Saudi Journal of Biological Sciences 28 (2021) 5579–5584

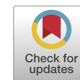
Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Pharmacological and pharmacognostical valuation of *Canna indica* leaves extract by quantifying safety profile and neuroprotective potential



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ARTICLE INFO

Article history:
Received 7 December 2020
Revised 26 May 2021
Accepted 27 May 2021
Available online 1 June 2021

Keywords:
Canna indica
Neuroprotective
Acetylcholinesterase
Safety profile

ABSTRACT

The current study primarily focused on the pharmacognostical and phytochemical screening of *Canna indica* and further analyzing the leaves extract for toxicological profile and neuroprotective potential. The microscopic, dry powder properties of the leaf material and phytochemical, physicochemical analysis was evaluated for pharmacognostical assessment. Dry leaves of *C. indica* were extracted using methanol and then further studied for both in vitro and in vivo toxicological study. The acute toxicity was measured by estimating the antioxidant defense system and anatomical impairment in the rat's organs. Also, the neuroprotective activity of the plant extract was assessed using anticholinesterase enzymatic inhibitory assay. The extract was found to be hemocompatible and showed absences of induction of behavioural changes. Likewise, no changes were seen on the anatomical structure of the rat's organs. The methanolic extract portrayed a significant upsurge in the reduced glutathione level and showed a comparable acetylcholinesterase inhibition in a dosedependent manner with an IC50 value of 14.53 µg/mL compared to the standard Donepezil with an IC50 value of 13.31 µg/mL. *C. indica* has compelling pharmacognostical characteristics, good safety reports, and significant antioxidant as well as the neuroprotective potential that shows great potential for its further in-depth research for pharmacological use.
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1. Introduction

Universally herbal formulations from natural sources used as medications for numerous diseases from antiquated times. Considering the long-established understanding from Ayurveda, a few likely products presented that are at concurrent use for modern clinical treatments. An ongoing factual report of the World Health Organization (WHO) has uncovered that most populations approximately 70–80% globally depend on eastern herbal-derived medicines in their healthcare systems (Chauhan et al., 2015). Even though naturally sourced medications portrayed various clinical benefits, nonetheless several herbaceous species have endured unexplored for their curative properties (Chigurupati, 2020).

Canna indica (Family Cannaceae), commonly known as Indian shot, is a well-known ornamental flowering plant with considerable remedial and commercial use (Al-Snafi, 2015). The plant is indigenous to the South (Andes), West Indies, Mexico, Europe, Africa, and Asia. The growing interest in Canna was mainly attributed to its various traditional uses for treating different diseases (Kanase and Vishwakarma, 2018; Darsini et al., 2015).

C. indica especially leaves and branched rootstocks traditionally used to treat malaria, dysentery, diaphoretic, diuretic, dropsy fever, and wound healing and AIDS (Anh et al., 2021). In addition, flowers can treat several eye diseases. Nevertheless, the roots able to treat amenorrhea and gonorrhea and powdered mixture of leaves and seeds used to treat dermatosis (Odugbemi et al., 2007; Thepouyporn et al., 2012). *C. indica* plant possess many chemical

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https://doi.org/10.1016/j.sjbs.2021.05.072
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constituents like betulinic acid, oleonolic acid and traraxer-14-en-3- one, 5, 8- henicosdine, tetracosane and tricosane (Bachheti et al., 2013). The hemostatic effect of *C. indica* showed a significant reduction in the permeability of abdominal capillaries, clotting and bleeding time in mice (Al-Snafi, 2015).

Hence, an investigation on the toxicity and safety profile of *C. indica* leaves must be done to affirm further use of plant extract for natural drug discovery. In the current study, we researched the pharmacognostical profile of *C. indica* leaves using fluorescence and organoleptic microscopical assessments, counter-reaction with various tested reagents, total moisture content, ash value, foreign organic material, phytochemical and physicochemical properties. In addition, the pharmacological studies, including toxicity and safety profiles as well as anticholinesterase enzymatic inhibitory assay, researched.

2. Material and methods

2.1. Sample collection and extraction of C. indica leaves

The *C. indica* leaves collected from Ronzai farms, Saudi Arabia, in September (2019). The authentication of the plant affirmed from the Department of Pharmacognosy, Qassim University, Saudi Arabia (Ref. No.: QA/FOP/06). Approximately 50 g of grounded dried leaves added with 200 mL of methanol and macerated for 5 days. A systematic extraction was done from the residual plant material, and the process was repeated until a colorless supernatant liquid was acquired. Accordingly, the extract solution was filtered using a muslin cloth and subjected to the rotary evaporation and the obtained *C.indica* leaves extract of (CILE) was freeze-dried. The percentage yield of the extract was calculated CILE (Chigurupati et al., 2018).

2.2. Pharmacognostical studies - Fluorescence and organoleptic microscopical assessment

The fluorescence observation of CILE was examined by adding various reagents, i.e. sulphuric acid, sodium hydroxide, and nitric acid. As for organoleptic observation, CILE was examined by adding various reagents, i.e. neutral, acidic, and basic reagents. The microscopical observation for both fluorescence and organoleptic of CILE was recorded (Chase and Pratt, 1949).

2.3. Phytochemical analysis

Phytochemical analysis was carried out for CILE as per the standard methods (Majid et al., 2015; Roopashree et al., 2008). A series of phytochemical tests on the extract used to identify the presence of constituents such as saponin, flavonoid, gum, tannin, glycoside, protein, phenol, starch and carbohydrate (Chigurupati et al., 2017).

2.4. Physicochemical analysis

The physicochemical analysis was carried out for CILE as per the standard methods. The ash value for CILE was calculated by measuring the content of the inorganic residue after ignition at 650–700 °C (Roy et al., 2013). The percentage of foreign organic material and total ash was further measured. The moisture content was identified by the loss upon drying in terms of grams.

2.5. Anticholinesterase enzymatic inhibitory assay

Different concentrations (0.01–100 mg / mL) of CILE and Donepezil (standard) were prepared using 70% ethanol. The prepared samples were incubated with 1.5 mL of sodium phosphate buffer (0.1 M, pH 8.0) and 2 mL of acetylcholinesterase (AChE) solution (0.1 U/mL) at 25 °C for 15 min. Then 1 mL of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB 10 mM) and acetylcholine iodide (14 mM) were added into the reaction mixture. The reaction mixtures were incubated at room temperature for 10 min. The absorbance (Abs.) was taken at 410 nm (Chigurupati et al., 2016). The percentage of AchE inhibition was calculated using Eq. (1) and half-maximal inhibitory concentration (IC_{50}) is obtained from the non-linear regression graph plotted between percentage inhibitions (x-axis) versus extract concentration (y-axis).

$$\text{AchE Inhibition } (\%) = (\text{Abs. Control} - \text{Abs. Sample})/\text{Abs. Control} \times 100 \quad (1)$$

2.6. Statistical analyses

The experimental values were expressed as the mean ± Standard error mean (SEM). All the IC_{50} values for AchE inhibition assay were computed using the Graph Pad Prism Software (Version 5)

3. *In vitro* evaluation for toxicological and safety study

3.1. Hemolysis assay

The hemolytic rate of CILE and standard Triton X (negative control) was equilibrated normal saline (positive control) incubated with blood samples. The extracted 100 μL of rat's blood was incubated with an equilibrated amount of normal saline, CILE, and Triton X. Then, the reaction mixture was incubated for 1 h and the absorbance was taken at 450 nm (Suhag et al., 2017). The hemolytic rate (%) was calculated using Eq. (2).

$$\textit{Hemolytic rate } (\%) = (\text{Abs. Sample - Abs. Positive Control})/(\text{Abs. Positive Control - Abs. Negative Control}) \times 100 \quad (2)$$

3.2. Red blood cells (RBC) agglutination assay

The freshly extracted rat's blood was centrifuged for 10 min at 2000*g*. Pellets were then suspended with normal saline (1:9). 100 mL of the resuspended solution was then added with 600 mL of normal saline prepared as a stock solution. Normal saline and an equal quantity of CILE were added with 2 mL of stock solution. The reaction mixture was incubated at 37 °C for 1 h and coated cell suspension was viewed using a microscope (Shakeel et al., 2017).

3.3. In vivo evaluation for toxicity and safety study

The toxicological and safety evaluation of CILE was performed on experimental rodents, approved by the Institutional Animal Ethics Committee as per the guidelines of CPCSEA, New Delhi India (Approval No: RITS/IAEC/2016/07/07). Wistar albino rats of either gender weighing 200 g to 225 g were used in the experimental study. The rats were subdivided into groups: Normal control and CILE treated groups. For 2 weeks, the control group rats were given normal saline and treated groups received CILE (300 mg/kg per p.o). The functional observational battery (FOB) parameters were measured at 0, 10, 30, and 60 min after a single administration of treatments. The histological and biological evaluations were done on the 14th day of the experimental period.

3.4. Evaluation of the functional observational battery

Instantaneous detrimental effects of a single administration of CILE and normal control were recorded by FOB for behavioral changes (Suhag et al., 2017).

3.5. Reduced glutathione (GSH) estimation

The following rat's organs including the kidney, brain, liver, and heart were dissected for GSH estimation. Tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer, pH 7.4, and centrifuged with 5% trichloroacetic acid. 50 µL sample was added with 150 µL of 0.1 mM DNTB, 0.1 M phosphate (pH 6.0), 0.24 mM NADPH, 2 mM Ethylenediamine tetra acetic acid (EDTA) and 0.4 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer. The reaction mixture was vortexed and incubated for 25 min. The absorbance was taken at 412 nm. The GSH level was measured in µ mol/g wt of tissue and done in triplicated (n = 3).

3.6. Histological assessment

The isolated rat's organs including the kidney, brain liver, and heart were kept in 10% formalin solution for fixation and paraffin-embedded the tissues. The section was sliced into thin slices and stained in hematoxylin and eosin using standard procedure. The sections were observed under a microscope.

4. Results

4.1. Phytochemical and physicochemical screening

The present study explored several phytoconstituents, safety profile and demonstrated the neuroprotection activity of *C. indica.* Methanolic extraction of *C. indica* by maceration is more convenient, cost-effective, and produces more yield, the yield was found to be 14.6%. Presence of phytochemicals constants are shown in Table 1. The total ash value, foreign organic matter of CILE was 20% and 0.5%, respectively. As for loss on drying (Gravimetric method), the moisture content was found to be 0.1 w/w.

4.2. Pharmacognostical screening

CILE treated with various reagents, including sulphuric acid, sodium hydroxide, and nitric acid under different UV radiations, the fluorescence exhibited different color observations, as illustrated in Table 2. Likewise, the organoleptic properties of CILE treated with various reagents, namely, iron trichloride, glacial acetic acid, potassium hydroxide, iodine solution, sodium hydroxide, sulphuric acid, nitric acid, and hydrochloric acid displayed different colour and powder reactions, as illustrated in Table 3.

Table 1
Phytochemical analysis of CILE.

Phytochemical constituents	CILE Response
Saponin	–
Flavonoid	+
Gum	+
Tannin	+
Glycoside	+
Protein	+
Phenol	+
Alkaloid	+
Carbohydrate	+
Starch	+

Table 2
CILE fluorescence microscopical assessment.

Chemical reagent used	Fluorescence observations		
	Under ordinary light	Under UV light (366 nm)	Under UV light (254 nm)
50% Nitric acid	Golden-yellow	No observation	None
50% Sulphuric acid	Pale green	No observation	Green
1 N Sodium hydroxide (prepared in methanol)	Opaque green	No observation	Green
No chemical reagent used	Green	No observation	None

Table 3
CILE dried powder organoleptic microscopical assessment.

Reagent used	Organoleptic observation	
	Powder reaction	Color reaction
Glacial acetic acid	Powder descends down gradually	Green
Hydrochloric acid	Powder descends down gradually	Greenish-black
5% Sodium hydroxide (aqueous)	Powder descends down gradually	Red
Sulphuric acid	Powder descends down gradually	Black
Nitric acid	Powder descends down gradually	Brown
Iodine solution	Powder descends down instantaneously	Reddish-brown
5% Iron(III) chloride (aqueous)	The powder remains on the surface	Pale green
5% Potassium hydroxide (aqueous)	The powder remains on the surface	

4.3. Anticholinesterase enzymatic inhibitory assay

As illustrated in Fig. 1, CILE showed a comparable anticholinesterase inhibition in a dose-dependent manner with an IC_{50} value of 14.53 µg/mL compared to the standard Donepezil with an IC_{50} value of 13.31 µg/mL.

4.4. In vitro evaluation for toxicological and safety analysis

The hemolysis rate after an hour of CILE incubation as well as Triton X was quantified as compared with normal control saline. The result displays a significant increase in RBC hemolysis in the

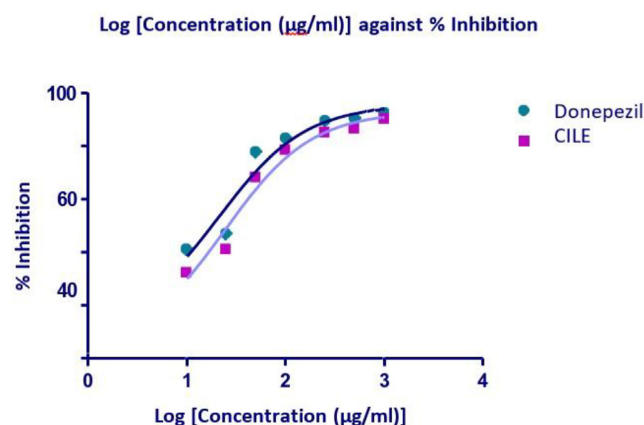

Fig. 1. AChE inhibition assay of CILE and Donepezil.

Triton X than normal saline. In contrast, CILE did not show significant agglutination in the hemolysis rate, 96.93%. Also, the RBC morphology (blood cell agglutination) is illustrated in Fig. 2.

4.5. In vivo evaluation for toxicological and safety analysis

The single oral administration of CILE (300 mg/kg) is unable to display any abnormal behavior, for instance, tremors, diarrhea, convulsions, fasciculations, vocalization, posture change, irregular urination, and urination. During handling and open-field activity, there was no adverse behavioral response seen. Thus, no significant behavioral change was seen in the FOB study upon CILE treatment when contrasted with the control group (Table 4).

The glutathione estimation outcomes illustrated absences of any changes in the reduced glutathione level within CILE tested. The results indicated no changes in the reduced glutathione level within CILE tested when contrasted with normal control, as portrayed in Fig. 3.

Histological results have observed an insignificant change in the organ morphology that was pre-treated with CILE when contrasted with normal control groups. Also, the photomicrograph indicated insignificant morphology change in the heart nuclei and myocytes myofibrils, brain hippocampal (CA1) region, kidney glomeruli, tubules, and parenchyma, liver hepatocytes and central vein when assessed with normal control rats (Fig. 4).

5. Discussion

Traditional approach including application of medicinal plants in the treatment of several diseases need to be explored for their various curable properties. The phytochemical analysis of CILE showed the presence of flavonoid, gum, tannin, glycoside, protein, phenol, alkaloid, starch, and carbohydrate. Especially phenol, alkaloid, tannin, and flavonoid are well known for their incredible properties to treat several diseases. However, phenols have been reported to treat related inflammatory disorders, wound healing, skin disorders, burns, and vascular abnormalities (Działo et al., 2016). Flavonoid is a well-known anti-oxidant to act against free radicals, diarrhea, inflammation, and hyperglycemia (Jung et al., 2014; Pietta, 2000). Proteins and carbohydrates are the fundamental molecular and cellular building blocks (Dimitrov, 2012). Additionally, carbohydrate and starch is a significant source of caloric consumption for metabolism and has a crucial function in the protein folding, immune defense, and blood clotting (Qureshi et al., 2011; Sunasee et al., 2014).

In pharmacognostical screening, distinct observations were made that can be used as authentication mark for CILE for further studies. Acetylcholinesterase inhibitor widely used in the treatment of Alzheimer's disease by inhibiting the breakdown of acetylcholine by acetylcholinesterase enzyme. CILE showed good anti-acetylcholinesterase potential when compared it with

Table 4
Effect of CILE on FOB parameters.

Categories	Normal control	CILE
Home cage		
Spontaneous activity level	3	3
Posture	2	2
Convulsions	Absent	Absent
Tremors	Absent	Absent
Fasiculations	Absent	Absent
Tonus	Absent	Absent
Clonus	Absent	Absent
Vocalization	Absent	Absent
Straubs tail	Absent	Absent
Writhing	Absent	Absent
Retropulsion	Absent	Absent
Diarrhea	Absent	Absent
Handheld		
Excitation	2	2
Salivation	0	0
Lacrimation	0	0
Piloerection	Absent	Absent
Fur appearance	Absent	Absent
Ptosis	Absent	Absent
Exophthalmia	Absent	Absent
Open cage		
Supported rears	5	5
Unsupported rears	0	0
Spontaneous activity level	4	4
Gait	1	1
Posture	2	2
Arousal	4	4
Convulsions	Absent	Absent
Straubs tail	Absent	Absent
Writhing	Absent	Absent
Retropulsion	Absent	Absent
Diarrhea	Absent	Absent
Stereotypy	Absent	Absent
Auditory response	3	3
Somatosensory response	3	3
Visual approach	Present	Present
Olfactory response	Present	Present
Pinna reflex	Present	Present
Extensor reflex	Present	Present
Palpebral reflex	Present	Present
Visual placing	Present	Present
Surface righting	Present	Present
Aerial righting	Present	Present
Pupil reaction	Present	Present
Tail pinch response	Present	Present
Urination spots	Present	Present

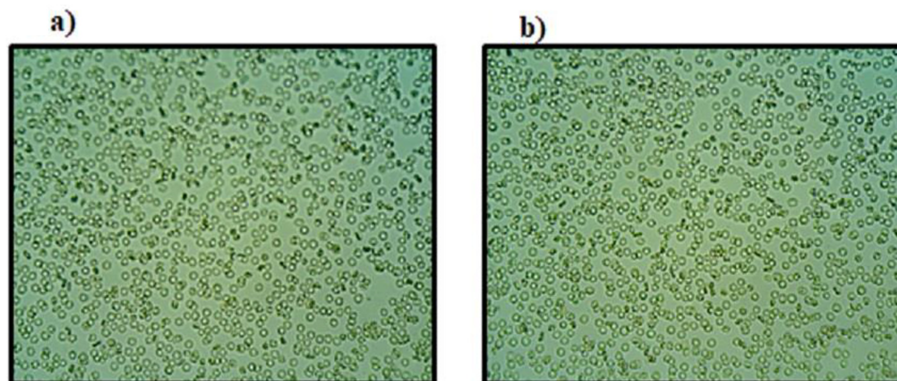

Fig. 2. Microscopy RBC examination for assaying hemagglutination for (a) normal control (b) CILE.

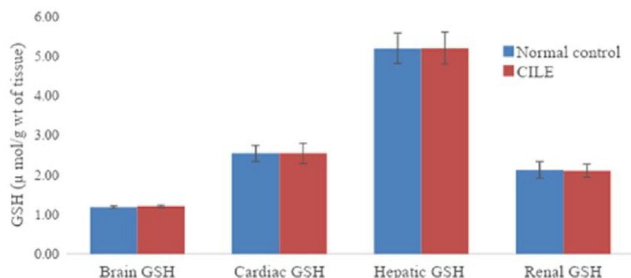

Fig. 3. The GSH level on different organs: brain, heart, liver, and kidney. All values are expressed in mean ± SD.

donepezil, that is known for its acetylcholinesterase inhibiting activity. So, CILE could be studied further for its anti-acetylcholinesterase activity against Alzheimer's disease.

In vitro hemolysis assays of CILE were performed to confirm that the treatment should not disrupt the integrity of RBCs. It was observed that CILE has not hasten the hemolysis rate and maintained the biconcave shape of RBCs. These results show that CILE does not affects the circulatory system and thus portrayed for the further biological activity without producing any toxic effect (Gong et al., 2010). Glutathione is a well-known anti-oxidant that possesses glutamate, cysteine and glycine and assists with retaining the enzymatic redox-sensitivity (Bulleid, 2012). Hence, glutathione can counteract the high cellular by-product (oxidative stress) that is linked with tissue damage susceptibility. The glutathione level was evaluated in kidney, brain, liver, and heart tissues to distinguish the detrimental effect of CILE on the natural antioxidant defense capability in vital organs (KumaráSharma, 2015). No reduction in glutathione level was observed in various tissue sections showing. Thus, CILE does not interfere with the redox system of body. The microscopic observation of several organs from different groups of rats were observed at 40x magnification for whichever indications of injury or damage. In histological study, some of insignificant morphological changes were observed in different tissue sections portraying that CILE has change the morphology of tissue samples to some extent. however some studies has shown the protective effect of methanolic extract of *C. indica* on tissues including study *by* Joshi et al. (2009) have concluded that aerial methanol extract of *C. indica* protected the CCl4 induced rat's liver against carbon tetrachloride-induced hepatotoxicity. So, further study needs to be performed for CILE protective action on morphology of different tissues.

6. Conclusion

The pharmacognostical description of *C. indica* leaves is evident identification as a candidate drug. The toxicity and safety findings of *C. indica* uncovered a non-detrimental effect of the methanolic extract on various organs seen. Likewise, the antioxidant potential of methanolic leaf extract attains significant stochastic therapeutic agents as oxidative stress is the primitive drawback of numerous incurable pathological circumstances and perturbed cellular signaling. The presence of various phytoconstituents within methanolic extract which lead to more prospect of applicable pharmacological mechanisms. The anticholinesterase enzymatic inhibitory assay by the extract is relatively comparable to standard donepezil, thus has a potential function as a neuroprotective agent. With this remark, the CILE has shown good antioxidant and neuroprotective potential in natural drug discovery Consequently, the plant extract must be examined further for isolation and classification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to the Deanship of Scientific Research, Qassim University, for funding publication of this project.

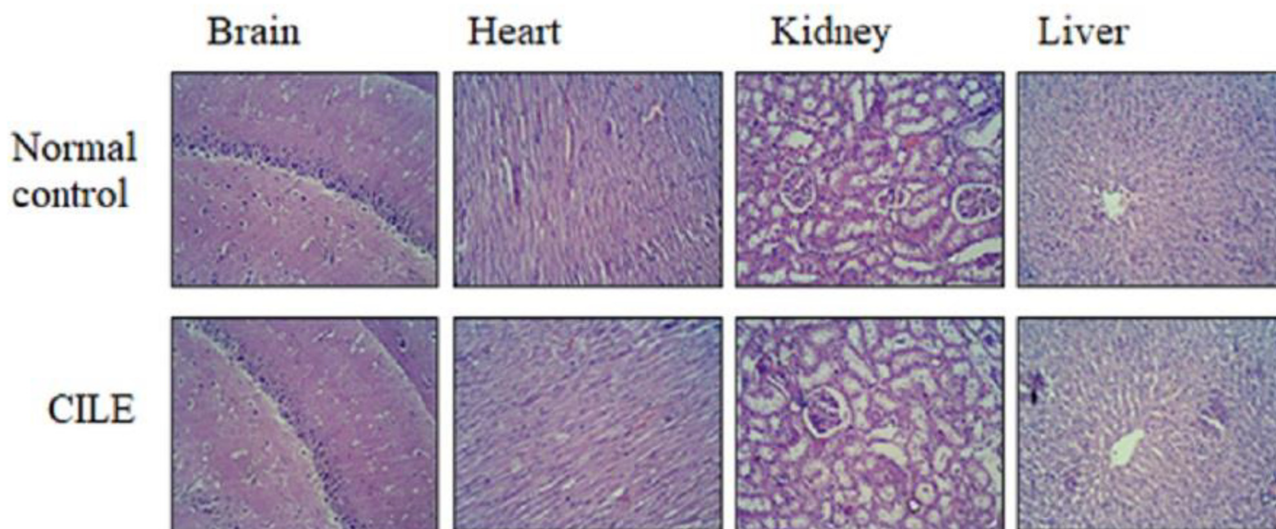

Fig. 4. The histological assessment of brain, heart, kidney, and liver obtained from each group of normal control and CILE treated animals at 40×.

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