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EVALUATION OF ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITIES OF DIFFERENT EXTRACTS OF *CLEOME CHELIDONII* ROOT IN ALBINO RATS

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ABSTRACT

The present study was carried out to evaluate the anti-inflammatory and hepatoprotective activities of different (Hydro-alcoholic, Methanolic, Ethylacetate, Hexane) extracts of *Cleome chelidonii* root against carrageenan induced inflammation and Carbon Tetrachloride (CCl_4) induced liver intoxication in rats. The extracts of *C.chelidonii* root showed a dose dependent anti-inflammatory and hepatoprotection in rats. Among all the extracts, methanolic extracts produced maximum anti-inflammatorty activity (Percentage inhibition of maximal paw edema 53.39±1.2) and hepatoprotection (81.02%) at a dose of 400 mg/kg. The results were statistically significant ($P<0.001$) when compared to control group. The results of the present investigation clearly indicates that all the extracts of *C.chelidonii* root possess dose dependent anti-inflammatory, hepatoprotective activities and this effect may be the result of presence various chemical constituents.

KEYWORDS: ***Cleome chelidonii*, Root, Anti-inflammatory, Hepatoprotective, Carbon Tetrachloride, Carrageenan, Oedema.**

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INTRODUCTION

There has been global resurgence of interest in herbal drugs in the recent past. Though herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used. Therefore herbal drugs deserve detailed studies in the light of modern medicine. A majority of population in India suffer from hepatic and inflammatory disease due to various reasons. The modern system of medicine still lack in providing suitable medicament for a large number of diseases even though tremendous advances were made in the medicine. The development of effective hepatoprotective and antiinflammatory drugs were one of the major thrust areas of research currently. *Cleome* is the largest genus from family *Cleomaceae* comprising 180 to 200 species of herbaceous annual or perennial plants and shrubs widely distributed in tropical and subtropical regions. *Cleome chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis, and rheumatism[1-6]. The present work has been undertaken to investigate the different extracts (Hydro-alcoholic, Methanolic, Ethylacetate and Hexane) of *Cleome chelidonii* roots for their anti-inflammatory, hepatoprotective activities in rats.

MATERIALS AND METHODS

Plant material and Preparation of extract

Cleome chelidonii roots were collected at Marteru region, East Godavari district, A.P. India and authenticity of the plant was confirmed by Taxonomist Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam, India. Freshly collected roots were dried under shade and were made into coarse powder. Coarse powder of *C. chelidonii* roots was extracted separately with 70% v/v ethanol, Methanol, Ethylacetate and Hexane using a soxhlet apparatus. The extracts thus obtained were dried under reduced pressure at a room temperature not exceeding 40^{o}C to get the extracts.

Drugs and Chemicals

Carbon tetrachloride (CCl_4), Riboflavin, Deoxyribose, Carrageenan and Silymarin was purchased from Sigma chemicals, USA. Serum glutamate pyruvate transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphatase (ALP) and Serum Total Bilirubin (T.Bil) assay kits were purchased from Span diagnostics Ltd, Gujarat, India. All other chemicals used were of analytical grade.

Animals

Adult albino Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 150-200 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2^{0}C having 50±5% relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local 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 (CPCSEA), Government of India.

Acute Toxicity Study

The acute toxicity study was conducted for Hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *C. chelidonii* root as per OECD (Organisation for Economic Co-operation and Development) guidelines 420 (OECD.2001).

Quantification of Phenolic and Alkaloidal Content

Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteau reagent[7]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to

the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE)[8].

Total Alkaloidal content[9]

The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG (Bromocresol Green) solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

All the experiments were performed thrice and the results were averaged and reported in the form of Mean ± S.E.M.

Free Radical Scavenging Activity

All the extracts of *C. chelidonii* root were investigated for free radical scavenging activity against Superoxide[10], Hydroxyl[11] and DPPH[12] free radicals.

Anti-inflammatory Activity in Carrageenan Induced Rat paw Oedema Model

All extracts of *C. chelidonii* root were investigated for anti-inflammatory activity in carragenan induced rat paw edema model at three doses levels (100, 200 and 400 mg/kg).The rats were given doses orally with extracts at different dose levels 18 h and 2 h prior to the induction of 0.1 ml of 1% carrageenan subcutaneously (*SC*) into the subplantar tissue of the hind paw of each rat and monitored the oedema progression by using the Zeitlin Isotonic Lever[13] for measuring paw thickness. The drug effects were estimated by comparing the maximal oedema response during 6 h in the drug as extract treated group and the area under the time-course (AUC) as total oedema response with that of vehicle treated group as control.

Hepatoprotective Activity against Carbon Tetrachloride Induced Hepatotoxicity Model (Prophylactic)

In the present work the hepatoprotective activity of Hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* root were tested against carbon tetrachloride (CCl_4) induced hepatotoxicity by measuring biochemical enzymes(SGOT, SGPT, ALP and T.BIL). An increase in the enzymes levels of these biochemical parameters is a sensitive index of hepatic damage. The standard and test group animals were treated with 50 mg/kg dose of Silymarin and 100, 200, 400 mg/kg doses of hydro-alcoholic, methanolic, ethylacetate and hexane extracts of *C. chelidonii* for 6 days. On 6^{th} day, 1hr after treatment with standard drug and selected plant extracts, the animals were intoxicated with CCl_4 in liquid paraffin (1:1 v/v, 0.75 ml of CCl_4/kg, i.p.). Serum was separated by centrifugation at 37 °C and used for estimation of various biochemical parameters. Biochemical parameters like Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT), Serum alkaline phosphatase (ALP), Serum Total bilirubin (T.Bil) were estimated by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy).

RESULTS

Acute Toxicity Study

Acute toxicity studies in mice revealed that the extracts up to 2000 mg/kg produced no sign of toxicity or mortality.

Phytochemical Screening

All the extracts of *C. chelidonii* root screened for the presence various phytoconstituents and quantified the total phenolic and alkaloidal contents. Qualitative phytochemical screening of different extracts of *C. chelidonii* root revealed the presence of steroids, terpenoids, glycosides, tannins, alkaloids, flavonoids, phenols, oils and carbohydrates and showed negative to quinines and amino acids. Methanolic extract showed positive to oils and

saponins and the remaining extracts showed negative to oils and saponins. The phenolic content of various extracts of *C. chelidonii* root was ranging from 14.56±0.86 to 38.95±0.39 (mg/gm). The methanolic extract possesses more phenolic content (38.95±0.39 mg per gm) than other extracts. Alkaloid content in extracts was ranging from 16.55±0.23 to 36.86±0.52 (mg/gm). The methanolic extract contains more alkaloid content (36.86±0.52 mg/gm) than other extracts. The results were given in Table 1.

Table1
Total Phenolic and alkaloid content (mg/gm) of different extracts of Cleome chelidonii root

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	14.56±0.86	16.55±0.23
Ethyl acetate	26.38±0.44	29.41±0.64
Methanolic	38.95±0.39	33.29±0.48
Hydro-alc.ext	31.22±0.64	36.86±0.52

Free Radical Scavenging Activity

In the present study, hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *C. chelidonii* root were found to possess concentration dependent scavenging activity against superoxide, Hydroxyl and DPPH free radicals. The mean IC_{50} (50% Inhibition Concentration) values for superoxide radical scavenging activity were found to be 130.00±1.4, 101.00±1.2, 177.00±2.2 and 552.5±3.4 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 53.5±1.2 µg.

The mean IC_{50} values for hydroxyl radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* roots were found to be 193.00±2.2, 136.5±1.2, 353.00±3.1 and 544.00±2.5 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 67.8±2.3 µg. The mean IC_{50} values for DPPH radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* were found to be 99.2±1.4, 74.8±1.4, 181.00±1.2 and 307.00±2.4 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 18.5±1.5 µg. Based on IC_{50} values, methanolic extract showed better inhibition of free radical scavenging activity compared other extracts of *C. chelidonii* root (Fig 1).

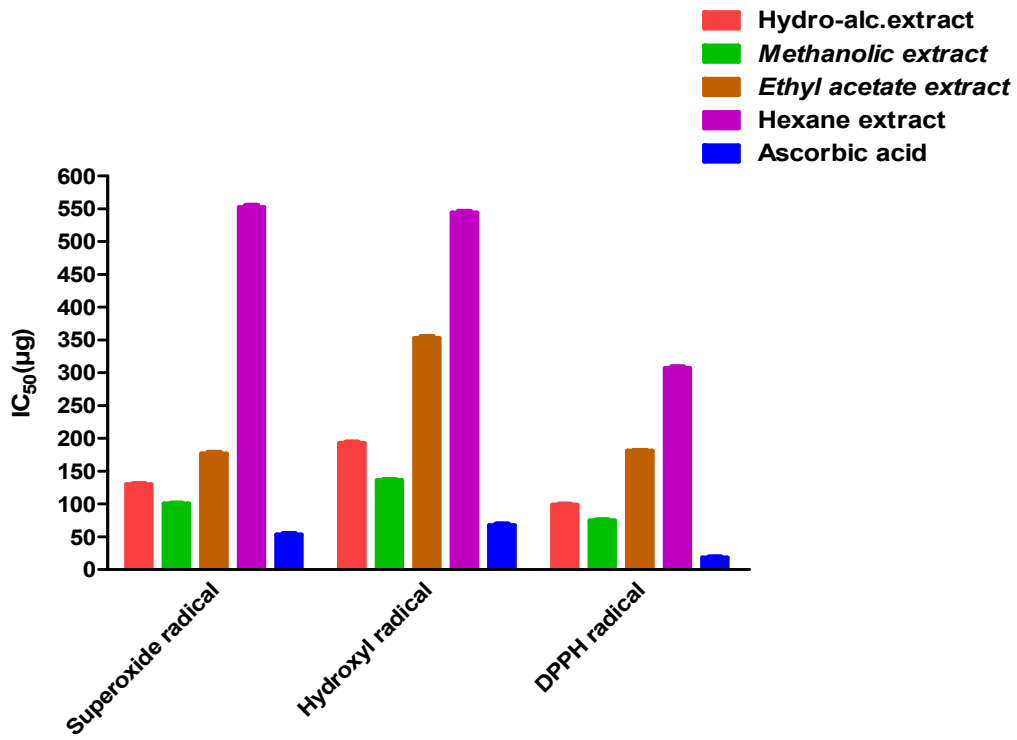
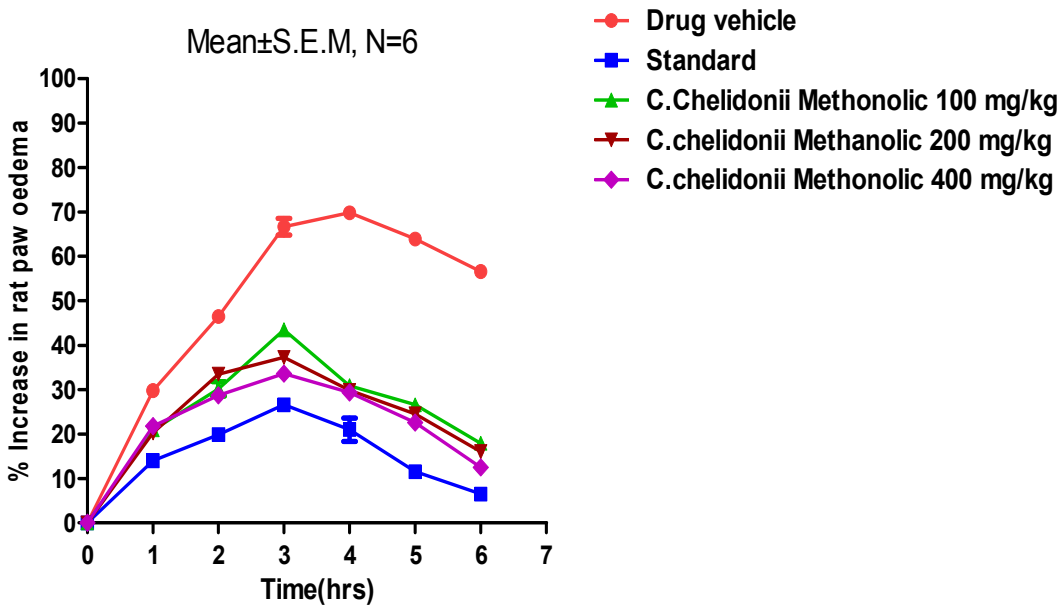


Figure 1
50% Inhibition concentrations (IC_{50}) of different extracts of Cleome chelidonii root against Superoxide, Hydroxyl and DPPH radicals

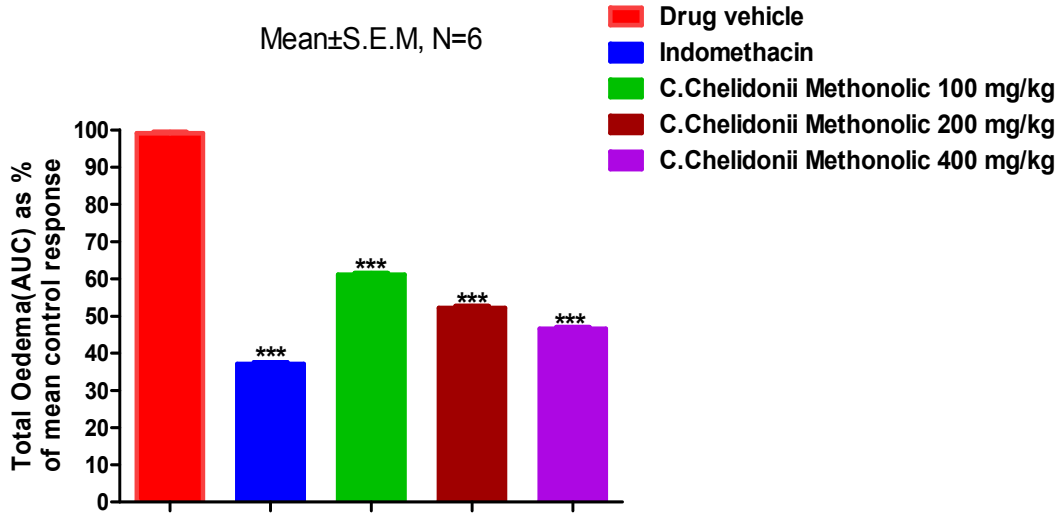
Anti-inflammatory Activity in Carrageenan Induced Rat paw Oedema Model

The Indomethacin at a dose of 5 mg/kg and hydro-alcoholic extract of *C. chelidonii* root at doses 100, 200 & 400 mg/kg significantly inhibited the maximal oedema response and the percentage inhibition was found to be 62.92±1.5, 37.41±0.8, 44.65±1.2 and 50.28±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 68.4±1.2, 45.71±0.8, 47.81±1.3 and 51.25±1.1% respectively over 6 h when compared to the control group treated with drug vehicle. Methanolic extract of *C. chelidonii* significantly inhibited the maximal oedema response and percentage inhibition was found to be 38.91±0.5, 47.76±1.1 and 53.39±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 47.15±1.2, 49.68±0.5 and 53.32±0.6% respectively over 6 h when compared to the control group treated with drug vehicle (Fig 2). Ethyl acetate extract of *C. chelidonii* root significantly inhibited the maximal oedema response and percentage inhibition was found to be 34.92±1.1, 43.08±0.6 and 48.65±1.3% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 44.1±1.2, 46.04±0.5 and 49.45±1.2% respectively over 6 h when compared to the control group treated with drug vehicle. Hexane extract of *C. chelidonii* root significantly inhibited the maximal oedema response and percentage inhibition was found to be 32.58±1.2, 36.92±1.1 and 44.17±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 41.55±1.2, 42.66±1.4 and 46.23±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

A)



B)



***p<0.001.Results were analysed by using One-Way ANOVA followed Dunnett's post-hoc test.
All groups were compared with drug vehicle group.

Figure 2

Effect of methanolic extracts of C.chelidonii root, at 100,200 & 400 mg/kg along with Indomethacin (5 mg/kg) on A) the maximal and B) the total paw oedema in carrageenan induced rats.

Hepatoprotective Activity

Based on SGPT levels, all the extracts are showed dose dependent hepatoprotective activity against CCl_4 liver intoxication in rats and the 400 mg/kg dose (Hydro-alc.ext 67.45%, Methnolic.ext 81.02%, Ethylacetate.ext 60.69%, Hexane.ext 41.81%) showed maximum percentage of protection. Among

four extracts, methanolic extract showed maximum percentage protection (81.02%) and the results were given in Table 2.

Table 2
Effect of different extracts of Cleome chelidonii root on serum SGOT (U/L), SGPT (U/L), ALP (U/L) and Total bilirubin level (mg/dL) and percentage of protection against CCl_4 induced liver toxicity in rats.

Treatment	Dose (mg/kg,p.o)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	T.Bil (mg/dL)
Vehicle Control	1 ml/kg	85.00±0.86 [a]	56.00±1.46 [b]	217.50±1.06 [c]	0.26±0.01 [d]
CCl_4	0.75 ml/kg,i.p	214.0±1.46	159.67±1.89	557.00±2.68	1.33±0.04
Silymarin	50	108.0±1.24[a] (81.96%)	65.50±1.31[b] (90.40%)	252.17±3.19[c] (89.83%)	0.31±0.01[d] (94.84%)
Hydro-alc.extract	100	161.17±0.60 [a] (38.75%)	106.17±0.95 [b] (49.92%)	335.00±1.51 [c] (65.49%)	0.57±0.0 [d] (71.43%)
	200	153.33±1.84 [a] (44.66%)	96.67±0.42 [b] (58.70%)	329.50±1.61 [c] (67.74%)	0.53±0.0 [d] (75.04%)
	400	145.83±0.95 [a] (50.62%)	87.50±0.67 [b] (67.45%)	316.83±1.47 [c] (71.94%)	0.51±0.01 [d] (76.25%)
Methanolic extract	100	151.33±0.99 [a] (46.59%)	85.67±0.33 [b] (69.27%)	308.17±2.01 [c] (74.24%)	0.50±0.01 [d] (78.06%)
	200	142.50±1.18 [a] (54.03%)	81.67±0.61 [b] (74.05%)	291.17±1.78 [c] (79.39%)	0.46±0.01 [d] (82.05%)
	400	133.0±1.9 [a] (62.47%)	75.00±0.77 [b] (81.02%)	280.0±2.16 [c] (82.36%)	0.40±0.01 [d] (87.01%)
Ethyl acetate extract	100	171.33±0.67 [a] (31.41%)	112.33±0.56 [b] (44.31%)	352.50±0.89 [c] (61.20%)	0.63±0.01 [d] (65.73%)
	200	164.33±0.49 [a] (36.03%)	100.67±0.71 [b] (55.92%)	337.50±1.45 [c] (65.59%)	0.59±0.01 [d] (69.65%)
	400	157.0±1.03 [a] (42.43%)	95.33±0.61 [b] (60.69%)	327.67±0.84 [c] (68.25%)	0.54±0.0 [d] (74.06%)
Hexane extract	100	177.67±0.42 [a] (25.74%)	127.50±0.43 [b] (28.81%)	327.17±1.19 [c] (54.52%)	0.72±0.01 [d] (57.66%)
	200	174.0±0.37 [a] (29.13%)	121.50±0.43 [b] (34.28%)	361.0±0.97 [c] (58.13%)	0.68±0.01 [d] (61.32%)
	400	162.83±0.6 [a] (37.44%)	114.17±0.31 [b] (41.81%)	354.0±0.86 [c] (60.00%)	0.64±0.0 [d] (64.26%)

[a,b,c,d] $P < 0.001$. a-compared with SGOT values of CCl_4, b-compared with SGPT values of CCl_4 group, c- compared with ALP values of CCl_4 group and d-compared with T.bil values of CCl_4 group. Values are mean ± S.E.M., *n* = 6 animals per group. Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 x *(*values of CCl_4 control − values of treatment)/(values of CCl_4 control − values of before treatment on 6th day).

DISCUSSION

The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, reactive oxygen species propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[14]. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins[15]. Qualitative investigation showed the presence of bioactive compounds like Flavonoids, Phenolic compounds, Tannins, Alkaloids, Glycosides, Sterols in different extracts of *C. chelidonii* root. There is variability in phenolic content of hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of the selected plant drugs. Among the hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *C. chelidonii* root, methanolic extract showed better superoxide anion scavenging activity (IC_{50} 101.0±1.2µg, hydroxyl radical scavenging activity (IC_{50} 136.5±1.2 µg) and DPPH radical scavenging activity (IC_{50} 74.8±1.4 µg). Based on percentage inhibition of maximal paw oedema during 6 hours and the percentage inhibition of total AUC paw oedema at a dose of 400 mg/kg, methanolic extracts showed better anti-inflammatory activity against carrageenan induced rat paw oedema than the hydro-alcoholic, ethyl acetate and hexane extracts. The order of percentage

inhibition of maximal rat paw oedema during 6 hours as follows: methanolic extract > hydro-alcoholic extract> Ethylacetate extract> hexane extract. Based on the SGPT levels at a dose of 400 mg/kg, among the extracts of four extracts methanolic extracts showed better hepatoprotection against CCl_4 intoxication .The order of percentage protection as follows: methanolic extract > hydro-alcoholic extract> Ethylacetate extract> hexane extract. Thus the study clearly indicated that the selected four extracts of *C. chelidonii* root possess dose dependent good anti-inflammatory, hepatoprotective activities and the protection produced by the extracts may be due to their free radical scavenging activities of different chemical constituents of these extracts. The variation in the effect of different extracts might be the distribution different chemical constituents quantitatively in diffident extracts of *C. chelidonii* root.

CONFLICT OF INTEREST STATEMENT

conflict of interest declared none.

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