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Antioxidants and Hepato Protective Potentials of Oral Cleome *Brachycarpa* Hydroalcoholic Extract: An Organ Toxicity Assessment in Mice

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Abstract:

Aims and Objectives : *Other species of cleome have showed hepato protective properties but there is no study on safety profile and xenobiotic induced hepato protective activities of Cleome brachycarpa which have showed many other pharmacological properties .This study aimed to evaluate the acute and subchronic oral toxicity of the hydroalcoholic extract of the aerial parts of Cleome brachycarpa to provide its safe dose and to compare the possible antioxidant and hepato protective potentials on acetaminophen-induced hepatotoxicity with Silybum Marianum as an approved hepatoprotective agent in global markets.*

Method and Materials: *After providing the hydroalcoholic extract, acute and repeated doseoral toxicity assessments were performed by OECD 425 and 407guidelines. In hepatoprotection assessments, animals were divided to 4 gorups for 6 days interventions.Negative and positive controls received 10 ml/kg normal saline and 500 mg/kg acetaminophen respectively .In 2 treatment groups animals received 100 mg/kg from the hydroalcoholic extract of C.brachycarpa and 200 mg/kg Silybum marianum (Silymarin) extract .All treatment groups received 500 mg/kg acetaminophen on the 5^{th} day, one hour after the last pretreatment with two different hepato protective agents. On the 6^{th} day, liver injuries and the quality of hepato protection were assessed by ALT, AST, ALP, SOD, CAT, and MDA in liver tissues and histopathologicalstudies.*

Results: *Acute test didn't show any sign of toxicity in doses up to 5000 mg/kg and in repeated dose test, no sign of organ toxicity was detected in doses up to 250 mg/kg .Pretreatment with C. brachycarpa extract (100 mg/kg) exhibited a significant reduction in ALT, AST, ALP and MDA ($p < 0.05$) levels when compared with the acetaminophen-only treated group. At the same time, pretreatment with C.brachycarpa extract (100 mg/kg) caused a significant increase in the levels of SOD and Catalase ($p < 0.05$) in comparison to the acetaminophen-treated group. Silymarin treated mice showed similar effects except for Catalase levels which were better controlled by C. brachycarpaextract. The complete histopathological study and liver scores confirmed the biochemical data.*

Conclusion: *This study has revealed the safety and hepato protective effects of Cleome brachycarpa herbal extract as a possible new pharmaceutical dosage form for future studies which could be considered as a new effective supplement in xenobiotics-induced liver damages.*

Keywords: *Cleome brachycarpa; Hepatotoxicity, Hepato protection, oxidative stress; MDA; Silymarin*

List of Abbreviations:

ALP: Alkaline phosphatase

ALT: Alanine Amino transferase

APAP: N-Acetyl-p-Aminophenol

AST: Aspartate Aminotransferase

CAT: Catalase

CCAC: Canadian Council on Animal Care

DPPH: 1, 1-Diphenyl-2-picryl-hydrazyl

FRAP: Fluorescence recovery after photobleaching

H&E: Hematoxylin and Eosin dye

IAUPS: Islamic Azad University, Pharmaceutical Sciences Branch
MDA: Malondialdehyde
SOD: Superoxide Dismutase
OECD: Organization for Economic Co-operation and Development
OBWI: Organ Body Weight Index
NAPQI: N-acetyl-p-benzoquinone imine
NIH: National Institutes of Health

BACKGROUND

More than 10% of the human populations are affected by sustained exposure to certain hepatotoxic agents , like alcohol, viruses, parasites, toxic substances and bio transformed metabolites which may finally develop to cirrhosis or hepatocellular carcinoma (HCC) [1]. Liver is a high-risk organ for toxic reactions because liver injuries are closely connected to inflammation, which is the innate defense system of the body for removing harmful stimulus [2].As the pathogenesis of liver diseases is associated with inflammation and Oxidative stress, herbals with anti-inflammatory and anti-oxidative properties may have potential values in prevention and protection from toxic injuries and they may calledas the main sources of new chemical substances with potential therapeutic effects in xenobiotic-induced liver injuries[3].

*Cleome brachycarpa*from the family of Cleomaceae has a wide distribution in sandy regions of Africa and Asia, especially in North Africa, Egypt, Saudi Arabia, Afghanistan, Pakistan, India, and Hormozgan province of Iran [4]. These species are called Spider flowers because of their special shape of flowers but they are becoming rare nowadays and only small populations from this plant with a restricted distribution have remained in India.

Other species of *cleome*s howed vermicide, analgesic and antiemetic effects [5] as well as hepato protective properties [6] , [7].They have been used in Iranian traditional medicine for treatment of jaundice, diarrhea, fever, infections, bronchitis, scabies, malaria, heart failure, immune system deficiency, seizure and rheumatoid arthritis [8] moreover in a recent study antioxidant effects of *C.brachycarpa* ethanolic extract from stems, roots and leaves have been suggested [9]. Total phenolic and flavonoid contents in all three parts of the plant showed high DPPH radical scavenging and FRAP activities [6].Furthermore, another research has established in vitro antibacterial effects of aerial parts[8], memory boosting effects in Alzheimer [10], and anxiolytic effects in CNS [11]. As the flavonoid and sesquiterpene constituents of the aerial parts of *C. brachycarpa* have shown cytotoxic effects, this plant might be considered as anantitum or agent in the future [12].

N-Acetyl-p-Aminophenol (APAP) known as acetaminophen is a widely used nonprescription analgesic and antipyretic drug that has a very low rate of liver toxicity at normal therapeutic doses; however, it causes hepatic and renal injuries in human and experimental animals when administered in high-risk people or at high dose levels [13].The liver metabolizes acetaminophen in the form of glucuronide and sulfate with urinary excretion [14] , but small fractions of the administered dose could be metabolized by CYP450 isotypes (cyp2 E1) to a reactive free radical, n-acetyl-p-benzoquinone imine (NAPQI) [15] . This metabolite is a strong electrophile with oxidizing properties which normally detoxified by reduced glutathione (GSH) in the liver [16]. In acetaminophen overdoses, drug interactions,CYP450 inductions by alcohol and drug consumptions and presences of other background factors the glucuronidation and sulfation pathways become saturated, and more acetaminophen becomes available for activation by the CYP450 isoenzymes, which produces a large amount of NAPQI that causes rapid depletion of GSH levels. Then, NAPQI metabolite binds covalently to cellular macromolecules that result in massive hepatic necrosis [17], [18].

Objectives : Herbal products with hepatoprotective and antioxidants properties have played valuable roles in the treatment of chemical-induced liver toxicities such as Silymarin which is obtained from *Silybum Marianum* [19].This study was carried out to evaluate the toxic potentials of this extract as well as to compare the antioxidant and hepato protective potentials of hydroalcoholic extract of *C.brachycarpa* on acetaminophen-induced hepatotoxicity with *Silybum marianum* as an approved hepatoprotective agent in global markets.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Aerial parts of *Cleome brachycarpa*were obtained in October 2015 from Hormozgan province, south of Iran. The voucher specimen of the plant was issued at the Herbarium of Islamic Azad University pharmaceutical Sciences Branch (IAUPS), Tehran, Iran, under the voucher number of 1573_AUPF.

Chemicals

- Acetaminophen powder was obtained from DarouPakhsh Mfg. Co. (St. Darou Pakhsh, Tehran, Iran).
- Silymarin was obtained from *Silybum Marianum* extracts product, (Liver gol®) from GolDaru Mfg. Co. (St. Shiraz, Isfahan, Iran). Liver gol® (batch No.1132) coated tablets contain dried extract of *Silybum marianum* 190-195 mg (equivalent to 140 mg Silymarin).
- All other chemicals and kits were purchased from Merck laboratories, Darmstadt, Germany. Solvents used throughout this investigation obtained from the highest commercially available analytical grade while the water was glass distilled.

Plant Extract Preparation

Aerial parts of the plant were extracted by Maceration method. In this method, the plant material was placed in a container full of menstruum (ethanol 80˚) and remained for three to four days and were shaken frequently until complete extraction of plant material. After this phase of extraction, the plant material was strained and the remaining solid was squeezed to remove all the remaining liquid. The obtained liquid was clarified by filtration. Then the extract was placed at room temperaturesince all amount of solvent evaporated and solid extract kept in the refrigerator [20]. For oral administrations, this extract dissolved in normal saline then used by gavages according to the required doses of 100 mg/kg body weight for 5 continues days.

Experimental Animals and Housing Conditions

Studies were carried out using male mice (6-8 weeks old, 25-30 g), obtained from Animal house of Pasteur Institute of Iran. Mice were kept in cages under standard condition (temperature 25±2˚C) with 12 hours light/dark cycle. They were provided with standard pellet diet and free access to drinking water ad libitum. The animals were acclimatized to the environment for a week before the commencement of the experiment. An investigation using experimental animals contained a statement confirming the adherence of the research to the Principles of Laboratory Animal Care published by NIH [21] and approved by the ethical committee of IAUPS.

Part I: Toxicological Evaluations

Acute Qral Toxicity Test

In this study, single oral doses ofplant extract (5000 mg/kg) were administered by oral gavages to 6 female and 6 male mice afterthe randomized division of animals to two groups(6 animals in each group). Mice were observed for mortality and any sign of toxicity for 14 hours and also signs of toxicity and weight changes were investigated during 14 daily follow-up protocol. This study was done according to OECD 425 toxicity assessment guideline [22,23].

Repeated Dose Oral Toxicity Study

Twenty-fivemice were randomly divided into 5 groups (5 mice in each group; 25,250,500,1000mg/kg and control).All the groups were administered orally from the plant extract on the basis of their body weights once daily for 6 days per week over a period of 28 days .Control groups received equal volumes of distilled water daily.This study was done according to OECD 407 toxicity assessment guideline [24].

Part II: Study of Hepato Protective Potentials

Grouping of Animals

In order to investigate the hepatoprotective effects of *C.brachycarpa* hydro alcoholic extract in mice, the mice were divided into the following groups each containing 6 mice:Group1: negative control group: which were fed (gavage) normal saline 10ml/kg for5 days; Group2:acetaminophen treated group: which were fed normal saline 10ml/kg for5 days, on the fifth day one hour after the last administration, administered orally 500mg/kg acetaminophen; Group3: positive control group which

were fed Silymarin 200mg/kg for5 days, on the fifth day one hour after the last administration, administered orally 500mg/kg acetaminophen. Group4: extract treated group, orally received a hydroalcoholic extract of *C. brachycarpa* 100mg/kg for5 days, on the fifth day one hour after the last administration, administered orally 500mg/kg acetaminophen. Mice weights recorded through 6days, then on the sixth day animals were sacrificed.

Administration of Acetaminophen

The acetaminophen liver toxicity model was previously described by Forouzandeh Hossein et al [25] with necessary modifications. Acetaminophen was first dissolved in normal saline at 70˚C and then cooled down to 37˚C for oral administration. It was administered in a single oral dose of 500 mg/kg on the 5th day of interventions just one hour after the last administration of study subjects.

Preparation of Plasma and Liver Homogenate

On the 6th day of study, animals were anesthetized with ether and sacrificed instantly. Three blood samples were undertaken from the hearts of 3 animals of all treatment groups and collected in sterilized centrifuge tubes. The blood samples were then allowed to coagulate at 30˚C for 45 minutes. Serum portion was separated from each sample by centrifugation at 25000g at 30˚C for 10 minutes and subjected to the biochemical investigation to assess liver function tests [26].Then the livers were dissected immediatelyand weighed for liver Organ Body Weight Index (OBWI) estimations and at the second, livers were divided into two equal parts. One part was homogenized in 50mM phosphate buffer (PH 7.4) and centrifuged at 32000g for 20min at 4˚C, the supernatant was separated, which were used to measure enzymes and tissue peroxidation [27].The remaining parts of the liver were used for histological studies.

Biochemical Analyses

Biochemical analyses were carried out on Serum Amino transaminases (ALT and AST) and Alkaline phosphatase (ALP). ALT and AST in serums were determined by the method of Reitman-Frankel. In this procedure, transaminase reacts with 2, 4-dinitrophenyl hydrazones reagent and produces acolor complex that is proportional to the AST and ALT levels [28] .ALP has estimated also according to end point method.

Antioxidant Assays

The Malondialdehyde (MDA) factor, an index of lipid peroxidation factor was determined by Buge& Aust method, (with thiobarbituric acid) [29].The Superoxide dismutase (SOD) factor was also calculated according to Maklund S. Ref method [30] .Finally, the Catalase (CAT) levels were determined according to Goth L. method [31].

Histopathological Assessments

Table 1. *Evaluation criteria and scoring method for the assessment of liver histopathological effects.*

Histopathological effects	Evaluation criteria	scores
Vacuolar degeneration	<5%	1
	5%-33%	2
	33%-66%	3
	>66%	4
Necrosis	No necrosis	1
	1-3 Necrotic cells	2
	3-6 Necrotic cells	3
	>6 Necrotic cells	4
Inflammation & Increase of kupffer cells	No inflammation	1
	Mild	2
	Moderate	3
	Severe	4
Total	-	-

Liver sections from different groups of mice were fixed in 10% (w/v) buffered formalin and used for histological studies. For histopathological studies, liver tissues were dehydrated with ethanol solutions, cut into5-micron sections by microtome, stained with Hematoxylin and Eosin dye (H&E) and observed under a photomicroscope by x100 and x400 magnifications. The observed morphological changes included: cell necrosis, vacuolar degeneration, inflammation and increased number of kupffer cells. The methodof scoringwas described in Table 1.

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS for windows.The results were expressed as mean± SD. One-way ANOVA followed by Tukey post-test were applied for statistical analysis with the level of significance set at $p<0.05$.

RESULTS

Part I: Toxicity Evaluations

Acute Oral Toxicity Studies

Recording clinical signs, no death and no sign of toxicity were recorded in the first 24 hours of administrations using plant extract. Normal physical activities were recorded in all animals during the next 14 days follow-up period.

Repeated Dose Toxicity Study

Survival and Clinical Signs

Animals survived healthy in dose groups of 250,500 and1000 mg/kg for 28 days period of study in water as a solvent group. Normal physical activities were recorded in all animals during this period of study.

Total Body Weights

The trends of weight gains were similar in all low, medium and high dose treatment groups when compared with control(Fig 1).The levels of water and food consumption during this 18 day study was similar(*P>0.05*).

Necropsy Studies

After careful considerations, absolute and relative weights of heart, kidney, liver, ovaries, lungs and uterus were recorded at the endpoint of study. The absolute and relative weights of all organs remained unchanged from corresponding control groups and no gross changes wereobserved.

Histopathological Studies

Pathological studies were performed at day 28 on all resected organs of 1000,500,250 mg/kg doses.

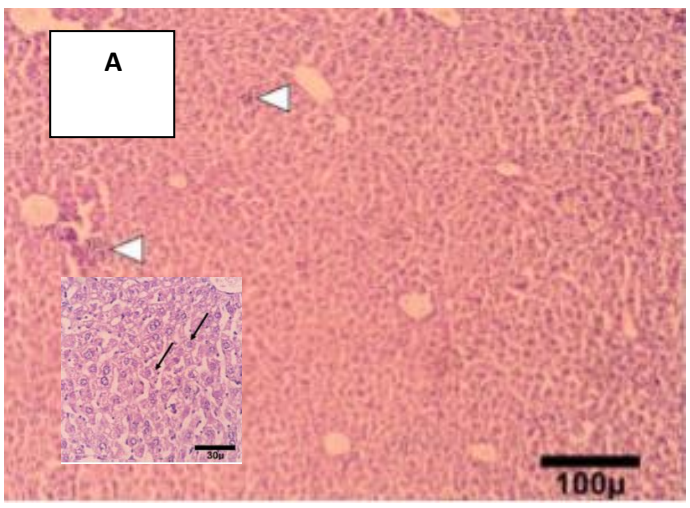
- **Liver**

In hepatic tissues of high dose group (1000mg/kg) partial necrosis of hepatocytes were seen in one animal the rest of animals didn't show any sign of liver toxicity (Fig 1A, 1B).

Fig 1. *Organ effects of Cleome Brachycarpahydro alcoholic extract in low, medium and high dose groups.*

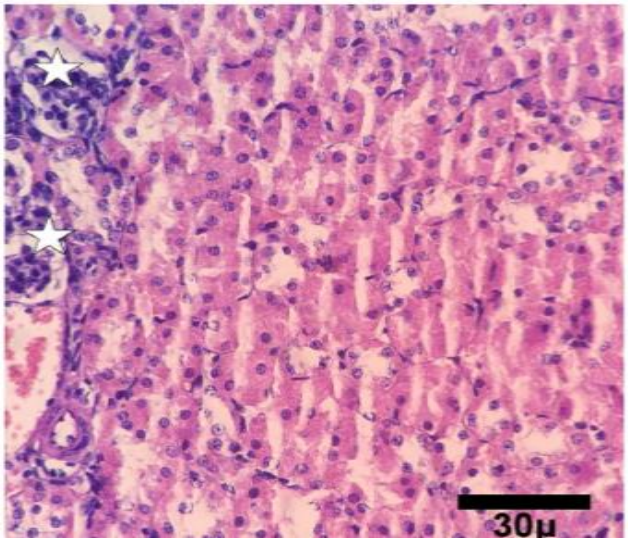
A: Liver

Liver showed normal structure without focal infiltration of mononuclear cells. Liver sections showed increased kupffer cells and normal nucleuses even in high dose groups (H&E x100, 400)



B: Kidney

Normal structure of kidney in high dose group without histopathological abnormality (H& E x100)

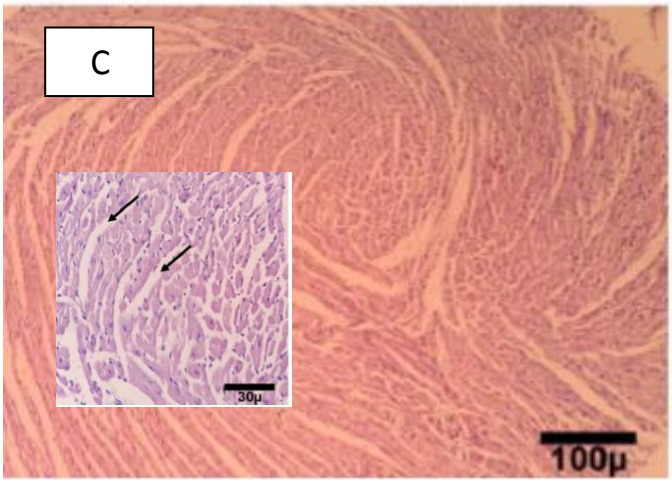


- **Lungs**

In pulmonary tissues of high dose group (1000mg/kg) accumulation of mononuclear cells in the alveolar duct and mild congestion were detected.Pulmonary tissues of lower doses remained normal (Fig 1C).

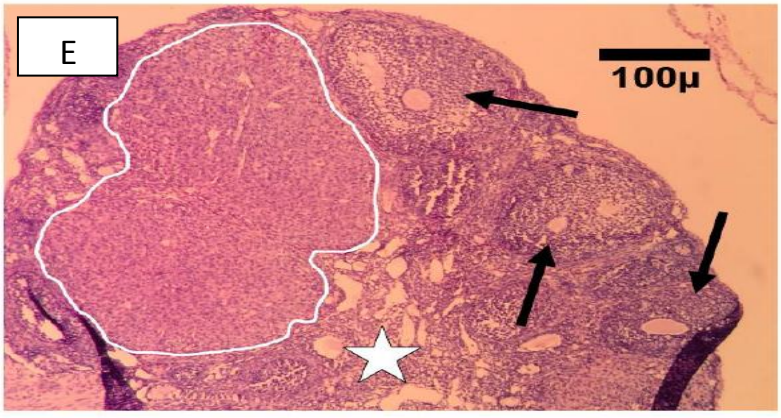
C: Heart

Normal structure of heart in high dose group without histopathological abnormality (H&E x100, 400)



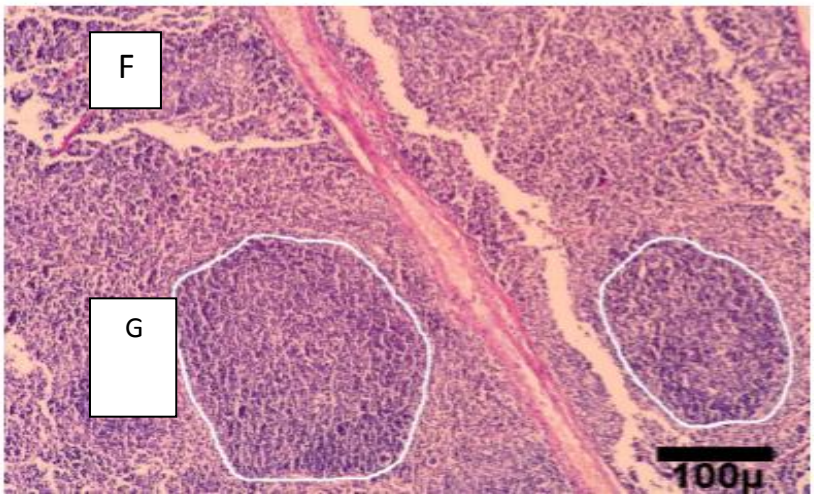
E: Ovary

Normal structure of Ovaries in high dose group ((H&E x100)



F: Spleen

Normal structure of spleen in high dose group without any histopathological feature (H&E, X100



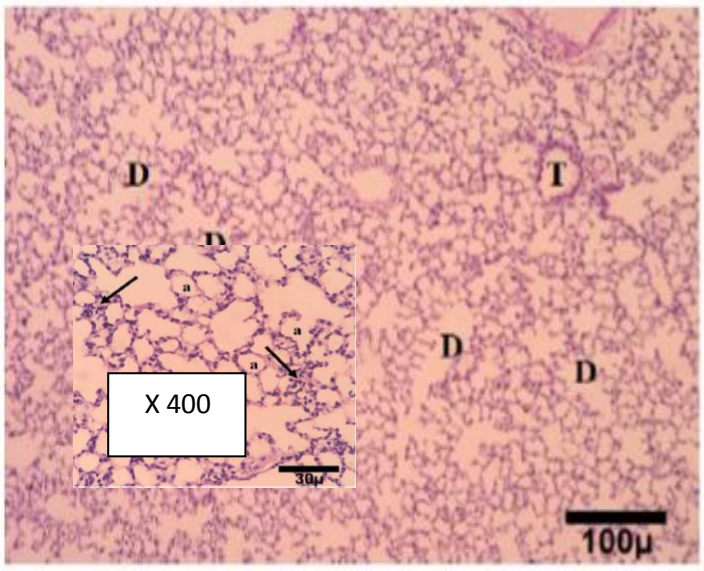
G: Uterus

Normal structures of endometrium (E), myometrium(M) and perimetrium (P) of high dose group.

- **Kidneys**

Mild degeneration of proximal tubules and renal tissue with epithelial necrosis in proximal tubule was recognized in high dose group. Hydropic degeneration of proximal tubules in 500 mg/kg and multifocal mononuclear cells infiltration in 250mg/kg dose groups were identified too (Fig 1D).

D: Normal structure of lungs in high dose group (H&E x100, 400)



- **Hearts**

Normal structures were seen in the hearts of animals in different dose groups.

- **Uterus**

Although mild edema in endometrium and moderate edema in perimeter layer of low and medium doses observed, respectively, the surface of endometrial epithelium was hyperplastic in high dose group.

- **Ovary**

Part II: Hepato Protection and Antioxidant Assessment

Plasma Marker Enzymes

As described in Table 1, serum levels of AST, ALT and ALP were considered as biomarkers of liver function due to acetaminophen-induced hepatotoxicity. Table 1 shows the acetaminophen-induced liver toxicity by significant raise of three mentioned biomarkers. Acetaminophen-treated mice developed significant hepato cellular damages as they are evident from significant ($p < 0.05$) increases in the serum activities of ALT, AST, ALP when compared with the negative control group. At the same time pretreatment with *C.brachycarpa* extract 100 mg/kg as test material and Silymarin 200 mg/kg as a standard drug, exhibited significant ($p < 0.05$) reductions in the levels of AST, ALT, and ALP when compared with the acetaminophen-treated group.

Antioxidant and Lipid Peroxidation Biomarkers

Table 2 shows MDA, SOD and CAT levels in acetaminophen-induced hepatotoxicitymodel:

Table 2. *Protective roles of hydroalcoholic extract of the aerial parts of Cleome brachycarpa against Acetaminophen induced liver toxicity*

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	SOD (U/mg-Protein)	CAT (µMol/mg-Protein)	MDA (nMol/gProtein)
1.Negetive control	Normal saline 10 ml/kg	$148.37^{b}\pm4.22$	$103.10^{b}\pm7.73$	$343.50^{b}\pm5.77$	$20.08^{b}\pm1.10$	0.28 ± 0.01	$1.93^{b}\pm0.09$
2.Acetaminophen treated	Normal saline 10 ml/kg+Acetaminophen 500 mg/kg	$164.01^{a}\pm4.58$	$125.80^{a}\pm2.31$	$379.37^{a}\pm9.50$	$16.36^{a}\pm0.57$	0.21 ± 0.03	$3.05^{a}\pm0.07$
3.Positive control	Silymarin 200 mg/kg+ Acetaminophen 500 mg/kg	$143.81^{b}\pm7.28$	$100.48^{b}\pm2.45$	$311.50^{ab}\pm19.56$	$19.70^{b}\pm0.23$	0.26 ± 0.00	$1.84^{b}\pm0.15$
4.*C.brachycarpa* extract	*C.brachycarpa* extract 100 mg/kg+ Acetaminophen 500 mg/kg	$144.10^{b}\pm9.80$	$101.66^{b}\pm3.05$	$320.07^{b}\pm8.65$	$18.41^{b}\pm0.46$	$0.34^{bc}\pm0.04$	$1.71^{b}\pm0.11$

Each value represents the mean±SD for six mice. [a]: Significantly different from the negative control group at $p < 0.05$. [b]: Significantly different from acetaminophen treated group at $p < 0.05$. [c]: Significantly different from the positive control group at $p < 0.05$.

MDA: After quantitative measurements, MDA analysis showed asignificant increase in comparison to negative control group ($p < 0.05$). At the same time, pretreatment with *C. brachycarpa* extract at doses of 100 mg/kg and Silymarin pretreatment at doses of 200 mg/kg exhibited significant reductions in MDA levels ($p < 0.05$).

SOD: The result of SOD analysis showed asignificant decrease when compared with negative control group ($p < 0.05$) but pretreatment with *C.brachycarpa* extract and Silymarin exhibited significantly and similar reductions in SOD concentrations($p < 0.05$).

CAT: The result of CAT analysis didn't show any significant change in all groups even in Silymarin treated mice in comparison to the negative control group. Pretreatment of mice with *C.brachycarpa* extract caused asignificant increase in CAT levels incomparison to Acetaminophen and Silymarin treated groups ($p<0.05$).

Liver Weights and Organ Body Weight Index (OBWI)

Liver weights of mice measured at the 6th day of study. Table 3 demonstrates liver weights of mice in acetaminophen-induced hepatotoxicity. The results exhibited that the Liver OBWI in the acetaminophen-treated group was significantly decreased in comparison to the normal liver in the control group (42 ± 0.001 mg *vs.* 61 ± 0.008 mg, $p<0.05$). Conversely, pretreatments with Silymarin 200 mg/kg and *C.brachycarpa* 100 mg/kg resulted in normalization of the Liver OBWI when compared with acetaminophen treated group (70 ± 0.005 mg *vs.* 60 ± 0.004mg,$p<0.05$). This could possibly be the result of the incidence of acute necrosis in the hepatocytes in the absence of adequate levels of hepatoprotective agents. In fact, both plant extracts supported the liver parenchymal cells growth and normalizedthe Liver OBWI.

Table 3. *Effect of hydroalcoholic extract of the aerial parts of Cleome brachycarpa on Acetaminophen induced changes in the liver and body weights.*

Groups	Treatment	Liver OBWI(mg)	Average body weight pre administration (g)	Average body weight post administration (g)
1.Negetive control	Normal saline 10 ml/kg	$61^{b}\pm0.008$	24.49±2.78	23.21±3.02
2.Acetaminophen treated	Normal saline 10 ml/kg+ Acetaminophen 500 mg/kg	$42^{a}\pm0.001$	23.65±1.86	18.74±1.07
3.Positive control	Silymarin 200 mg/kg+ Acetaminophen 500 mg/kg	$70^{b}\pm0.005$	26.21±2.63	27.17±2.09
4.*C.brachycarpa* extract	*C.brachycarpa* extract 100 mg/kg+ Acetaminophen 500 mg/kg	$60^{b}\pm0.004$	21.32±1.90	22.15±1.39

Each value represents the mean±SD for six mice. [a]*: Significantly different from the negative control group at p < 0.05.* [b]*: Significantly different from acetaminophen treated group at p < 0.05.*

Body Weight

Through the experiment, mice weights recorded regularly. Table 4 showed average body weight pre and post administrations in all groups. Although statistical analysis didn't show any significant difference *(p > 0.05)* in treatment groups and negative control, acetaminophen treated group showed asignificant reduction in total body weight ($p < 0.05$) due to acetaminophen-induced toxicity. Two pretreatment groups with Silymarin 200 mg/kg and *C. brachycarpa* 100 mg/kg showed significant ($p<0.05$) increase in their total weights probably because of protective effects of these plants in acetaminophen toxicity, therefore, the mean weights of these two groups were not significantly different from the negative control group.

Table 4. *Comparisons of histopathological scores in liver tissue histopathological studies***.**

Groups	Administrations	Mean histopathological lesions	*P*-value	
1.Negetive control	Normal saline10ml/kg	5.33(±1.53)	Group2	0.037
			Group3	0.993
			Group4	0.993
2.Acetaminophen treated	Normal saline10ml/kg + Acetaminophen500mg/kg	9.67(±1.53)	Group1	0.037
			Group3	0.026
			Group4	0.026
3.Positive control	Silymarin200mg/kg + Acetaminophen500mg/kg	5.00(±2.00)	Group1	0.993
			Group2	0.026
			Group4	1.000
4.*C.brachycarpa* extract	*C.brachycarpa* extract100mg/kg + Acetaminophen500mg/kg	5.00(±1.00)	Group1	0.993
			Group2	0.026
			Group3	1.000

Histological Assessment of the Liver:

Table 4 compares the histological scores of liver lesions in all 4 study groups.The levels of liver lesions were comparable without observing any significant difference between *C.brachycarpa,* Sylimarin and negative control groups *(p>0.05).* Figure 2 (a) and 2(b) shows the photomicrographs from the hepatic morphology of *C. brachycarpa* and acetaminophen groups. The histopathological study of liver sections in the negative and positive control groups, as well as the *C.brachycarpa* group showed that mice livers remained healthy and all of the microscopic structuresremained clearly identifiable with mean histological lesions of 5.00(±1.00) in *C.brachycarpa* group. Although in few cases of Silymarin and *C. brachycarpa* groups the cores remained dark, hepatocyte necrosis was not recognized and Central vein and hepatic lobules were detectable in all cases (Figures 2a).

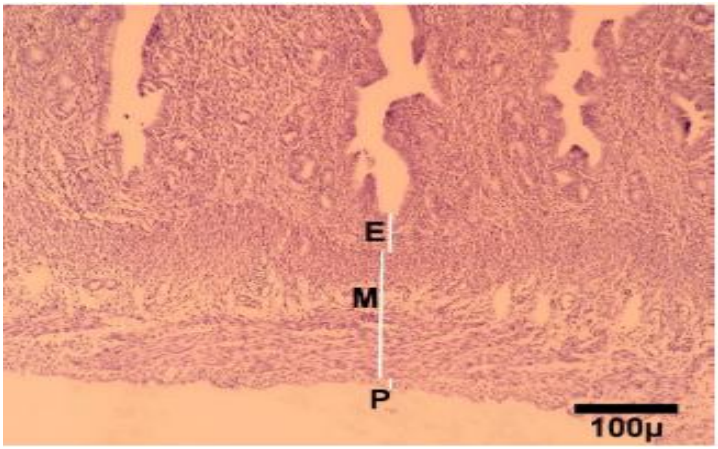
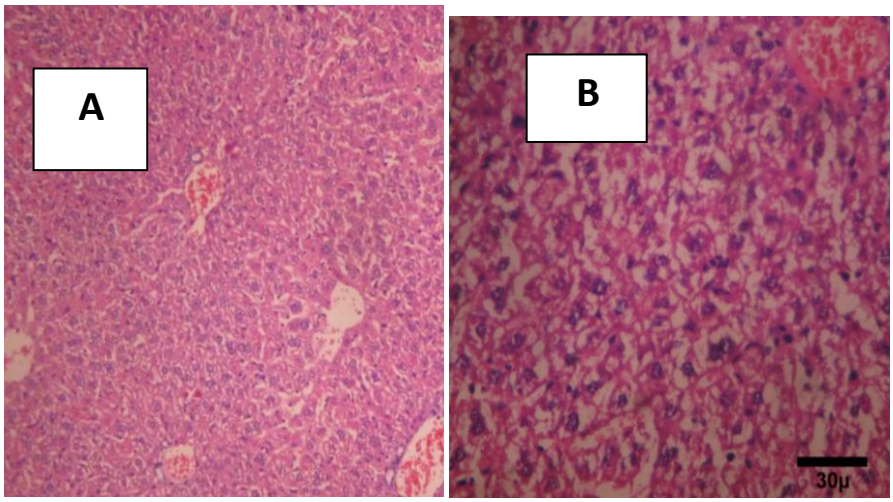


Figure 2. *Photomicrograph of mice liver sections (x400)*

Fig 2A. *Liver section of C. brachycarpa group (x100), liver tissue remained healthy and all of microscopic structures remained clearly identifiable. Although in a few cases the cores remained dark, hepatocyte necrosis was not recognized and Central vein and hepatic lobules was detectable.*

Fig 2B. *Show scattered necrosis and severe vacuole degeneration of hepatocytes with increased kupffer cells in Acetaminophen exposed group of animals.*



In acetaminophen group, scattered necrosis and moderate vacuole degeneration of hepatocytes with increasedkupffer cells observed with mean histological lesions of 9.67(±1.53).

DISCUSSION

Liver is one of the largest organs in the human body and the major site for metabolism and excretion. It has a wide range of functions including, detoxifications, protein synthesis, and production of biochemical's necessary for digestion. Liver disease has become one of the major causes of morbidity and mortality in human all over the world and hepatotoxicity due to drugs appears to be the most common contributing factor in this regard [32].N-Acetyl-p-Aminophenol (APAP), also known as acetaminophen, is the most commonly used over-the-counter antipyretic and analgesic drug. APAP-induced toxicity is considered as one of the primary causes of acute liver failure; numerous scientific reports have focused majorly on APAP hepatotoxicity. A variety of rodent models of hepatoprotection has been described in which the levels of tolerance to acetaminophen-induced hepatotoxicity could be determined [33]. In fact protection against acetaminophen-induced toxicity has been used as an effective screening test for potential hepatoprotective agents by several investigations [34] which were the major concern of present study.

There are three specific and sensitive hepatotoxicity biomarkers [Aspartate amino transferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP)] which could be considered as promising factors because they elevate significantly and dose dependently in the serum of hepatotoxic agent treated animals when compared to control group [35].In fact, any rise in serum levels of AST, ALT, and ALP could be attributed to structural hepatocellular damages because they are normally located in

the cytoplasm of hepatocytes and could be released into the circulation after cellular damages [36].In present study and in an attempt to identify the hepatoprotective efficacy of *C.brachycarpa* , the extract was prepared and subjected to the acetaminophen-induced liver injury model in mice and thehepatoprotective effects of *C. brachycarpa* were shown for the first time similar to Silymarin.

Other than the efficacy of *C. brachycarpa* in normalization of hepatotoxicity biomarkers , this extract showed significant efficacy in reducing the extent of oxidative stress by reversing the levels of MDA and SOD to normal ranges.Since hepatic damages induced by acetaminophen are mediated by its free radical metabolites, antioxidant activity or inhibition of the generation of free radicals is a very important mechanism in the body protection against acetaminophen-induced liver injury [37]). It has been reported that *C.brachycarpa* extract had higher antioxidant activities with concomitant higher total phenolic and flavonoid levels in all three parts (leaves, stem, root) of the plant[38]. Furthermore established hepatoprotective effects of another species of *Cleome* such as *viscosalinn*[6] *cleome viscosa*[39]have been suggested on the basis of their antioxidant activities. These properties motivated us to study the antioxidant activity of *C.brachycarpa* in acetaminophen-induced liver toxicity.

Our results in present provided strong evidence on safety of oral administration in acute and subchronic models as well as significant potencies and inhibitory effects of that *C. brachycarpa* extract exertson the acute liver toxicity induced by high doses of acetaminophen in mice as shown by significant reductions of MA and SOD. In the present experiment, MDA level decreased significantly in the treatment group and antioxidant enzymes level (SOD, Catalase) significantly increased in plant extract group. Moreover, liver histopathology, mice weights, and Liver OBWI findings confirmed the protective activity of this extract, too. The histopathological studies on the livers confirmed the protective effects of this plant against acetaminophen as it was evident from the reversal changes in scattered necrosis, vacuole degeneration and in kupffer cells production. In some cases scattered necrosis, moderate vacuole degeneration and increased kupffer cells observed confirmed its protective role. To compare the *C.brachycarpa* and silymarin extracts, according to studied variables, it could be concluded that there was no significant difference between silymarin and *Cleome brachycarpa (p > 0.05),* except the efficacy of Silymarin in controlling the Catalase concentration. Catalase level was significantly ($p < 0.05$) increased in plant extract group. In histopathological analysis Silymarin and plant, extract groups showed asignificant difference ($p < 0.05$) with the group treated with acetaminophen, that demonstrated the effectiveness of these two agents in liver protection but the histopathological results were equal for silymarin and plant extract ($p > 0.05$).

CONCLUSION

In general, it can be concluded on the basis of al reviewed factors both extracts (Silymarin 200 mg/kg and *Cleome brachycarpa*100 mg/kg) have similarhepatoprotective efficacies but in terms of potency, *Cleome brachscarpa*extract was twice more potent than Silymarin. In conclusion, this safe herbal extract has a protective effect on the liver and can be used as a supplement in liver damages on the basis of its lack of organ toxicity for regulatory purposes and possible commercialization as an effective supplement for liver injuries.

ETHICAL APPROVAL

The investigation was performed according to the Local Animal Ethics Committee guidelines for the use of experimental animals and was approved by ethical committee of Islamic Azad University of Pharmaceutical Sciences branch, IAUPS.

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