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In Vitro Antioxidant Activity and Hepatoprotective Potential of *Ceropegia spiralis* Against Paracetamol Induced liver injury

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ABSTRACT

Ceropegia spiralis is a usually used remedial herb in herbal remedies to delight diverse diseases. The plan of the current investigation is to assess the *in vitro* free radical scavenging activity and hepatoprotective activity of *Ceropegia spiralis* against paracetamol induced liver damage in preventive and curative models. The *Ceropegia spiralis* was evaluated for in vitro antioxidant activity by 2, 2-diphenyl-1-picryl hydrazyl (DPPH), hydroxyl and superoxide radical scavenging activity and inhibition of lipid peroxide and ascorbic acid was used as a standard. In two separate studies, the 100, 200 and 400 mg/kg body weight of *Ceropegia spiralis* extract, and 100 mg/kg body weight of Silymarin in both studies were given orally. The hepatic damage was done by oral administration of 2 g/kg body weight of paracetamol. The IC_{50} values of *Ceropegia spiralis* in the superoxide, 2, 2-diphenyl-1-picryl hydrazyl (DPPH), hydroxyl radical scavenging activity and inhibition of lipid peroxidation was found to be 365.64, 381.13, 461.32 and 469.39 µg/ml correspondingly. The hepatic damage in rats induced by paracetamol as evidence by elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels and decreased serum total proteins. The administration of *Ceropegia spiralis* and Silymarin in both preventive and curative models decreases the toxic effect of paracetamol on the above selected serum parameters.

INTRODUCTION

The liver has good impact in the regulation of homeostasis in the body. It maintains different biochemical pathways which are involved in the regulation of body growth, protect from certain disorders, provide nutritional support, production of energy and maintain reproductive consequences (Rajkiran *et al.*, 2015; Nelson.1990).

The liver is the first target organ of xenobiotics which will alter the normal physiological functions and produce harmful effect. The liver protects the body from such type of unwanted toxic effects. The two main hepatotoxic events leads to mortality are Jaundice and hepatitis (Vermeulen *et al*., 1992).

Free radicals are the important inducing agents for the lipid peroxidations, cellular aging and diseases causing (Halliwell and Gutteridge, 1984). The Antioxidants are the substances which scavenge the free radicals like reactive oxygen species (ROS) and maintain the cytoskeleton of tissues (Barros *et al*., 2007; Sreenivasan *et al*., 2010). Nowadays the researchers are mainly focusing on the evaluation of various hepatoprotective agents for their paramount importance in the maintenance of the various metabolic activities and major protective site from different hazardous. A variety of literatures are indicating of a constant research on herbal hepatoprotective agents associated with antioxidant principle (Nithianantham *et al*., 2011; Soundararajan *et al.*, 2012). *Ceropegia spiralis (Famaly: Apocynaceae)* is a small perennial herb, 20-30 cm tall, with weak, erect stem. Tubers are 1-2 cm, spherical. Traditionally, this plant is used in the treatment of fever, indigestion and liver diseases (Yadav *et al*., 2006). The present investigation is to enumerate the hepatoprotective activity of methanolic extracts of *Ceropegia spiralis*, in higher dose of paracetamol induced hepatotoxicity.

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MATERIALS AND METHODS

Drug and chemicals

2-deoxy-D-ribose, Nitrobluetetrazolium (NBT) and 2, 2-diphenyl-1-picrylhydrazil (DPPH) were procured from SISCO Research laboratories Pvt Ltd, Mumbai and Sigma Chemical Co. (St. Louis, MO, USA) respectively. Paracetamol and Silymarin were obtained as a gift sample from Sri Krishna Pharmaceuticals, Mumbai, India and Micro Labs, Bangalore, India. The liver diagnostic kits like Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Total bilirubin (TBR) and Total protein (TP) were procured from Agappe Diagnostics, Kerala, India. All additional chemicals and reagents used were of analytical grade.

Preparation of methanolic extract

The whole plant of *Ceropegia spiralis* were freshly collected and were subjected to shade dried. The dried whole plants were milled to coarse powder. One kg of the coarse powder and one liter of methanol (95%) were taken in a round bottomed flask for the maceration process up to 24 hours at room temperature. The liquid extract was collected from soxhlet apparatus after successive extraction with 3 liter of methanol (95%). The obtained liquid extract was subjected to rotary evaporator under reduced pressure until a soft mass obtained. The extracts were properly air dried to remove all traces of the solvent (Meharunnisha and Raj Kiran, 2016).

In vitro antioxidant activity

In present study inhibition of lipid peroxidation, Hydroxyl radical, Superoxide radical and DPPH radical scavenging activity were determined by Ohkawa *et al*., 1979, Elizabeth and Rao., 1990, Robak and Gryglewski *et al*., 1988 and Braca *et al*., 2003 respectively.

Animals

The male Albino Wistar rats (180 ± 200 g) were selected for experimental work, which are procured from the Mahaveer Enterprises, Hyderabad, India. They were maintained in a constant environment temperature of (23 ± 2) ºC, humidity of 50 % and 12 h: 12 h of light and dark cycles, respectively. All these animals were fed with the marketed pellet diet (Rayon's Biotechnology Pvt Ltd, India). The animals were access to water *ad libitum*. The experiment was supervised under the Institutional Animal Ethics Committee and is approved by CPCSEA, Government of India (Regd. No. 516/PO/C/01/ CPCSEA).

In vivo Hepatoprotective Study (Eswar Kumar *et al*., 2013; Rajkiran *et al.*, 2015)

Preventive study

The male Albino Wistar rats were separated into six groups and each group has 6 rats.

Group A: Administered with 1% sodium CMC for 3 days.

Group B: Administered with 2 g/kg body weight of paracetamol for a period of 3 days.

Group C: Administered with 2 g/kg body weight of paracetamol and *Ceropegia spiralis* extract (100 mg/kg body weight) concurrently for 3 days.

Group D: Administered with 2 g/kg body weight of paracetamol and *Ceropegia spiralis* extract (200 mg/kg body weight) concurrently for 3 days.

Group E: Administered with 2 g/kg body weight of paracetamol and *Ceropegia spiralis* extract (400 mg/kg body weight) concurrently for 3 days.

Group F: Administered with 2 g/kg body weight of paracetamol and *Silymarin* (100 mg/kg body weight) concurrently for 3 days.

Curative Study (Shenoy *et al*., 2002)

Group A: Control rats treated with 1% sodium CMC for 10 days.

Group B: Treated with paracetamol (2 g/kg body weight) for first 3 days and then next seven days treated with 1% sodium CMC.

Group C: Treated with paracetamol (2 g/kg body weight) for first 3 days and then next seven days treated with *Ceropegia spiralis* extract (100 mg/kg body weight).

Group D: Treated with paracetamol (2 g/kg body weight) for first 3 days and then next seven days treated with *Ceropegia spiralis* extract (200 mg/kg body weight).

Group E: Treated with paracetamol (2 g/kg body weight) for first 3 days and then next seven days treated with *Ceropegia spiralis* extract (400 mg/kg body weight).

Group F: Treated with paracetamol (2 g/kg body weight) for first 3 days and then next seven days treated with *Silymarin* (100 mg/kg body weight).

All groups received paracetamol, *Ceropegia spiralis* and *Silymarin* orally. Silymarin was selected as standard agent for hepatoprotective activity. In preventive (0^{th} and 4^{th} day) and curative study (0^{th}, 4^{th} and 11^{th} day) blood samples were collected from rats retro-orbital plexus. The serum was isolated from blood by centrifugation at 3000 rpm for 15 min for assessment of serum biochemical parameters.

The selected serum parameters like ALT, AST, ALP, serum Total bilirubin and Total protein estimated by Semi-auto analyzer (Agappe). After completion of the both studies the animals were sacrificed for isolation of livers for histopathological study and estimation of liver weight and volume.

Calculation of Percentage Hepatoprotection

The following formula used for to calculate the percentage of liver protection produced by Ceropegia spiralis and Silymarin (Sintayehu *et al*., 2012).

$$H = \left(\frac{T - B}{T - C}\right) \times 100$$

Where T = Group B mean value of marker.
B = Group C, Group D, Group E and Group F Mean value of marker.
C = Group A mean value of marker.
H = Percentage of liver protection.

Statistical analysis

The data was indicated as Mean ± SEM produced from six animals. The Statistical analysis was done by using Prism-5 Graph Pad software. The one way analysis of variance (1 way ANOVA) with Bonferroni's multiple comparison test was used to compare the groups. The difference was less than 0.05 was considered to be statistically significant (P<0.05).

RESULTS

***In vitro* antioxidant activity**

Superoxide scavenging activity

The *Ceropegia spiralis* and standard ascorbic acid showed concentration dependent scavenging activity on superoxide radical at different concentrations (10-1000 µg).

The *C. spiralis* needed 365.64 µg for 50% scavenging of superoxide radicals; whereas standard ascorbic acid needed 156.50 µg (Table 1 & Histogram 1).

Lipid peroxidation inhibiting activity

The *Ceropegia spiralis* and standard ascorbic acid showed concentration dependent inhibition of lipid peroxidation at different concentrations (10-1000 µg). The *C. spiralis* needed 469.39 µg for 50% inhibition of lipid peroxidation; whereas standard ascorbic acid needed 125.69 µg (Table 1 & Histogram 1).

Hydroxyl radical scavenging activity

The *Ceropegia spiralis* and standard ascorbic acid showed concentration dependent scavenging activity on hydroxyl radical at different concentrations (10-1000 µg). The *C. spiralis* needed 461.32 µg for 50% scavenging of hydroxyl radicals; whereas standard ascorbic acid needed 237.49 µg (Table 1 & Histogram 1).

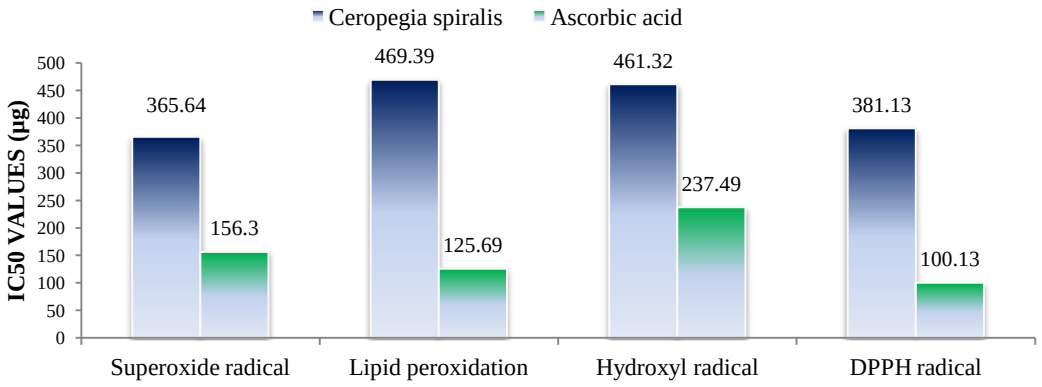
DPPH radical scavenging activity

The *Ceropegia spiralis* and standard ascorbic acid showed concentration dependent scavenging activity on DPPH radical at different concentrations (10-1000 µg). The *C. spiralis* needed 381.13 µg for 50% scavenging of DPPH radicals; whereas standard ascorbic acid needed 100.13 µg (Table 1 & Histogram1).

Table 1: *In vitro* antioxidant activity of methanolic extract of *Ceropegia spiralis* & Ascorbic acid.

Conc. (µg/ml)	Percentage Inhibition of free radicals							
	Super oxide radical		Hydroxyl radical		Lipid peroxidation		DPPH radical	
	Ascorbic acid	*Ceropegia spiralis*	Ascorbic acid	*Ceropegia spiralis*	Ascorbic acid	*Ceropegia spiralis*	Ascorbic acid	*Ceropegia spiralis*
10	25.68±0.22	15.26±0.62	19.56±0.47	20.23±4.05	31.51±0.07	24.26±0.09	24.72±0.84	18.20±1.81
25	34.58±0.08	24.11±0.36	28.26±1.01	26.55±4.25	34.73±0.07	30.34±0.09	35.06±1.79	29.85±0.06
50	47.48±0.44	37.36±0.64	36.98±0.63	35.65±4.14	48.23±0.08	36.50±0.06	49.91±0.45	36.83±0.04
100	59.44±0.66	46.76±0.06	54.46±0.87	47.10±2.41	60.18±0.10	39.73±0.05	63.83±0.36	47.58±0.03
250	66.62±4.24	55.39±0.04	77.99±0.66	57.68±5.15	66.36±0.05	51.92±0.03	72.58±0.07	54.77±0.06
500	73.47±0.09	66.30±0.90	80.65±0.57	49.68±6.94	74.56±0.09	59.21±0.03	75.26±0.08	62.62±0.05
1000	83.49±0.10	73.52±0.05	82.63±0.55	61.81±2.18	78.92±0.03	61.65±0.07	85.52±0.10	68.20±0.04
IC_{50}	**156.30**	**365.64**	**237.49**	**461.32**	**125.69**	**469.39**	**100.13**	**381.13**

Values are mean ± S.D three replicates.

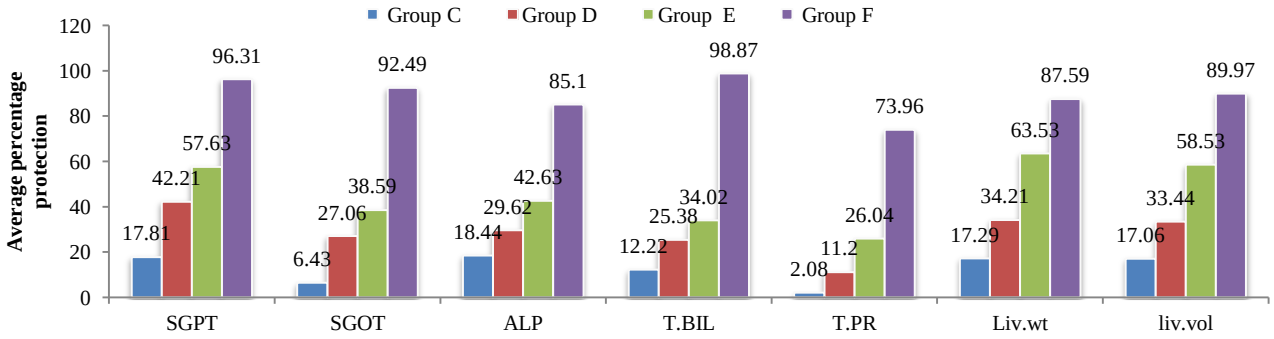


Histogram 1: *In vitro* 50% inhibition concentration (IC_{50}) of methanolic extracts of *C. spiralis* and ascorbic acid on free radicals scavenging activity.

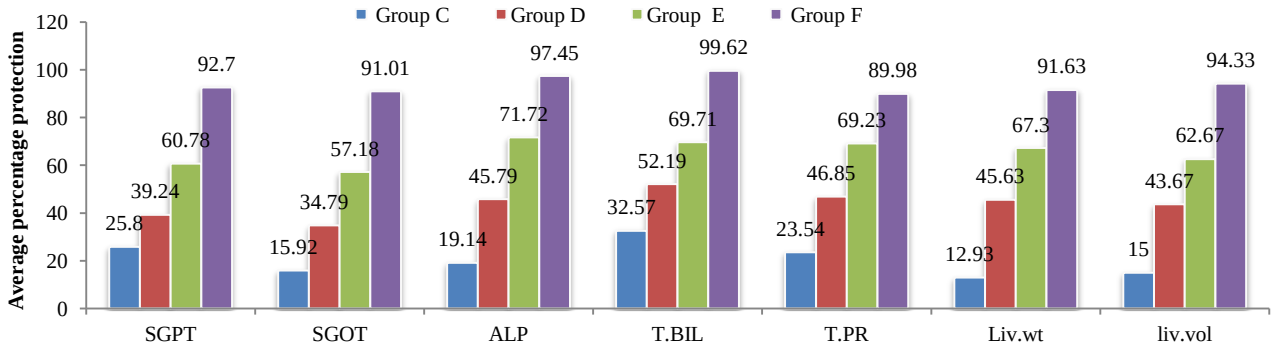
Table 2: Effect of methanolic extract of *Ceropegia spiralis* and Silymarin on serum biochemical parameters on paracetamol induced hepatotoxicity in rats (preventive study).

Group/	SGPT(IU/L)		SGOT(IU/L)		ALP(IU/L)		T.BIL(mg/dl)		T.PR(g/dl)	
Treatment	0^{th} Day	4^{th} Day	0^{th} Day	4^{th} Day	0^{th} Day	4^{th} Day	0^{th} Day	4^{th} Day	0^{th} Day	4^{th} Day
Group A	55.28±3.47	59.35±2.62	82.92±3.03	83.78±2.67	146.25±2.71	144.98±2.69	0.41±0.02	0.39±0.02	7.57±0.22	7.78±0.25
Group B	60.38±3.71	233.73±4.35$^{###}$	86.71±4.31	319.18±8.65$^{###}$	149.44±4.85	388.35±3.65$^{###}$	0.36±0.03	5.71±0.31$^{###}$	7.97±0.35	3.94±0.22$^{###}$
Group C	62.73±2.46	202.68±4.72**	91.22±3.45	304.04±5.74^{ns}	149.65±2.16	343.48±4.13***	0.38±0.04	5.06±0.24^{ns}	7.93±0.44	4.02±0.16^{ns}
Group D	61.66±3.97	160.12±5.67***	87.42±2.98	255.49±7.38***	138.61±4.94	316.27±6.29***	0.39±0.04	4.36±0.25*	7.91±0.45	4.37±0.31^{ns}
Group E	56.63±3.41	133.23±3.69***	89.08±4.17	228.33±6.62***	140.51±4.60	284.59±5.17***	0.35±0.04	3.90±0.20***	8.02±0.30	4.94±0.29^{ns}
Group F	58.36±3.41	65.79±4.69***	88.19±2.95	101.46±5.18***	152.46±3.71	181.25±5.29***	0.39±0.03	0.45±0.11***	7.07±0.34	6.78±0.23***

Values are the Mean± S.E.M. of six rats for each treatment; $^{###}$ Significance P <0.001 when Group B compared with Group A; ***Significance P <0.001, **P<0.01,*P<0.05 when rest of Groups compared with PCM treated group (Group B). NOTE: Group A: Normal 1%Sod.CMC, Group B: Treated with Paracetamol(2g/kg), Group C: Treated with Paracetamol(2g/kg) + MECS (100mg/kg), Group D: Treated with Paracetamol(2g/kg) + MECS (200mg/kg), Group E: Treated with Paracetamol(2g/kg) + MECS (400mg/kg) and Group F: Treated with Paracetamol(2g/kg) + Silymarin 100mg/kg).



Histogram 2: Average percentage protection produced by methanolic extract of *Ceropegia spiralis* and Silymarin against PCM induced hepatotoxicity (preventive study). **NOTE**: **Group C**: Treated with Paracetamol (2g/kg) + MECS (100mg/kg), **Group D**: Treated with Paracetamol (2g/kg) + MECS (200mg/kg), **Group E**: Treated with Paracetamol (2g/kg) + MECS (400mg/kg) and **Group F**: Treated with Paracetamol (2g/kg) + Silymarin 100mg/kg).



Histogram 3: Average percentage protection produced by methanolic extract of *Ceropegia spiralis* and Silymarin against PCM induced hepatotoxicity **(**curative study). **NOTE**: **Group C**: Treated with Paracetamol (2g/kg) + MECS (100mg/kg), **Group D**: Treated with Paracetamol (2g/kg) + MECS (200mg/kg), **Group E**: Treated with Paracetamol (2g/kg) + MECS (400mg/kg) and **Group F**: Treated with Paracetamol (2g/kg) + Silymarin 100mg/kg).

In vivo Hepatoprotective Activity

Estimation of Serum Biochemical Parameters

The paracetamol treated group (Group-B) showed significant (p<0.01) increase in selected serum biochemical parameters namely AST, ALT, ALP and total bilirubin and serum levels of total protein significantly decreased when compared with normal group. However, the administration of *Ceropegia spiralis* at different doses and Silymarin showed significant (p<0.01) reduction in serum biochemical parameters like AST, ALT, ALP and total bilirubin levels and serum levels of total protein significantly elevated when compared to paracetamol treated group in both preventive and curative study (Table 2 to 4).

The results of the study based on levels of AST, ALT, ALP, T. BIL and T. PR biochemical parameters the rats treated with 400 mg/kg body of Ceropegia *spiralis* produced better recovery 57.63%, 38.59%, 42.63%, 34.02% and 26.04% against paracetamol intoxication respectively in preventive study and 60.78%, 57.18%, 71.72%, 69.71% and 69.23% respectively in curative study (Histogram 2 & Histogram 3).

Estimation of physical parameters

In our study the paracetamol treated group showed significant ($p<0.01$) increase in levels of physical parameters like liver weight and volume when compared with normal rats. However, the rats treated with different doses of *Ceropegia spiralis* and Silymarin showed significant ($p<0.01$) reduction in physical parameters like liver weight and volume when compared to paracetamol treated group in both preventive and curative study (Table 5).

The based on the liver weight and volume the 400 mg/kg body weight of *Ceropegia spiralis* produced better recovery 63.53% and 58.53% against paracetamol intoxication respectively in preventive study and 67.30% and 62.67% respectively in curative study (Histogram 2 & Histogram 3).

Table 3: Effect of methanolic extract of *Ceropegia spiralis* and Silymarin on serum biochemical parameters on paracetamol induced hepatotoxicity in rats (curative study).

Group/ Treatment	SGPT(IU/L)			SGOT(IU/L)			ALP(IU/L)		
	0^{th} Day	4^{th} Day	11^{th} Day	0^{th} Day	4^{th} Day	11^{th} Day	0^{th} Day	4^{th} Day	11^{th} Day
Group A	62.43± 1.94	58.15±1.95	60.19±2.80	83.52±1.77	85.26±3.31	85.29±2.42	140.17±3.42	140.89±2.06	137.54±2.17
Group B	57.57±2.64	221.32±3.75	210.25±2.91$^{###}$	86.20±3.94	320.71±5.85	311.17±6.36$^{###}$	151.48±3.83	396.91±6.85	372.33±7.52$^{###}$
Group C	65.91±1.67	227.06±3.52	171.54±3.33***	81.22±2.68	339.46±3.55	275.22±5.35***	154.80±6.48	410.23±3.54	327.39±3.91***
Group D	60.76±2.58	220.73±6.14	151.37±3.67***	83.65±1.93	329.11±6.91	232.59±5.48***	160.98±2.74	408.60±5.56	264.81±7.52***
Group E	59.54±0.66	227.66±5.52	119.04±5.77***	84.20±1.59	321.21±4.39	182.02±4.98***	141.96±3.14	426.48±5.20	203.95±6.21***
Group F	59.83±2.72	234.06±3.64	71.14±3.43***	94.93±2.09	338.93±3.64	105.59±3.30***	142.51±2.74	408.31±2.15	143.53±3.30***

Values are the Mean± S.E.M. of six rats for each treatment; $^{###}$ Significance P <0.001 when Group B compared with Group A; ***Significance P <0.001, **P<0.01,*P<0.05 when rest of Groups compared with PCM treated group (Group B). NOTE: Group A: Normal 1%Sod.CMC, Group B: Treated with Paracetamol(2g/kg), Group C: Treated with Paracetamol(2g/kg) + MECS (100mg/kg), Group D: Treated with Paracetamol(2g/kg) + MECS (200mg/kg), Group E: Treated with Paracetamol(2g/kg) + MECS (400mg/kg) and Group F: Treated with Paracetamol(2g/kg) + Silymarin 100mg/kg).

Table 4: Effect of methanolic extract of *Ceropegia spiralis* and Silymarin on serum biochemical parameters on paracetamol induced hepatotoxicity in rats (curative study).

Group/ Treatment	T.BIL(mg/dl)			T.PR(g/dl)		
	0^{th} Day	4^{th} Day	11^{th} Day	0^{th} Day	4^{th} Day	11^{th} Day
Group A	0.34±0.04	0.43±0.07	0.37±0.03	8.04±0.38	8.01±0.32	8.13±0.43
Group B	0.32±0.13	5.78±0.32	5.69±0.37$^{###}$	8.47±0.35	3.72±0.17	3.84±0.37$^{###}$
Group C	0.27±0.05	5.90±0.41	3.98±0.32***	7.32±0.27	4.19±0.18	4.85±0.24^{ns}
Group D	0.38±0.07	6.16±0.38	2.95±0.13***	8.84±0.46	3.86±0.31	5.85±0.30***
Group E	0.35±0.07	6.69±0.31	2.03±0.32***	8.02±0.38	3.92±0.34	6.81±0.23***
Group F	0.38±0.05	6.04±0.30	0.46±0.10***	8.00±0.35	4.50±0.19	7.70±0.13***

[a] Values are the Mean± S.E.M. of six rats for each treatment; $^{###}$ Significance P <0.001 when Group B compared with Group A; ***Significance P <0.001, **P<0.01,*P<0.05 when rest of Groups compared with PCM treated group(Group B). NOTE: Group A: Normal 1%Sod.CMC, Group B: Treated with Paracetamol(2g/kg), Group C: Treated with Paracetamol(2g/kg) + MECS (100mg/kg), Group D: Treated with Paracetamol(2g/kg) + MECS (200mg/kg), Group E: Treated with Paracetamol(2g/kg) + MECS (400mg/kg) and Group F: Treated with Paracetamol(2g/kg) + Silymarin 100mg/kg).

Table 5: Effect of methanolic extract of *Ceropegia spiralis* and Silymarin on physical parameters on paracetamol induced hepatotoxicity in rats.

Group/ Treatment	Preventive study		Curative study	
	Liv.wt (g/100g)	Liv.vol (ml/100g)	Liv.wt (g/100g)	Liv.vol (ml/100g)
Group A	3.24±0.06	3.57±0.07	3.38±0.08	3.71±0.07
Group B	5.90±0.19$^{###}$	6.56±0.21$^{###}$	6.01±0.12$^{###}$	6.71±0.16$^{###}$
Group C	5.44±0.14^{ns}	6.05±0.16^{ns}	5.67±0.08^{ns}	6.26±0.13^{ns}
Group D	4.99±0.18**	5.56±0.19**	4.81±0.13***	5.40±0.19***
Group E	4.21±0.11***	4.81±0.14***	4.24±0.12***	4.83±0.12***
Group F	3.57±0.09***	3.87±0.10***	3.60±0.13***	3.88±0.15***

Values are the Mean± S.E.M. of six rats for each treatment; $^{###}$ Significance P <0.001 when Group B compared with Group A; ***Significance P <0.001, **P<0.01,*P<0.05 when rest of Groups compared with PCM treated group (Group B). NOTE: Group A: Normal 1%Sod.CMC, Group B: Treated with Paracetamol(2g/kg), Group C: Treated with Paracetamol(2g/kg) + MECS (100mg/kg), Group D: Treated with Paracetamol(2g/kg) + MECS (200mg/kg), Group E: Treated with Paracetamol(2g/kg) + MECS (400mg/kg) and Group F: Treated with Paracetamol(2g/kg) + Silymarin 100mg/kg).

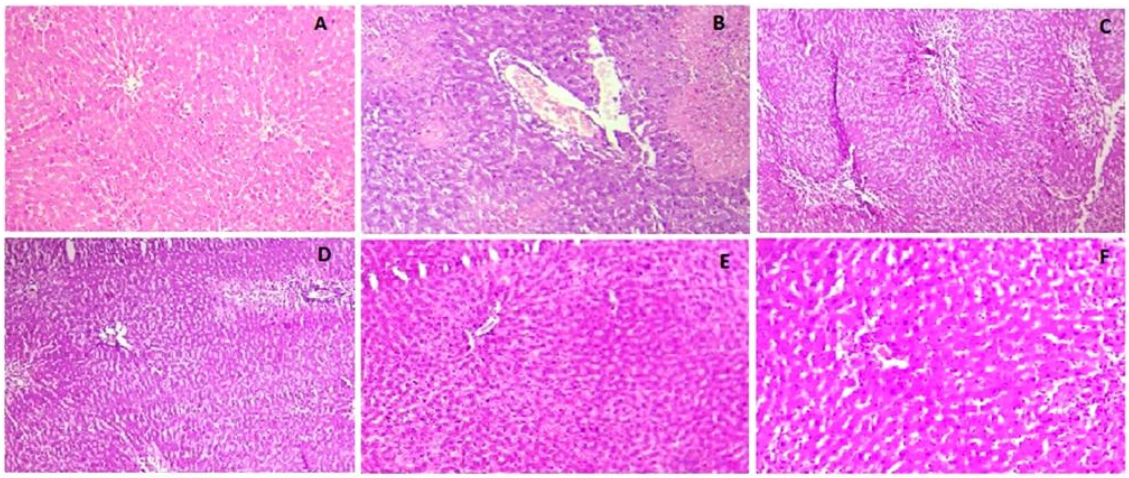


Figure No.1: Histopathology of liver tissues in control and experimental groups of rats (Preventive study). (A) Normal control group: showed the well defined architecture and vesicular nuclei. (B) Hepatotoxic group: showed degenerative changes in hepatocytes and necrosis occurred. (C) Group treated with MECS 100 mg/kg: showed proliferative hepatocytes no degenerative changes and increased inter cellular space. (D) Group treated with MECS 200 mg/kg: showed proliferative hepatocytes no degenerative changes and increased inter cellular space (E) Group treated with MECS 400 mg/kg: showed better architecture and well defined nuclei when compared with group A. (F) Group treated with Silymarin 100 mg/kg: section showed normal architecture with well defined nucleus.

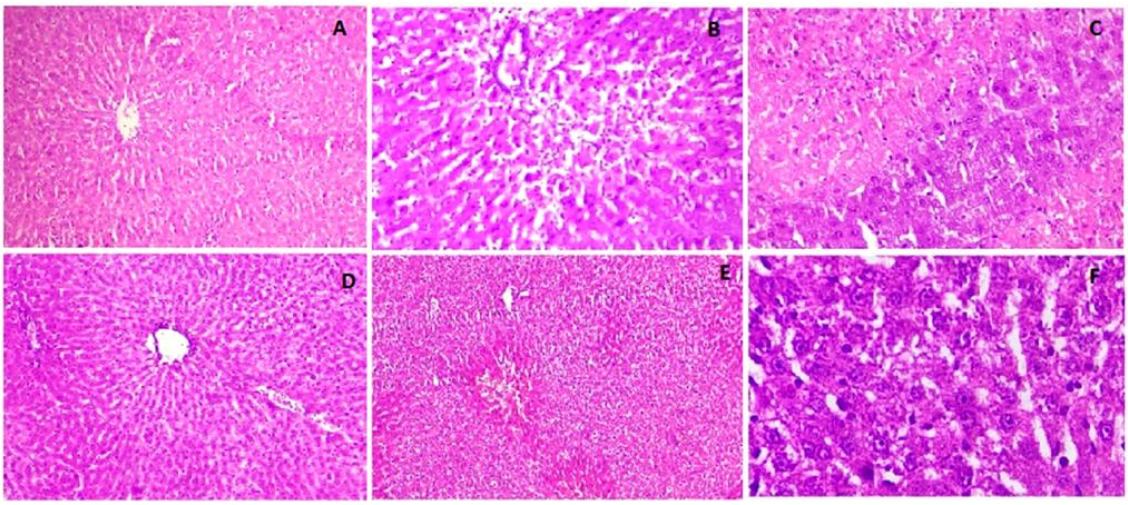


Figure No.2: Histopathology of liver tissues in control and experimental groups of rats (Curative study). (A) Normal control group: showed the well defined architecture and vesicular nuclei. (B) Hepatotoxic group: showed degenerative changes in hepatocytes and necrosis occurred. (C) Group treated with MECS 100 mg/kg: showed proliferative hepatocytes no degenerative changes and increased inter cellular space. (D) Group treated with MECS 200 mg/kg: showed proliferative hepatocytes mild degenerative changes and increased inter cellular space (E) Group treated with MECS 400 mg/kg: showed clear cells, nucleated, follows normal structure and architecture with sign of very mild inflammation when compared with group A. (F) Group treated with Silymarin 100 mg/kg: section showed normal architecture with well defined nucleus.

Histopathological studies

The histopathological study of liver was done as a supportive parameter for the hepatoprotective action shown by the methanolic extract of Ceropegia *spiralis* in both preventive and curative studies. The normal architecture of hepatocytes contains number of hexagonal/polygonal lobules, with more eosinophilic cytoplasm (Naidu et al., 2007). The same features were observed in normal control group (Fig. no. 1 & 2).

The histopathological study of paracetamol treated group (Group B) in both preventive as well as curative studies shown a high derangement of hepatic cords, ballooning, necrosis, cellular infiltration and loss of cell boundaries without any sign of regeneration (Fig. no. 1 & 2), whereas *Ceropegia spiralis* treated groups showed decrease in these abnormalities in dose dependent manner with poor regeneration (Fig. no. 1 & 2).

DISCUSSION

A low dose of Paracetamol is widely used as antipyretic and analgesic agent but a higher dose can produces hepatic damage in humans as well as in rodents. It is a well adapted model for screening of hepatoprotective agents. The N-acetyl-p-benzoquinoneimine (NAPQI) is the metabolic product of Paracetamol, which trigger the hepatotoxicity (Lee *et al*., 1991) by generating the reactive oxygen species (ROS). Consequently oxidative stress is overwhelming and leads to hepatic injury and hepatic death (McGill *et al.*, 2012; Yanpallewar *et al*., 2002). The enzymes present in cytoplasm are rushed into the systemic circulation in order to damage of the liver cell membrane, (Ramaiah. 2007). Hence, the elevated serum cytosolic enzymes level indicates the quantitative biomarkers of hepatic damage.

The laboratory findings of paracetamol (PCM) induced hepatotoxicity are similar as other acute hepatic inflammation and enhancement of liver ailment with major increase of AST, ALP, ALT, LDH, cholesterol, bilirubin and decrease of TP (Davidson and Eastham, 1996). The experimental data clearly referred the elevation of serum hepatic enzymes level such as AST, ALP, ALT, total bilirubin, and reduce level of Total Protein, which conveying the hepatic damage in the PCM induced hepatotoxic rats. The Methanolic extract of *Ceropegia spiralis* at various doses of 100, 200 and 400 mg/kg significantly ($p < 0.05$ to $p < 0.01$) lowered the AST, ALP, ALT, total bilirubin and physical parameters, and total protein levels were elevated in these PCM intoxicated rats and also offered maximum recovery at a dose of 400 mg/kg body weight in both studies.

The data obtained from the liver function tests were analyzed with histopathological changes from photomicrographs taken. The centrilobular hepatic necrosis, cell degeneration and infiltrating lymphocytes were well marked in PCM intoxicated group. Treatment with methanolic extract of *Ceropegia spiralis* prevented these PCM induced histopathological changes. These results of the study showed inhibition of hepatic damage and elevated hepatic function markers may participate in the protective effect of the methanolic extract of *Ceropegia spiralis* against paracetamol induced hepatotoxicity in both preventive and curative studies.

The results further indicates among these two studies the methanolic extract of *Ceropegia spiralis* showed better results in curative study against paracetamol induced hepatotoxicity. The hepatoprotective activity of methanolic extract of *Ceropegia spiralis* might be due to its free radical scavenging property, which reduce the oxidative stress imposed by paracetamol.

CONCLUSION

The present investigation concludes that, the methanolic extract of *Ceropegia spiralis* have hepatoprotective activity in preventive and curative study, which is due to its antioxidant activity. A further investigation has to carry out for the identification of active principle responsible for hepatoprotective action.

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