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Cardioprotective Potential of A Polyherbal Combination Of *Tinospora Cordifolia*, *Boerhaviadiffusa*, And *Glycyrrhiza Glabra* Against Doxorubicin-Induced Cardiotoxicity

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Abstract: Cardiovascular diseases remain a leading cause of global morbidity and mortality, with drug-induced cardiotoxicity posing a significant clinical challenge. The present study investigates the cardioprotective potential of a polyherbal combination consisting *of Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra* against doxorubicin-induced cardiotoxicity. The plant materials were collected, authenticated, and subjected to extraction using suitable solvents, followed by phytochemical screening and pharmacological evaluation. In vitro antioxidant activity of the polyherbal extract was assessed using lipid peroxidation, nitric oxide scavenging, DPPH free radical scavenging, and hydroxyl radical scavenging assays. Among various combinations, the optimized ratio demonstrated significant antioxidant potential. Acute toxicity studies confirmed the safety of the extract up to 2000 mg/kg, and doses of 200 mg/kg and 400 mg/kg were selected for in vivo studies. Cardioprotective activity was evaluated using doxorubicin-induced myocardial toxicity models in Wistar rats. Treatment with the polyherbal extract resulted in a significant reduction in serum cardiac biomarkers such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein levels compared to disease control groups. Histopathological examination further confirmed the protective effects, showing preservation of myocardial architecture and reduced cellular damage. The results suggest that the polyherbal formulation possesses significant cardioprotective activity, likely mediated through its antioxidant properties and ability to mitigate oxidative stress. This study supports the therapeutic potential of plant-based formulations in managing drug-induced cardiotoxicity.

Keywords: Polyherbal formulation, Cardioprotective activity, Doxorubicin-induced cardiotoxicity, Antioxidant activity, *Tinospora cordifolia*, *Boerhavia diffusa*, *Glycyrrhiza glabra*

1. INTRODUCTION

1.1 Medicinal Plants and Their Therapeutic Significance

Medicinal plants have been used since ancient times as a primary source of therapeutic agents for the treatment and prevention of various diseases. Traditional systems of medicine such as Ayurveda, Unani, and Siddha have extensively utilized plant-based formulations due to their accessibility, affordability, and minimal side effects. According to the World Health Organization, nearly 80% of the global population relies on herbal medicines for primary healthcare needs [1]. Over the years, scientific validation of traditional knowledge has led to the discovery of several modern drugs derived from plants, including aspirin, digoxin, and morphine [2]. The increasing interest in natural products has further accelerated research into plant-derived compounds as potential alternatives to synthetic drugs.

Medicinal plants are rich in bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds, which contribute to their diverse pharmacological activities. These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties, making them valuable in the management of chronic diseases [3]. The growing concern over adverse effects and resistance associated with conventional drugs has prompted researchers to explore herbal formulations as safer therapeutic options.

1.2 Cardiovascular Diseases and Drug-Induced Cardiotoxicity

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide, accounting for a significant proportion of global deaths. Myocardial infarction (MI), commonly known as heart attack, is a major contributor to cardiovascular-related deaths and is often associated with oxidative stress and inflammation [4]. The pathogenesis of CVDs involves complex mechanisms including lipid peroxidation, endothelial dysfunction, and accumulation of reactive oxygen species (ROS), which ultimately lead to cardiac tissue damage [5] .Doxorubicin, an anthracycline antibiotic widely used in cancer chemotherapy, is highly effective against various malignancies such as breast cancer, ovarian cancer, and lymphomas. However, its clinical use is limited due to its severe cardiotoxic effects, which can result in cardiomyopathy and congestive heart failure [6]. The mechanism of doxorubicin-induced cardiotoxicity is primarily attributed to the generation of free radicals, oxidative stress, mitochondrial dysfunction, and apoptosis of cardiac cells [7]. Similarly, isoproterenol-induced myocardial necrosis is a well-established experimental model used to study cardiac injury and evaluate cardioprotective agents [8]. Despite advances in pharmacotherapy, current treatments for cardiotoxicity are limited and often associated with adverse effects. Therefore, there is an urgent need to identify novel therapeutic agents that can effectively protect the heart with minimal side effects.

1.3 Role of Antioxidants in Cardioprotection

Oxidative stress plays a crucial role in the development of cardiovascular diseases and drug-induced cardiotoxicity. Reactive oxygen species (ROS) can cause damage to cellular components such as lipids, proteins, and DNA, leading to impaired cardiac function [9]. Antioxidants are substances that neutralize free radicals and prevent oxidative damage, thereby offering protection against cardiovascular disorders [10]. Plant-derived antioxidants, particularly polyphenols and flavonoids, have been shown to exhibit significant cardioprotective effects by reducing lipid peroxidation, improving endothelial function, and enhancing antioxidant defense mechanisms [11]. The use of natural antioxidants has gained considerable attention as they provide a safer alternative to synthetic antioxidants, which may have potential toxicity.

1.4 Therapeutic Potential of Selected Medicinal Plants

The present study focuses on three important medicinal plants, namely *Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra*, which have been widely used in traditional medicine for their diverse pharmacological properties. *Tinospora cordifolia* (Guduchi) is a well-known medicinal plant in Ayurveda, recognized for its immunomodulatory, antioxidant, anti-inflammatory, and cardioprotective properties. It has been traditionally used for the treatment of fever, diabetes, liver disorders, and infections [12].*Boerhavia diffusa* (Punarnava) is another important medicinal herb known for its diuretic, anti-inflammatory, hepatoprotective, and nephroprotective activities. It contains various bioactive compounds such as alkaloids, flavonoids, and rotenoids that contribute to its therapeutic effects [13]. *Glycyrrhiza glabra* (Licorice) is widely used for its anti-inflammatory, antioxidant, antiviral, and hepatoprotective properties. It also exhibits cardioprotective effects by reducing oxidative stress and improving lipid metabolism [14]. The combination of these plants in a polyherbal formulation is expected to produce a synergistic effect, enhancing their individual pharmacological activities and providing better therapeutic outcomes.

Taxonomical Classification

(i) *Tinospora cordifolia*

Kingdom: Plantae (Plants)

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: Tinospora

Species: *Tinospora cordifolia*

(ii) *Boerhaviadiffusa*

Kingdom: Plantae (Plants)

Division: Angiosperm

Class: Eudicots

Order: Caryophyllales

Family: Nyctaginaceae

Genus: Boerhavia

Species: *Boerhaviadiffusa*

(iii) *(III) Glycyrrhiza glabra*

Kingdom: Plantae (Plants)

Division: Magnoliophyta

Class: Magnoliopsida (Dicotylédones)

Order: Fabales

Family: Fabaceae

Genus: Glycyrrhiza

Species: Glycyrrhiza glabra

1.5 Rationale of the Study

Polyherbal formulations are gaining popularity due to their synergistic effects, improved efficacy, and reduced toxicity compared to single-drug therapies. The integration of multiple medicinal plants allows for targeting different pathways involved in disease progression, thereby enhancing therapeutic effectiveness [15]. In the context of cardioprotection, combining plants with antioxidant, anti-inflammatory, and cytoprotective properties can provide comprehensive protection against cardiac injury. Although individual plants such as *Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra* have been studied for their pharmacological properties, limited research has been conducted on their combined cardioprotective potential, particularly against doxorubicin-induced cardiotoxicity.

1.6 Aim of the Study

Therefore, the present study was designed to evaluate the cardioprotective activity of a polyherbal formulation containing *Tinospora cordifolia*, *Boerhaviadiffusa*, and *Glycyrrhiza glabra* against doxorubicin-induced cardiotoxicity using in vitro antioxidant assays and in vivo experimental models. The study aims to provide scientific validation for the traditional use of these medicinal plants and explore their potential as natural therapeutic agents for cardiovascular disorders.

Objective:

1. The combination of *Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra* is highly regarded for its therapeutic benefits.
2. Benefits for heart protection may involve diminishing inflammation, decreasing blood pressure, and alleviating oxidative damage.
3. The procedure consisted of verifying the authenticity and managing the materials obtained from the combination of *Tinospora cordifolia, Boerhavia diffusa*, and *Glycyrrhiza glabra*.

4. The technique involves purifying, drying, and milling the mix of *Tinospora cordifolia, Boerhavia diffusa*, and *Glycyrrhiza glabra*.
5. Potential heart protective effects of a mix of *Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra* against cardiotoxicity caused by Doxorubicin.
6. This research focuses on the heart protective potential of a mix of *Tinospora cordifolia*, *Boerhavia diffusa*, and *Glycyrrhiza glabra* in relation to Doxorubicin-induced cardiotoxicity.

2. MATERIAL AND METHODS

2.1 Collection and Authentication of Plant Materials

The plant materials, Tinospora cordifolia (stem), Boerhaviadiffusa (root), and Glycyrrhiza glabra (root), were procured from local herbal sources and authenticated by a qualified botanist. The collected materials were washed thoroughly with distilled water to remove adhering impurities, shade-dried at room temperature, and pulverized into coarse powder using a mechanical grinder. The powdered materials were stored in airtight containers for further use.

2.2 Preparation of Polyherbal Extract

The dried powders of *Tinospora cordifolia*, *Boerhavia diffusa,* and *Glycyrrhiza glabra* were mixed in suitable proportions to prepare a polyherbal formulation. The combined powder was subjected to extraction using a Soxhlet apparatus with hydroalcoholic solvent (ethanol:water). The extraction process was continued until complete exhaustion of the plant material. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a semisolid mass. The dried extract was stored in a desiccator and used for further studies.

Tinospora cordifolia: The leaves were collected and shade dried and powdered by mechanical grinder. About 250 gm of powder was extracted by ethanol (50%) using Soxhlet apparatus for 3 days and the mixture was subsequently filtered and concentrated at 40-60^{0}C. Extract was dried and preserved in desiccators.

Boerhavia diffusa: The leaves were collected and shade dried and powdered by mechanical grinder. About 250 gm of powder was extracted by ethyl acetate using Soxhlet apparatus for 3-4 days and the mixture was subsequently filtered and concentrated at 50-60^{0}C. Extract was dried and preserved in desiccators.

Glycyrrhiza glabra: The leaves were collected and shade dried and powdered by mechanical grinder. About 300gm of powder was extracted by ethanol (50%) using Soxhlet apparatus for 2-3days and the mixture was subsequently filtered and concentrated at 40-60^{0}C. Extract was dried and preserved in desiccators. Some part of the total extract of individual plants was used for Phytochemical investigation and remaining of the extract was used for Pharmacological screening.

2.3 Phytochemical Screening

Preliminary qualitative phytochemical analysis of the polyherbal extract was carried out using standard methods to identify the presence of bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, and steroids.

2.4 In Vitro Antioxidant Studies

The antioxidant activity of the polyherbal extract was evaluated using standard in vitro assays:

- **DPPH Free Radical Scavenging Assay**
- **Nitric Oxide Scavenging Assay**
- **Hydroxyl Radical Scavenging Assay**
- **Lipid Peroxidation Assay**

Different concentrations of the extract were tested, and the percentage inhibition of free radicals was calculated. Ascorbic acid was used as the standard antioxidant.

2.5 Experimental Animals

Healthy Wistar albino rats (150–200 g) of either sex were used for the study. The animals were obtained from an approved animal house facility and maintained under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55–65%, 12-hour light/dark cycle). Animals were fed with standard pellet diet and water ad libitum. All experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and CPCSEA regulations.

2.6 Acute Toxicity Study

Acute oral toxicity studies were conducted according to OECD guideline 423. The polyherbal extract was administered orally at doses up to 2000 mg/kg body weight. Animals were observed for 14 days for signs of toxicity or mortality. No significant toxic effects were observed, indicating the safety of the extract. Based on these findings, doses of 200 mg/kg and 400 mg/kg were selected for further studies.

2.7 Evaluation of Cardioprotective Activity

2.7.1 Doxorubicin-Induced Cardiotoxicity Model

Animals were divided into four groups (n = 6):

- **Group I:** Normal control
- **Group II:** Doxorubicin control
- **Group III:** Polyherbal extract (200 mg/kg) + Doxorubicin
- **Group IV:** Polyherbal extract (400 mg/kg) + Doxorubicin

Doxorubicin was administered intraperitoneally to induce cardiotoxicity. The polyherbal extract was administered orally for a specified duration prior to and during doxorubicin treatment.

2.8 Biochemical Analysis

At the end of the experimental period, blood samples were collected and serum was separated by centrifugation. The following biochemical parameters were estimated using standard diagnostic kits:

- Creatine phosphokinase (CPK)
- Lactate dehydrogenase (LDH)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Total protein

2.9 Histopathological Examination

Heart tissues were excised, washed with saline, and fixed in 10% formalin. The tissues were processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathological changes such as myocardial necrosis, edema, and inflammatory infiltration were observed under a microscope.

2.10 Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

a) Phytochemical Investigation:

S. No	Phytoconstituents	*Combination Of Tinospora Cordifolia, BoerhaviaDiffusa, And Glycyrrhiza Glabra*
		Aqueous Extract ($D.H_2O$)
1.	Carbohydrate	Present
2.	Glycosides	Absent
3.	Alkaloids	Present
4.	Steroids	Absent
5.	Tannins	Present
6.	Flavonoids	Present
7.	Phenols	Present
8.	Saponins	Absent
9.	Flavanoglycosides	Absent
10.	Gums	Absent
11.	Resins	Present
12.	Carboxylic acid	Absent
13.	Protein	Present
14.	Biuret	Absent

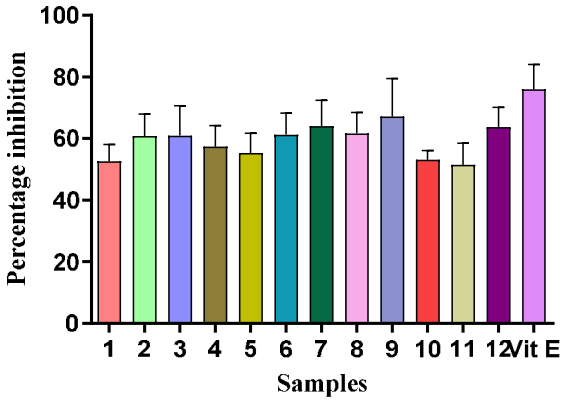
b) Pharmacological Investigations:

Tinospora cordifolia, *Boerhaviadiffusa*, *and Glycyrrhiza glabra,*

Acute Oral Toxicity Studies:

- The ethanolic extract (50%) of *Tinospora cordifolia* and *Boerhavia diffusa,* ethyl acetate extract of *Glycyrrhiza glabra,* poly herbal extract with a combination no.9 (mentioned in table no.1:-125:50:60:80) was found 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.
- The animals were observed continuously for general behavioural, neurological and autonomic profiles which are mentioned in the table no.3.

Graph No.1 Histogram showing the effect of the Poly herbal extract on Lipid Peroxidation activity



Each value represents Mean ± SEM; n =13 observation

p< 0.01: p< 0.05: NS = Non significant compared to standard.

Samples 1, 10 and 11 showed no significant change in the percent lipid peroxide inhibition when compared to standard. Samples 2, 3, 4, 5 showed slightly significant ($p< 0.05$) decrease in the activity when compared to standard. Samples 6, 7, 8,9,12 showed highly significant ($p<0.01$) decrease in the activity when compared to standard. Almost all the samples showed good percent inhibition of lipid peroxidation, amongst the entire above samples, sample 9 showed maximum inhibitory activity than other samples with respect to standard(values are from table no.4).

b) Nitric Oxide scavenging activity:

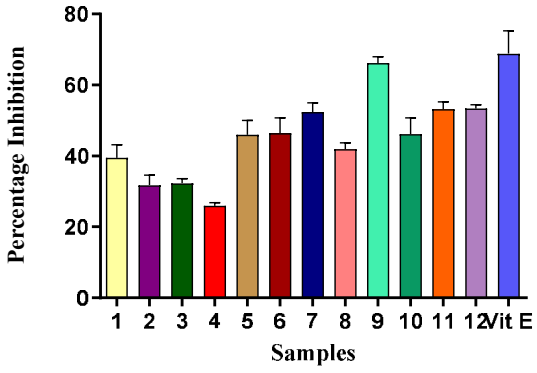
Nitric oxide scavenging activity was carried out on the poly herbal extract where the different combinations of the extracts (mentioned in table no.1) were compared with the standard (Vitamin E) to find the maximum inhibitory activity of the samples. The results are mentioned in the form of a graph (mentioned in graph no. 2).

Table No.1 Effect of different ratios of poly herbal extracts on Nitric oxide scavenging activity:

Sample No.	Conc. 10µg	Conc. 50µg	Conc. 100µg	Conc. 200µg	Conc. 400µg	Conc. 800µg	Conc. 1000µg	Mean ± S.E.M	P-Value
1	34.00	32.23	42.17	57.55	49.36	44.56	30.67	49.50±3.63	NS
2	33.22	36.66	43.55	24.09	33.53	40.04	18.45	38.21±4.92	NS
3	24.77	30.99	35.45	38.12	37.58	41.72	32.64	42.32±1.27	NS
4	22.14	29.99	29.54	23.37	25.73	33.67	25.23	45.96±0.89	NS
5	46.55	54.56	57.60	58.45	36.73	48.37	30.34	46.01±4.05	$p< 0.05$
6	50.35	63.34	53.78	43.00	38.73	53.56	29.34	46.45±4.24	$p< 0.05$
7	43.34	47.07	57.55	54.56	59.75	61.59	55.78	49.38±2.57	$p< 0.05$
8	34.34	43.35	46.56	48.65	42.58	53.56	40.45	45.92±1.74	$p< 0.05$
9	61.34	67.41	78.45	72.49	68.74	65.45	60.45	64.20±1.77	$p< 0.01$
10	38.56	48.56	64.68	48.56	32.57	44.56	54.67	48.16±4.53	$p< 0.05$
11	43.45	47.67	55.81	54.06	54.65	66.57	51.78	53.14±2.06	$p< 0.05$
12	51.45	53.79	57.56	58.00	56.71	62.09	47.71	56.34±1.10	$p< 0.05$
Vitamin-E	48.00	57.68	52.04	72.70	76.08	69.53	96.66	65.81±6.52	

All the values are expressed as Mean±S.E.M. analysed by student t test and $p<0.05$, $p<0.01$, is considered slightly significant, very significant, NS= non significant respectively when compared to standard.

Graph No.2 Histogram showing the effect of the poly herbal extract on Nitric Oxide Scavenging activity



Each value represents Mean ± SEM; n =13 observation

$p < 0.01$: $p< 0.05$: NS = Non significant compared to standard.

Samples 1, 2, 3 and 4 showed no significant change in the percent nitric oxide inhibition when compared to standard. Samples 5, 6, 7, 8, 10, 11, 12 showed slightly significant ($p< 0.05$) decrease in the activity when compared to standard. Sample 9 showed highly significant ($p<0.01$). Amongst all, sample 9,

showed highly significant and showed potent nitric oxide inhibitory activity than other samples with respect to standard(values are from table no.5).

c) Free Radical scavenging by DPPH method:

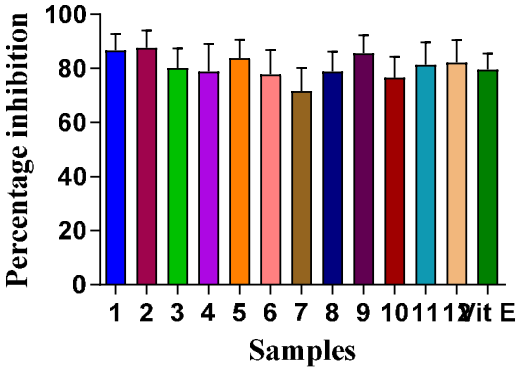
Free Radical scavenging by DPPH method was carried out on the poly herbal extract where the different combinations of the extracts (mentioned in table no.1) were compared with the standard (Vitamin E) to find the maximum inhibitory activity of the samples. The results are mentioned in the form of a graph (mentioned in graph no. 3).

Table No.2 Effect of different ratios of poly herbal extracts on DPPHpercentage inhibition:

Sample No.	Conc. 10µg	Conc. 50µg	Conc. 100µg	Conc. 200µg	Conc. 400µg	Conc. 800µg	Conc. 1000µg	Mean ±S.E.M	P-value
1	52.12	84.56	94.55	96.62	95.51	93.54	90.23	86.73±5.97	p< 0.01
2	50.21	86.32	96.34	97.21	94.34	93.43	95.52	87.62±7.24	p< 0.01
3	46.34	60.34	82.45	93.45	94.51	93.45	90.45	80.14±7.24	p< 0.01
4	23.32	64.52	84.55	96.67	96.12	95.34	91.42	78.85±10.20	p< 0.05
5	45.43	77.89	94.71	95.42	93.33	90.27	89.64	83.80±6.77	p< 0.01
6	33.45	57.31	80.05	96.06	93.19	93.67	90.21	77.81±8.99	p< 0.05
7	33.45	53.31	63.64	78.57	94.45	91.15	86.54	71.59±8.49	p< 0.05
8	42.34	63.00	82.48	94.75	94.23	87.79	87.78	78.91±7.31	p< 0.05
9	46.41	90.53	94.60	96.34	93.64	86.11	91.34	85.57±6.64	p< 0.01
10	42.34	52.94	76.95	91.61	92.39	89.00	90.41	76.52±7.79	p< 0.05
11	32.33	82.34	91.22	92.35	94.4	87.56	89.11	81.33±8.29	p< 0.01
12	33.33	83.55	95.15	93.67	93.36	88.37	87.65	82.15±8.28	p< 0.01
Vitamin.E	54.78	64.55	74.98	82.93	86.34	94.76	98.18	79.50±5.96	p< 0.01

All the values are expressed as Mean±S.E.M. analysed by student t test & p<0.05, p<0.01, is considered slightly significant, very significant respectively when compared to standard.

Graph No.3 Histogram showing the effect of the poly herbal extract on Free Radical scavenging by DPPH method



Each value represents Mean ± SEM; n =13 observation

p< 0.01: p< 0.05: NS = Non significant compared to standard.

All the Samples showed significant change in the DPPH percent inhibition, Samples 1, 2, 3 5, 9, 11 and 12 showed highly significant (p<0.01) inhibitory activity when compared with standard. Samples 4, 6, 7, 8 and 10 also showed slightly (p<0.05) significant decrease in the activity when compared to standard (values are from table no. 6). So all the plant extracts under investigation are with potent inhibitor of DPPH.

d) Scavenging of Hydroxyl Radical (Deoxyribose Method):

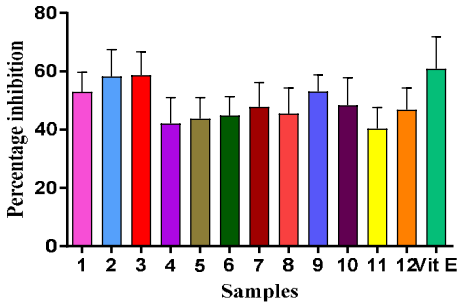
Scavenging of Hydroxyl Radical by Deoxyribose Methodwas carried out on the poly herbal extract where the different combinations of the extracts (mentioned in table no.1) were compared with the standard (Vitamin E) to find the maximum inhibitory activity of the samples. The results are mentioned in the form of a graph (mentioned in graph no.4).

Table No.3 Effect of different ratios of poly herbal extract on Hydroxyl radical percentage inhibition

Sample No.	Conc. 10µg	Conc. 50µg	Conc. 100µg	Conc. 200µg	Conc. 400µg	Conc. 800µg	Conc. 1000µg	Mean ± S.E.M	P -value
1	33.57	32.45	46.49	45.56	62.51	75.43	74.23	52.89±6.79	p< 0.01
2	32.83	34.45	43.34	50.43	62.52	85.33	95.12	58.15±9.31	p< 0.01
3	37.32	38.49	42.76	53.64	66.22	83.47	88.67	58.65±8.03	p< 0.01
4	22.58	22.55	22.49	34.43	47.51	61.45	83.59	42.09±8.90	NS
5	25.38	21.49	35.66	42.61	44.35	64.43	72.63	43.79±7.17	NS
6	24.63	31.64	32.51	43.39	45.42	62.48	73.07	44.73±6.62	NS
7	23.46	28.45	28.54	52.46	51.34	73.41	77.34	47.86±8.31	p< 0.05
8	24.67	27.34	28.75	32.66	55.04	65.46	84.34	45.47±8.73	NS
9	31.09	33.44	55.62	54.16	64.67	64.89	67.64	53.07±5.67	p< 0.01
10	18.52	25.22	36.84	43.67	55.96	66.77	91.25	48.32±9.53	p< 0.05
11	33.13	26.76	32.39	34.75	31.35	41.64	82.66	40.38±7.24	NS
12	24.67	26.73	33.67	42.64	59.32	68.21	72.47	46.82±7.49	p< 0.05
Vitamin E	18.92	21.63	66.33	71.32	72.35	84.11	91.60	60.89±10.97	

All the values are expressed as Mean±S.E.M. analysed by student t test & p<0.05, p<0.01, is considered slightly significant, very significant respectively when compared to standard.

Graph No.4 Histogram showing the effect of the Poly herbal extract on Hydroxyl Radical Activity



Each value represents Mean ± SEM; n =13 observation

p < 0.01: p < 0.05: NS = Non significant compared to standard.

Samples 4, 5, 6, 8, 11 showed no significant change in the percent hydroxyl radical inhibition when compared to standard. Samples 7, 10, 12 showed slightly significant (p < 0.05) decrease in the activity when compared to standard. Samples 1, 2, 3 and 9 showed highly significant (p<0.01), potent nitric oxide inhibitory activity than other samples with respect to standard (value are from table no.7).

Doxorubicin (Dox) treated groups:

Table No. 8 shows the 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 CPK, LDH, AST, ALT, and TP. On treatment with the poly herbal extract for 2 weeks a significant decrease in the levels of CPK, LDH, AST, ALT, and TP was observed in Group III and Group IV as compared to disease control (Group II) (Table No. 8). These results of the serum myocyte markers and biochemical parameters presented in the

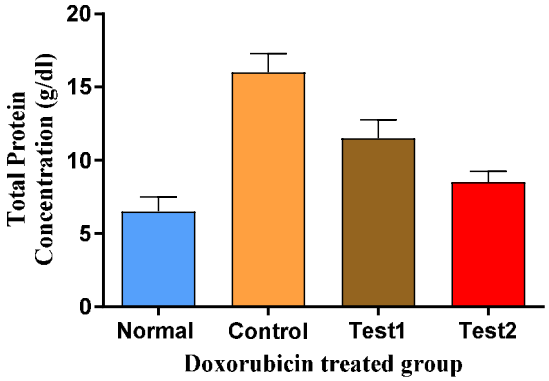
form of graph (mentioned in the graph no. 5, 6, 7, 8, 9)data clearly support that the poly herbal extract for cardioprotective activity.

Table No.4 Effect of the poly herbal extract against Doxorubicin on serum myocytes marker and biochemical parameters.

S.No	Treatment groups	Creatine Phospho Kinase (CPK)-IU/L	Lactate Dehydrogenase (LDH)-IU/L	SGOT (AST)-IU/L	SGPT (ALT)-IU/L	Total Protein (TP)-g/dl
1.	Normal	189±8.60	231±8.42	86±4.66	38±3.54	7.5±0.99
2.	Disease Control	368±7.0	458±9.08	219±8.11	112±5.56	26±1.29
3.	Polyherbal extract (200mg/kg)	238±5.15**	324±9.68**	163±6.35**	74±3.82**	21.5±1.25*
4.	Polyherbal extract (400mg/kg)	186±6.63***	281±8.06***	98±5.97***	33±3.36***	8.3±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.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 $p<0.05$. (Values are from table no. 8).

5.7. Histopathological Studies:

The Histopathological study of the heart sections also supported the above results. The heart tissue sections were fixed in 10% formalin. The specimens were processed by standard procedure and embedded in paraffin wax. The blocks were sectioned from the ventricular portion and stained according to the hematoxylin and eosin method and were examined by light microscopy under magnification (10X). Normal group (Dox) mentioned in (fig no. 15, 19) showed intact architecture of the myocardial fibres. Sharp demarcation between the myocardial fibres was seen. However, the disease control group treated with the Doxorubicin mentioned in (fig no. 16, 20) showed thrombus formation, loss of myocardial fibres, vacuolization of the cytoplasm, degeneration of myocardial tissue and fragmentation of the nuclei, were observed. In the Treatment groups of Dox, which are treated with poly herbal extract 200mg/kg b.w. mentioned in (fig no. 17,21) and 400mg/kg b.w. mentioned in (fig no. 18,22) showing decreased thrombus formation, less extensive vacuolization of the cytoplasm and no myocardial fibres loss.

5.7.1. Micro photograph of Heart Section after Dox Induced Cardiotoxicity against treated Poly herbal extract magnification under (10X).

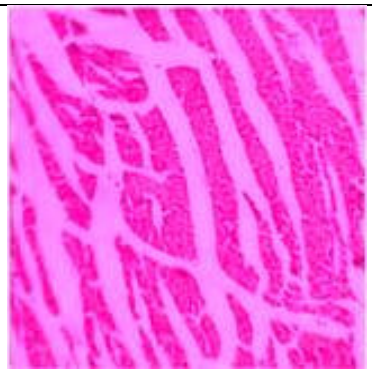


Fig.No.6. Normal

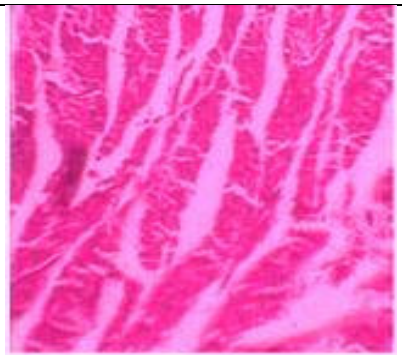


Fig.No.7. Disease Control (Dox)

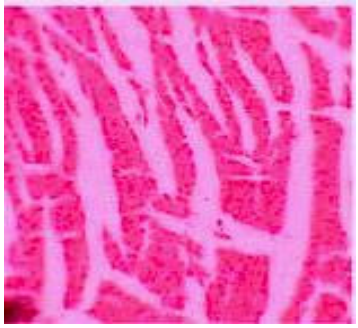


Fig.No.8 Polyherbal Extract (200mg/kg)

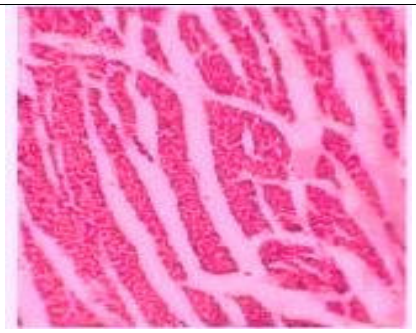


Fig.No.9 Polyherbal Extract (400mg/kg)

6. DISCUSSIONS

Tinospora cordifolia, *Boerhavia diffusa*, and *Glycyrrhiza glabra,* In the present study, Poly herbal extract which includes dried leaves of *Tinospora cordifolia*, dried leaves of *Boerhavia diffusa*, dried leaves of *Glycyrrhiza glabra.* Individual plant extracts were obtained. The dried leaves of *Tinospor*a *cordifolia* were extracted with 50% ethanol and its percentage yield was obtained as 18%. Dried leaves of *Boerhavia diffusa* were extracted with ethylacetate and its percentage yield was obtained as 8%. Dried leaves of *Glycyrrhiza glabra* were extracted with 50% ethanol and its percentage yield was found to be 8.34%.The phyto chemical screening of the phytoconstituents was performed in all the plant extracts. It showed the presence of alkaloids, carbohydrates, flavonoids, proteins, aminoacids, 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 combinations of plant extracts mentioned (table no.1). The results of the methods has shown that combination 9 which includes the plant extract in ratio of (125:50:60:80) has shown maximum inhibition activity.Acute toxicity as per OECD guideline 425 was carried out and no mortality was found. The maximum dose for pharmacological studies was taken as 400mg/kg b.w.p.o. and 200mg/kg b.w.p.o. ($1/5^{th}$ and $1/10^{th}$ of maximum tested dose, 2000mg/kg b.w.).In the present study, Doxorubicin was used to induce cardiotoxicity in the rats. In the first model, Dox was used to induce the cardiotoxicity by administration of 2.5mg/kg b.w.i.p. with six equal injections during two weeks, making it a cumulative dose of 15mg/kgb.w. Animals are treated with poly herbal extract (200, 400mg/kg) through oral route of administration in both the models. In this model, Dox treated groups, the poly herbal extract (200, 400mg/kg) were administered for a period of 15 days. Enzyme biomarkers such as Creatine Phosphokinase(CPK), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase(AST), Alanine aminotransferase(ALT) and Total Protein were monitored. The Dox treated group showed significant decrease in the level upon treatment with poly herbal extract (200, 400mg/kg) Creatine Phospho kinase(CPK)($p<0.01$, $p<0.001$), Lactate dehydrogenase (LDH)($p<0.01$, $p<0.001$), Aspartate amino

transferase(AST)($p<0.01$, $p<0.001$), Alanine amino transferase (ALT)($p<0.01$, $p<0.001$), Total protein ($p<0.05$, $p<0.01$) respectively The mechanism of cardiotoxicity induced by Dox is not clearly understood from the present study. Largely the evidences indicate towards formation of oxygen free radicals which can damage cells by lipid peroxidation. Cardiac tissue damage may be due to increased oxidative stress and depletion of antioxidants as reported earlier[32].The histopathological examination, of the Dox treated group, demonstrated thrombus formation, loss of myocardial fibres, and fragmentation of the nuclei. Pretreatment with poly herbal extract (200, 400mg/kg) has a potential to inhibit the cardiotoxic effects induced by Dox and ISO and possess significant results.

7. CONCLUSION

The Phytochemical screening of the phytoconstituents was performed in all the plant extracts. 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 combinations of plant extracts mentioned (table no.1). The results of the methods has shown that combination 9 which includes the plant extract in ratio of (125:50:60:80) has shown maximum inhibition activity. The Pharmacological screening included evaluation of Cardioprotective activity using Dox induced model in rats. Increased levels of serum marker enzymes such as CPK, 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 antioxidant property. Further work is necessary to isolate the active constituents responsible for the cardioprotective activity and further studies on larger animal models and on humans is warranted to draw final conclusions.

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