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Research Article

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF ROOT OF *COLEUS EDULIS* AND ITS CORRELATION WITH *IN VIVO* HEPATOPROTECTIVE ACTIVITY IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: People of Ethiopia chew the root of *Coleus edulis* (Lamiaceae) for the treatment of liver disorders. Since free radical mechanism are reported to be involved in causing such disorders, the aqueous extract of root of *C. edulis* was screened for its antioxidant activity by *in vitro* and for *in vivo* hepatoprotective activity against paracetamol (PCM) induced liver toxicity in rats.

Methods: The antioxidant activity was evaluated by estimating the lipid peroxidation inhibition, superoxide, and hydroxyl radical scavenging activities. Hepatotoxicity was induced in rats by oral administration of 2 g/kg body weight of PCM for 3 days. Blood samples were collected and estimated the alkaline phosphatase (ALP), glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and total bilirubin (T.BIL) in serum.

Results: The amount of extract and ascorbic acid needed for 50% scavenging of superoxide and hydroxyl radicals was found to be 65.56 µg, 42.86 µg, 930.47 µg, and 817.06 µg, respectively. The amount needed for 50% inhibition of lipid peroxide was 773.26 µg (extract) and 807.70 µg (ascorbic acid). The preventive and curative treatment with the aqueous extract of root of *C. edulis* (300 mg/kg) found to be decrease the rise in serum ALP, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and T.BIL levels due to PCM treatment. The results of the extract are comparable with standard silymarin (100 mg/kg).

Conclusion: The lipid peroxidation inhibition activity coupled with free radical scavenging activity of the extract might be responsible for its protective activity against PCM induced hepatotoxicity in rats.

Keywords: *Coleus edulis*, Hepatoprotective activity, Lipid peroxidation, Paracetamol.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, the fight against the disease, nutrient supply, energy provision and reproduction [1]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [2].

The role of the free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, diabetes etc., and the compounds that can scavenge free radicals have great potential in ameliorating these disease processes [3]. Antioxidants play an important role to protect the human body against damage by reactive oxygen species [4]. Human body has endogenous molecules such as superoxide dismutase, glutathione peroxidase, catalase, and vitamin E (α-tocopherol) to reduce free radical induced injury. Sometimes these protective molecules are found to be insufficient compared to the insult produced to the body and the exogenous antioxidants like vitamin C are administered [5] and hence the search for exogenous antioxidants is continued.

Much attention has been focused on antioxidant compounds present in edible plants because of safety concerns associated with synthetic antioxidants [6]. In Asia and Africa, a number of plants are used in the treatment of a number of diseases such as liver cirrhosis, diabetes, arthritis, and cancer. Even though, there is a lack of sufficient scientific proof. Many plant extracts and plant products have been known to have significant antioxidant activity [7-9]. Ethiopia is a land of natural resources. The majority of the production depends on traditional medical systems. People in northern Ethiopia chew the root of *Coleus edulis* (family: Lamiaceae) for the treatment of peptic ulcer, jaundice, etc. So, it is planned to evaluate the aqueous root extract of *C. edulis* for its *in vitro* antioxidant activity and *in vivo* protective activity against paracetamol (PCM) induced hepatotoxicity in rats and compare with the known antioxidant ascorbic acid *in vitro* studies and silymarin *in vivo* studies.

METHODS

Drug and chemicals

Nitroblue tetrazolium (NBT) was purchased from SISCO Research Laboratories Pvt. Ltd. Mumbai, 2-deoxy-D-ribose was purchased from Sigma Chemical Company, USA. Alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and total bilirubin (T.BIL) are supplied by med source, Faridabad, India. PCM and silymarin were supplied by Sri Krishna Pharmaceuticals, Hyderabad and Micro Labs, Bengaluru respectively as gift samples. All other chemicals and reagents used were of analytical reagent quality.

Plant material and preparation of extract

The dried aqueous extract of root of *C. edulis* was prepared in the University of Gondar, Ethiopia and the plant specimen was authenticated by Dr. M. Venkaiah, Associate Professor, Department of Botany, Andhra University, Visakhapatnam. The dried powdered material of root of *C. edulis* of 670 g was extracted with boiling water (3 L) for 30 minutes. The filtrate was evaporated below 70°C in a vacuum dryer to give a final yield of 44 g. The crude extract was redissolved in distilled water when necessary for assessment of *in vitro* antioxidant and *in vivo* hepatoprotective activity.

Animals
Albino rats of either sex weighing between 170 and 220 g obtained from M/S. Mahaveer Enterprises, Hyderabad, India were used in the study. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12 hrs light and dark cycle. The use and care of the animals in the experiment protocol has been approved by the Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

Qualitative photochemical studies
The extract was tested for carbohydrates, alkaloids, glycosides, phytosterols, phenols, tannins and proteins by standard chemical procedures AOAC (1990).

Acute toxicity studies
Acute toxicity study for an aqueous extract of roots of *C. edulis* was performed by using albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extract was administered orally in increasing dose and found safe up to a dose of 2000 mg/kg for extract.

***In vitro* antioxidant activity**

Determination of superoxide radical scavenging activity

Riboflavin photo reduction method: Superoxide scavenging activity of the extract was determined by McCord and Fridovich method [10] which depends on light-induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained 0.3 ml of different concentrations of the extract and 0.2 ml ethylenediaminetetraacetic acid (6 µM containing 3 µg NaCN), 0.1 ml NBT (50 µM), 0.05 ml riboflavin (2 µM) and 2.35 ml phosphate buffer (58 mM, pH 7.8) to give a total volume of 3 ml. The tubes were uniformly illuminated with an incandescent light for 115 minutes, and the optical density was measured at 560 nm. The percentage inhibition by the extract of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes.

Determination of hydroxyl radical scavenging activity

Deoxyribose method: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radical generated from the Fe^{3+}/ascorbate/ethylenediaminetetraacetic acid/H_2O_2 system. The hydroxyl radical attacks deoxyribose and eventually results in the formation of thiobarbituric acid reacting substances (TBARS) [11]. The reaction mixture containing deoxyribose (2.8 mM), ferric chloride (0.1 nm), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), phosphate buffer (20 mM, pH 7.4) and 0.2 ml different concentrations of the extract in a final volume of 1 ml was incubated for 1 hr at 37°C. Deoxyribose degradation was measured as TBARS by the method of Ohkawa *et al*. [12] and percentage of inhibition was calculated from the control where no test compound was added.

Determination of lipid peroxidation inhibiting activity

Induction by Fe^{2+}/ascorbate system: Inhibition of lipid peroxidation was determined by the thiobarbituric acid method. 0.1 ml of different concentrations of the extract was incubated at 37°C with 25% (w/v) rat liver tissue homogenate (0.1 ml) containing Tris-HCl buffer (40 mM, pH 7.0), 0.1 ml KCl (30 mM), 0.1 ml ascorbic acid (0.06 mM) and 0.1 ml ferrous ion (0.16 mM) in a total volume of 0.5 ml for 1 hr. At the end of the incubation period, 0.4 ml of the reaction mixture was treated with 0.2 ml sodium dodecyl sulfate (8.1%), 1.5 ml thiobarbituric acid (0.8%) and 1.5 ml acetic acid (20%, pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 95°C for 1 hr. After the mixture had been cooled, 1 ml distilled water, and 5 ml of butanol-pyridine (15:1 V/V) were added. Following vigorous shaking, the tubes were centrifuged, and the absorbance of the upper layer containing the chromophore was read at 532 nM. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control and experimental tubes [12].

Calculation of percentage inhibition
The percentage inhibition by the extract and ascorbic acid was calculated by using the formula:

$$\text{Percentage inhibition} = \frac{\text{Average of the control OD} - \text{Test sample OD}}{\text{Average of the control OD}} \times 100$$

Calculation of 50% inhibition concentration
The optical density obtained with each concentration of the extracts and ascorbic acid was plotted on a graph taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the concentration needed for 50% inhibition.

PCM induced hepatotoxicity

Procedure

Rats were divided into six groups, each group containing of six rats.

Group 1: Control rats which received 2% gum acacia orally for 3 days.

Group 2: Received PCM 2 g/kg body weight orally once daily for 3 days.

Group 3: Received PCM 2 g/kg body weight orally and *C. edulis* extract 300 mg/kg orally simultaneously for 3 days (preventive study).

Group 4: Received PCM 2 g/kg body weight orally and silymarin 100 mg/kg body weight orally simultaneously for 3 days (preventive study).

Group 5: Received PCM 2 g/kg body weight orally for first 3 days and *C. edulis* extract 300 mg/kg body weight orally from 4^{th} to 10^{th} day (curative study).

Group 6: Received PCM 2 g/kg body weight orally for first 3 days and silymarin 100 mg/kg body weight orally from 4^{th} to 10^{th} day (curative study).

Biochemical determinations
The biochemical parameters like serum enzymes: SGOT (aspartate aminotransferase), SGPT (alanine aminotransferase) (Reitman and Frankel, 1957), serum ALP (King, 1965) and T.BIL (Malloy and Evelyn, 1937) were assayed using assay kits (Spam Diagnostic, Surat) and semi auto analyzer (Screen Master 3000).

Statistical analysis
Results were expressed as mean ± standard error of mean, (n=6). Statistical analysis was performed with one-way analysis of variance (one-way) followed by Tukey multiple comparison tests using Graph Pad Prism-5 software. $p<0.05$ was considered to be statistically significant. $^{###}p<0.001$ when Group B compared with Group A and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ and ns = not significant when rest of groups compared with Group B.

RESULTS

Phytochemical study
The preliminary phytochemical screening of aqueous extract of *C. edulis* showed the presence of carbohydrates, alkaloids, phytosterols, phenols, and tannins.

Acute toxicity studies
The aqueous extract did not show any sign and symptom of toxicity and mortality up to 2000 mg/kg dose.

In vitro antioxidant activity

Superoxide scavenging activity

The extract of *C. edulis* was found to scavenge the superoxide generated by photoreduction of riboflavin. The extract and ascorbic acid at various concentrations (7.5-120 µg) were found to be inhibit superoxide radicals in a dose-dependent manner (Table 1). The quantity of extract/ ascorbic acid needed for 50% scavenging of superoxide was found to be 65.56 µg and 42.86 µg respectively. The extract was found to produce comparable (Fig. 1) activity with that of ascorbic acid in scavenging superoxide radical.

Inhibition of lipid peroxidation

Lipid peroxides generated by the induction of Fe^{2+}/ascorbate on liver homogenate was found to be inhibited by the addition of the extract and ascorbic acid at different concentrations (100-800 µg) in a dose-dependent manner (Table 2). The quantity of extract and the ascorbic acid need for 50% inhibition of peroxidation was found to be 773.26 µg and 807.70 µg respectively. This shows both extract and ascorbic acid exhibit comparable (Fig. 2) potency in inhibiting lipid peroxidation.

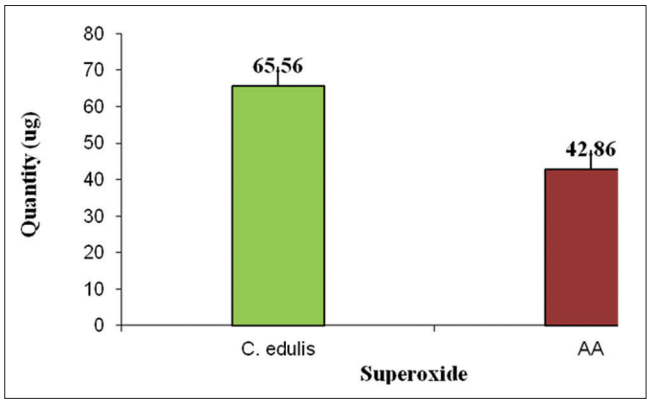

Fig. 1: The quantity of the extract and ascorbic acid (AA) required for 50% inhibition (scavenging) of superoxide radicals in *in vitro* studies (mean ± standard error of mean)

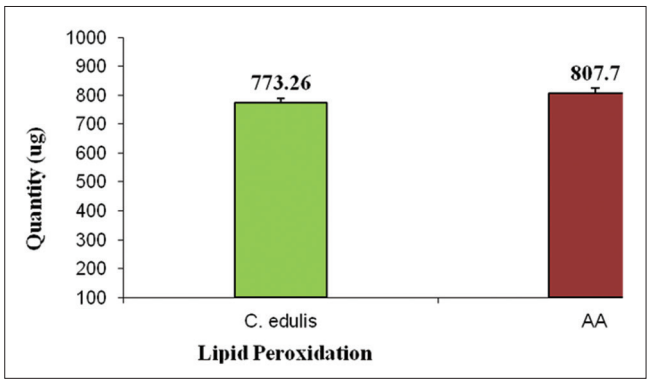

Fig. 2: The quantity of the extract and ascorbic acid (AA) required for 50% inhibition of lipid peroxidation in *in vitro* studies using rat liver homogenate (mean ± standard error of mean)

Hydroxyl radical scavenging activity

The degradation of deoxyribose mediated by hydroxyl radical generated by Fe^{2+}/ascorbate/EDTA/H_2O_2 system was found to be inhibited by the extract. The extract and ascorbic acid at different concentration (100-1000 µg) scavenged the hydroxyl radicals in a dose dependent manner (Table 3). The quantity of extract and ascorbic acid needed for 50% inhibition of hydroxyl radical was found to be 930.47 µg and 817.06 µg respectively. This shows that both the extract and ascorbic acid have comparable (Fig. 3) potency in scavenging hydroxyl radical.

In vivo hepatoprotective effect

The preventive and curative treatment given with 300 mg/kg body weight of *C. edulis* was found to reduce liver damage as shown by reduced enhancement of ALP, SGOT, SGPT and T.BIL when given preventive dose of extract orally for 3 days treatment with PCM and after 3 days treatment with PCM the curative dose of extract was administered orally from 4th to 10th day. The results were comparable with the results of silymarin 100 mg/kg body weight a standard drug administered orally. The results are compiled in Tables 4-9 and graphically represented in the histogram (Figs. 4 and 5).

DISCUSSION

Free radicals such as superoxide radical, peroxide radical and hydroxyl radical play an important role in the genesis of various

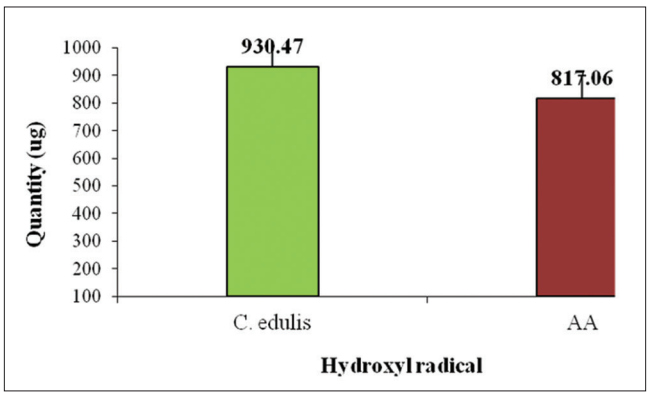

Fig. 3: The quantity of the extract and ascorbic acid (AA) required for 50% inhibition (scavenging) of hydroxyl radicals in *in vitro* studies (mean ± standard error of mean)

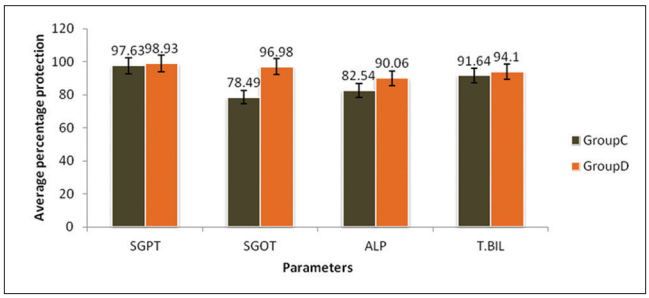

Fig. 4: Average percentage protection produced by *Colies edulis* and silymarin against paracetamol induced hepatotoxicity on 4th day (preventive study)

Table 1: *In vitro* percentage inhibition of superoxide radicals in riboflavin photoreduction method

Treatment	Quantity (µg)				
	7.5	15	30	60	120
Aqueous extract of *C. edulis*	20.64±0.77	36.18±1.23	44.58±1.38	51.42±1.09	65.22±1.37
Ascorbic acid	21.12±0.51	42.36±1.99	46.34±1.32	69.36±1.40	79.16±1.12

Mean values of five samples±standard error of mean. *C. edulis: Coleus edulis*

Table 2: *In vitro* percentage inhibition of lipid peroxidation using thiobarbituric acid method

Treatment	Quantity (µg)				
	100	**200**	**400**	**500**	**800**
Aqueous extract of *C. edulis*	13.02±1.73	21.74±1.28	28.16±1.89	36.76±1.39	51.30±1.74
Ascorbic acid	18.82±0.94	25.84±1.12	30.26±1.62	33.88±1.66	51.60±1.82

Mean values of five samples±standard error of mean. *C. edulis: Coleus edulis*

Table 3: *In vitro* percentage inhibition of hydroxyl radicals using deoxyribose degradation method

Treatment	Quantity (µg)			
	100	**200**	**400**	**1000**
Aqueous extract of *C. edulis*	20.46±0.45	27.76±0.95	36.80±1.02	50.92±0.49
Ascorbic acid	13.00±1.02	33.48±1.63	44.86±1.12	52.66±0.82

Mean values of five samples±standard error of mean. *C. edulis: Coleus edulis*

Table 4: Basal levels of selected serum biochemical parameters in all groups of rats for PCM induced toxicity on 0 day (preventive study)[a]

S. No	Group/treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)
1	Group A treated with 2% gum acacia	41.50±6.42	150.33±10.66	222.33±13.79	1.07±0.14
2	Group B treated with PCM (2 g/kg)	46.52±6.08	148.83±9.40	215.17±13.41	0.87±0.14
3	Group C treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	43.26±7.21	143.25±9.82	232.08±10.26	0.87±0.11
4	Group D treated with PCM (2 g/kg)+silymarin (100 mg/kg)	40.05±6.97	156.47±12.74	225.84±12.48	0.94±0.12

[a]Values are the mean±standard error of mean of six rats, *C. edulis: Coleus edulis*, PCM: Paracetamol, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin

Table 5: Influence of selected doses of plant extracts on serum biochemical parameters in all groups of rats, on day 4th in preventive study of PCM induced toxicity (preventive study)[a]

S. No.	Group/treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)
1	Group A treated with 2% gum acacia	43.00±4.32	165.33±9.67	229.83±12.79	1.30±0.24
2	Group B treated with PCM (2 g/kg)	850.50±16.46###	861.33±19.92###	868.33±19.18###	9.43±0.57###
3	Group C treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	62.17±10.02***	336.00±14.18***	341.33±15.16***	1.98±0.31***
4	Group D treated with PCM (2 g/kg)+silymarin (100 mg/kg)	51.67±10.41***	186.33±9.30***	293.32±17.21***	1.78±0.22***

[a]Values are the mean±standard error of mean of six rats/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). PCM: Paracetamol, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin, *C. edulis: Coleus edulis*

Table 6: Basal levels of selected serum biochemical parameters in all groups of rats for PCM induced toxicity on 0 day (curative study)[a]

S. No.	Group/treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)
1	Group A treated with 2% gum acacia	41.50±6.42	150.33±10.66	222.33±13.79	1.07±0.14
2	Group B treated with PCM (2 g/kg)	46.52±6.08	148.83±9.40	215.17±13.41	0.87±0.14
3	Group C treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	43.26±7.21	143.25±9.82	229.84±15.04	0.87±0.11
4	Group D treated with PCM (2 g/kg)+silymarin (100 mg/kg)	40.05±6.97	156.47±12.74	219.79±10.53	0.94±0.12

[a]Values are the mean±standard error of mean of six rats. PCM: Paracetamol, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin, *C. edulis: Coleus edulis*

Table 7: Influence of selected plant extracts on serum biochemical parameters in all groups of rats, on day 11th in preventive study of PCM induced toxicity (curative study)[a]

S. No.	Group/treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)
1	Group A treated with 2% gum acacia	45.17±5.52	155.50±7.54	225.00±15.76	1.55±0.26
2	Group B treated with PCM (2 g/kg)	631.00±16.43###	722.33±9.33###	725.67±15.20###	8.52±0.63###
3	Group E treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	68.17±7.12***	272.33±13.53***	300.00±13.66***	1.68±0.24***
4	Group F treated with PCM (2 g/kg)+silymarin (100 mg/kg)	53.33±9.10***	192.17±4.48**	268.33±10.46***	1.57±0.40***

[a]Values are the mean±standard error of mean of six rats/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). PCM: Paracetamol, SGOT: Serum glutamate, oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin

diseases such as cirrhosis of the liver, arthritis, rheumatism, cataract, and ischemia [13]. Herbal drugs containing antiradical constituents are gaining importance in prevention and treatment of such diseases and free radical scavengers like phenolics are well known for their therapeutic activity [7]. It is reported that the terpenoids and flavonoids having glycosidic linkage are likely to be extracted into aqueous extracts [14,15]. The presence of flavonoids and polyphenolic structures are reported to have antioxidant activity [16]. It was found that the potential of the extract to scavenge oxygen radicals depended on the type of radicals encountered [17]. The preliminary studies with ferric chloride test, the aqueous extract of root of *C. edulis* revealed the presence of phenolics compounds, the antioxidant activity of extract may be due to the presence of polyphenolic compounds, the aqueous extract of root of *C. edulis*

Table 8: Percentage protection of aqueous extract of *C. edulis* and silymarin against PCM induced liver damage in rats on 4th day (preventive study)

S. No.	Group/treatment	SGPT	SGOT	ALP	T.BIL
1	Group C treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	97.63	78.49	82.54	91.64
2	Group D treated with PCM (2 g/kg)+silymarin (100 mg/kg)	98.93	96.98	90.06	94.10

C. edulis: Colies edulis, PCM: Paracetamol, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin

Table 9: Percentage protection of aqueous extract of *C. edulis* and silymarin against PCM induced liver damage in rats on 11th day (curative study)

S. No.	Group/treatment	SGPT	SGOT	ALP	T.BIL
1	Group E treated with PCM (2 g/kg)+*C. edulis* (300 mg/kg)	96.07	79.39	85.02	98.13
2	Group F treated with PCM (2 g/kg)+silymarin (100 mg/kg)	98.61	93.53	91.35	99.71

C. edulis: Colies edulis, PCM: Paracetamol, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, T.BIL: Total bilirubin

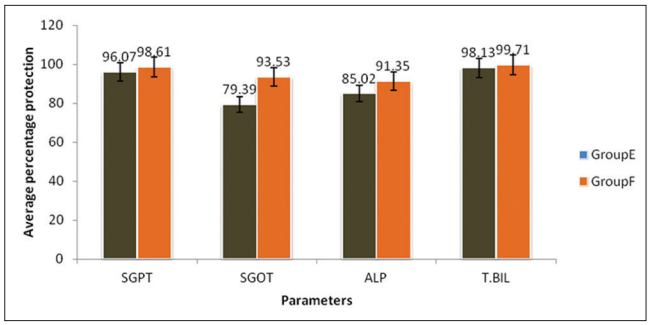

Fig. 5: Average percentage protection produced by *Colies edulis* and silymarin against paracetamol induced hepatotoxicity on 11th day (curative study)

administered orally as prophylactic, exhibited protective activity of root against PCM induced liver damage.

PCM a commonly used antipyretic agent gets metabolized in the liver to an active metabolite, N-acetyl-p-benzoquinoneimine by the cytochrome P-450 microsomal enzyme system, which results in an oxidative stress producing liver glutathione and glycogen depletion and hepatic necrosis [18,19]. The metabolite also oxidizes the sulfohydrxyl groups of the protein resulting in lipid peroxidation induced by a decrease in glutathione in the liver as the cause of hepatotoxicity [20,21]. In the assessment of liver damage by PCM the determination of enzyme levels SGPT, SGOT and ALP and bilirubin is commonly used. Necrosis of membrane damage release the enzyme into circulation and therefore it can be measured in serum. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [22]. Lipid peroxidation has been postulated as being the destructive process in liver injury due to PCM administration [23].

The preventive and curative treatment with the aqueous extract of root of *C. edulis* found to prevent a rise in the serum ALP, SGOT, SGPT and T.BIL levels due to PCM treatment. The extract showed the better effect in inhibiting the lipid peroxidation activity compared to standard antioxidant ascorbic acid *in vitro* studies which might be partly responsible for its beneficial action against PCM induced liver damage. It also exhibited free radical scavenging activity against superoxide and hydroxyl radicals. Hence, inhibition of lipid peroxidation coupled with free radical scavenging activity might be responsible for its protective activity against PCM induced liver injury.

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