kg) induced myocardial infarction. Ascorbic acid (20mg/kg) was given as standard reference drug.

Results: Effect of METCL on serum enzymes and biomarkers

Rats intoxicated with Doxorubicin exhibited significant elevation in the levels of Aspartate amino Transferase (AST), Alkaline Phosphatase (ALP), Creatine kinase (CK), CK-MB and Lactate Dehydrogense(LDH) as compared to normal group. Animals pre-treated with graded doses of (100, 250 and 500 mg/kg) METCL significantly reduced the elevated levels of AST, ALP, CK, CKMB and LDH in a dose dependent manner as compared to Doxorubicin treated group.

Effect of METCL on serum cholesterol and triglycerides

Significant increase in the levels of serum cholesterol and triglycerides monitored in Dox administered group compared to normal control. Dose dependent significant reversal of these two lipids demonstrated in animals pre-treated with METCL compared to Doxorubicin treated group.

Conclusion: The results of the present study conclude that methanolic extract of Terminalia catappa leaves possesses significant cardioprotective activity against Doxorubicin induced cardiotoxicity in albino rats. This observed activity may be due to the presence of flavonoids and tannins in the test extract and may be linked to its antioxidant property.

INTRODUCTION

Myocardial infarction (MI) is the interruption of blood supply to part of the heart, causing heart cells to die, commonly due to occlusion of a coronary artery. It creates a major cause of morbidity and mortality in developing countries due to increased high prevalence of risk factors and also aging of their populations [1]. Causative factors for cardiovascular disease are smoking, hypercholesterolemia, high low density lipoprotein and low high density lipoprotein, diabetes, high blood pressure, older age and obesity. Complications of MI include arrhythmias, congestive heart failure, cardiogenic shock, pericarditis and pulmonary embolism [2].

According to WHO 17.3 million peoples died from cardiovascular diseases, more than 80% of CVD death took place in low and middle income countries [3]. It is estimated that by 2030 more than 23 million peoples in world 2.6 million people in India will die annually from CVDs [4, 5]. Apart from drug therapy and life style modifications, dietary changes and supplementation play an important role in the conservative treatment of CVDs. Current interest has focused on plant based natural drug treatment. *Terminalia catappa* belongs to the family Combretaceae and is popularly known as 'Deshi Badam'. It is a well-known herb in Ayurvedic system of medicine. Juice of young leaves are employed in preparation of ointment for leprosy, scabies and also used internally for colic and headache [6].

T. catappa fruits has been investigated for its effect on fasting sugar level and serum parameters. Various pharmacological studies have reported that the extract of *T. catappa* leaves and fruits have antioxidant, anticancer, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic effects and hepatoprotective activities [7]. Earlier researchers demonstrated that medicinal plant extract possessing antioxidant activity exhibits cardioprotective efficacy [8]. *T. catappa* leaves also possesses antioxidant activity, in this context, the present research work was undertaken.

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MATERIAL AND METHODS

Plant material

The leaves of *T. catappa* were collected from in and around the Vijayapur, city after sample of plant material identified by Dr. M. B. Mulimani**,** Professor of botany, S.B. Arts and K.C.P Science college vijayapur. The specimen was preserved in the herbarium of the college.

Preparation of extract

Fresh leaves of *Terminalia catappa* were shade dried at room temperature, coarse powdered and extracted with methanol by Soxhlet's method. Then the extract was concentrated by removing excess of solvent using rotary flash evaporator. The yield of the test extract was found to be 15.79%. The extract was stored in airtight container in refrigerator below 10^0C. The preliminary phytochemical screening of methanolic extract of *Terminalia catappa* leaves revealed the presence of Carbohydrates, Flavonoids, Glycosides, Tannins and saponins.

Experimental animals

The Albino rats (Wistar Strain) 150-200 g of either sex were used in the experimentation. The animals were procured from Shri B M Patil Medical College Central Animal house (113/1999/CPCSEA). After randomization into different groups, animals were acclimatized for period of 10 days under standard husbandry conditions: room temperature: 27 ± 3º, relative humidity: 65 ± 10%, 12 hr. light/dark cycle. All the animals were given rodent pellet diet (VRK Nutritionals Industries, Pune, India) and water *ad libitium* under. All procedures were performed according to the Institutional Animal Ethics Committee's approval.

Determination of acute toxicity (LD_{50})[9]

An acute toxicity of METCL was conducted on female albino mice (20-30 g). The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method of CPCSEA was adapted for toxicity studies. $1/25^{th}$, $1/10^{th}$and $1/5^{th}$ LD_{50}cut off value of the extract were selected as screening dose for the cardioprotective activity.

Experimental Design

The Wistar albino rats of either sex were divided into 6 groups of 6 animals (n=6) each. Group I served as normal control received normal saline 5ml/kg, body weight. Group II served as cardiotoxic control (Doxorubicin 2.5 mg/kg, body weight, i. p.) in 6 equal Injections alternatively for two weeks to make a total cumulative dose of 15 mg/kg body weight. Group III received Standard (Ascorbic acid 20mg/kg body weight, p.o. daily two weeks pre-treatment) + Doxorubicin 2.5 mg/kg body weight, i. p. in 6 equal injections alternatively for two weeks (one hour after drug treatment) to make a total cumulative dose of 15 mg/kg body weight. Similar procedure has been followed for groups IV, V and VI. Group IV received test extract 100mg/kg, body weight, p.o. daily two weeks + Doxorubin. Group V received test extract 250mg/kg, body weight + Doxorubicin. And Group VI – METCL 500mg/kg, body weight + Doxorubicin.

Estimation of cardiac biomarkers and lipids

Thirty six hour after the last treatment, orbital blood samples were obtained under light ether anesthesia using heparinized microcapillaries for the estimation of cardiac biomarkers namely CK, CK-MB, LDH, ALP, AST and also lipid parameters such as serum cholesterol and triglycerides. Normal as well as treated animals were observed for a period of three weeks after the last injection of Dox for the general appearance, behavior and mortality.

Statistical analysis

The results are expressed as mean ± S.E.M. The results were analyzed using one-way ANOVA followed by Tukey's Krammer Multiple Comparison Test. Data was computed for statistical analysis using Graph Pad Prism 5 Software.

RESULTS

Effect of METCL on serum enzymes and biomarkers

The results of effects of methanolic extract of *Terminalia catappa* leaves on Doxorubicin intoxicated rats are shown in table no. 1. Rats intoxicated with Dox exhibited significant elevation in the levels of AST, ALP, CK, CKMB and LDH as compared to normal group. Animals pre-treated with graded doses of (100, 250 and 500 mg/kg) test extract significantly reduced the elevated levels of AST, ALP, CK, CKMB and LDH in a dose dependent manner as compared to Dox treated group. The protection offered by test extract was found to be less potent than the reference standard drug (Ascorbic acid)

Effect of METCL on serum cholesterol and triglycerides

The results of effects of methanolic extract of *Terminalia catappa* leaves on Dox intoxicated rats are shown in table no. 2. Significant increase in the levels of serum cholesterol and triglycerides monitored in Dox administered group compared to normal control. Dose dependent significant reversal of these two lipids demonstrated in animals pre-treated with test extract compared to Dox treated group. The protection offered by test extract was found to be less potent than the reference standard drug (Ascorbic acid).

DISCUSSION

Doxorubicin mediated myocardial toxicity have been well documented in experimental animals as well as in patients. The present study was undertaken to evaluate the effect of METCL on Dox induced myocardial infraction. The results of research work suggest that METCL prevents Dox induced cardiomyopathy in rats. Following statements of evidence can be drawn from present study.

General Observations

In the Dox treated group, the animal fur became scruffy and in the later days of observation period red exudates around the eyes and nose was noticed. Necrosis was also seen at the site of Dox injection. These changes were less pronounced in case of standard and METCL pretreated groups. There was 50% mortality in Dox treated group, where as in pretreated group there was no mortality in standard and METCL at doses of 500 & 250 mg/Kg groups and only 30 % mortality in METCL at 100 mg/Kg.

Serum Enzyme Markers

Doxorubicin is a well-known cardiotoxic agent it will destruct myocardial cells. As a result, Lactate Dehydrogenase (LDH), transaminase (AST, ALT) and Creatine kinase (CK) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage [10]. The amount of these cellular enzymes present in the blood reflects the alteration in plasma membrane integrity and/or permeability.

In the current study, Dox treated rats showed significant increase in the levels of these diagnostic marker enzymes (AST, ALP, CK, CKMB and LDH). Moreover, increased levels of these enzymes are an indicator of the severity of Dox

Table No 1 Effect of METCL on cardiac biomarkers in Dox induced cardiotoxicity in rats

Sl. No.	Treatment	AST(IU/L)	ALP(IU/L)	CK(IU/L)	CK-MB(IU/L)	LDH(IU/L)
1	Normal	45.61±2.89	109.65±6.68	122.24±3.42	9.61±1.20	182.20±6.46
2	Dox	248.86±10.62$^{@}$	218.90±7.58$^{@}$	568.59±16.63$^{@}$	420.65±18.53$^{@}$	424.64±10.32$^{@}$
3	Std Ascorbic Acid20 mg/kg	65.51±3.13***	130.21± 4.18***	160.18± 2.98***	40.30± 2.12***	195.87± 3.10 ***
4	METCL 100 mg/kg	108.84±4.62***	180.86±12.88*	346.36±10.98 ***	147.59±8.99***	316.64±16.95 ***
5	METCL 250 mg/kg	90.64± 2.48***	162.84± 8.69***	294.60±9.50***	104.30±6.82***	268.40±6.94***
6	METCL 500 mg/kg	80.54±2.64***	150.34±6.43***	240.68±6.20 ***	60.42±4.62***	230.43±8.23***

Results are Mean ± SEM, n =6, $^{@}$p<0.001 compared to Normal control and ** p < 0.01 and *** p < 0.001 v/s Dox control

Table No 2 Effect of METCL on serum cholesterol and triglycerides in Dox induced cardiotoxicity in rats

Sl. No.	Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)
1	Normal	20.68± 2.26	59.50± 4.42
2	Dox	89.64± 3.85$^{@}$	102.26± 4.23$^{@}$
3	Std	25.34± 2.40***	47.32± 2.19***
4	METCL 100mg/kg	46.20± 2.42***	79.46± 4.20***
5	METCL 250mg/kg	38.72± 2.62***	71.21± 3.20***
6	METCL 500mg/kg	30.48±3.20***	66.22± 2.54***

challenged myocardial necrosis in rats. The prior administration of METCL showed significant reduction in Dox mediated elevated serum marker enzymes. This reduction in the enzyme level confirms that the test extract is responsible for maintenance of normal structural and architectural integrity of cardiac myocytes, thereby restricting the leakage of these enzymes, which can be accounted for membrane stabilizing property of METCL.

In the present study, Dox treated rats showed significant elevation in the levels of serum transaminases such as AST and ALP after extensive tissue damage have been associated with liver injury or myocardial infarctions[10]. This result implies that the Dox when taken for long period of time could cause both liver and heart injury.

The existing experimental evidence suggests that Dox oxidative stress is due to the generation of free radicals [11]. Which may cause cellular cholesterol accumulation; (a) by increasing cholesterol biosynthesis and its esterification, (b) by decreasing cholesteryl ester hydrolysis and (c) by reducing cholesterol efflux [12]. Hence, elevation in cholesterol levels may be due to increase in biosynthesis and decrease in its utilization. Triacylglycerol's are degraded by the lipoprotein lipase (LPL) to fatty acids [12]. The administration of METCL showed dose dependent reduction in Dox induced elevated serum cholesterol and triglyceride. Increase in the level of triglycerides could be due to the alterations in LPL activity. This reduction in levels of cholesterol and triglyceride confirms that the antihyperlipidemic action of METCL is responsible to alleviate Dox induced hyperlipidemic cardiomyopathy.

Earlier research papers claimed that medicinal plant extract possessing antioxidant activity exhibits cardioprotective efficacy [8]. *Terminaliacatappa* leaf extract also reported for its antioxidant activity [13] and this could be the reason for exhibiting the cardioprotective activity in the present study. Triterpinoids, flavonoids and tannins present in the plant extract are reported to be having potent antioxidants and/or cardioprotective properties [14]. The presence of flavonoids and tannins in METCL may be also the reason for protective effect against Dox induced cardiotoxicity.

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