REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: July 2024; Revised: August 2024; Accepted: September 2024

Mitigating Doxorubicin-Induced Cardiotoxicity: The Role Of Polyherbal Extracts In Albino Rat Models

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Abstract:
This study was conducted to evaluate the cardioprotective effects of an ethanolic (50%) extract of a polyherbal mixture containing the leaves of *Bryophyllum pinnatum*, leaves of *Piper betle*, and aerial parts of *Ocimum sanctum* against Doxorubicin-induced heart toxicity in albino rats. The antioxidant properties of this polyherbal extract were assessed using various in vitro methods, including lipid peroxidation, nitric oxide scavenging, DPPH scavenging, and hydroxyl radical activity. Cardiotoxicity was induced in rats through the administration of a cumulative dose of Doxorubicin (15 mg/kg intraperitoneally for 2 weeks). The polyherbal extract was then administered at doses of 200 and 400 mg/kg body weight for 15 days. Observations included measurements of serum biomarkers, such as lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), alongside biochemical parameters like aspartate transaminase (AST), alanine transaminase (ALT), total protein.
Histopathological analysis of heart tissues was also performed. The in vitro antioxidant assays demonstrated significant inhibition potential of the polyherbal extract. In the in vivo studies, the administration of Doxorubicin led to substantial cardiotoxicity, as evidenced by elevated levels of biomarkers such as LDH, CK-MB as well as increased AST and ALT levels. However, pretreatment with the polyherbal extract provided notable protection to the myocardium, as indicated by the normalization of these biomarkers and biochemical parameters, along with improved histopathological outcomes. The findings, supported by comprehensive serum biomarker analysis, lipid profiles, biochemical data, and histopathology, suggest that the polyherbal extract offers substantial cardioprotective effects against Doxorubicin-induced heart damage.

Keywords: Cardiotoxicity, Doxorubicin, polyherbal extract, *Bryophyllum pinnatum*, *Piper betle*, *Ocimum sanctum*, lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB)

INTRODUCTION:

In India, Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy are the officially recognized alternative medicine systems, each with a well-documented history of safe, sustained use of various herbal drugs. Despite the absence of a distinct category for herbal drugs or dietary supplements in the Indian Medicines Act, a significant proportion of prescription pharmaceuticals—approximately 25%—continue to incorporate plant-derived components. However, the ascendancy of the pharmaceutical industry in the early 20th century led to a decline in the use of traditional and herbal medicines (Sharma, 2005). Nonetheless, in many developing nations, herbal remedies remain a primary source of healthcare, contributing to their importance in basic healthcare services. The World Health Organization has also endorsed the safe and effective application of herbal medicines, recognizing their value in contemporary medical practices (WHO, 2002). Traditional medical systems catalog over 2,000 plants, some of which are particularly efficacious in treating cardiovascular conditions like ischemic heart disease and hyperlipidemia (Patel et al., 2010).
Bryophyllum pinnatum, commonly referred to as the "Mother of Thousands," is a medicinal plant prevalent in tropical and subtropical regions. Its pharmacological potential has been well-documented, particularly its antioxidant, anti-inflammatory, analgesic, antimicrobial, and wound-healing properties (Akinmoladun et al., 2007). The therapeutic efficacy of Bryophyllum pinnatum is largely attributed to its bioactive compounds, including flavonoids, alkaloids, and phenolic compounds (Singh et al., 2012).
Betel Leaves (Piper betle) are another example of a widely used medicinal plant, particularly in South and Southeast Asia. Known for their carminative, antiseptic, anti-inflammatory, and analgesic properties, Betel leaves have traditionally been employed to address digestive disorders, oral hygiene issues, and respiratory conditions such as coughs and colds (Dasgupta et al., 2010).
Ocimum sanctum, commonly known as Holy Basil or Tulsi, holds significant spiritual and therapeutic importance in traditional medicine. This herb exhibits a range of pharmacological activities, including antioxidant, anti-inflammatory, adaptogenic, and antimicrobial properties. Holy Basil is widely used to support general health, enhance immunity, alleviate stress, manage respiratory disorders, and promote cardiovascular health (Mondal et al., 2009).

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MATERIAL AND METHODS:
Leaves of *Bryophyllum pinnatum*, leaves of *Piper betle*, and aerial parts of *Ocimum sanctum* were collected from Chittoor district and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati.

EXTRACTION OF THE PLANTS:
A total of 200 grams of dried plant material, in a **1:1:1 ratio**, was extracted using **50% ethanol.** The resultant mixture from the three plants was then evaporated on a water bath until it reached a semisolid consistency, after which it was stored in an airtight container for future use.

Phytochemical analysis:
The percentage yield of Ethanolic (50%) extract of polyherbal mixture was found to be 18.75%, with Dark green color and sticky consistency.

Qualitative Phytochemical analysis:
Polyherbal mixture of ethanolic (50%) extract was found to contain Phenolics, alkaloids, saponins, Tannins, Flavonoids, Cardiac glycosides, Gums and mucilages

Table 1. Qualitative profile of Phytochemicals of Polyherbal mixture

S.No.	Phytochemicals	+ / -	Type of Test
1.	Phenolics	+	Ferric chloride test
2.	Flavonoids	+	Alkaline reagent
3.	Tannins	+	Lead acetate
4.	Alkaloids	+	Wagner's reagent
5.	Cardiac glycoside	+	Baljet test

+ = present and - = absent

Animals:
Healthy adult albino rats (100-180g each) aged 60-90 days were used for the study. The rats were housed in polypropylene cage and maintained under standard conditions (12h light/12h dark cycle; 25± 3 ^{o}C; 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum.* The study was approved by the Institutional Animal Ethical committee of Deccan School of Pharmacy, Hyderabad, Telangana.

Invitro anti-oxidant acitivity:
The polyherbal extract of *Bryophyllum pinnatum, Piper betle, Ocimum sanctum* taken in ratio of **(1:1:1)** has shown maximum inhibition activity in the following methods of antioxidant activity such as Lipid peroxidation activity, Nitric oxide scavenging activity, Free radical scavenging by DPPH method. Scavenging of Hydroxyl radical (deoxyribose method).

Cardioprotective activity of Poly herbal extract in Doxorubicin (Dox) induced myocardial necrosis
The cardioprotective activity in Doxorubicin induced myocardial necrosis was carried out as per the method (Swamy et.al. 2011) . 24 albino rats were subdivided into four groups containing six animals in each group.
Group I: Received normal saline (1ml/kg, p.o.)
Group II: Animals were treated with Doxorubicin (2.5 mg/kg b.w. i.p.) in 6 equal injections on alternate days for two weeks to make a total cumulative dose of 15mg/kg body weight.
Group III: Animals were treated with poly herbal extract (200mg/kg b.w. p.o.)+ Doxorubicin (2.5 mg/kg b.w. i.p.) in 6 equal injections alternate days.
Group IV: Animals were treated with poly herbal extract (400mg/kg b.w. p.o.) +Doxorubicin (2.5 mg/kg b.w. i.p.) in 6 equal injections on alternate days.
Group III and Group IV received 200mg/kg b.w. and 400mg/kg b.w. poly herbal extract were administered orally to each animal for 2 weeks and Dox (2.5 mg/kg b.w. i.p.) in 6 equal injections on alternate days for two weeks to make a total cumulative dose of 15mg/kg body weight. The animals were sacrificed 24hr after administration of Dox under chloroform anesthesia; blood sample were collected through retro orbital sinus, hearts were excised and immediately processed for biochemical parameters and histopathological studies.

Blood Biochemistry:
Albino Rats were sacrificed by cervical dislocation. Blood samples were withdrawn by cardiac puncture in heparinized tubes and were centrifuge at 3000-4000 rpm for 10 min to obtain serum. The myocytes injury markers such as CK-MB,

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LDH, AST, ALT, ALP and Total Protein were measured according to the standard procedures given along with the kits purchased.

Histopathological studies:
For microscopic examination, the hearts were preserved in a 10% formalin solution. The tissues were then dehydrated through an ascending ethanol series (70%, 80%, 96%, 100%), cleared with xylene, and embedded in paraffin. Thin tissue sections of 5µm thickness were stained with hematoxylin and eosin. At least 10 fields per heart slide were analyzed to assess the loss of myocardial fibers and the formation of thrombi. Tissue samples were photographed under a microscope at 10X magnification.

RESULTS:
Acute toxicity studies:
- The polyherbal extract of *Bryophyllum pinnatum*, *Piper betle*, *Ocimum sanctum* taken in ratio of **(1:1:1)** has shown to be safe and no mortality was observed up to a dose of 2000mg/kg body weight, p.o. The maximum tolerated dose was taken as 2000mg/kg body weight. The doses for pharmacological studies were taken as 400mg/kg, 200 mg/kg body weight, p.o. i.e. $1/5^{th}$, $1/10^{th}$ of the maximum tolerated dose i.e. 2000mg/kg body weight.

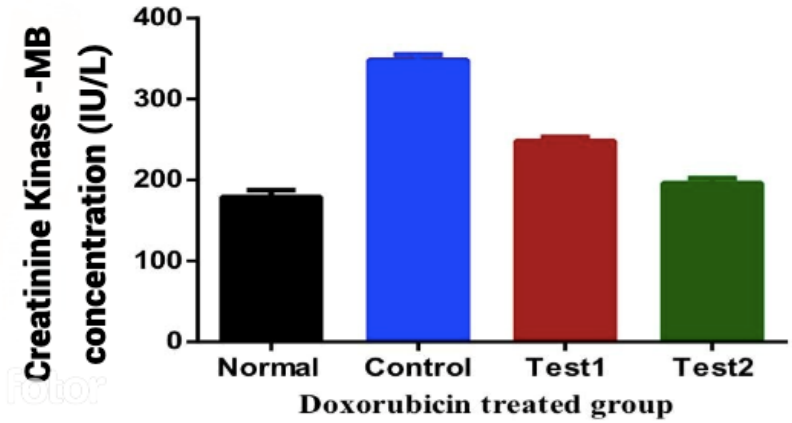
Doxorubicin (Dox) treated groups:
The table no.1give details of the levels of the serum myocyte markers and biochemical parameters in the normal, Disease control and treated animals. Doxorubicin was administered for 2 weeks in the control and treated animals which results in the increased levels of CK-MB, LDH, AST, ALT, and TP. On treatment with the poly herbal extract for 2 weeks a significant decrease in the levels of CK-MB, LDH, AST, ALT, and TP was observed in Group III and Group IV as compared to disease control (Group II) . These results of the serum myocyte markers and biochemical parameters presented in data clearly support that the poly herbal extract for cardioprotective activity.

Effect of the poly herbal extract against Doxorubicin on serum myocytes marker and biochemical parameters.

S.No	Treatment groups	Creatine kinase myocardial band (CKMB)-IU/L	Lactate Dehydrogenase (LDH)-IU/L	SGOT (AST)-IU/L	SGPT (ALT)-IU/L	Total Protein (TP)-g/dl
1.	Normal	179±8.60	221±8.42	76±4.66	28±3.54	6.5±0.99
2.	Disease Control	348±7.0	448±9.08	209±8.11	102±5.56	16±1.29
3.	Polyherbal extract(test-1) (200mg/kg)	248±5.15**	314±9.68**	153±6.35**	71±3.82**	11.5±1.25**
4.	Polyherbal extract(test-2)(400mg/kg)	196±6.63***	261±8.06***	95±5.97***	39±3.36***	8.5±0.76**

All values are expressed as a mean ± SEM, n=6, analysed by ANOVA followed by Dunnett's Test. $p < 0.05$ was considered as significant. * $p<0.05$, ** $p< 0.01$, *** $p<0.001$ when compared to control group.

Graph No.1 Histogram showing the effect of Poly herbal extract on Serum Creatine kinase -MB Levels



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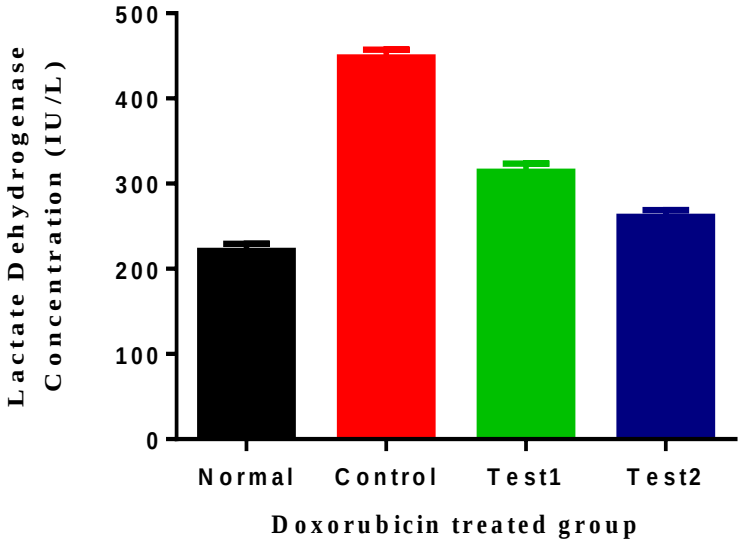
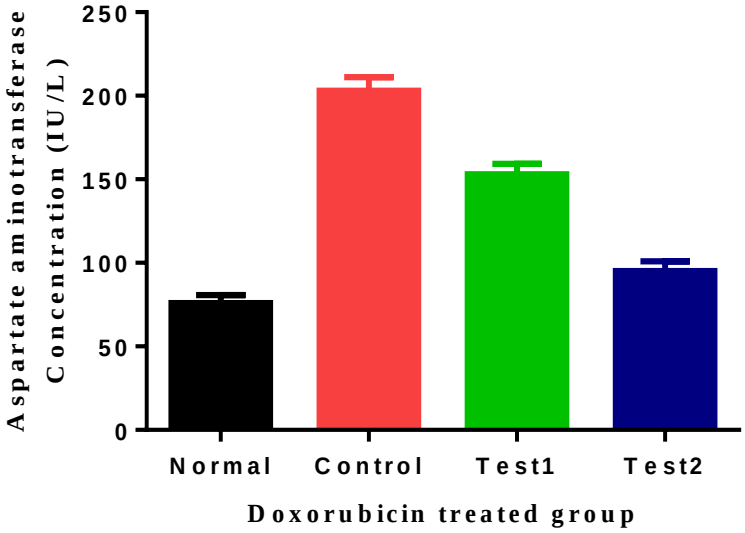
Data were expressed as Mean ± S.E.M. The differences between the control and treatment groups were compared using one-way ANOVA followed by Dunnett's test using Graph Pad Prism Software (version 6). The results were considered significant when $p<0.05$

Graph No.2 Histogram showing the effect of Poly herbal extract on Serum Lactate Dehydrogenase Levels



Data were expressed as Mean ± S.E.M. The differences between the control and treatment groups were compared using one-way ANOVA followed by Dunnett's test using Graph Pad Prism Software (version 6). The results were considered significant when $p<0.05$.

Graph No.3 Histogram showing the effect of Poly herbal extract on Serum Aspartate Aminotransferase Levels



Data were expressed as Mean ± S.E.M. The differences between the control and treatment groups were compared using one-way ANOVA followed by Dunnett's test using Graph Pad Prism Software (version 6). The results were considered significant when $p<0.05$.

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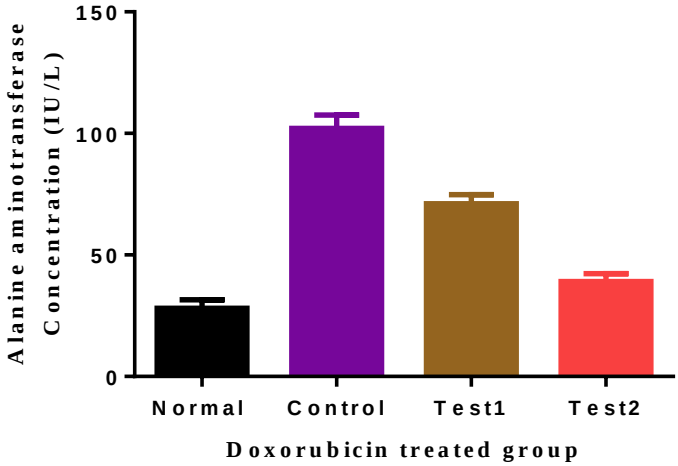
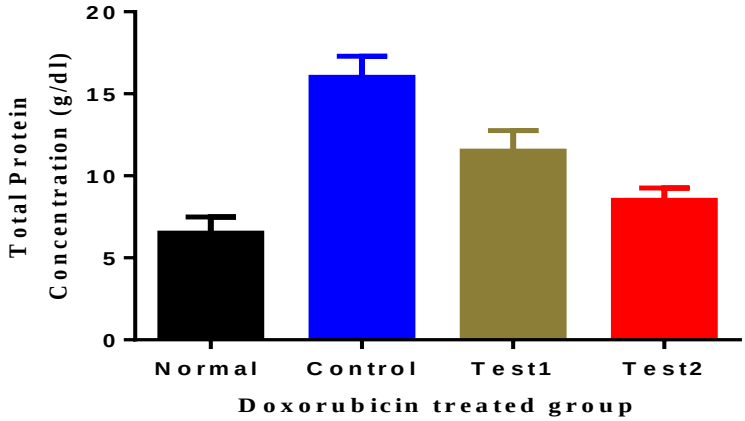
Graph No.4 Histogram showing the effect of Poly herbal extract on Serum Alanine Aminotransferase Levels



Data were expressed as Mean ± S.E.M. The differences between the control and treatment groups were compared using one-way ANOVA followed by Dunnett's test using Graph Pad Prism Software (version 6). The results were considered significant when $p<0.05$.

Graph No.5 Histogram showing the effect of Poly herbal extract on Serum Total Protein Levels



Data were expressed as Mean ± S.E.M. The differences between the control and treatment groups were compared using one-way ANOVA followed by Dunnett's test using Graph Pad Prism Software (version 6). The results were considered significant when $p<0.05$.

Histopathological Studies:

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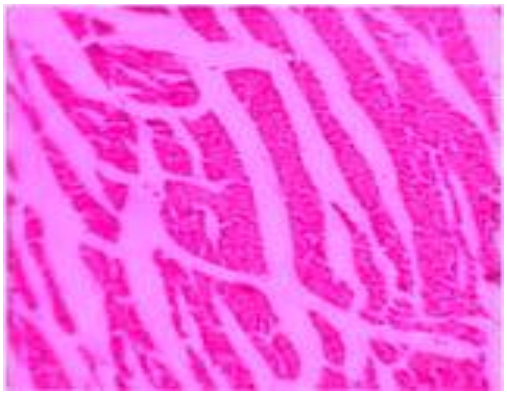

Fig 1. Microarchitecture of heart sections of Group -I (normal- DOX) received a single oral dose of normal saline 10ml/kg only.

It showed intact architecture of the myocardial fibres & sharp demarcation(at 10X)

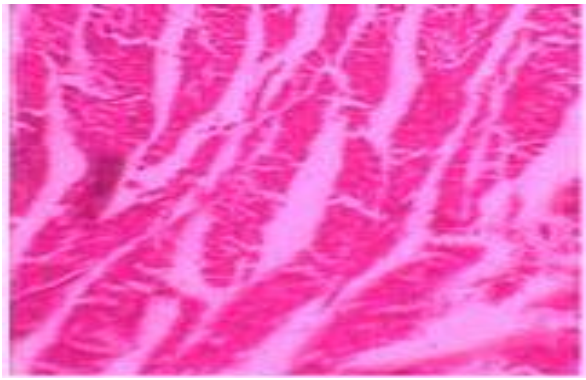

Fig 2. Microarchitecture of heart sections of Group-II received a single toxic dose of Doxorubicin (2.5mg/kg b.w. i.p)

It shows thrombus formation, loss of myocardial formation fibres, degeneration of myocardial tissues fragmentation of nuclei. (at 10X).

Fig 3. Microarchitecture of heart sections of Group-III received a single toxic dose of Doxorubicin (2.5mg/kg b.w. i.p)+ 200mg/kg b.w. of polyherbal extract(test-1).

It shows decreased thrombus formation,less extensive vacuolization of the cytoplasm,myocardial fibres are intact. (at 10X).

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Fig 4. Microarchitecture of heart sections of Group-IV received a single toxic dose of Doxorubicin (2.5mg/kg b.w. i.p)& + 400mg/kg b.w. of polyherbal extract(test-2).

It depicts decreased thrombus formation, less extensive vacuolization of the cytoplasm, no loss of myocardial fibres. (at 10X).

CONCLUSION:

- The Phytochemical screening of the phytoconstituents was performed in the combined mixture of polyherbal extract. It showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, saponins, polyphenols and tannins.
- In vitro antioxidant activity of the poly herbal extract was performed by different methods such as Lipid Peroxidation activity, Nitric oxide scavenging activity, Free radical scavenging activity, Scavenging of hydroxyl radical by using different combination. The results of the methods has shown that the polyherbal extract in ratio of (1:1:1) has shown maximum inhibition activity.
- The Pharmacological screening included evaluation of Cardioprotective activity using Dox and ISO induced model in rats. Increased levels of serum marker enzymes such as CK-MB, LDH, AST, ALT, Total protein were significantly decreased by oral administration of poly herbal extract (200, 400 mg/kg) for 15 days in Dox treated group. In support to the enzyme markers, the histopathological examination demonstrated a prominent effect and prevented the damage to the myocardium of the rats. Histopathological findings also showed and supported the present study.
- The mechanism underlying this effect is still unknown but the possible mechanisms of action for cardioprotective activity of poly herbal extract may be due to the presence of flavonoids, polyphenols, which has potent anti-oxidant property.

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