

# Effects of Cleome Brachycarpa Ethanol Extract on Liver and Kidney Tissues of Rats: A Prospective Study Focusing on Histopathological Variations

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## ABSTRACT

**Objective:** To know the histological and morphological changes after treating Cleome brachycarpa ethanol extract on liver and kidney tissues in rats.

**Study Designs:** Experimental/interventional comparative study.

**Place and Duration of Study:** This study was conducted at the Department of Pharmacology, Baqai Medical College University, Karachi from 20 June 2014 to 15 Oct 2015.

**Materials and Methods:** Albino rats (200±5 grams) were collected from a maintained and well established animal house of Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi. The animals were housed for 12/12 hours light/dark cycle in the animal house of Baqai Medical University at a temperature of about 25±2°C. Animals in Group A (Control, n=09), albino rats (200±5 grams) were given normal saline orally. In Group B: (Test, n=09) in this group albino rats (200±5 grams) received Cleome brachycarpa extract 200 mg/kg orally for fifteen days.

**Results:** Few morphological and pathological changes were observed in the liver & kidney tissues of treated animals at the dose of 200 mg/kg. Normal parenchyma (B) cells of liver were found, but in kidney tissues figure 2(B) mild tubulitis but in figure 2(C) intra-tubular neutrophilic cast (A) was found in figure 2(D) & no any other changes observed. Significant congestion of portal tract (A) was found. Significant lobular inflammation (A) was found. Inflammatory cells were present in central vein and portal tract (A) Figure 1(d). No any fatty alteration and necrotic changes were found. Fibrotic changes, Steatosis and Cholestasis were not found but in kidney tissues figure 2(b) and c showed patchy tubulitis. Intratubular neutrophilic cast (A) was found in figure 1(d) and no any other change observed.

**Conclusion:** This study established that Cleome brachycarpa do not have marked effect on histopathological features of liver and kidneys. However, mild inflammation was seen in liver and kidneys which may be beneficial in remedial progression. Therefore the extract of Cleome brachycarpa is less toxic and further studies are required to determine its human consumption.

**Key Words:** Cleome brachycarpa, liver and kidney tissues

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## INTRODUCTION

Cleome brachycarpa is an annual woody herb up to 50 cm high. It is branched with leaflets of about 5-15 mm long, 2-4 mm broad.

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Petioles of Cleome brachycarpa are long up to 25-35 mm and its smooth pedicels are 10-15 mm long. Flowers are bright yellowish and 6-8 mm across. Sepals are 2-3 mm while petals are usually 6-8 mm elongated and 2-2.5 mm wide with tiny scale-like basal attachment (Flora of Pakistan)<sup>1</sup>.

The herb of Cleome brachycarpa usually recognized as Ponwar, bitter in taste and is used in the treatment of different diseases like arthritis, inflammation, itching, leprosy, leukoderma, rheumatism, scabies, swelling etc. Its different extracts used in treating wide range of medical problems e: g it works best as an antioxidant agent. Pakistan, Iran and India are chief countries of its cultivation. Different parts of the plant Cleome brachycarpa are frequently used including leaves, root and flowers in managing a number of ailments. (Herbopathy)<sup>2</sup>.

Ahmad et al. (1986)<sup>3</sup> worked on *Cleome brachycarpa* and isolated a new terpenoid, recognized as trinortriterpenoid. Ahmad et al. (1990)<sup>4</sup> evaluated different parts of the *Cleome brachycarpa* and alienated a new triterpenoid, named as, cleocarpone from it. Different species of *Cleome* including *droserifolia*, *amplyocarpa*, *brachycarpa*, *chrysantha* were searched by Sharaf et al. (1992)<sup>5</sup> and they isolated 10 different methylated flavonoids from the diverse parts of the plant. General pollen characteristics and morphology of *Cleome brachycarpa* was studied by Perveen and Kaiser (2001)<sup>6</sup> with the help of light and scanning microscope. Rahman et al. (2004)<sup>7</sup> enlightened the anti-inflammatory property of *Cleome brachycarpa* leaves in the rheumatic pain management. The use of whole plant of *Cleome brachycarpa* in joint pain and inflammation was demonstrated by Mushtaq et al. (2006)<sup>8</sup>. In Dera Ismail Khan, District the use of whole plant of *Cleome brachycarpa* was described. According to their research it has great potential of treating scabies and abdominal pain. Local name of *Cleome brachycarpa* is Dhanar Khathuri reported by Rahmatullah et al. (2010)<sup>9</sup>. They also described the utilization of whole plant of *Cleome brachycarpa* in the joint pain and in inflammation. Ali et al. (2012)<sup>10</sup> testified the anti-oxidant action of ethanol extract of *Cleome brachycarpa*. Mohammad and Salman (2013)<sup>11</sup> choose chicks as experimental animals, to evaluate the anti-emetic property of *Cleome brachycarpa* and *Cleome viscosa* leaves. Schmelzer and Gurib-Fakim (2013)<sup>12</sup> defined the whole plant of *Cleome brachycarpa* and described the use of the different parts of the plant, *Cleome brachycarpa*, in different regions of the world. The dried powder of the herb, *Cleome brachycarpa*, used for the controlling of infants fever and in reducing inflamed body parts. Other uses of the plant was mentioned as an appetizer and as animal feed. In Pakistan *Cleome brachycarpa* is used in the powder form in stomach problems, scabies, rheumatism, abdominal pain and inflammation treatment and management.

## MATERIALS AND METHODS

**Animal and plant material:** Albino rats of weight  $200 \pm 5$  grams were gathered from a conserved and well reputable animal stock of Baqai Medical University and were kept in a light/dark cycle of 12/12 hours at approximately  $25 \pm 2^\circ\text{C}$  temperature. Ethical Committee of Baqai Medical University approved the experimental work before the start of the research. Experimental animals were provided with a balanced diet and were weighted approximately  $200 \pm 5$  grams one week earlier to the study. Fifty four (54) albino rats of weight approximately  $200 \pm 5$  grams were selected and histopathological effects of *Cleome brachycarpa* extract were evaluated. All the research animals (Wister strain albino rats) were separated into two groups (A and B)

comprising of 9 rats in each group. The experiment was repeated three times for the accuracy purpose.

The plant *Cleome brachycarpa* was taken from department of pharmacognosy, faculty of pharmacy, University of Karachi.

### Experimental design:

**Treatment:** Control group A of animals consisting of 9 rats ( $n=09$ ) of  $200 \pm 5$  grams weight were treated with only normal saline orally for fifteen days. The test Group B containing up of 9 rats ( $n=09$ ) of  $200 \pm 5$  grams weight treated with the ethanolic extract of *Cleome brachycarpa* 200 mg/kg orally for fifteen days.

**Sacrifice of animals:** Anesthesia was produced through chloroform inhalation and dissection was performed to remove the organs like liver and kidney after opening the abdominal region. Sectioning and staining of both organs were performed and well-preserved in 10% formalin solution. Histopathological variations produced after the treatment were noticed and examined by making slides of the preserved treated organs (liver and kidney).

**Tissue processing:** Samples of treated rat's organs including liver and kidney were kept in formalin solution for about 24-48 hours then dehydrated with 60%-70% ethanol and then to 90%-95% ethanol for one hour approximately till the clear stage was attained. The absolute alcohol and xylene gave element transparency to organ tissues. Tissues of experimental animals, albino rats were surrounded by liquefied parablax at  $58^\circ\text{C}$  in metal fragments. The blocks prepared were cooled, labeled and sliced by Rotary microtome to five (5) microns thickness. Sections were mounted on clean gelatinized slides and placed on hot plates at about  $37^\circ\text{C}$  for 24 hours for suitable fixation. The H and E stains (Hematoxylin and Eosin stains) were used to stain the sides.

The dried and labelled slides were reserved in plastic and wooden boxes (Bancroft and Stevens, 1990) and were studied under microscope through 10x, 20x and 40x objectives.

## RESULTS

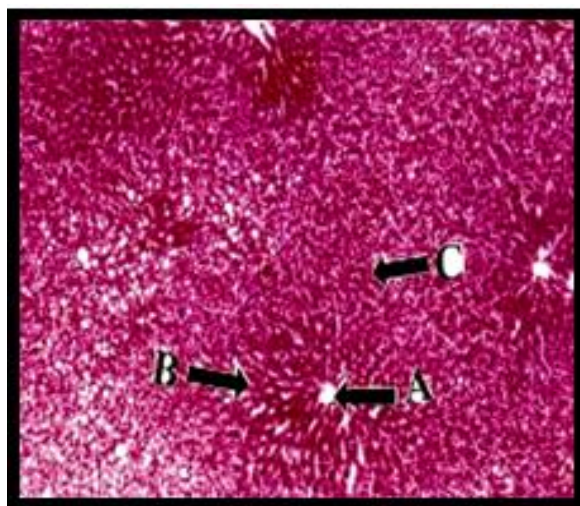
In this research effort, liver and kidney of experimental animals were taken part to perceive the differences in the different parameters including morphological and histological structures after giving the dose of *Cleome brachycarpa* at 200mg/kg/body weight to the experimental animals (rats). Treated and untreated experimental animals (albino rats) were inspected for their morphologically and histologically changes. The treated outcomes were observed and listed in table 1

**Liver histological and pathological changes after *Cleome brachycarpa* dosing on rats:** Liver and kidney tissues of treated animals (Albino rats, Wister Strain) and untreated group of experimental animals (Albino rats, Wister Strain) were examined and compared to note the changes after treatment. The liver

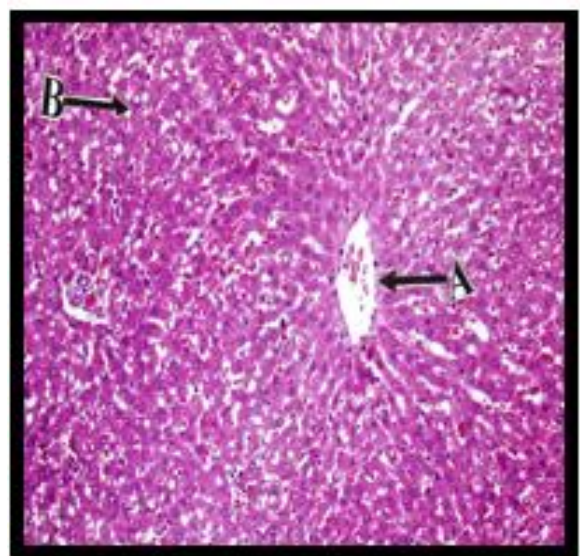
of experimental animals of control group comprises up of lobules, each lobule is hexagonal and is positioned on hepatic vein named as central vein moreover sinusoids and hepatocytes were also seen, mentioned and labelled as (A), (B) and (C) respectively as shown in Figure 1(a)

Some changes were observed in the morphology of liver tissues of the treated rats at the dose of 200 mg/kg. Parenchymal cells of liver were found normal mentioned as (B) in Figure 1(b, c). Congestion was noticed in the portal tract which was quite significant and mentioned as (A) in Figure 1(b). Significant inflammation was found in the lobular region of the liver mentioned as (A) in Figure 1(c)

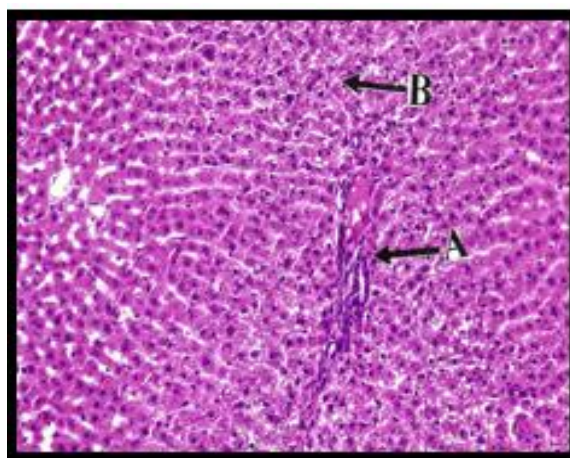
Some of the inflammatory cells were seen in central vein and portal tract which were mentioned as (A) in Figure 1(d). Certainly not any fatty and necrotic modification were found. Cholestasis, Steatosis and Fibrotic modification were not bring into being after the treatment.



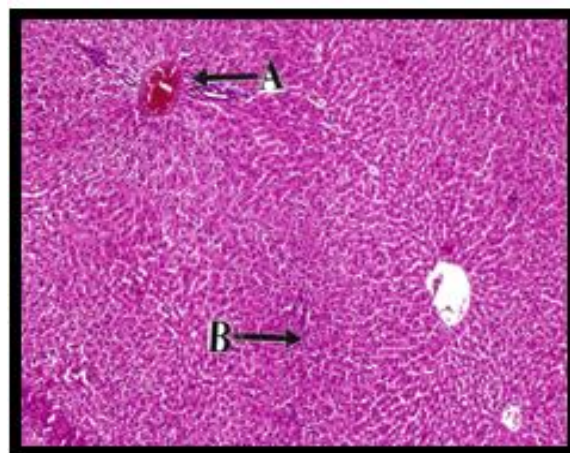
**Figure No.1(a): Control**



**Figure No.1(b): Treated**



**Figure No.1(c): Treated**



**Figure No.1(d): Treated**

Figure No.1: Sections (a) of liver of rat (control) showing normal central vein (A), sinusoids (B) and hepatocytes (C); (b) portal tract congestion (A), Normal parenchyma (B); (c) lobular inflammation (A), Normal parenchyma (B); (d) portal tract inflammation (A) following treatment of rats with cleome brachycarpa

#### **Kidneys histological and pathological changes after Cleome brachycarpa dosing on rats:**

General architecture of the kidney through microscopic examination was found normal in the control group of experimental animals (Albino rats, Wister Strain) including normal renal corpuscle and renal tubules mentioned as (A) and (B) respectively in Figure 2 (a).

Some changes were observed in the morphology of kidney tissues of the treated rats at the dose of 200 mg/kg of Cleome brachycarpa.

Glomeruli of the kidney was found normal and mentioned as (A) but mild to moderate patchy tubulitis was also established which was marked as (B) in Figure 2 (b). Insignificant tubulitis was also found in another slide and was mentioned as (A) in Figure 2 (c). No any shrinkage of glomeruli of the kidney was noticed but intratubular neutrophilic cast, marked as (A) was found



in Figure 2 (d). Hemolysis and edema were not observed after treatment moreover no fibrotic variations in interstitial was initiated.

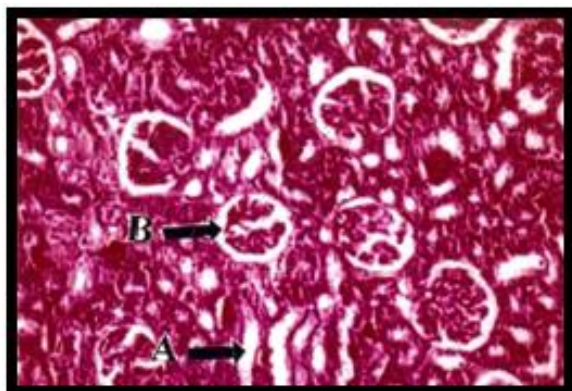


Figure No.2(a): Control

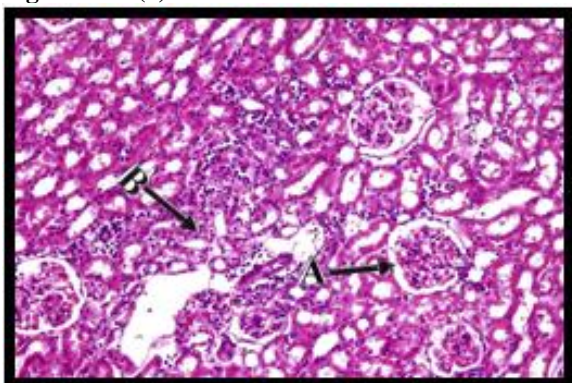


Figure No.2(b): Treated

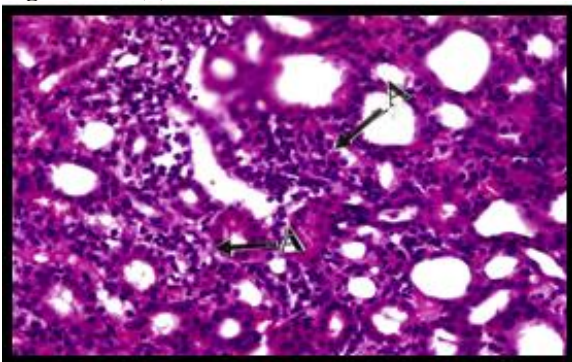


Figure No.2(c): Treated

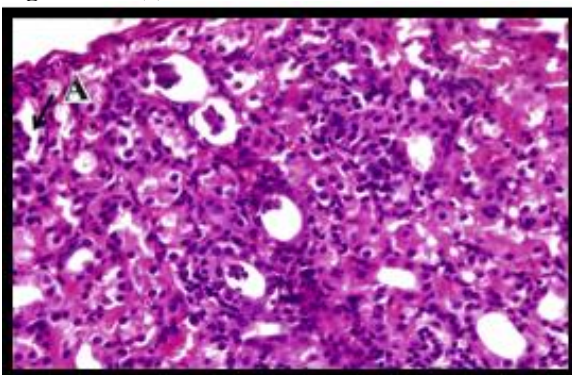


Figure No.2(d): Treated

Figure No.2: Sections (a) of rat kidney (control) showing renal corpuscle (A) and tubules (B); (b) showing normal glomeruli (A), mild-moderate patchy tubulitis (B); (c) Mild tubulitis (A) and (d) showing Intratubular neutrophilic cast (A) following treatment of rats cleome brachycarpa (200mg/kg)

Table No.1: Gross Features of Liver and Kidney Tissues of Rats

Features	Control	Treated
<b>Liver Tissues</b>		
General Architecture	Normal and intact	Normal and intact
Central Vein	No congestion and delation was present	Dilatation of portal tract was found
Inflammatory cells	Not present	No any necrotic changes were observed
Necrosis	Not present	No any necrotic changes were observed.
Fibrotic changes	Not present	Fibrotic changes were not seen.
Fatty changes	Not present	No any fatty alteration was found.
Portal tract	No congestion and dilation	Significant congestion of portal tract was found.
<b>Kidney Tissues</b>		
General Architecture	Normal and intact	Normal and intact
Renal tubules	Normal	Congestion is renal tubulus were found Milk tubulitis and intratubular neutrophilic cast was found.
Renal Corpuscles	Normal	Normal
Glomeruli	Normal	Normal glomeruli was found without any shrinkage.
Blood vessels	Normal in size and appearance	No haemolysis was found and no any edema was present in interatitium.
Necrosis	Not found	Not found
Inflammatory cells	Not present	Not present
Cellularity	Normal	Cellularity was normal.
Basement membrane	Normal	Normal

## DISCUSSION

To the best of our acquaintance, the pathological effects of the ethanoic extract of Cleome brachycarpa plant on the liver and kidney tissues of the experimental animals (albino rats, Wister strain) are being testified for the first time in this experimental work.

In this research homework, different effects including histopathological effects, of Cleome brachycarpa at 200 mg/kg dose on the Wister strain albino rats divided in

two groups (control and treated) were evaluated. The effects and changes in the liver and kidney tissues of albino rats in treated (group B) animals were observed after dosing and were compared with the same organs of the normal group A (control) of experimental albino rats.

Oladele and Abatan (2003)<sup>13</sup> appraised biochemical and histopathological parameters of *Cleome viscosa* leaves extract in albino rats and reported that the plant is nephrotoxic.

The general building and morphology of liver and kidney tissues were found normal through microscopic examination after the administration of ethanolic extract of *Cleome brachycarpa* for 15 days to group B (treated) rats.

Consistent parenchymal cells marked as (B) were seen. Inflammation of portal tract was initiated and labelled as (A) in figure 1(b). Inflammation of lobules were also noticed and mentioned as (A) in figure 1(c). Congestion of portal tract was found significant and mentioned as (A) in figure 1(d).

Levibovitch et al. (1991)<sup>14</sup> evaluated the activity of serum alkaline phosphatase in the renal damage.

Elimination of metabolites is one of the most important function of kidney. So, in this prevailing research work kidney histopathological discrepancies were evaluated after 200 mg/kg dosing of *Cleome brachycarpa* for 15 days to Wistar strain albino rats of group B (treated).

Microscopic inspection disclosed the standard overall architecture of kidney after treatment of 15 days. Glomeruli was found absolutely normal without any contraction mentioned as (A) in figure 2(b) and (c). Patchy tubulitis (B) was also recognized in figure 2(b) and (c). Intratubular neutrophilic cast (A) was also an important feature mentioned in figure 1(d). Renal parenchyma was found absolutely normal with not any significant changes.

## CONCLUSION

According to this present study the extract of *Cleome brachycarpa* is less toxic. In past no any histopathological studies were done on *Cleome brachycarpa*. The plant is used as herbal medicine in many countries as it produced negligible side effects. Further investigations are required for its human consumption.

### Author's Contribution:

Concept & Design of Study:	Hira Naeem
Drafting:	Rehana Parveen
Data Analysis:	Ijaz Hussain Zaidi
Revisiting Critically:	Mohammad Mahmood
Final Approval of version:	Hira Naeem

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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