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"SCREENING OF CARDIOPROTECTIVE AND ANTI-OXIDANT ACTIVITY OF *TERMINALIA CORIACEA*"

Sanjib Kumar Yadav* and Dr. Nagrathna P.K.M.

PG Scholar, Karnataka College of Pharmacy, Thirumenahalli Bangalore-560064.

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***Corresponding Author**
Sanjib Kumar Yadav
PG Scholar, Karnataka College of Pharmacy, Thirumenahalli Bangalore-560064.

ABSTRACT

Objective: The aim of the current study was to evaluate the cardioprotective effect of the methanolic extract of *Terminalia coriacea* on doxorubicin induced cardiotoxicity. **Methodology:** For cardiotoxicity, thirty rats were evenly divided into 5 groups. Groups-1 served as controls, Group-2 standard drug treated group, Group-3 vitamin-E treated group, Group-4 test drug(low dose) treated group, Group-5 test drug(high dose) treated group and treatment is given for 7 days, On 8^{th} and 9^{th} day need to induce cardiotoxicity with doxorubicin, then on 10^{th} day need to sacrifies rat then blood samples is taken for lipid profile test and the rat heart and liver for histopathology were obtained under inhaled diethyl ether anesthesia. **Result:** administration of doxorubicin in control rats showed a significant increase serum total cholesterol(TC), triglycerides(TG), lowdensity lipoprotein(LDL) and decrease in high density lipoprotein (HDL). Rats treated with hydromethanolic extract of *Terminalia coriacea* (200 mg/kg and 100 mg/kg) showed decreased TC,TG,LDL and increases HDL levels. The histopathological studies also showed that the plant extract significantly minimized the damage induced by doxorubicin. **Conclusion:** thus, *Terminalia coriacea* provide cardioprotection against doxorubicin induced MI in rates.

KEYWORD: cardiotoxicity, *Terminalia coriacea*, doxorubicin, MI, propranolol, vit-E and Albino rats.

1. INTRODUCTUION

Cardiotoxicity has been extensively reviewed with the use of anthracyclines.[1,2,3,4] Anthracyclines have been reported to cause cardiomyopathy, congestive heart failure and

ECG alterations (e.g. nonspecific ST-T changes, decreased QRS voltage and prolongation of QT interval).

Doxorubicin

Doxorubicin is an anthracycline antibiotic having anti-tumor action and produced by the fungus Streptococcuspeucetius var. caesius. Doxorubicin produces clinically useful responses in a variety of human cancers. However the toxicity of doxorubicin has limited its usefulness. This side effect is mainly due to the doxorubicin–mediated free radical formation.[5] It is capable of causing breaks in DNA strands by activating topoisomerase-II and generating quinine type of free radicals. It produces cardiotoxicity as a unique adverse effect.[6] Tissues with less developed antioxidant defense mechanism such as the heart are highly susceptible to injury by anthracycline–induced oxygen radicals.[7] Many investigators have described the role of reactive oxygen species including hydroxyl radical in DOX–induced cardiotoxicity.[8,9,10]

Both early and late onset cardiac effects are reported.

Early onset effects occur within one year after start of the anthracycline therapy and can be acute, subacute or chronically progressive. In children, early onset cardiotoxicity seems to occur less frequently than late onset clinical cardiotoxicity. Late onset effects can occur up to 20 years after completion of anthracycline therapy.

Acute toxicity

Acute or subacute cardiotoxicity with anthracyclines is rare and will occur during or immediately following infusion, is usually transient (e.g. electrocardiographic abnormalities such as nonspecific ST-T changes and QT prolongation, pericarditis–myocarditis syndrome and ventricular dysfunction with congestive heart failure) and will attenuate after discontinuation of the therapy.

Chronic toxicity

The chronic effects start with early cardiac abnormalities which can progress to overt cardiac disease. Chronic effects persist after discontinuation of the anthracyclines and the clinical symptoms may include all signs of cardiomyopathy such as electrophysiologic changes, decrease of left ventricular function, changes in exercise-stress capacity and overt signs of congestive heart failure.

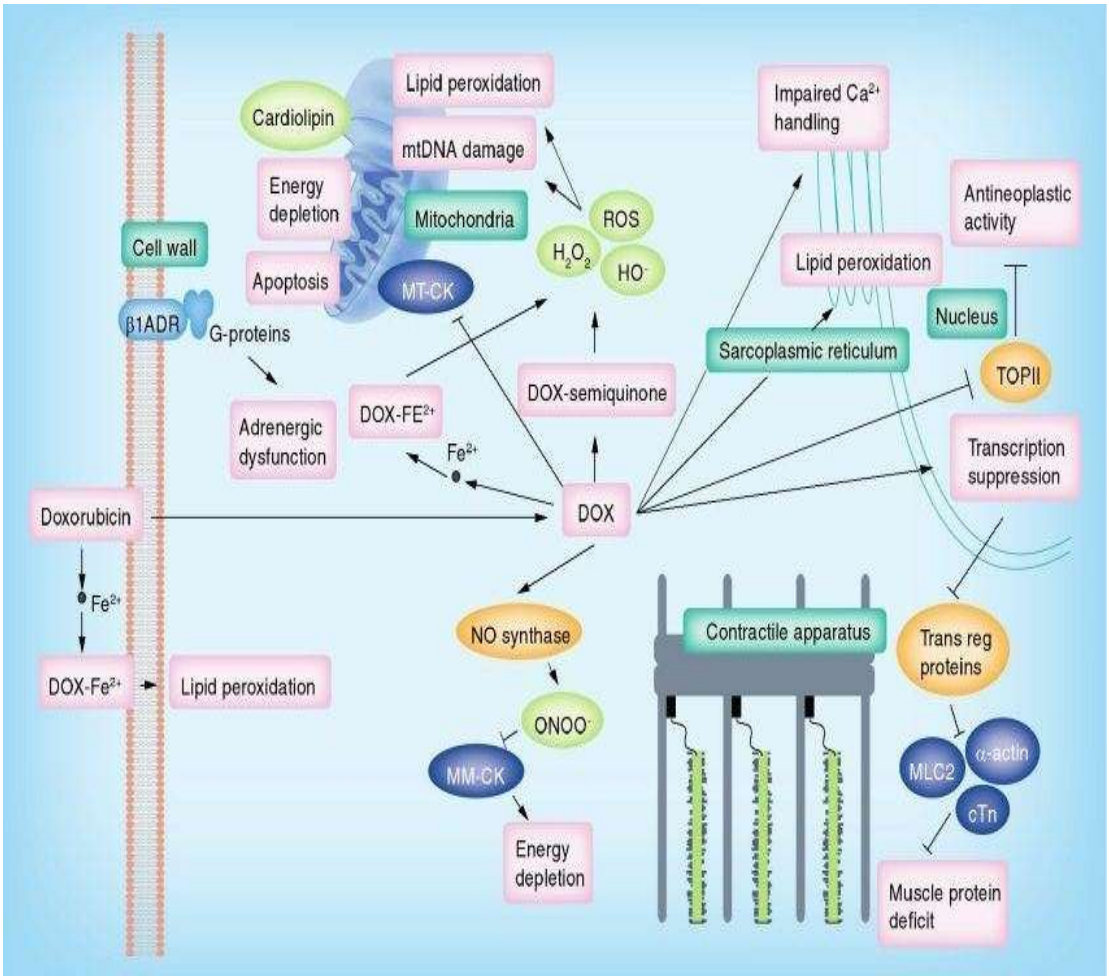


Figure-1: Toxic activity of doxorubicin inside cell.

Terminalia coriacea[11,12,13,14,15,16,17,18,19]

Terminalia is a genus of large trees of the flowering plant family Combretaceae, comprising around 100 species. Distributed in tropical regions of the world. *Terminalia* native to southern and Southeast Asia in India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Cambodia and Vietnam. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. It is found mainly on Andhra Pradesh and Tamil Nadu, states of India.

The crude methanol extract of *Terminalia coriacea* contains pharmacologically active substances like Alkaloids, aminoacids, carbohydrates, flavonoids, glycosides, resins, saponins, sterol, tannins, triterpenoids and phenolic compounds.

Traditionally the stem bark and leaf of the plant is used: As cardiac stimulant, Anti-epileptic, Anti-nociceptive, In wound healing, In treatment of atonic diarrhea & callous ulcer (Chetty et al., 2008), Exhibits high anti-oxidant activity, Have hair-growth promoting property, Having

anti-pyretic activity (TCSBAE), against yeast induced hyperpyrexia, As purgative, In treatment of upper respiratory tract infection, In treatment of dyspepsia, cough, bronchitis. Therefore planned to study the effects of flavonoids of *Terminalia coriacea* against doxorubicin induced cardiotoxicity.

2. MATERIAL AND METHODS

2.1 Collection and authentication of plant material

The leaf of plant *Terminalia coriacea* was collected from thirupati forest region, thirupathi district, Andhra Pradesh, India in the month of September, 2016, the plant species were authenticated by Dr. K. Mahadev Chetty, Assistant professor, Department of botany, Sri venkateswara university, thirupati, Andhara Pradesh India. The plant was identified by a botanist and voucher specimen was deposited in Rajiv Gandhi University Of Health Science and copy has been preserved for, future reference at Karnataka College Of Pharmacy, Department Of Pharmacology, the collected leaf was washed thoroughly with water to remove adhering soil, mud and debries. then the leaf was dried in shade at room temperature, then the plant material was powdered with blender, the powder was stored in an airtight container and protected from light.

2.2 preparation of extract

250 gm powdered plant material was subjected to successive extraction in a reflux condenser using 1.5 litre methanol for 3 hour at temperature of 80^{0c},separate the supernatant layer and remaining portion mixed with 1 litre of methanol and heated at 80^{0c} for another 1 and half hour again separate the supernatant layer and remaining portion is mixed with 1 litre methanol and heated at 80^{0c} for another 1 and half hour and separate the supernatant layer, finally mix all the three layers of extract and distillated and final product is evaporated to dryness to get constant weight.

2.3 Experimental animals

Wister albino rat having weight (150-200gm) were purchased from NIMHANS Bangalore. They were housed, six per poly propylene cage with paddy husk bedding. Animal will maintain under standard laboratory conditions at room temperature (25°C± 2°C) with 12h light / dark cycle. The animals were provided with pellet chow and water. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Karnataka College of pharmacy, Bangalore.

2.4 Experimental design

Animals are divided into five group containing six in each group.

Group-1: control (normal saline for 7 days) +doxorubicin (48 hr before scarification)

Group-2: standard drug (for 7 days) +doxorubicin (48 hr before scarification)

Group-3: vitamin-E (for 7 days) +doxorubicin (48 hr before scarification)

Group-4: *Terminalia coriacea* extract, low dose (for 7 days) +doxorubicin (48 hr before scarification)

Group-5: *Terminalia coriacea* extract, high dose (for 7 days) +doxorubicin (48 hr before scarification)

Here, to control group normal saline was given at the dose of 10 ml/kg and to standard drug treatment group propranolol was given at the dose of 10 mg/kg and to vitamin-E treatment group vitamin-E was given at the dose of 4 mg/kg and to TC treatment group(low dose) 100 mg/kg TC was given and to TC treatment group(high dose) 200 mg/kg TC was given.

All above drugs was given for seven days by oral route. Then on 8^{th} and 9^{th} day doxorubicin was given at the dose of 10 mg/kg by IV route.

2.5 Biochemical analysis[20,21,22,23]

On the 9th day, the rats were fasted overnight. On the 10th day the fasted rats were sacrificed under diethyl ether anesthesia and blood samples were collected into plain sample bottles. Blood samples were collected via retro-orbital puncture or by cardiac puncture with 21G needle mounted on 5ml syringe. The animals were analysed according to standard methods for effect of the extract on various biochemical parameters of rats such as TC,TG,LDL and HDL.

2.6 Histopathological analysis

On the 9th day, the rats were fasted overnight. On the 10th day the fasted rats were sacrificed under diethyl ether anesthesia and portion of heart and liver of rats was collected from all group rats (normal, std. drug +doxorubicin, vit-E +doxorubicin, low dose of TC+ doxorubicin, high dose of TC+doxorubicin) and fixed in 10% formalin (10 ml of formaldehyde added to 90 ml of water). Then it was send for histopathological study to diagnostic centre.

2.7 Antioxidant activity of *Terminalia coriacea*

SUPEROXIDE DISMUTASE(SOD)[24]

Procedure

The sample i.e. leaves (0.5g), were ground with 3.0ml of potassium phosphate buffer, centrifuged at 2000 RPM for 10 minutes and the supernatants were used for the assay.

The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of Phenazine methosulfate, 0.3ml of Nitroblue tetrazolium, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer. In the case of sample and standard(ascorbic acid) assay they are taken at various concentration i.e. 10,20,30,40,50 µg/ml respectively but in the case of control all reagent without standard or sample.

% scavenging= [Absorbance of control-Absorbance of test sample/Absorbance of control] X 100

DPPH[25]

Procedure

4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517 nm for control reading. 100 µl of various concentrations(10,20,30,40 and 50 µg/ml) of *Terminalia coriacea* compounds as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min,at 517 nm using methanol as blank on UV-visible spectrometer.

The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging=[Absorbance of control -Absorbance of test sample/Absorbance of control] X 100

CATALASE(CAT)[26]

Procedure

20% homogenate of the leaf was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

The assay mixture contained1.0 ml of phosphate buffer and 0.4 ml of water and 0.2 ml of the enzyme was added but in the case of standard ascorbic acid instead of enzyme to initiate the reaction, 2.0 ml of the dichromate / acetic acid reagent was added after 1 minute of incubation. The tubes were then heated for 10 min. and then color developed was read at 610 nm.

In the case of sample and standard(ascorbic acid) assay they are taken at various concentration i.e. 10,20,30,40,50 µg/ml respectively but in the case of control all reagent without standard or sample.

% scavenging= [Absorbance of control-Absorbance of test sample/Absorbance of control] X 100

LIPIDPEROXIDATION(LP)[27]

Procedure:

20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the leaves of the plant, clarified by centrifugation and the supernatant was used for the assay.

To 1.0 ml of the sample supernatant or standard(ascorbic acid), 2.0 ml of Trichloricacetic acid- Thiobarbituric acid-HC1 reagent was added and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1,000 RPM for 10 min. The absorbance was determined at 535nm spectrometer.

In the case of sample and standard(ascorbic acid) assay they are taken at various concentration i.e. 10,20,30,40,50 µg/ml respectively but in the case of control all reagent without standard or sample.

% scavenging= [Absorbance of control-Absorbance of test sample/Absorbance of control] X 100

3. REASULTS

3.1. Biochemical parameters

Group	Total cholesterol(mg/dl)	Triglyceride(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Control	250	125	35	179
Standard	225	60	53	128
Vitamin-E	230	75	56	145
Low dose	220	52	55	125
High dose	210	47	54	112

administration of doxorubicin in control rats showed a significant increase serum total cholesterol(TC),triglycerides(TG), lowdensity lipoprotein(LDL) and decrease in high density lipoprotein (HDL).Rats treated with hydromethanolic extract of *Terminalia coriacea* (200 mg/kg and 100 mg/kg) showed decreased TC,TG,LDL and increases HDL levels.

3.2. Histopathology of doxorubicin induced myocardial necrosis

Microscopy

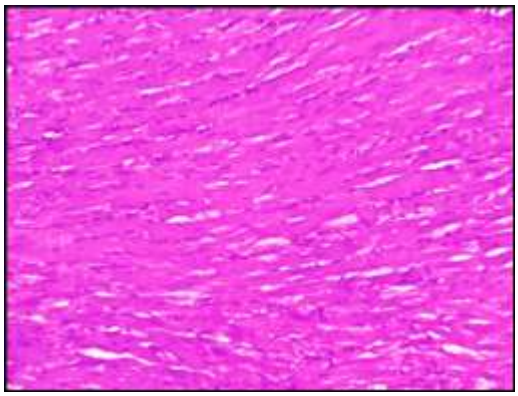


Fig-2: Normal control

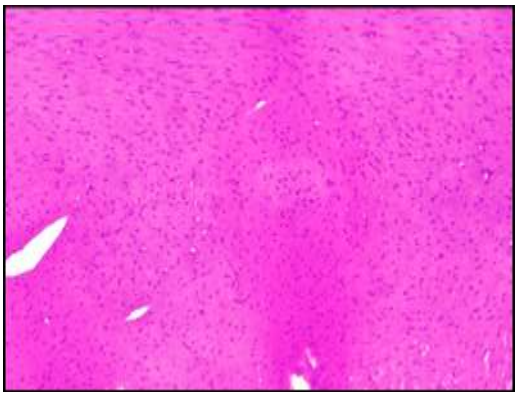


Fig-3: Standard drug

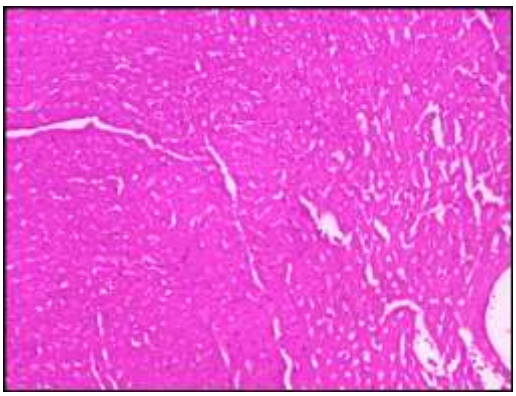


Fig-4: Vitamin-E

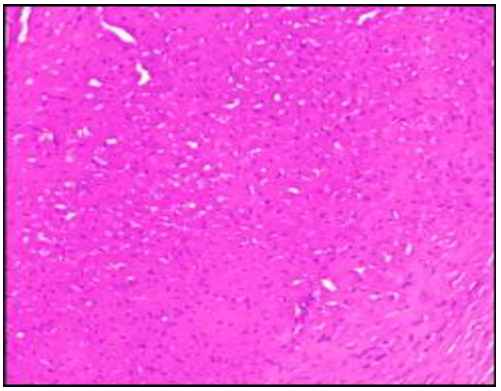


Fig-5: Low dose

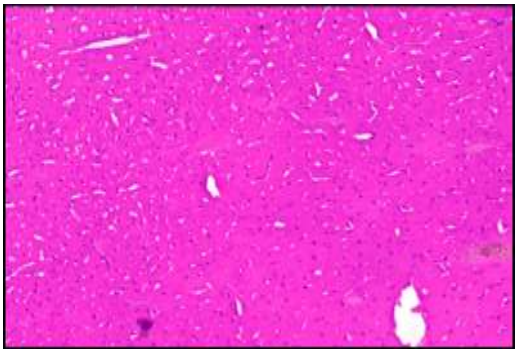


Fig-6: High dose

By performing histopathological study it shows that the cardiac tissue damage is more in control group rats than that of test, standard and vitamin-E treated group.

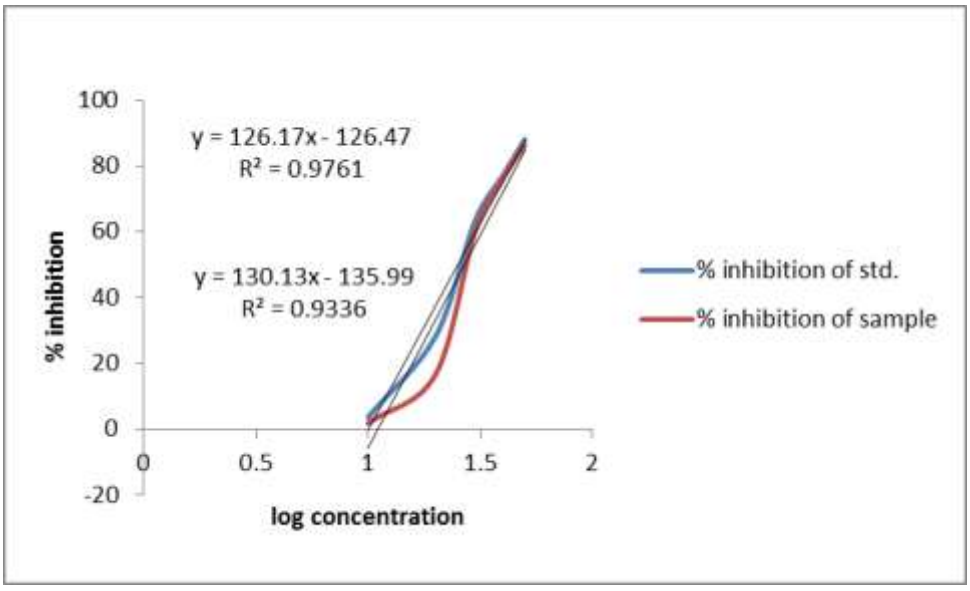
3.3 Anti-oxidant study

3.3.1. Anti-oxidant activity of catalase

Table-1

Concn.	Log concn.	Absorbance of standard	Absorbance of test	% inhibition of standard	% inhibition of test
10	1	0.245	0.247	1.2096	0.40
20	1.301	0.180	0.198	27.41	20.16
30	1.477	0.092	0.192	62.90	22.58
40	1.6020	0.045	0.047	81.85	81.04
50	1.6989	0.021	0.025	91.53	89.91

Absorbance of control = 0.431



Graph-1: Percentage inhibition in different concentration.

From above graph IC50 value:

Calculated by formula Y= mx+c or Y= mx-c

Here, Y= % inhibition, C= constant, m = coefficient

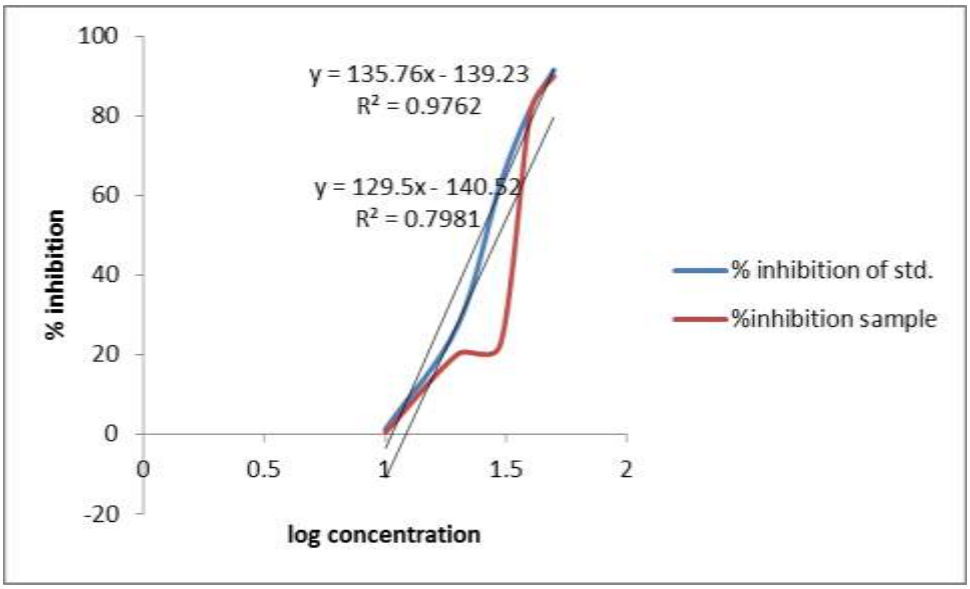
So, IC50 value for sample = 0.6608 µg/ml and IC50 value for standard = 0.6060 µg/ml

3.3.2. Anti-oxidant activity of DPPH

Table-2

Concn.	Log concn.	Absorbance of standard	Absorbance of test	% inhibition of standard	% inhibition of test
10	1	0.245	0.247	1.2096	0.40
20	1.301	0.180	0.198	27.41	20.16
30	1.477	0.092	0.192	62.90	22.58
40	1.6020	0.045	0.047	81.85	81.04
50	1.6989	0.021	0.025	91.53	89.91

Absorbance of Control = 0.248



Graph-2: Percentage inhibition in different concentration.

From above graph IC50 value:

Calculated by formula Y= mx+c or Y= mx-c

Here, Y= % inhibition, C= constant, m = coefficient

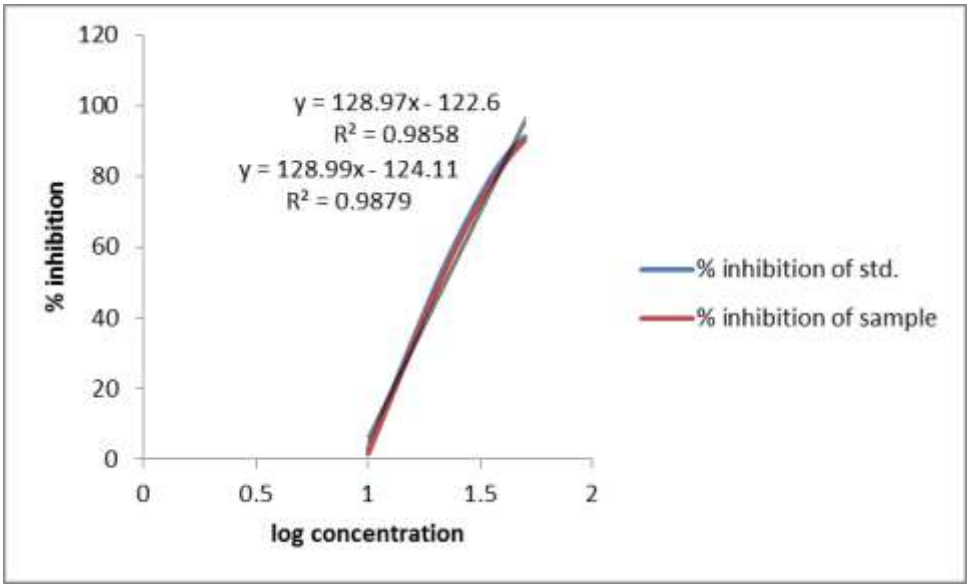
So, IC50 value for sample = 0.6572 µg/ml and IC50 value for standard = 0.6989 µg/ml.

3.3.3 Anti-oxidant activity of Lipid Peroxidation

Table-3

Concn.	Log concn.	Absorbance of standard	Absorbance of test	% inhibition of standard	% inhibition of test
10	1	0.392	0.397	2.72	1.48
20	1.301	0.205	0.212	49.13	47.39
30	1.477	0.113	0.120	71.96	70.22
40	1.6020	0.061	0.068	84.86	83.12
50	1.6989	0.035	0.039	91.31	90.32

Absorbance of control = 0.403



Graph-3: Percentage inhibition in different concentration.

From above graph IC50 value:

Calculated by formula Y= mx+c or Y= mx-c

Here, Y= % inhibition, C= constant, m = coefficient

So, IC50 value for sample = 0.5745 µg/ml

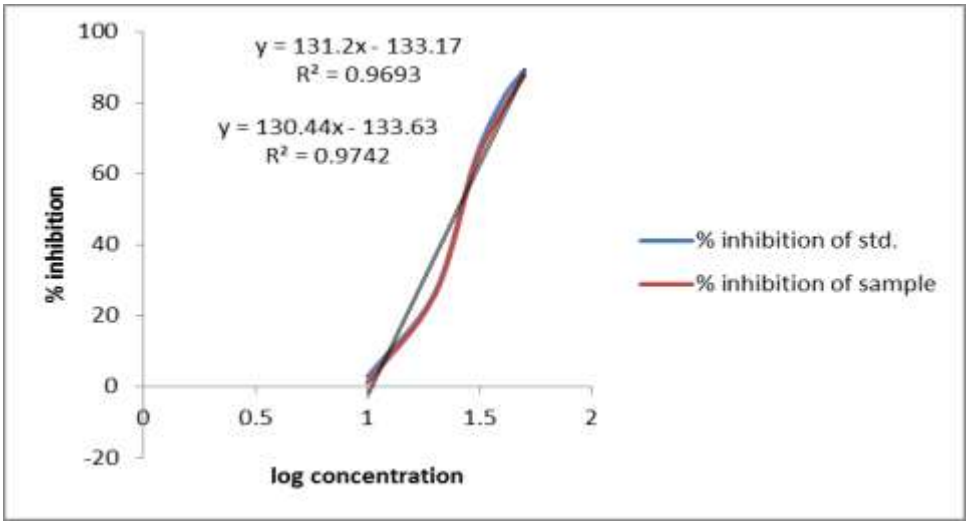
IC50 value for standard = 0.5629 µg/ml.

3.3.4 Anti-oxidant activity of superoxide dismutase

Table-4

Concn.	Log concn.	Absorbance of standard	Absorbance of test	% inhibition of standard	% inhibition of test
10	1	0.519	0.528	2.80	1.12
20	1.301	0.392	0.395	26.59	26.02
30	1.477	0.195	0.201	63.48	62.35
40	1.6020	0.102	0.118	80.89	77.90
50	1.6989	0.058	0.065	89.13	87.82

Absorbance of control = 0.534.



Graph-4: Percentage inhibition in different concentration.

From above graph IC50 value:

Calculated by formula Y= mx+c or Y= mx-c

Here, Y= % inhibition, C= constant, m = coefficient

So, IC50 value for sample = 0.6339 µg/ml and IC50 value for standard = 0.6411 µg/ml.

4. DISCUSSION

Medicinal plants have long been valued as sources of new compounds with therapeutic activity. The present study was under taken to find out the cardioprotective and anti-oxidant activity of *Terminalia coriacea* extract on doxorubicin induced cardio-toxicity (in rat model).

4.1. Doxorubicin- induced myocardial necrosis in rats

In this study animal feed with normal diet for 7 days. Then DOX is given on 8th and 9th day, On the 10th day the fasted rats were sacrificed under diethyl ether anesthesia and blood samples were collected into plain sample bottles. Blood samples were collected via retro-orbital puncture or by cardiac puncture with 21G needle mounted on 5ml syringe. The animals were analysed according to standard methods for effect of the standard drug, vit-E, and extract on various biochemical parameters of rats such as TC,TG,LDL and HDL.

At the same time the heart and liver also removed and preserved on 10% formalin and send for histopathological test, to observe the tissue damage on heart and liver and compared.

4.2. Antioxidant property

Terminalia coriacea was investigated in comparision with known antioxidant ascorbic acid(AA) following in-vivo studies.

The absorbance, % inhibition and IC50 value of *Terminalia coriacea* determined on anti-oxidant studies by spectrophotometer and compared with standard i.e. ascorbic acid.

5. CONCLUSION

The present study was aimed to explore the cardioprotective and anti-oxidant activity of *Terminalia coriacea* on doxorubicin induced cardio-toxicity in rats.

From the experimental studies carried out, extract of leaves of *Terminalia Coriacea* at two different administered doses (100 mg/kg and 200 mg/kg) showed dose dependent cardioprotective and antioxidant activity. The higher dose 200 mg/kg showed significant protection compared to lower dose 100 mg/kg.

The damage of cardiac musculature was also demonstrated and confirmed by histopathological observation, there is less pathological scores on standard drug, vitamin-E and test drug treated group so, we can say this plant is having cardioprotective activity.

This study determined that Methanolic extract of leaves of *Terminalia coriacea* showed better antioxidant potential by DPPH, SOD, Catalase, Lipidperoxidation method when compare to standard ascorbic acid by calculating IC50 value for both ascorbic acid and alcoholic extract So, we can say this plant is having antioxidant activity.

Further studies need to be carried out to isolate the potential chemical constituents of leaves of *Terminalia Coriacea* and to find its mechanism of action in the treatment.

The cardioprotective and antioxidant effect may be due to the presence of cardiac glycoside and flavonoids.

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