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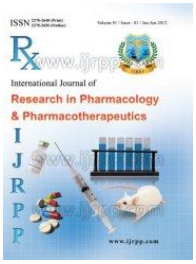

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Research article ***Open Access***

Evaluation of Cardioprotective activity of Poly herbal extract on Dox and ISO induced Cardiac toxicity in Albino rats

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ABSTRACT

The activity of the poly herbal extract was studied by inducing cardiotoxicity with cumulative dose administration of Doxorubicin (15mg/kg i.p.for 2 weeks). The poly herbal extract was administered in doses of 200, 400 mg/kg b.w. for 15 days. In another model Isoproterenol induced myocardial infarction (necrosis) in rats. Animals were treated with poly herbal extract in doses of 200, 400 mg/kg b.w. for 30 days. On 31st, 32nd days the animals were treated with Isoproterenol (85mg/kg b.w. s.c.) to induce myocardial infarction. The general observations were serum myocytes biomarkers like LDH, CPK, biochemical parameters such as AST, ALT, total protein, and histopathology were done. It was found that in the invitro antioxidant activity the poly herbal extract of 9th combination which include a ratio as (125:20:60:80) has shown more percentage inhibition. The administration of Dox and ISO induced cardiotoxicity associated with increased level of biomarkers. Pretreatment with the polyherbal extract significantly protected myocardium from the toxic effects of Dox and ISO by reducing elevated levels of biomarker enzymes like LDH, CPK, and biochemical parameters such as AST, ALT, total protein were back to normal.

Keywords: Cardiotoxicity, Doxorubicin, Isoproterenol, Polyherbal extract.

INTRODUCTION

Medicinal herbs and plant extracts are now generally considered as effective medicines to be respected, appreciated and they play a major role in modern pharmacy. World Health Organization estimated that about 80% of the world's population relies on herbs for their primary healthcare needs. There has been an explosion of scientific information concerning plants, crude plant extracts and various substances from plants as medical agents during the last 20 to 30 years. Although, herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored. Numbers of plants are claimed to have medicinal uses and many researches are going on in this view. Cardiotoxicity occurs during therapy with several cytotoxic drugs and may be the dose limiting factor in cancer treatment and hence tumour response. Furthermore, cardiotoxicity can also be responsible for long term side effects and may cause severe morbidity in surviving cancer patients, which may be relevant especially in pediatric oncology.

Cardiotoxicity from cytotoxic treatment is known to have a high prevalence. Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. In literature different mechanisms of chemotherapy induced cardiotoxicity are postulated including cellular damage due to the formation of free oxygen radicals and the induction of immunogenic reactions with the presence of antigen presenting cells in the heart. [2] The present study plans to systematically evaluate combination of the plant i.e. poly herbal extract (which includes dried leaves of *Camellia sinesis,* dried leaves of *Mangifera indica*, dried leaves of *Ocimum sanctum*, dried fruit peels of *Punica granatum)* having various medicinal properties, which are widely used in Ayurveda. In this study, the detailed account of the individual plant along with its phytochemical screening, including the study of polyherbal extract against Doxorubicin (Dox) and Isoproterenol (ISO) induced cardiac toxicity in rats, serum myocytes biomarkers, biochemical parameters and histopathological evidences were used to support the study. [3-5]

MATERIAL AND METHODS

Collection and authentication of the plants

The leaves of plant *Camellia sinesis,* leaves of *Mangifera indica*, leaves of plant *Ocimum sanctum*, peel of the fruit of plant *Punica granatum* were collected and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, SV University, Tirupati.

EXTRACTION OF THE PLANTS

Camellia sinensis

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.

Mangifera indica

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.

Ocimum sanctum

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.

Punica grantum

The Peels of the fruits were collected and shade dried and powdered by mechanical grinder. About 250 gm of powder was extracted by methanol using Soxhlet apparatus for 3 days and the mixture was subsequently filtered and concentrated at 50-60^{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. [6-8]

PHARMACOLOGICAL ASSESSMENT

Experimental animals

51 Healthy adult Wistar rats of either sex, weighing about 150-200 gm were used in the experiments. Animal were housed in polypropylene cages maintained under standard condition (12 hrs light/dark; 25±3^{0}C) and had free access to standard rat feed (Hindustan Lever Ltd. India) and water. All the animals were acclimatized to laboratory condition for a week before commencement of the experiment. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) [9-11].

Chemicals

- Isoproterenol(ISO),
- Doxorubicin,
- Ethanol (AR grade),
- Methanol (AR grade).

Extract Used

The poly herbal extract was insoluble in water. Hence it was dissolved by using DMSO 20% (v/v) (Dimethyl sulfoxide) before administration to rats. Calculated quantity of extract was given to each animal in corresponding group, daily once by oral gavage.

Acute toxicity study

The acute oral toxicity study was carried out as per the guidelines set by Organization for economic co-operation and development (OECD) (Guidelines 425) received from Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animals (female rats) were fasted overnight prior to the experiment. The group was treated after fasting overnight with oral dose of 2000mg/kg body weight with the poly herbal extract. The extract was given to the animals and was observed continuously for 4-6 hrs for general, behavioral, neurological, autonomic profiles and finally death within 24 hrs. There was no mortality and no sign of toxicity. The maximum tolerated dose was taken as 2000 mg/kg body weight.

Selection of dose

Two dosed levels were chosen of the poly herbal extract in such a way that, one dose was approximately $1/5^{th}$ (400mg/kg b.w.) and $1/10^{th}$ (200mg/kg b.w.) of maximum tolerated dose.

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 described by Swamy et.al. . 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.

Cardioprotective activity of Poly herbal Extract in Isoproterenol (ISO) induced myocardial necrosis

The cardioprotective activity in Isoproterenol induced myocardial necrosis was carried out as per the method described by Sharma et. al. [1]. 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 Isoproterenol (ISO) at a dose of (85mg/kg s.c.) induced myocardial necrosis
- Group III: Animals were treated with the poly herbal extract (200mg/kg b.w. p.o.)+ ISO (85mg/kg b.w. s.c.)
- Group IV: Animals were treated with the poly herbal extract (400mg/kg b.w. p.o.)+ISO (85mg/kg b.w. s.c.)
- Group III and Group IV received 200mg/kg b.w. and 400mg/kg b.w. of poly herbal extract were administered orally to each animal orally for 30 days and ISO (85mg/kg) administered subcutaneously twice with an interval of 24hrs (i.e. 31^{st} and 32^{nd} day) at the end of the animal study. The animals were sacrificed 24hr after administration of ISO under chloroform anesthesia, blood sample were collected through retro orbital sinus, hearts were excised and immediately processed for biochemical and histopathological studies.

HISTOPATHOLOGICAL EXAMINATION

For the microscopic evaluation, Hearts were fixed in 10% formalin solution. Following dehydration in ascending series of ethanol (70, 80, 96,100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5µm were stained with hematoxylin-eosin. A minimum of 10 fields for each

heart slide was examined for loss of myocardial fibres and thrombus formation. Tissue slices were photographed using microscope at 10X magnification.

STATISTICAL ANALYSIS

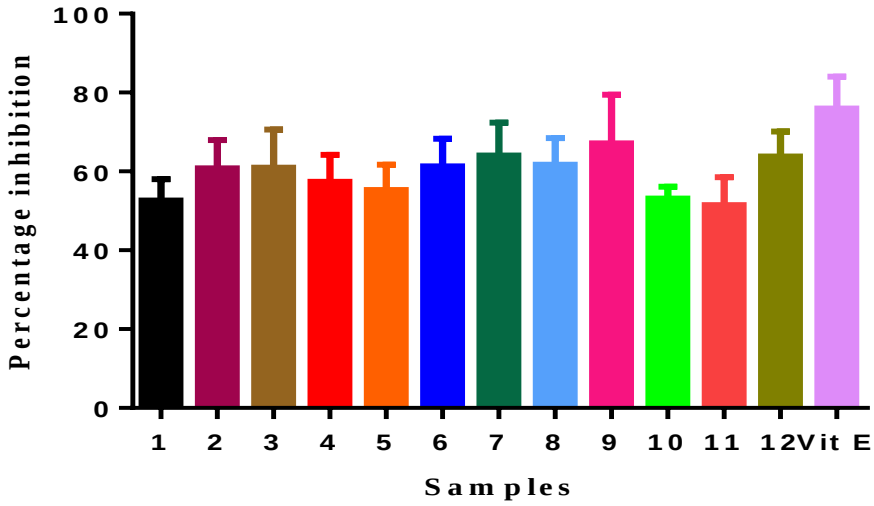
The data were expressed as Mean±S.E.M. The differences were compared using one way ANOVA followed by Dunnett's test using Graph Pad Prism software (version 6). The results were considered significant when p was <0.05.

RESULTS

Pharmacological Investigations

Acute Oral Toxicity Studies

- The ethanolic extract (50%) of *Camellia sinensis* & *Ocimum sanctum*, ethyl acetate extract of *Mangifera indica*, methanolic extract of *Punica granatum* and the poly herbal extract with a combination no.9 (mentioned in 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.



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.

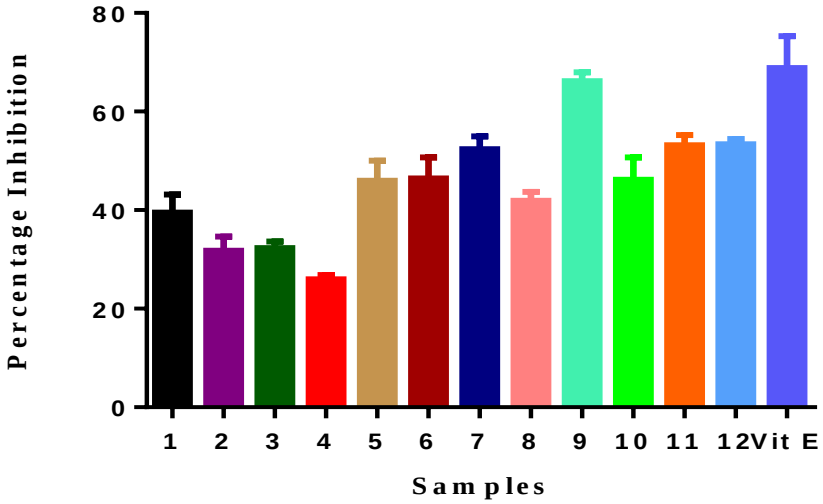
Nitric Oxide scavenging activity

Nitric oxide scavenging activity was carried out on the poly herbal extract where the different combinations of the extracts 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	36.00	30.23	40.17	55.55	49.34	34.56	30.67	39.50±3.(	NS
2	32.22	35.66	43.55	29.09	33.56	30.04	18.45	28.21±4.9	NS
3	26.77	29.99	33.45	34.12	37.56	31.72	32.64	32.32±1.2	NS
4	24.14	28.99	29.54	24.37	25.77	23.67	25.23	25.96±0.8	NS
5	48.55	53.56	56.60	57.45	36.77	38.37	30.34	46.01±4.(	p< 0.05
6	51.35	62.34	56.78	43.00	38.76	43.56	29.34	46.45±4.2	p< 0.05
7	42.34	44.07	54.55	58.56	59.75	51.59	55.78	52.38±2.5	p< 0.05
8	32.34	43.35	46.56	44.65	42.56	43.56	40.45	41.92±1.7	p< 0.05
9	60.34	65.41	70.45	72.49	68.78	65.45	60.45	66.20±1.7	p< 0.01
10	37.56	48.56	65.68	49.56	32.56	34.56	54.67	46.16±4.5	p< 0.05
11	44.45	47.67	57.81	59.06	54.65	56.57	51.78	53.14±2.(	p< 0.05
12	53.45	55.79	53.56	54.00	56.78	52.09	47.71	53.34±1.1	p< 0.05
Vitamin-E	46.00	54.68	58.04	70.70	76.07	79.53	96.66	68.81±6.5	

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.1).

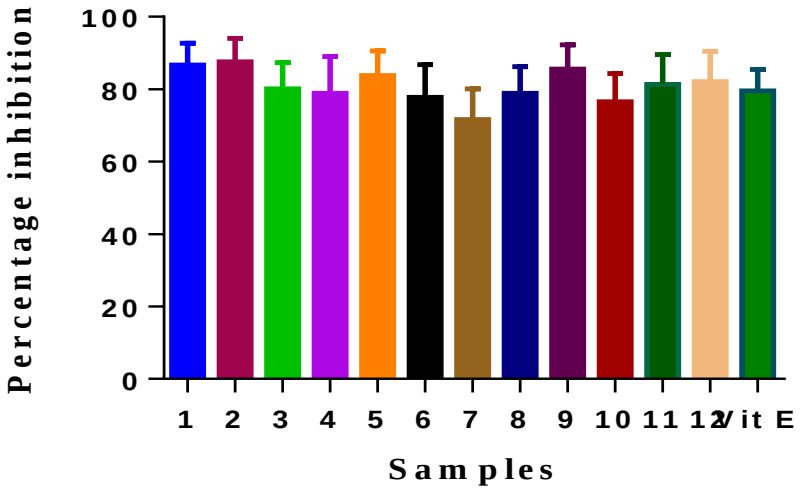
Free Radical scavenging by DPPH method

Free Radical scavenging by DPPH method was carried out on the poly herbal extract where the different combinations of the extracts 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.2 Effect of different ratios of poly herbal extracts on DPPH 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	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. 2). So all the plant extracts under investigation are with potent inhibitor of DPPH.

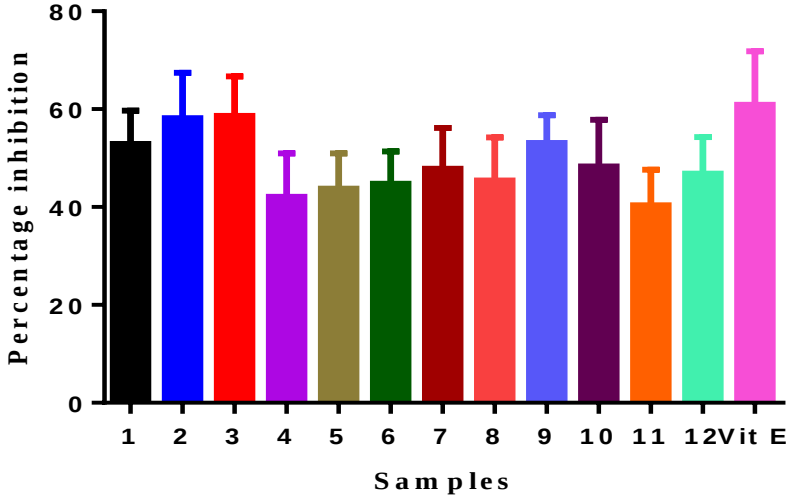
Scavenging of Hydroxyl Radical (Deoxyribose Method)

Scavenging of Hydroxyl Radical by Deoxyribose Method was carried out on the poly herbal extract where the different combinations of the extracts 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.3).

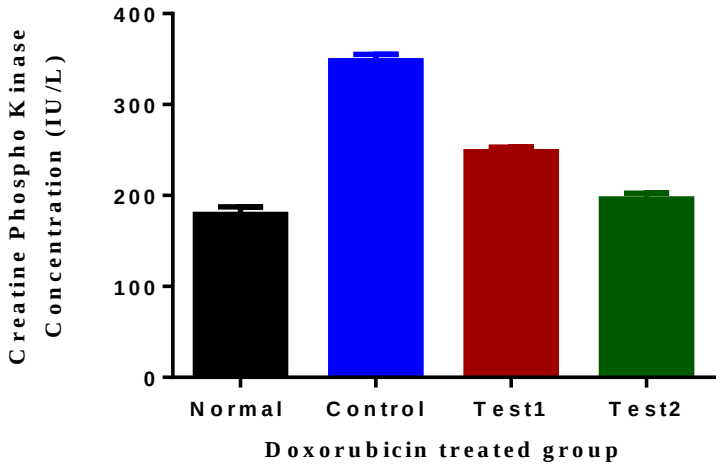
Doxorubicin (Dox) treated groups

Table No. 4 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.

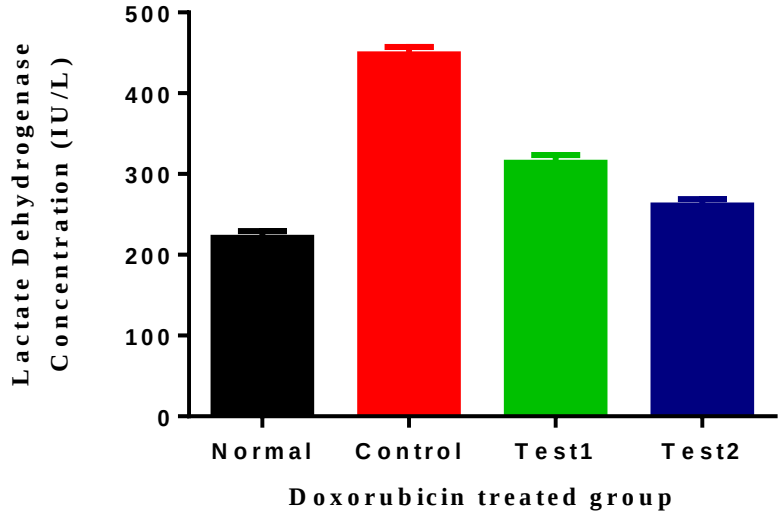
S.No	Treatment groups	Creatine Phospho Kinase (CPK)-IU/L	Lactate Dehydrogenase (LDH)-IU/L	SGOT (AST)-IU/L	SGPT (ALT)-IU/L	Total Protein (TP)-g/dl
1.	Normal	179±8.60	221±8.42	76±4.66	28±3.54	6.5±0.99
2.	Disease Control	348±7.0	448±9.08	209±8.11	102±5.56	16±1.29
3.	Polyherbal extract (200mg/kg)	248±5.15**	314±9.68**	153±6.35**	71±3.82**	11.5±1.25**
4.	Polyherbal extract (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.



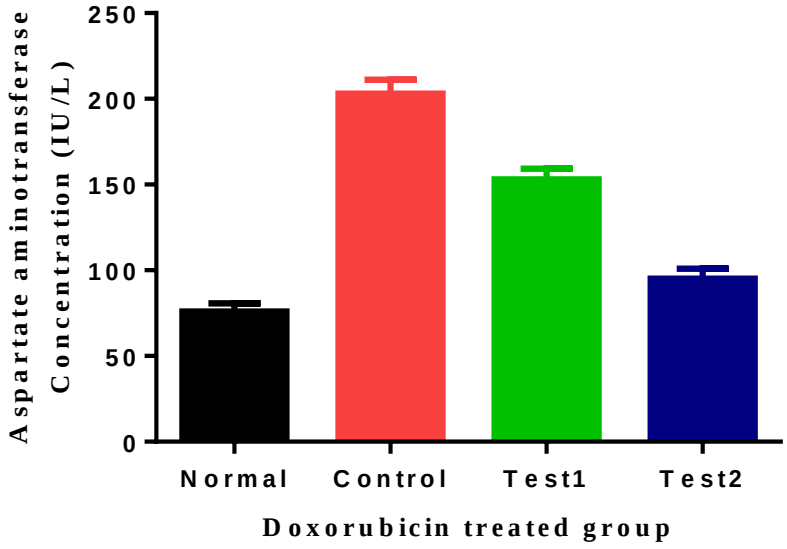
Graph No.5 Histogram showing the effect of Poly herbal extract on Serum Creatine Phospho Kinase Levels

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. 4).



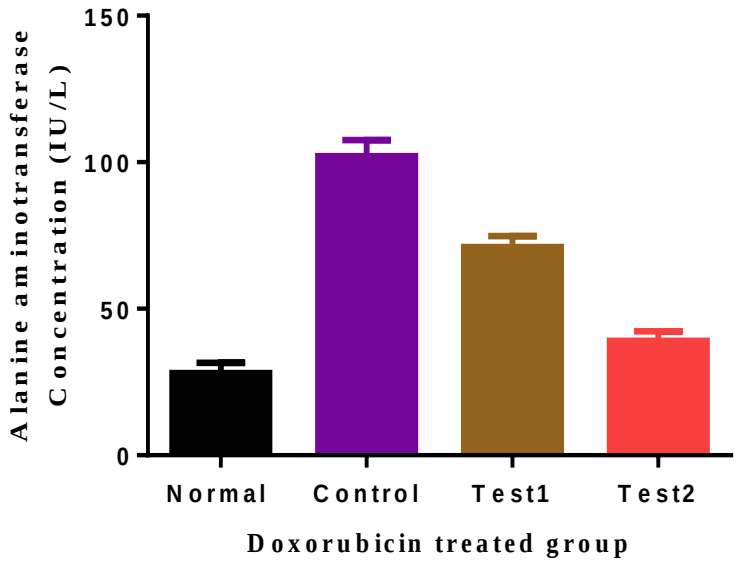
Graph No.6 Histogram showing the effect of Poly herbal extract on Serum Lactate Dehydrogenase Levels

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 $p<0.05$. (Values are from table no. 4).



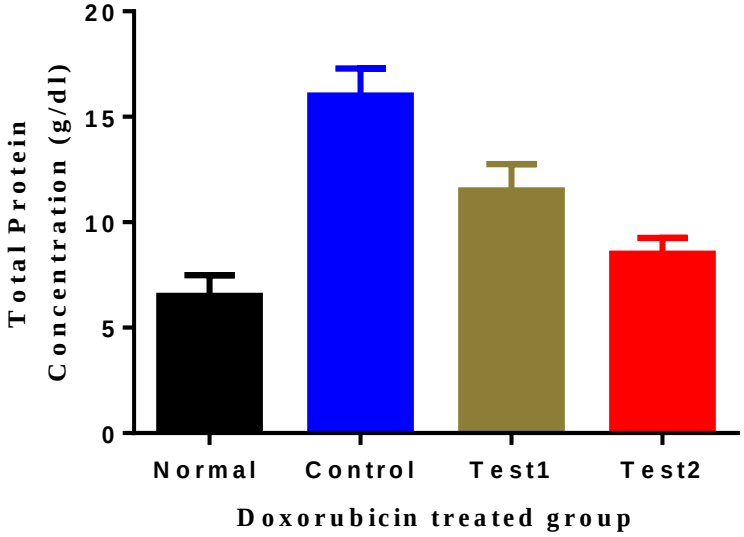
Graph No.7 Histogram showing the effect of Poly herbal extract on Serum Aspartate Aminotransferase Levels

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 $p<0.05$. (Values are from table no. 4).



Graph No.8 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 $p<0.05$. (Values are from table no. 4).



Graph No.9 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. 4).

Isoproterenol (ISO) treated groups

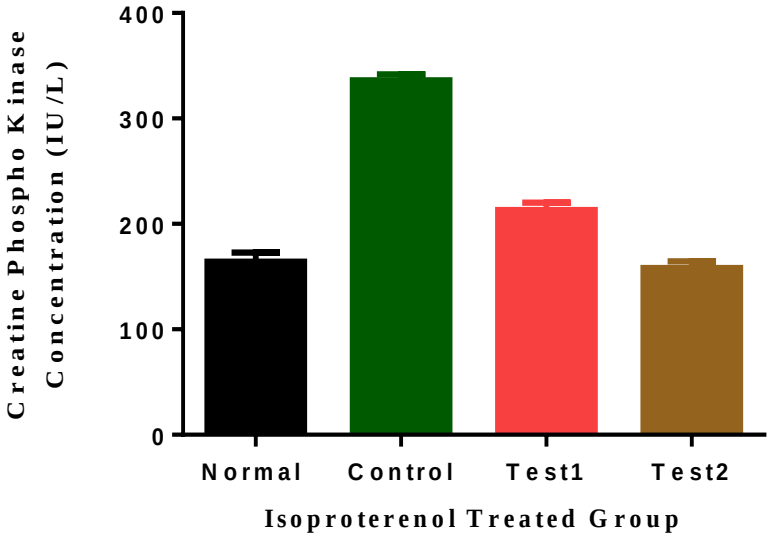
Table No.5 shows the details of the levels of the serum myocyte markers and biochemical parameters in the normal, Disease control and treated animals. Isoproterenol (85mg/kg b.w. s.c.) was administered

on the last days of animal study i.e. 31st and 32nd days 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 30 days 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. 5). These results of the serum myocyte markers and biochemical parameters presented in the form of graph (mentioned in the graph no. 10, 11, 12, 13, 14)data clearly support that the poly herbal extract for cardioprotective activity.

Table No.5 Effect of the polyherbal extract against ISO on serum myocytes marker and biochemical parameters.

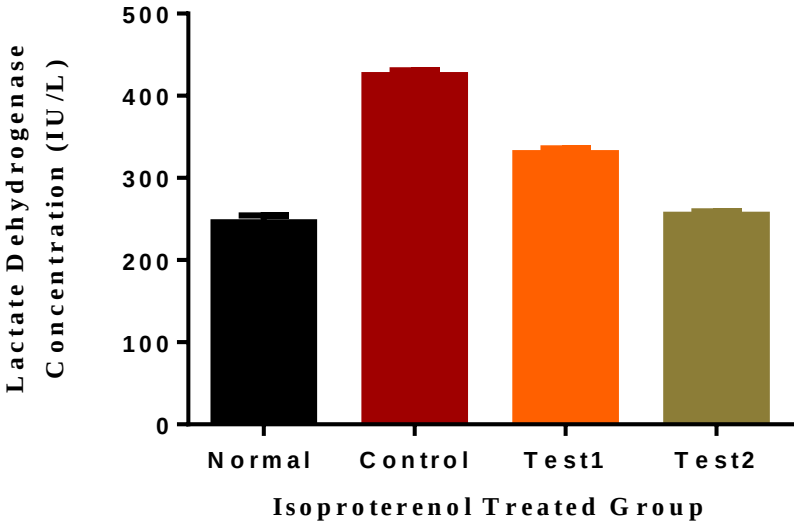
S.No	Treatment groups	Creatine Phospho Kinase (CK)-IU/L	Lactate Dehydrogenase (LDH)-IU/L	SGOT (AST)-IU/L	SGPT (ALT)-IU/L	Total Protein (TP)-g/dl
1.	Normal	164±8.74	246±8.54	68±5.15	40±3.98	5.0±0.68
2.	Disease Control	336±5.88	425±6.24	190±5.74	89±3.29	11.0±0.96
3.	Polyherbal extract (200mg/kg)	234±7.26**	330±5.91***	113±6.11**	54±4.03**	8.5±0.42*
4.	Polyherbal extract (400mg/kg)	158±6.60***	255±4.43***	83±5.71***	47±5.18**	7.0±0.57**

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 with control group.



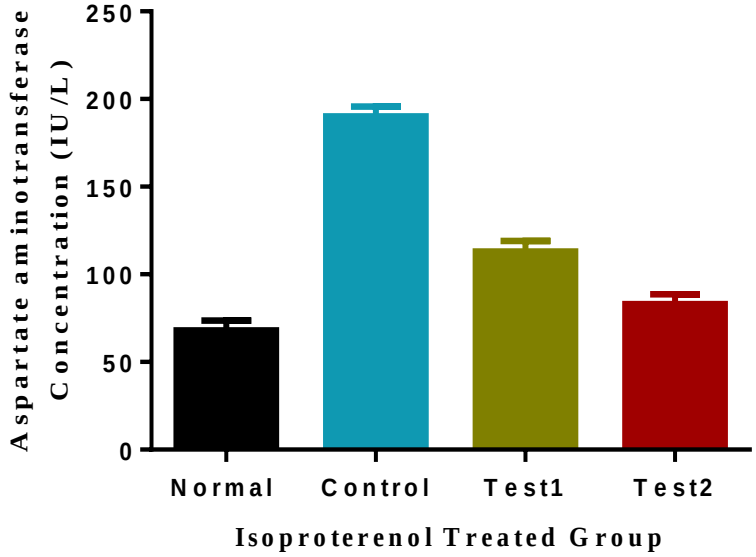
Graph No.10 Histogram showing the effect of Poly herbal extract on Serum Creatine Kinase Levels

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. 5).



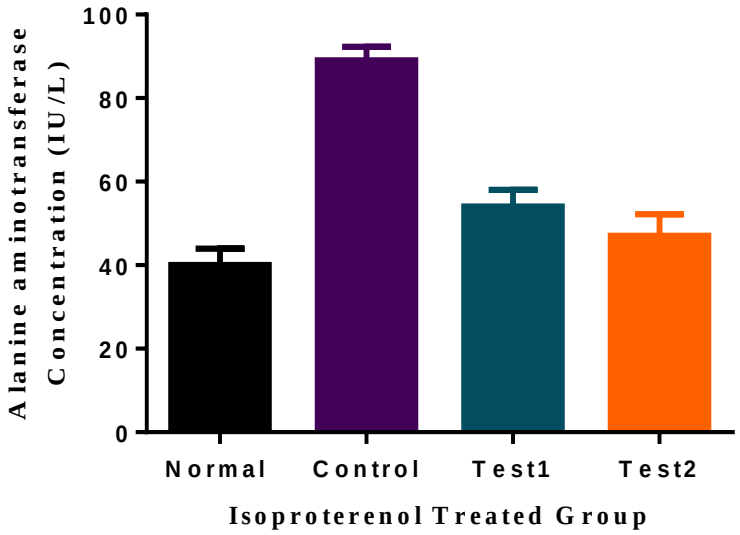
Graph No.11 Histogram showing the effect of Poly herbal extract on Serum Lactate Dehydrogenase Levels

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 $p<0.05$. (Values are from table no. 5).



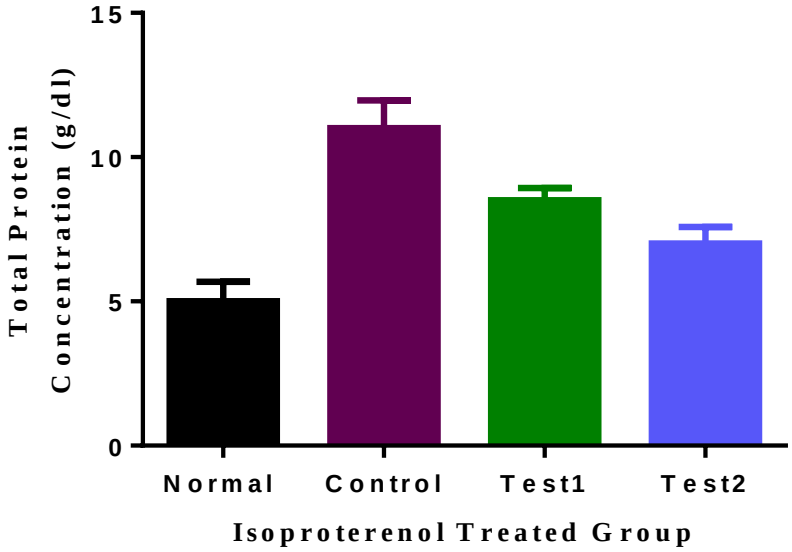
Graph No.12 Histogram showing the effect of Poly herbal extract on Serum Aspartate Amino transferase Levels

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. 5).



Graph No.13 Histogram showing the effect of Poly herbal extract on Serum Alanine Amino transferase 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. 5).



Graph No.14 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. 5).

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 and ISO) 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 and Isoproterenol 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 and ISO 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.

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

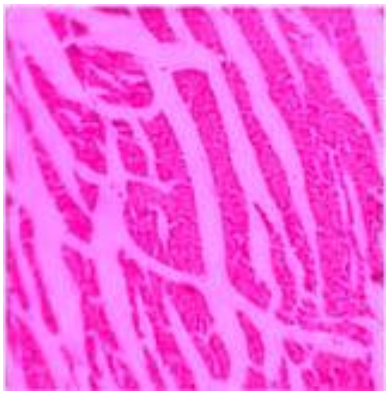


Fig.No.15. Normal

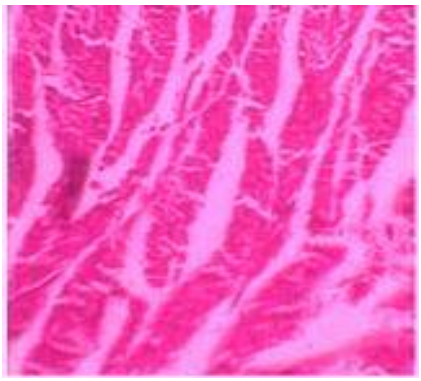


Fig.No.16. Disease Control (Dox)

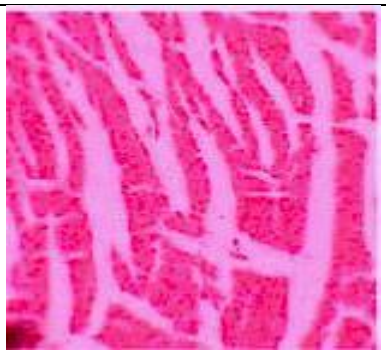


Fig.No.17 Polyherbal Extract (200mg/kg)

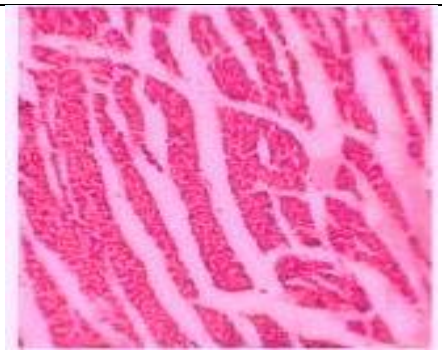


Fig.No.18 Polyherbal Extract (400mg/kg)

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

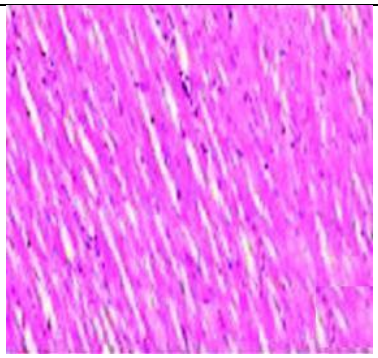


Fig.No.19. Normal

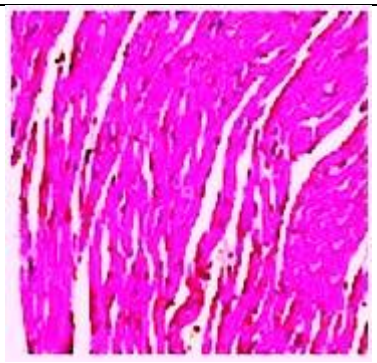


Fig.No.20. Disease Control (ISO)

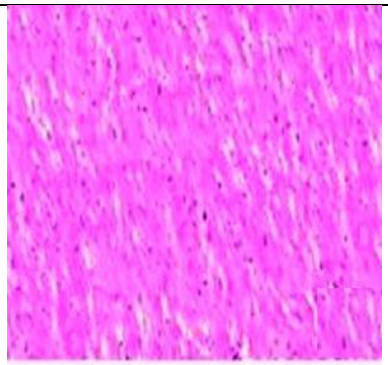


Fig.No.21. Poly herbal Extract (200mg/kg)

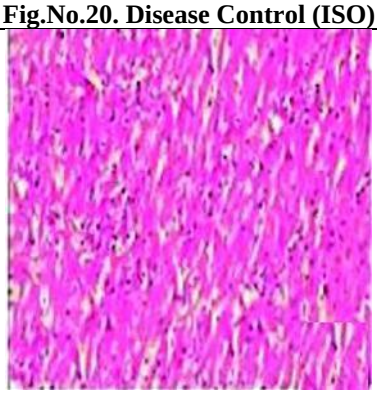


Fig.No.22. Poly herbal Extract (400mg/kg)

DISCUSSION

In the present study, Poly herbal extract which includes dried leaves of *Camellia sinensis*, dried leaves of *Mangifera indica*, dried leaves of *Ocimum sanctum*, fruit peel of *Punica granatum*. Individual plant extracts were obtained. The dried leaves of *Camellia sinensis* were extracted with 50% ethanol and its percentage yield was obtained as 18%. Dried leaves of *Mangifera indica* were extracted with ethylacetate and its percentage yield was obtained as 8%. Dried leaves of *Ocimum sanctum* were extracted with 50% ethanol and its percentage yield was found to be 8.34%. Dried fruit peel of *Punica granatum* was extracted with methanol and its percentage yield was obtained as 24%. 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. 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 and Isoproterenol were 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. In the second model, ISO was used to induce the myocardial infarction upon administration of 85mg/kg s.c. twice during the last days of experimental activity with a 24 hour interval between the two doses. Animals are treated with poly herbal extract (200, 400mg/kg) through oral route of administration in both the models. In the first model, Dox treated groups, the poly herbal extract (200, 400mg/kg) were administered for a period of 15 days.

In the second model, ISO treated groups, poly herbal extract (200, 400mg/kg) were administered for a period of 30 days. Enzyme biomarkers such as Creatine Phospho kinase(CPK), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase(AST), Alanine aminotransferase(ALT) and Total Protein were monitored. In the first model, the Dox treated group, the enzyme markers 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.

In the second model, the ISO treated group, the enzyme markers shown 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)($p<0.001$, $p<0.001$), Aspartate amino transferase(AST)($p<0.01$, $p<0.001$), Alanine amino transferase (ALT)($p<0.05$, $p<0.01$), Total protein ($p<0.05$, $p<0.01$) respectively. The mechanism of cardiotoxicity induced by Dox and ISO 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 and ISO 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.

CONCLUSION

The Phytochemical 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. The Pharmacological screening included evaluation of Cardioprotective activity using Dox and ISO 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 and 30 days in ISO 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. 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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