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Hypolipidemic and Cardioprotective Efficacy of *Peltophorum pterocarpum* Linn in Doxorubicin Induced Cardiotoxicity in Rats

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ABSTRACT

Background: Cardiovascular disease (CVD) refers to a common group of illnesses affecting the heart and blood arteries. It encompasses heart valve problems, atherosclerosis, angina, cardiomyopathy, coronary artery disease (CAD), and infections of the heart which are considered the main causes of death. Modern medications have some success but they typically come with unfavorable side effects. Researchers are paying attention to herbal remedies because of their limited or no negative effects. This study was conducted to find out the hypolipidemic and cardioprotective potential of the ethanolic extract of *Peltophorum pterocarpum (P. pterocarpum*) Linn leaves in doxorubicin-induced cardiac damage in rats.

Methods: The ethanolic extract of *P. pterocarpum* leaves was subjected to compound identification by GC-MS analysis. For *in vivo* analyses, the rats were divided into six groups. Cardiac damage was induced by doxorubicin at a dosage of 1.5 ml/kg b.wt. various dosages of extract (200 and 300 mg/kg b.wt.) were injected and propranolol (25 mg/kg b.wt.) is used as a standard drug. After the treatment of the extract, biochemical parameters were evaluated followed by in silico analysis.

Result: The levels of cardiac marker and lysosomal enzymes significantly increased in toxin-treated rats, and the mitochondrial enzyme levels were significantly reduced. After treatment with *P. pterocarpum* leaf extract, the levels significantly ($P<0.05$) returned to normal. These results may prove the cardiac protective effect of the ethanolic extract of *P. pterocarpum* leaves in DOX-induced cardiotoxicity rats.

Key words: Cardioprotective activity, Docking analysis, Doxorubicin, *Peltophorum pterocarpum,* Propranolol.

INTRODUCTION

Myocardial infarction (MI) occurs when fat deposits occur in the artery lining cells. The arteries that feed the heart with blood flow are impacted by the progressive accumulation of these fatty deposits known as atheroscelerosis. This can injure the heart muscles by delaying the blood supply to them. A myocardial infarction results from the total buildup of fat in the coronary arteries, which ultimately causes death. The World Health Organization (WHO) estimated that 18.6 million individuals worldwide lost their lives to CVD in 2019. Countries with low and moderate incomes account for more than 75% of deaths from CVD.

Three-quarters of the 17 million premature fatalities (dead before age 70) attributed to non-communicable illnesses in 2019 (CVDs) were caused by these disorders (WHO, 2021). They estimate that nearly 23 million people will die in 2030 due to the CVD. In India, 4.77 million (2020) people will die because of the CVD (Huffman *et al*., 2011). Allopathic drugs are available to overcome the MI disorders. While these medications work well, they have harmful side effects. Researchers are searching for plant-based medications that don't have any harmful effects.

P. pterocarpum (Fabaceae) is planted alongside roadsides. Studies on the phytochemical composition of *P. pterocarpum* have shown that it contains a variety of substances (Amala and Poonguzhali, 2015). Asia and a widely planted ornamental tree worldwide (Mariyappillai and Swaminathan, 2024). According to Devi and Battu (2018), *P. pterocarpum* has been shown to possess the

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following qualities like antimicrobial, anti-diabetic, anti-oxidant, anti-inflammatory and anti-arthritic. The present study aims to find out the hypolipidemic and cardioprotective potential of the ethanolic extract of *P. pterocarpum* Linn leaves in doxorubicin-induced cardiac damage in rats.

MATERIALS AND METHODS

Collection of plants and extract preparation

The leaves of *P. pterocarpum* were collected locally. Then the plant powder is subjected to ethanol extraction by using Soxhlet apparatus. GC-MS analysis was carried out.

Ethical issue, animals, housing and experimental design

The adult male Albino Wistar rats weighed 150–165 gm. Every experimentation procedure and animal handling technique was authorised by the Institutional Animal Ethics Committee of Srimad Andavan College of Arts and Science, Trichy, Tamil Nadu, India.

Rats were randomly divided into 6 groups of 6 rats each. The animal groups were divided into six categories: G1 received saline water as the control group; G2 received doxorubicin (1.5 ml/kg b.w.) as a negative control; G1 was supplemented with 300 mg/kg of *P. pterocarpum* ethanolic leaf extract (G3); The toxin treated rats treated 200 mg/kg of *P. pterocarpum* ethanolic leaf extract (G4); the DOX treated rats received 300 mg/kg of *P. pterocarpum* ethanolic leaf extract (G5). and toxin treated rats received propranolol (25 mg/kg b.w.) (G6).

Biochemical evaluation in the serum and tissue samples

At the end of the experimental period, rats were sacrificed, and the samples were collected with a standard procedure. The samples were introduced to find the following lipid parameters (Zak *et al.,* 1953; Foster and Dum, 1973), cardiac marker enzymes (Apple *et al*., 1998; Okinaka *et al*., 1961; Burtis and Ashwood, 1996), lysosomal enzymes (King, 1965; Kawai and Anno, 1971; Moore and Morris, 1982; Sapolsky *et al*., 1973) and mitochondrial enzymes (Bell and Baron, 1960; Slater and Bonner, 1952; Mehler *et al*., 1948; Tsoo *et al*., 1967; Pearl *et al*., 1963) were evaluated.

In silico analysis

Pubchem and the protein data bank (PDB) database provided the protein and ligand structures. Using a graphical user interface and automated docking, the Auto Dock tools were used to create grids, determine the dock score, and assess the conformers of activators bound to protein targets in the active site.

RESULTS AND DISCUSSION

GC-MS analysis

The GC-MS analysis of the ethanolic extract of *P. pterocarpum* leaves is displayed in Fig 1. Eight peaks were detected over the retention period, which spanned from 11.30 to 44.24 minutes. The compounds are psoralene, cyclohexasiloxane-dodecamethyl, hexadecanoic acid, cycloheptasiloxane-tetradecamethyl, 4-ethyl-8-quinolinol, docosanoic acid, canthaxanthin, vitamin E acetate.

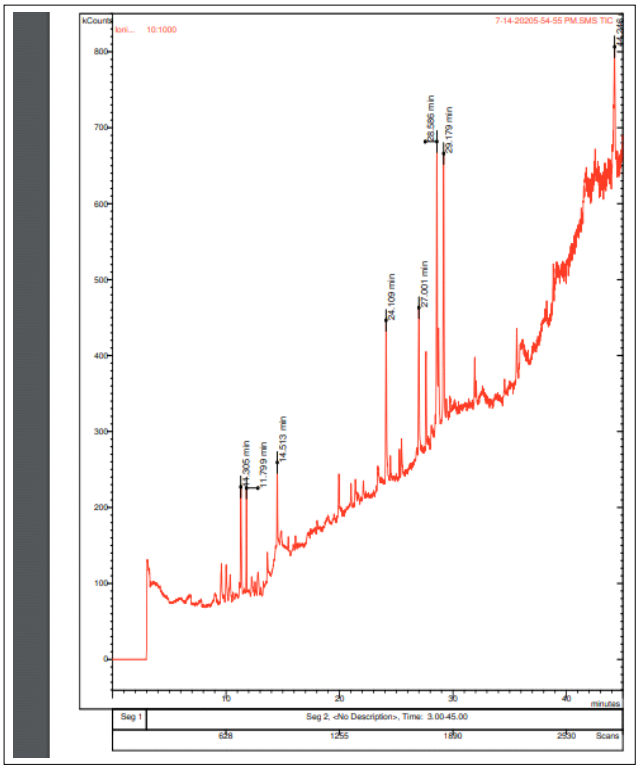

Fig 1: The GC-MS of *P. pterocarpum* leaf extract.

Effect of *P. pterocarpum* leaves on lipid profiles in doxorubicin induced rats

The results of the serum lipid profile levels in control and experimental groups were shown in the in Table 1. The level of lipid profiles except HDL, were increased in doxorubicin induced rats. The levels of the all parameters were normalized nearer to the control when the treatment of ethanolic extract of *P. pterocarpum* leaves and standard drug propranolol treated groups.

The increasing concentration of cholesterol in heart tissues is induces the oxidative stress and it induces the formation of free radicals and it leads the cellular injury (Gokkusu and Mostafa Zadeh, 2003). Anandan *et al.* (2007) demonstrated the effect of doxorubicin, which raises the cholesterol level in the rats. This proved in the present study also. After the treatment of *P. pterocarpum* leaves the levels returned to normal. This could be because the phytocompounds slow down the production of free radicals and shield the cells from injury.

The increasing level of cholesterol may induce the synthesis of fatty acids and TG levels in the liver and heart tissues. The increasing concentration of TG is directly proportional to the cardiovascular disease (Fungwe *et al.*, 1993). In the present study, the level of TG is elevated in the

Table 1: Effect of ethanolic extract of *P. pterocarpum* on total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein of the toxin induced and non-toxic rats.

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I	151.43±0.92a	68.15±0.75a	91.28±0.24a	47.19±0.78a	13.09±0.24a
Group II	390.13±0.83b	348.51±1.26b	40.15±0.38b	293.92±0.46b	71.35±0.29b
Group III	150.53±1.01a	67.17±0.83a	92.13±0.54a	46.17±1.09a	12.19±0.23a
Group IV	291.92±0.89c	201.14±0.83c	68.25±0.58c	167.13±0.95c	51.23±0.17c
Group V	192.97±1.17d	101.13±0.97d	83.92±0.86d	65.13±1.26d	25.78±0.51d
Group VI	172.09±1.29a,d	78.19±1.64a	87.38±0.89a,d	53.67±0.13a	23.16±0.48d

Values are expressed as means ± SD for six rats in each group

Values not sharing a common marking (a,b,c……) differ significantly at $p \leq 0.05$ (DMRT.

Table 2: Effect of *P. pterocarpum* leaves extract on the lysosomal enzymes in the doxorubicin toxin induced and control rats.

Parameters/ Groups	Acid Phosphatase (IU/L)	β-D-glucosaminidase (μ mol/h/100 mg protein)	β-N-acetyl glucuronidase (μ mol/h/100 mg protein)	Cathepsin D μ mol/h/100 mg protein)
Group I	123.16±0.94a	22.02±0.45a	49.01±1.17a	23.56±0.65a
Group II	181.19±1.03b	39.39±0.39b	78.91±1.09b	50.73±0.84b
Group III	123.08±0.87a	21.90±0.54a	49.23±0.75a	24.34±0.78a
Group IV	147.39±0.74c	33.85±1.03c	60.64±1.38c	39.75±1.29c
Group V	130.25±0.82a,d	25.64±0.95a	51.93±1.04a	27.85±0.87a,d
Group VI	128.93±1.04a,d	25.18±0.89a	51.24±1.06a	25.15±0.98a

doxorubicin treated rats. After the treatment of *P. pterocarpum* leaves the level of TG was normalized. This might be due to the cholesterol reduction because the reducing level of cholesterol may reduces the fatty acid synthesis in serum. The previous study of Subashini *et al*. (2007) proves that the Nardostachys jatamansi extract reduces the TG level in the doxorubicin induced cardio toxicity rats.

The increased concentration of LDL is directly proportional to the risk of myocardial infarction. At the same time, the decreased level of TG may reduced the level of LDL and VLDL. In the present study, the level of LDL and VLDL was increased in the doxorubicin treated rats. After the treatment the level of LDL and VLDL was normalized. This demonstrates clearly that the plant extract lowered the risk of atherosclerosis. The VLDL level is directly correlated with the declining TG concentration. Sakthivel *et al.* (2010) and Manikandan *et al.* (2018) found comparable results to these. This study showed that when doxorubicin is used to treat cardiotoxicity-induced albino rats, the levels of LDL and VLDL in *Buchanania axillaris* are lowered. The previous study of Algefare *et al*. (2022) also proves the same.

The transfer of cholesterol from the tissues to the liver is greatly aided by this HDL. This is because lipoprotein lipase activity lowers HDL levels. In the current investigation, rats given doxorubicin had lower levels of HDL. The amount of HDL increased following treatment with the ethanolic extract of *P. pterocarpum* leaves. The level of HDL is indirectly proportional to the risk of cardiac damage.

Effect of *P. pterocarpum* leaves extracts on the lysosomal enzymes

In the doxorubicin induced rats the level of lysosomal enzymes were increased in the experimental rats when compared to the control rats. The treatment of ethanolic extract of *P. pterocarpum* leaves reduced the levels significantly (Table 2).

Myocardial cellular damage is caused by the release of the enzyme acid phosphatase from the lysosome into the cytosol by the acid hydrolases in the heart (Decker and Wildentha, 1980). In the present study, after the treatment of *P. pterocarpum* leaves the levels were reduced when compared to the disease control rats. This might be caused by lysosomal hydrolases' decreased activity. This work may corroborate the earlier research by Karthikeyan *et al.* (2007), which demonstrated that treatment with grape seed proanthocyanidins lowers serum levels of acid phosphatase.

In the current investigation, the level of cathepsin D was significantly decreased after the treatment of *P. pterocarpum* leaves in the cardiotoxicity rats. This might be as a result of the leaf extracts potent inhibition of lysosomal enzyme release, which may shield membranes from damage and boost lysosome stability. Arafa *et al.*, (2014) proved the cardioprotective properties of endangered Indian medicinal plants. Researchers demonstrated that ethanolic extracts of *Gmelina arborea* and *Grewiaum bellifera* considerably lower the activity of cathepsin D in rats given doxorubicin.

Table 3: Effect of ethanolic extract of *P. guajava* leaves on the mitochondrial enzymes in the experimental rats.

Parameters /Groups	ICH (NADH oxidized/h/mg protein)	Ketoglutarate dehydrogenase (nmoles of ferrocy anideformed /h/mg protein)	Succinate Dehydrogenase (nmoles of succinate oxidized/min/mg protein)	Malate Dehydrogenase (nmoles of NADH oxidized/min/mg protein)	NADH dehydrogenase (NADH oxidized/min/mg protein)	Cytochrome-C-oxidaseC-oxidase (nmol/min/mg protein
Group I	601.26±16.72a	140.48±8.23a	218.02±17.73a	300.18±18.16a	32.18±1.27a	7.93±0.94a
Group II	210.82±10.86b	89.53±9.84b	101.83±16.92b	160.64±10.47b	14.92±1.91b	3.29±0.46b
Group III	602.75±9.85a	141.82±9.01a	217.57±16.62a	299.86±11.75a	33.19±0.99a	7.01±0.68a
Group IV	475.94±10.17c	112.85±7.92c	169.34±15.39c	228.67±16.49c	23.34±1.53c	5.58±1.01c
Group V	579.94±9.91d	133.17±9.32a	204.58±11.12d	284.91±13.93d	29.47±0.86a	6.96±0.94d
Group VI	590.64±10.15a,d	136.82±10.02a	205.31±10.17d	289.01±20.68a,d	30.28±1.20a	7.01±0.76d

Table 4: Level of cardiac marker enzymes in the experimental rats.

Parameters/ Groups	CK-MB (IU/L)	CK (U/L)	Troponin (ng/L)
Group I	0.87±0.01a	36.19±0.92a	0.59±0.02a
Group II	3.95±0.14b	97.92±1.93b	1.96±0.32b
Group III	0.87±0.04a	36.29±0.71a	0.59±0.06a
Group IV	1.64±0.75c	62.92±0.28c	1.31±0.23c
Group V	0.95±0.27a	40.23±0.47a	0.64±0.08a
Group VI	0.90±0.07a	38.91±0.38a	0.63±0.04a

In the current investigation, the levels of β-D-glucuronidase and β-N-acetyl glucosaminidase levels were significantly reduced after the treatment of *P. pterocarpum* leaves in the cardiotoxicity rats. This could be attributed to plants anti-oxidant activity, as it scavenge for oxygen free radicals, which may have conserved the stability of lysosomes and cell membranes. This could stop the lysosomal enzymes from leaking out. Suchalatha and Devi (2004) demonstrated that *T. chebula* treatments in rats with isoproterenol-induced heart injury have lower levels of β-D-glucuronidase and β-N-acetyl glucosaminidase.

Effect of *P. pterocarpum* leaves extracts on the mitochondrial enzymes

The level of all enzymes (ICH, KDH, SDH, MDH, NADPH dehydrogenase and cytochrome C oxidase) were reduced in the doxorubicin induced rats. The treatment of ethanolic extract of *P. pterocarpum* leaves the levels were returned back to the normal compared to the control rats.

The level of ICH was reduced due to the damage of the mitochondrial by the action of doxorubicin. Because of the stress caused by the rising amounts of free radicals produced by the doxorubin-induced rats, the levels of tricarboxylic acid cycle enzymes are decreased. The current findings were synchronized with the *Ganoderma lucidum* versus CCl4 treated rats results (Sudheesh, 2011).

The tricarboxylic acid cycle enzymes activities are increased in the current study by the ethanolic leaf extract of *P. pterocarpum* and propranolol. This could strengthen the mitochondrial anti-oxidant defense system and alleviate the problems associated with the tricarboxylic acid cycle's lower function. Propranolol and *P. pterocarpum* leaf extract treatment may lessen oxidative damage to mitochondria and improve anti-oxidant status. Al-Assaf (2014) suggested that the decrease in mitochondrial anti-oxidants may be attributed to either feed-back inhibition or oxidative inactivation of enzyme proteins resulting from excessive ROS production.

Effect of *P. pterocarpum* leaves extracts on the cardiac marker enzymes

The levels of CK-MB, CK and troponin levels were increased in the doxorubicin induced rats when compared to the normal rats. After the treatment of ethanolic extract of *P. pterocarpum leaves the levels were return back to the normal when compared to the normal and standard drug treated rats*(Table 4).

In animal models, myocardial infarction is directly correlated with rising blood troponin levels (O'Brien *et al.*, 2006). Myofibrillarlysis, which results in the release of troponin, can be caused by decreased production of muscle-specific proteins, accelerated breakdown of myofibrillar components and suppression of protein synthesis. The level of troponin was elevated in the doxorubicin-induced rats; this could be because to tissue injury and inhibition of protein synthesis. The extract which decreased the protein breakdown and it favors the protein formation. This effect is reflected in the decreased release of troponin into the bloodstream from cardiac muscle; Herman *et al.* (1998) had shown the value of troponin as a marker for detecting cardio toxicity; significant ($P<0.05$) differences were noted between the treatment groups.

After the treatment of *P. pterocarpum* leaves the levels of CK was decreased. The toxins are directly attacks the plasma membrane permeability. The leaves of *P. pterocarpum* may reduces the damage and increases the plasma membrane permeability and this may reduces the CK secretion in the blood stream. Both the usual medication treated rats and the rats treated with the plant extract alone had levels that were comparable to those of the control group.

Table 5: Docking scores of creatine kinase - brain, creatine kinase - Muscle and troponin to the canthaxanthin ligand.

Protein names	Docking score (kcal/mol)	Glide energy (kcal/mol)	Glide emodel (kcal/mol)	Interiction
Creatine kinase Brain (3 dre)	-6.331	-17.842	-24.541	ARG96
Creatine kinase Muscle (1i0e)	-5.289	-23.118	-33.731	-
Troponin (1a2x)	-6.369	-32.142	-37.369	ASN141

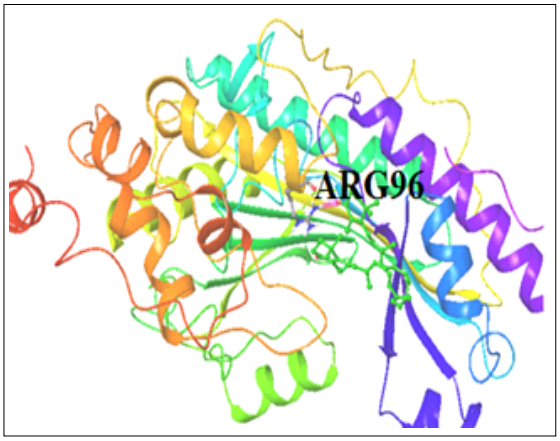

Fig 2: 3D Interaction canthaxanthin to the creatine kinase Brain.

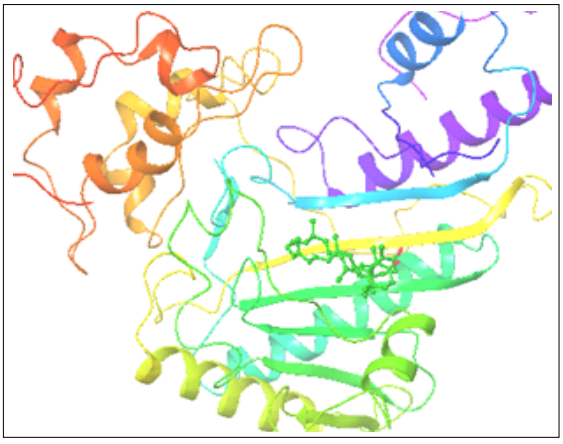

Fig 3: 3D Interaction canthaxanthin to the creatine kinase muscle.

In the time of myocardial damage, the CK-MB enzyme is released from the heart into the circulatory blood. The toxins rupture the cells and releases the CK-MB from heart into the blood. In the present study, after the treatment of *P. pterocarpum* leaves the levels are decreased. Significant (P<0.05) changes were noted in the treatment groups. *Grewia umbellifera* and *Gmelina arborea* ethanolic extracts reduce CK-MB activity in rats treated with doxorubicin, as demonstrated by Arafa *et al.* (2014).

In silico analysis of the compound canthaxanthin reported from *P. pterocarpum* leaves

In the present study, the *in silico* approach on phytochemicals canthaxanthin against cardiac target of

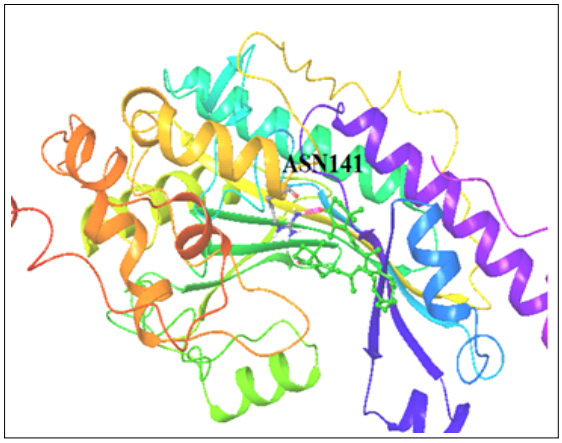

Fig 4: 3D Interaction canthaxanthin to the troponin.

creatine kinase-Muscle, Cretine kinase-Brain and troponin is carried out using virtual screening, molecular docking and ADMET methods. Virtual screening of canthaxanthin compound showed the binding affinity towards target creatine kinase-Muscle, Cretine kinase-Brain and troponin. The docking scores were canthaxanthin was found by -6.331, -5.289 and -6.369 Kcal/mol to the creatine kinase-brain, creatine kinase-muscle and troponin resptctively. (Table 5, Fig 2-4). The molecular docking of the hits showed the binding mode and interaction energy. The docking studies confirmed the inhibition of cardiac target protein creatine kinase-brain, creatine kinase-muscle and troponin to show the cardioprotctive activity of canthaxanthin.

CONCLUSION

In the present *in vivo* study clearly proved the action of *P. pterocarpum* leaves on the cardiac marker enzymes and mitochondrial enzymes of doxorubin induced cardiotoxicity rats. Further compound isolation and *in silico* analysis were warranted. In future it may be a potential drug for cardiac complications.

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Conflict of interest

The authors have no conflict of interest.

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