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IN VIVO INVESTIGATION OF ANTIDIABETIC, HEPATOPROTECTIVE, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF *CENTAUREA TOUGOURENSIS* BOISS. & REUT

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Centaurea species are widely used in traditional medicine to treat several illnesses, especially by Mediterranean populations due to their pharmacological properties. The present study aimed to evaluate for the first time some *in vivo* activities of n-butanol (n-BuOH) extract of the aerial part of *Centaurea tougourensis*. For this approach; the antidiabetic (streptozotocin-induced diabetes), hepatoprotective (paracetamol induced hepatotoxicity), anti-inflammatory (croton oil-induced ear edema assay) and antipyretic activities of this plant extract were tested. The pharmacological results suggest that *C. tougourensis* has a non-negligible anti-inflammatory effect on the formation of ear edema with a maximum inhibition percentage of (39.58%) for the highest tested concentration of 400 mg/kg. However, the antipyretic activity of the plant was remarkable for both tested concentrations (200 and 400 mg/kg) 5 h after treatment with a significant ($P < 0.05$) reduction of rectal temperature to (32.88 ± 0.23ºC) and (32.36 ± 0.18ºC) which correspond to a pyrexia inhibition of (78.9%) and (90.18%) respectively. *C. tougourensis* exhibited also a good anti-hyperglycemic effect which reached an inhibition percentage of (68.29%) at the end of the 3rd week of treatment for the tested concentration of 400 mg/kg and was considered almost similar to those of standard value (71.83%) at the same time. The n-BuOH extract *C. tougourensis* showed also a remarkable hepatoprotective effect which was confirmed by biochemical and histological approaches of note is that natural silymarin was also used as reference drug and showed a remarkable hepatoprotective effect. These encouraging results demonstrated once again the pharmacological potential of Centaurea species.

Key words: *plant extract, streptozotocin-induced diabetes, antidiabetic, anti-inflammatory, antipyretic, hepatoprotective, Centaurea tougourensis, silymarin,* glibenclamide

INTRODUCTION

To maintain an optimal body temperature, a delicate balance between the production and loss of heat must be maintained, and this thermoregulation is insured by a key gland called hypothalamus which acts as thermoregulator center (1). Many exogenous pyrogens, including microbes are responsible of fever, but other reasons could explain the abnormal increase in temperature like transplant rejection or tissue damage (2). Unfortunately, the use of antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) to treat hyperthermia has a lot of side effect (3).

The inflammatory response, which involve key pro-inflammatory mediators called cytokines; is considered crucial for the organism defense system (4), but may lead when deregulated to hazardous and sometimes irreversible complications especially those linked to cardiovascular system which may generate in long term some complex chronic pathologies like rheumatoid arthritis, diabetes, heart disease and even cancer (5, 6). The scientific community is aware about the side effect of using non-steroidal anti-inflammatory drugs (NSAIDs) even if it remains the best alternative to effectively treat inflammatory illnesses (7). The actual progress in science especially in pharmacognosy domain suggest that plant may significantly contribute to treat illnesses related to inflammation, especially those related to autoimmune disorders (8).

The liver is considered a key organ to regulate metabolism due to its capacity to catalyse several reactions especially those that break down fats to produce energy (9), but also involved in

carbohydrates and proteins metabolisms by converting the excess of these molecules into triglyceride and fatty acids to be stored in adipose tissue (10). This organ has also other physiological roles and it is considered as the main center of detoxification, and this could be explained by the fact that many toxic substances transit at its level (11), which considerably contribute for the elimination of undesirable xenobiotics from the body.

Diabetes mellitus is a group of metabolic disorders characterized by a chronic hyperglycemia which can be the result of abnormal insulin secretion, due to a defect in beta-cells function or metabolic abnormalities (12). According to actual statistics, nearly 463 million people around the world are suffering from diabetes, especially in low-and middle-income countries (13), and these alarming results brings us back to revise our conception of therapy.

The actual lack of pharmacological and phytochemical studies on *C. tougourensis* explains our interest to investigate this species and the actual study is a continuation of the previous studies, in which we revealed some *in vitro* and *in vivo* properties of this species (14, 15). Another key reason is the remarkable biological properties of some *Centaurea* species including antioxidant, anti-inflammatory, cytotoxic, immunomodulatory, hemostatic, herbicide and antidiabetic activities due to their richness in various phenolic compounds (16-18).

This study consists of evaluating for the first time the possible antidiabetic, hepatoprotective, anti-inflammatory and antipyretic activities of an endemic plant species from the Aures region (Algeria) named *Centaurea tougourensis*.

MATERIALS AND METHODS

Chemical and reagents

All solvents and standards used in this study were purchased from Sigma Aldrich, Steinheim, Germany. This includes; acetone (C_3H_6O), brewer's yeast, croton oil, eosin ($C_{20}H_6Br_4Na_2O_5$), ethyl acetate, ethanol, formaldehyde (CH_2O), hematoxylin ($C_{16}H_{14}O_6$), indomethacin ($C_{19}H_{16}ClNO_4$), industrial silymarin, methanol, n-butanol (n-BuOH), nicotinamide ($C_6H_6N_2O$), paracetamol ($C_8H_9NO_2$), streptozotocin ($C_8H_{15}N_3O_7$).

Plant material

Centaurea tougourensis was collected in spring 2019 at Belezma National Park in the municipality of Fesdis (Algeria) (GPS coordinates: latitude 35.621975; longitude 6.241327) and was identified by experts in the field from agronomic department of Batna-1 University (Algeria). A voucher specimen under the code (CT/2019/LPTPCMB) was deposited at the Laboratory of Improvement of the Phytosanitary Protection Techniques in Mountainous Agrosystems, Agronomy Department, Institute of veterinary and Agricultural sciences, University of Batna-1, Batna, Algeria.

Experimental animals

Male Swiss Albino mice (25 – 30 g) were purchased from Pasteur Institute (Algiers) and maintained at ambient temperature (22 ± 1ºC), 12 h light/dark cycle with free access to feed and water for two weeks to acclimatize with laboratory conditions.

The experiments were performed as recommended by the National Research Council guidelines (19) and approved by the Biology Animal Ethics Committee of University of Batna-2, Algeria (approval no. 14/DBO/FSNV/UB2/2017).

Preparation of plant extracts

The aerial parts of *C. tougourensis* were dried in a dry and ventilated place, away from the sun's rays and then ground to obtain (300 g) of fine powder. Maceration was carried out three times with 3L EtOH-H_20 (70:30) at room temperature for 3 days. After liquid-liquid extraction with hexane, ethyl acetate and n-butanol solvents, 1.58% of n-butanol, 1.03% of ethyl acetate and 0.42% of n-hexane extracts were obtained.

Croton oil-induced ear edema assay

In this test, the edema was caused at the level of the inner surface of the right or left animal ear *via* an application of 20 µl of a solution containing 80 µg of croton oil prepared in acetone-water solution (1: 1 V/V) which is considered as irritant solution. Mice's ear which received this application was considered croton oil control group while the other ear not treated with croton oil served as negative control and received only 0.9% normal saline solution. Another group served as positive control and received, in addition to the irritant solution, 0.5 mg indomethacin (20 mg/kg) which was considered as the standard. Two other groups received respectively in addition to the irritant, 2 mg of the n-butanolic extract of *C. tougourensis* (200 and 400 mg/kg). Noting that the diameter and weight of each ear were measured using a digital caliper before the treatment then 2h and 4h after the treatment (20).

The following equation was used to calculate the inhibition percentage:

$$\%\ \text{Inhibition} = [\frac{(\text{MEE}_{\text{cont}} - \text{MEE}_{\text{treat}})}{\text{MEE}_{\text{cont}}}]\text{x } 100$$

MEE_{cont}: represents the mean ear edema volume in the negative control group at a given time; MEE_{treat}: represents the mean ear edema in the extract or standard group at a given time.

Streptozotocin-induced diabetes

In order to evaluate this activity, the approach of Kim *et al.* (21) was used. Induced diabetes in mice was performed by a single intraperitoneal injection of streptozotocin (STZ) at the concentration of 50 mg/kg; preceded by another injection of nicotinamide (vitamin B3) at a concentration of 120 mg/kg to avoid total destruction of pancreatic β cells by STZ. After 72 h, the mice presenting a glycemia near or greater than 200 mg/dL were selected for this study. The diabetic mice were subdivided into four groups (n = 6) and received for 15 days *via* gastric gavage their respective treatment; Group 1: received NaCl solution (0.9%, 10 mL/kg, p.o.) and was considered streptozotocin-control group; Group 2: was considered standard group and received glibenclamide (5 mg/kg, i.p.), Group 3 and 4 received respectively a concentration of 200 and 400 mg/kg, p.o. of the n-butanolic extract of *C. tougourensis*. Noting that a 5th group was also used for this experiment and was not treated by streptozotocin and considered as negative control group. Blood samples were obtained from the tail vein and were measured before and then during the 1st, 5th, 10th, 15th days after induction of diabetes, using a glucometer (issucare model).

In addition, the following formula was used to calculate the inhibition percentage of hyperglycemia:

$$\%\ \text{Inhibition} = [\frac{(\text{A}_{\text{cont}} - \text{A}_{\text{treat}})}{\text{A}_{\text{cont}}}]\text{x } 100$$

A_{cont}: represents the average glycemia in the negative control group at a given time; A_{treat}: represents the average glycemia in the extract or standard group at a given time.

Hepatoprotective activity

This activity was tested for one week using paracetamol as hepatotoxic agent. For this purpose; two types of silymarin were used to compare their protective effects; an industrial one and another purified from *Silybum marianum* extract. Mice were subdivided into: Group 1: served as normal control and received only 0.9% normal saline solution (1 mL/kg per day, intraperitoneally) for 7 consecutive days, while the 2nd group was considered as paracetamol-control group and received only paracetamol (250 mg/kg) for 7 consecutive days. The 3rd and 4th received respectively the industrial and natural silymarin at a concentration of 100 mg/kg per day, for 7 consecutive days as well as paracetamol (250 mg/kg per day, intraperitoneally), 3 hours after administration of silymarin. In groups 5 and 6, mice received respectively two different concentrations of n-butanol extract of *C. tougourensis* (200 and 400 mg/kg per day, intraperitoneally) each day for 7 days, followed by paracetamol (250 mg/kg, intraperitoneally) 3 h after administration of the extracts (22).

Antipyretic assay

The possible antipyretic activity of *C. tougourensis* was evaluated using yeast-induced hyperthermia model (23). The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by dorsolateral injection of 20% aqueous suspension of Brewer's yeast (10 mL/kg, p.o.). All groups were fasted overnight with free accesses to water for 16 hours. Then, the rectal temperature of each mouse was recorded and only animals showing an increase in temperature of at least 0.5ºC were selected for the study. Mice were then divided into four groups (n = 6) as follows: Group 1: served as negative control and received NaCl (0.9%, 10 mL/kg, p.o.), Group 2: served as positive control and received paracetamol (150 mg/kg, i.p.), Group 3 and 4 received respectively 200 and 400 mg/kg, p.o. of n-butanol extract of *C. tougourensis*. After treatment, the rectal temperature of each group was recorded at 1 h, 2 h, 3 h, 4 h and 5 h and the following equation was used to calculate the inhibition percentage of pyrexia:

$$\% \text{ Inhibition } = \left(\frac{B - Cn}{B - A}\right) x\ 100$$

Where B represents the rectal temperature after pyrexia injection; Cn represents the rectal temperature after 1, 2, 3, 4 and 5 h of treatment; A = normal body temperature.

Measurement of biochemical parameters in antidiabetic and hepatoprotective assays

The blood samples of anesthetized mice were collected from the jugular vein using a needle mounted on a syringe and transferred into adequate tubes. The blood was analyzed for biochemical parameters (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), phosphatases alcalines (PAL), blood sugar level, urea, creatinine, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and cholesterol).

Histological examination

At the end of the antidiabetic, hepatoprotective and anti-inflammatory experiments; the mice were sacrificed and the pancreas, liver and ears tissues of each group were removed and fixed in 10% formaldehyde solution and then decalcified with Plank-Rychlo's solution. After that, they were processed with a graded mixture of ethanol and xylene, and then embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) as a preparation for histological evaluation.

Statistical analysis

Data obtained from this study were expressed as mean ± SEM and the statistical analyses were performed by One-way ANOVA followed by multiple Dunnet's test, using GraphPad Prism version 8 (California corporation, USA). Results were considered significant at $p < 0.05$, $p < 0.01$, $p < 0.001$.

RESULTS

In-vivo anti-inflammatory assay

As noted in *Table 1*, the n-BuOH extract of *C. tougourensis* showed a non-negligible suppression of ear edema induced by croton-oil with a maximum inhibition percentage of (39.58%) for the highest tested concentration of 400 mg/kg, while the indomethacin value was slightly higher (47.91%). These results were considered highly significant ($p < 0.001$) when compared to control group. Noting that a small weight variation (10.35%) was observed in the group of mice which were treated with n-BuOH extract (400 mg/kg), while those of control group was important (38.27%).

The histopathological investigation of ears revealed that groups of mice which were treated with standard (indomethacin) (20 mg/kg) (*Fig. 1C*) or both n-BuOH extracts (200 and 400 mg/kg) of *C. tougourensis* (*Fig. 1D* and *1E*) preserved ear thickness and integrity. However, an epidermal hyperplasia was observed in the group, which received croton-oil application in their right ears (*Fig. 1B*) and was boarded by an important neutrophil infiltration, which spread until cartilage and striated muscle areas. This suggest and important

Table 1. Effect of n-butanolic extract of *C. tougourensis* on croton-oil induced ear edema in mice.

Treatment	Dose	Ear diameter (mm)			Ear weight (mg)		
		Before treatment	After treatment		Left ear (witness)	Right ear (treated)	Weight variation (%)
			2 h	4 h			
Control	–	0.28 ± 0.02	0.45 ± 0.01	0.48 ± 0.01	37.15 ± 1.61	75.42 ± 2.79	38.27%
Indomethacin	20 mg/kg	0.23 ± 0.01[a]	0.26 ± 0.01[c] (42.22%)	0.25 ± 0.00[c] (47.91%)	37.82 ± 2.81[ns]	42.26 ± 2.06[c]	4.44%
n-BuOH extract	200 mg/kg	0.26 ± 0.01[ns]	0.36 ± 0.01[c] (20%)	0.35 ± 0.01[c] (27.08%)	36.11 ± 2.2[ns]	52.53 ± 3.25[b]	16.42%
n-BuOH extract	400 mg/kg	0.26 ± 0.01[ns]	0.32 ± 0.01[c] (28.96%)	0.29 ± 0.01[c] (39.58%)	33.56 ± 0.69[ns]	43.91 ± 2.66[c]	10.35%

Values are mean ± SEM, n = 6. One way ANOVA followed by multiple Dunnet's test. Level of Significance [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared with control group, [ns] is no significant. Values given in parentheses represent percentage inhibition of ear edema.

inflammatory response. We also noted the presence of several edema in dermal layer.

Antidiabetic assay

The results of antidiabetic activity of the plant are shown in *Fig. 2*. The n-BuOH extract of *C. tougourensis* has significantly reduced the hyperglycemia exerted by streptozotocin especially for tested concentration of 400 mg/kg. The blood glucose level was reduced gradually from the 1st day of treatment to reach the value of (93.2 ± 3.61 mg/dL) at the end of the 3rd week, which corresponds to an inhibition percentage of hyperglycemia of (68.29%). It is also interesting to see that these values are close to those expressed by the standard glibenclamide (5 mg/kg), which showed an average glycaemia of (82.8 ± 2.31 mg/dL), corresponding to inhibition percentage of (71.83%). Noting that these data were considered highly significant ($p < 0.001$) when compared to diabetic-control group.

The groups of mice treated by *C. tougourensis* (400 mg/kg) showed also a significant decrease ($p < 0.01$) of various biochemical parameters tested in this study, especially urea (0.26 ± 0.02 g/L), ASAT (270 ± 1.3 U/L) and ALAT (40.5 ± 2 U/L),

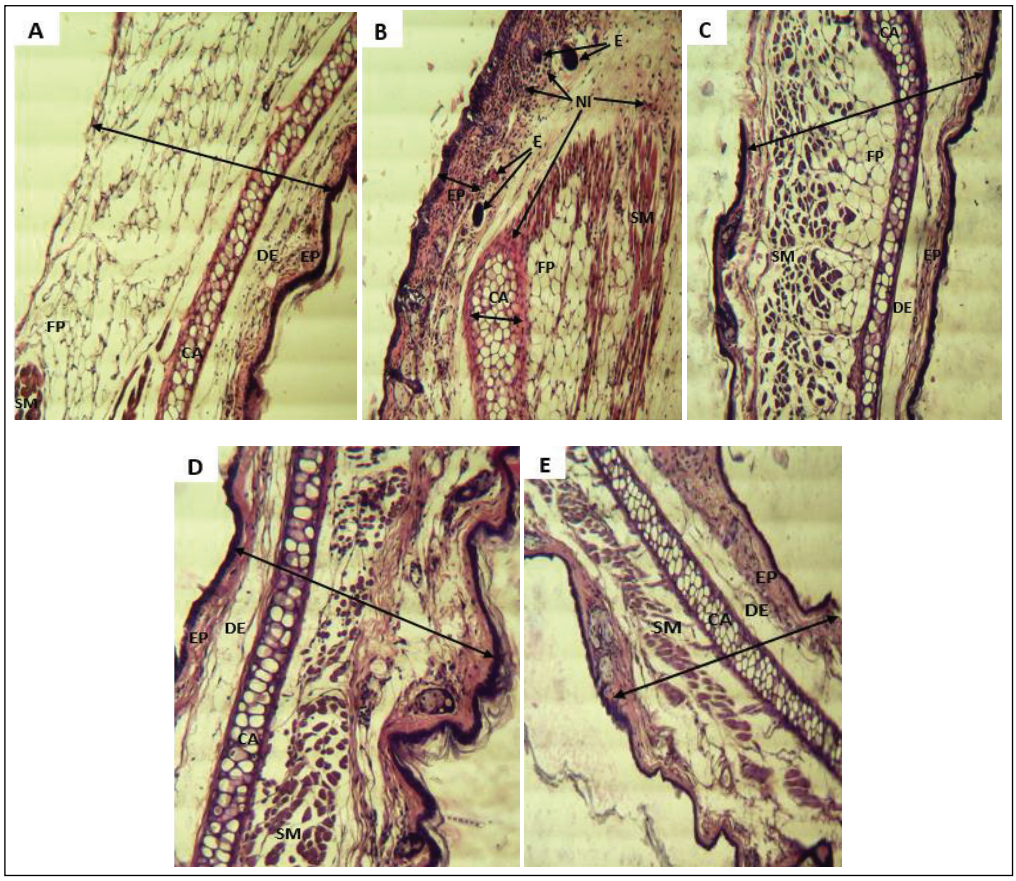

Fig. 1. Results of histopathologic examination of mice ear tissues (H&E stain, magnification × 100); (A): Normal control, (B): Croton oil-control, (C): Indomethacin (20 mg/kg), (D): n-BuOH extract (200 mg/kg), (E): n-BuOH extract (400 mg/kg).
CA - cartilage layer, DE - dermal layer, E - edema; EP - epidermal layer, FP - fatty part, SM - striated muscle, NI - neutrophil infiltration.

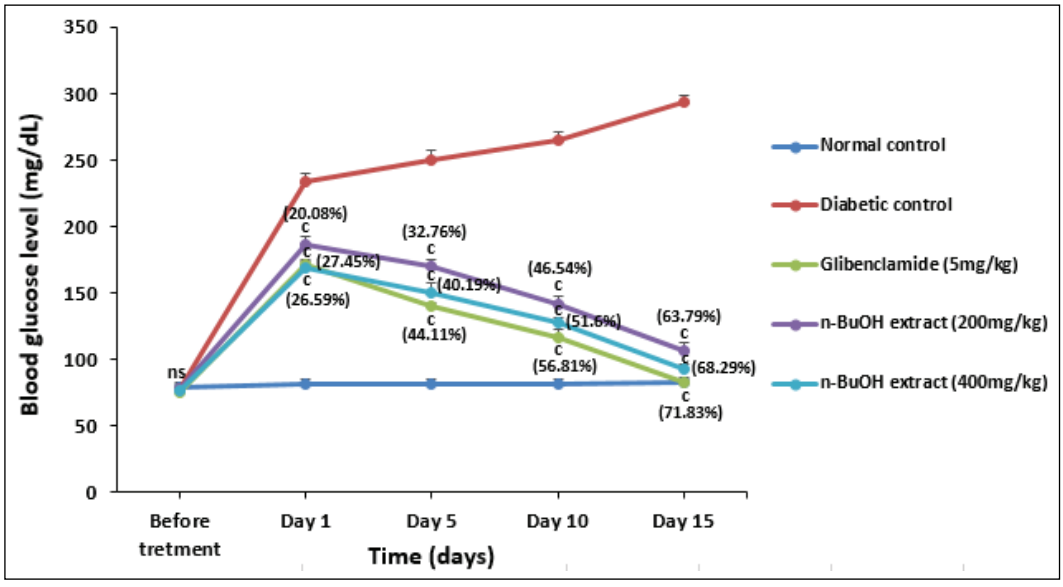

Fig. 2. Effect of *C. tougourensis* extracts on blood glucose level. Values are expressed as mean ± SEM (n = 6). One way ANOVA followed by multiple Dunnet's test. Level of significance $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ is statistically significant with a comparison to diabetic control group. Values given in parentheses represent percentage inhibition of hyperglycemia.

when compared to diabetic control group, and these values were considered more considerable than the tested standard glibenclamide. However, the values of triglycerides, total cholesterol, HDL-C and LDL-C were elevated in almost all treated groups (*Table 2*).

The histopathological investigation of liver tissues revealed that the groups of mice treated with standard glibenclamide (5 mg/kg), or n-BuOH extract at a concentration of 400 mg/kg of *C. tougourensis* didn't present any signs of inflammation, which is not observed in the sinusoids, but the presence of granulocytes (yellow arrow) around the central vein was noted, and even disseminated in the liver parenchyma (*Fig. 3C*). Another phenomenon was observed especially in the liver treated by the extract (400 mg/kg), which is expressed by a vacuolization explaining the installation of a sclerosis or the existence of a steatosis (*Fig. 3E*). We noticed an important neutrophil infiltration (yellow arrow) in diabetic-control group, a necrosis (Blue arrow) and a vascular congestion around the central vein (red circle) in this group (*Fig. 3B*).

Concerning the mice group which were treated with n-BuOH extract at a concentration of 200 mg/kg, the lesion shows a discrete congestion, which attenuation could be the result of plant action, whereas we noted the appearance of cell necrosis as it is shown by the blue arrows (*Fig. 3D*). This could be due to inflammatory response process, which suggests the onset of the immunological response.

Regarding the histopathological investigation of pancreas, it revealed that groups of mice which were treated with both n-BuOH extracts (200 and 400 mg/kg) of *C. tougourensis* (*Fig. 4D* and *4E*), or standard glibenclamide (5 mg/kg) (*Fig. 4C*) preserved the islets of Langerhans integrity (green arrow), and didn't present any signs of inflammation. However, several vascular congestions (red circle) were observed in diabetic-control group (*Fig. 4B*).

Table 2. Effect of *C. tougourensis* extract and glibenclamide on various biochemical parameters in streptozotocin-induced diabetic mice.

Biochemical parameters	**Normal control**	**STZ treated groups**			
		Diabetic-control	**Glibenclamide** (5 mg/kg)	**n-BuOH** (200 mg/kg)	**n-BuOH** (400 mg/kg)
Urea (g/L)	0.235 ± 0.01	0.34 ± 0.02	0.28 ± 0.04^{ns}	0.31 ± 0.04^{ns}	0.26 ± 0.02^{a}
Creatinine (mg/L)	4 ± 0.00	4 ± 0.00	4 ± 0.00^{ns}	4 ± 0.00^{ns}	4 ± 0.00^{ns}
ASAT (U/L)	238 ± 3.5	414 ± 2.81	325 ± 2.26^{a}	335 ± 1.65^{ns}	270 ± 1.3^{b}
ALAT (U/L)	30.5 ± 1.75	64 ± 2.48	48.5 ± 2.75^{b}	51 ± 2.96^{a}	40.5 ± 2^{b}
Triglycerides (g/L)	1.16 ± 0.04	1.73 ± 0.25	1.51 ± 0.46^{ns}	1.74 ± 0.25^{ns}	1.72 ± 0.28^{ns}
Total cholesterol (g/L)	0.85 ± 0.03	1.26 ± 0.03	0.94 ± 0.04^{a}	1.31 ± 0.13^{ns}	1.26 ± 0.17^{ns}
HDL-C (g/L)	0.52 ± 0.02	0.74 ± 0.02	0.51 ± 0.1^{a}	0.83 ± 0.11^{ns}	0.83 ± 0.09^{ns}
LDL-C (g/L)	0.05 ± 0.01	0.20 ± 0.04	0.19 ± 0.12^{ns}	0.16 ± 0.02^{ns}	0.08 ± 0.02^{b}

All the values are mean ± SEM, n = 6. One way ANOVA followed by multiple Dunnet's test. Level of significance $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared with diabetic-control group, ^{ns} is no significant. ALAT, alanine aminotransferase; ASAT, aspartate-aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

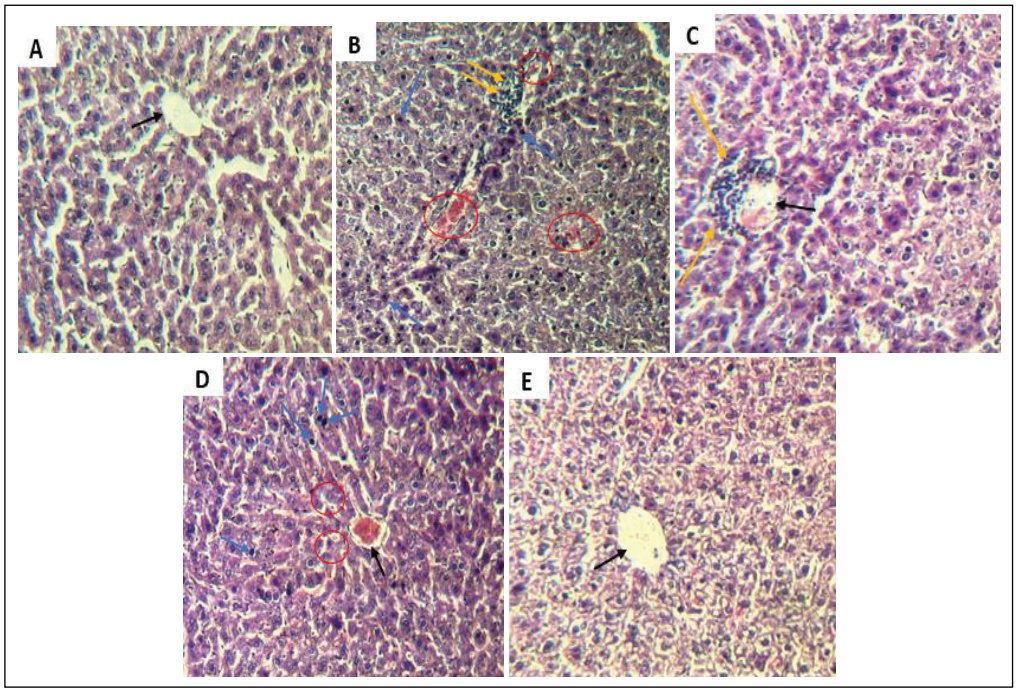

Fig. 3. Results of histopathologic examination of mice liver tissues (H&E stain, magnification × 100); (A): Normal control, (B): Diabetic-control, (C): Glibenclamide (5 mg/kg), (D): n- BuOH extract (200 mg/kg), (E): n- BuOH extract (400 mg/kg).

Hepatoprotective activity

Regarding the hepatoprotective activity, the biochemical results from this study clearly indicated that *C. tougourensis* has a good hepatoprotective effect with both tested concentration (200 and 400 mg/kg), but was considered more effective with the highest tested concentration, and this for all tested parameters when compared to paracetamol-control group (*Table 3*).

In this study, a natural silymarin was also tested and showed almost similar results with industrial silymarin especially for dosed parameters: urea, creatinine and ALAT. However, ASAT and PAL values were considered better for the group treated by industrial silymarin. Noting that the influence of natural silymarin on the four tested parameters related to lipid metabolism was also comparable with industrial silymarin values.

The histopathological investigation revealed that the groups of mice which were treated with both n-BuOH extract (200 and 400 mg/kg) of *C. tougourensis* didn't present any signs of inflammation (*Fig. 5E* and *5F*). However, a vascular congestion

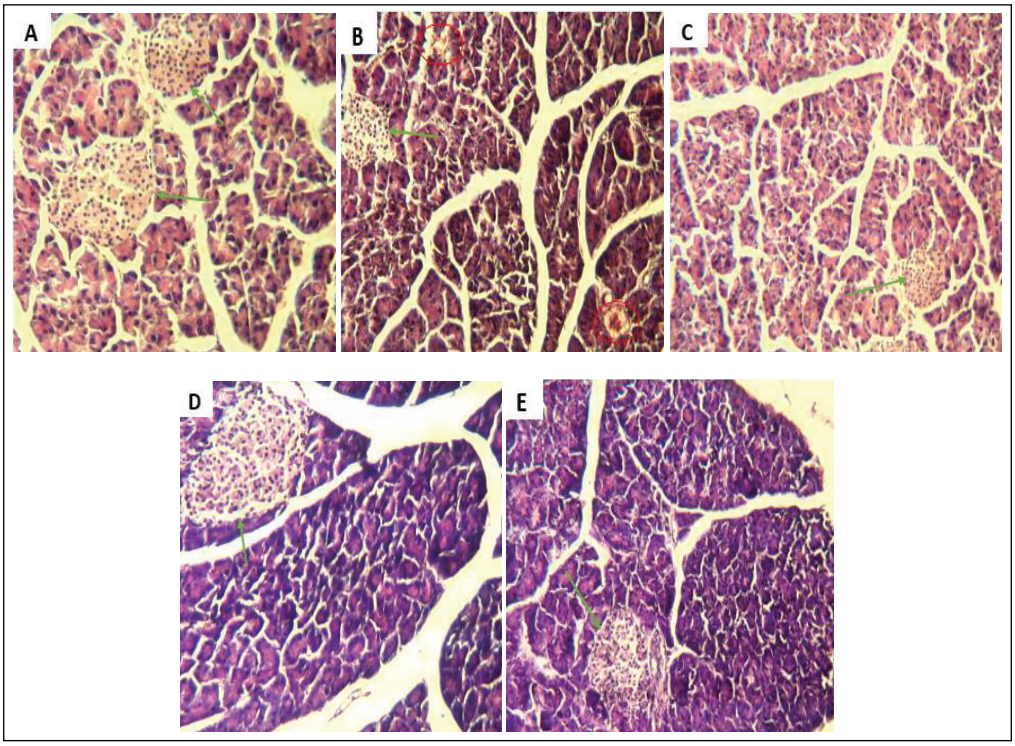

Fig. 4. Results of histopathologic examination of mice pancreas tissues (H&E stain, magnification × 100); (A): Normal control, (B): Diabetic-control, (C): Glibenclamide (5 mg/kg), (D): n-BuOH extract (200 mg/kg), (E): n-BuOH extract (400 mg/kg).

Table 3. Effect of *C. tougourensis* extract and two types of silymarin on various biochemical parameters in paracetamol-induced hepatotoxicity in mice.

Biochemical parameters	**Normal control**	**Paracetamol treated groups**				
		Paracetamol-control	**Industrial silymarin** (100 mg/kg)	**Natural silymarin** (100 mg/kg)	**n-BuOH** (200 mg/kg)	**n-BuOH** (400 mg/kg)
Urea (mmol/L)	0.10 ± 0.00	0.21 ± 0.01	0.16 ± 0.03^{ns}	0.15 ± 0.03^{a}	0.16 ± 0.01^{ns}	0.15 ± 0.01^{a}
Creatinine (mmol/L)	0.18 ± 0.05	0.26 ± 0.06	0.23 ± 0.1^{ns}	0.23 ± 0.04^{ns}	0.2 ± 0.1^{ns}	0.21 ± 0.02^{ns}
ASAT (U/L)	69.08 ± 1.57	94. 96 ± 1.6	64 ± 2.37^{b}	84.07 ± 1.82^{ns}	69.42 ± 0.25^{b}	67.19 ± 1^{b}
ALAT (U/L)	19.98 ± 1.11	30.4 ± 0.25	19.75 ± 0.35^{a}	22.2 ± 0.28^{ns}	19.43 ± 2.45^{a}	10.29 ± 1.35^{c}
Alkaline phosphatase (U/L)	35.65 ± 2.75	53.84 ± 1.4	48.05 ± 1.64^{ns}	59.27 ± 0.81^{ns}	43.96 ± 0.88^{a}	36.65 ± 1.15^{b}
Triglycerides (mmol/L)	0.20 ± 0.00	0.22 ± 0.02	0.21 ± 0.08^{ns}	0.23 ± 0.06^{ns}	0.3 ± 0.03^{ns}	0.2 ± 0.01^{ns}
Total cholesterol (mmol/L)	0.3 ± 0.04	0.5 ± 0.01	0.39 ± 0.07^{ns}	0.39 ± 0.05^{ns}	0.33 ± 0.02^{ns}	0.34 ± 0.09^{ns}
HDL-C (mmol/L)	0.25 ± 0.05	0.37 ± 0.05	0.26 ± 0.03^{ns}	0.28 ± 0.06^{ns}	0.27 ± 0.06^{ns}	0.21 ± 0.05^{a}
LDL-C (mmol/L)	0.03 ± 0.00	0.05 ± 0.00^{ns}	0.04 ± 0.01^{ns}	0.03 ± 0.00^{ns}	0.04 ± 0.00^{ns}	0.03 ± 0.00^{ns}

All the values are mean ± SEM, n = 6. One way ANOVA followed by multiple Dunnet's test. Level of Significance $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared with paracetamol-control group, ^{ns} is no significant. ALAT, alanine aminotransferase; ASAT, aspartate-aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

(red circle) was observed in the group treated with natural silymarin (100 mg/kg) but the general aspect of hepatocytes was normal and a few necrosis (blue arrow) were observed in the group treated with industrial silymarin (100 mg/kg) (*Fig. 5C* and *5D*) respectively.

A severe vascular congestion was observed in paracetamol-control group with an important neutrophil infiltration (green arrow) and a restrained necrosis (*Fig. 5B*).

Antipyretic activity

As shown in *Table 4*, the obtained results revealed that 16 h after fever induction by yeast; both *C. tougourensis* concentrations (200 and 400 mg/kg) showed an antipyretic effect but more considerable results were found for the highest tested concentration of (400 mg/kg), with a corresponding rectal temperature reduced to (32.36 ± 0.18ºC) 5 h after treatment. It is also interesting to observe that during the first hours of treatment, the percent inhibition of hyperthermia was almost the same for paracetamol (150 mg/kg) and *C. tougourensis* (400 mg/kg) groups, and was considered in general significant ($P < 0.05$) when compared to control group. However, during the 4 h and 5 h hours following the treatment; the antipyretic property of our plant was higher than paracetamol group at the concentration of (400 mg/kg) and reached (90.18%) for *C. tougourensis* group, while the value of paracetamol group was only (79.2%).

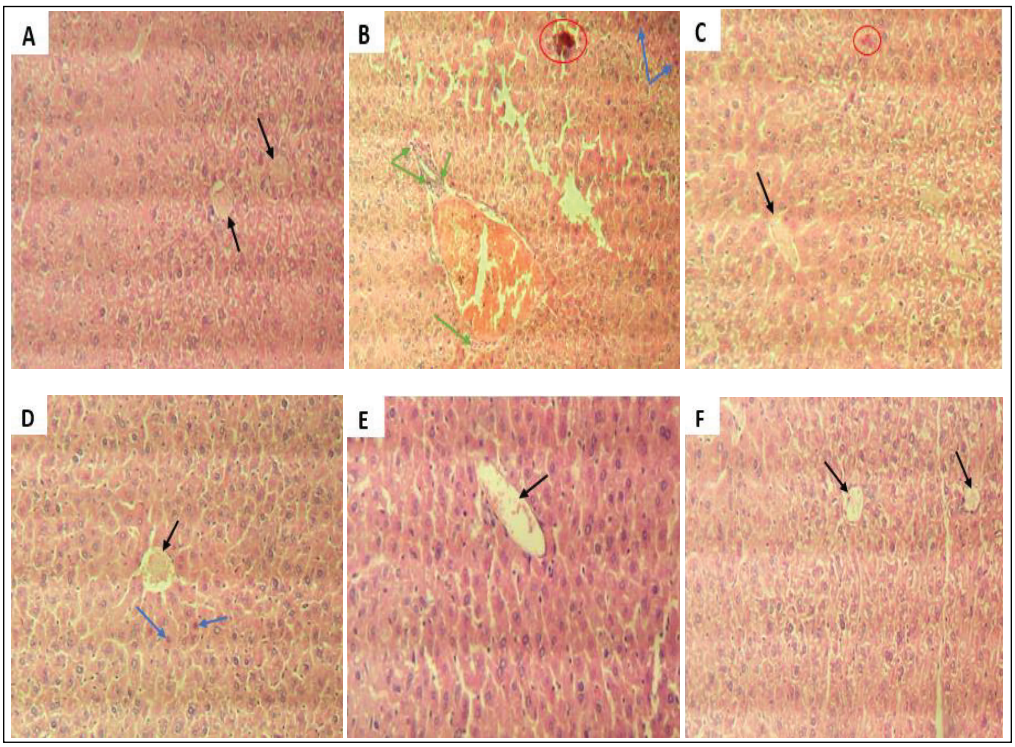

Fig. 5. Results of histopathologic examination of mice liver tissues (H&E stain, magnification × 100); (A): Normal control, (B): Paracetamol-control, (C): Natural silymarin (100 mg/kg), (D): Industrial silymarin (100 mg/kg), (E): n-BuOH extract (200 mg/kg), (F): n-BuOH extract (400 mg/kg).

Table 4. Effect of n-butanolic extract of *C. tougourensis* in yeast induced pyrexia before and after yeast injection.

Treatment	**Dose**	**Temperature** (°C)						
		Normal	**16 h after yeast injection**			**After treatment**		
				1 h	**2 h**	**3 h**	**4 h**	**5 h**
Control	–	32.66 ± 0.22	34.73 ± 0.24	34.66 ± 0.24	34.48 ± 0.26	34.41 ± 0.25	34.08 ± 0.27	33.56 ± 0.23
Paracetamol	**150** mg/kg	32.15 ± 0.23^{ns}	34.9 ± 0.23^{ns}	33.93 ± 0.11^{ns} (35.27%)	33.48 ± 0.16^{a} (51.63%)	33.13 ± 0.12^{b} (64.36%)	32.96 ± 0.14^{b} (70.54%)	32.73 ± 0.17^{a} (79.2%)
n-BuOH extract	**200** mg/kg	32.46 ± 0.24^{ns}	34.48 ± 0.18^{ns}	34.1 ± 0.23^{ns} (18.81%)	33.85 ± 0.26^{ns} (31.18%)	33.56 ± 0.3^{a} (45.54%)	33.21 ± 0.29^{a} (62.87%)	32.88 ± 0.23^{ns} (78.9%)
n-BuOH extract	**400** mg/kg	32.1 ± 0.22^{ns}	34.75 ± 0.27^{ns}	33.76 ± 0.31^{a} (37.35%)	33.43 ± 0.29^{a} (49.84%)	33.06 ± 0.26^{b} (63.77%)	32.53 ± 0.2^{c} (83.75%)	32.36 ± 0.18^{b} (90.18%)

All the values are mean ± SEM, n = 6. One way ANOVA followed by multiple Dunnet's test. Level of significance $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared with control group, ^{ns} is no significant. Values given in parentheses indicate percentage inhibition of pyrexia.

DISCUSSION

Plants are vital for the stability of ecosystem, and essential for humans and all living organisms, and their role is not just limited to providing us with oxygen and nutrients (24), but plays also a key role in biotechnology area, as a new source of energy called clean energy (25). Their pharmacological properties are also remarkable. The phytochemical screening of *C. tougourensis* revealed the richness of this species in various classes of secondary metabolites, including tannins, flavonoids, triterpenes, alkaloids, saponins, mucilage, quinones and anthocyanins. Noting that a high polyphenol yield was observed the n-BuOH extract of this plant (26). Several studies reported that flavonoids are able to prevent the activation of a key transcription factor called nuclear factor-kappa (NF-κB), that play a crucial role in the establishment of immune and inflammatory responses, but these flavonoids may also prevent the interaction of this factor with specific DNA binding sites of several pro-inflammatory mediators (27), since we know that NF-κB is responsible of many proinflammatory pathways and cytokines activation such as lipoxygenases (LOX), cyclooxygenase 2 (COX-2), tumor necrosis factor-alpha (TNF-α) and several interleukins (IL-1, IL-6 and IL-8). Goettert *et al.* (28) suspected that flavonoid may generated this anti-inflammatory effect *via* p38 MAPK pathway but could also prevent mRNA transcription of inducible nitric oxide synthase (iNOS) (29), which represent a key pro-inflammatory agent.

Serotonin is an important neurotransmitter that coordinate neurons communication, helping human controlling his emotion, overcome stress, digestion of nutrients, development of the brain functions of the fetus (30, 31), but is also considered a key pro-inflammatory agent (32), generating these effects by acting on his respective receptor called serotonin 1A receptor (5-HT1A) or on other subtypes of this receptor such as 5-HT2A and 5-HT6. A study made by Hassan *et al.* (33) on five medicinal plants, reported a highly significant anti-inflammatory effect of these species on the development of edema, and suspected that the high saponin proportions in their plants may inhibited the release of, histamine, serotonin and kinins, considered as the principal pro-inflammatory mediators during the first phase of the inflammatory response, which will automatically block the release of the inflammatory mediators of the second phase. Polyphenols can also exert a non-negligible immunomodulatory effect to reduce mast cells and neutrophils degranulation, since these cells contains a high proportions of histamine and serotonin, to prevent the onset of the inflammatory response related to these compounds (34, 35).

Plants are also known for their remarkable antidiabetic effect, especially species of the genus *Centaurea* such as *C. alexanderina* which demonstrated a remarkable ability to regulate blood glucose level in different diabetes pathologies especially type 2 diabetes which is marked by an abnormal elevation of glycemia level (36). Several studies (37, 38) reported that during diabetes, phytocompounds such as tannins, saponins and alkaloids may actually increase the sensitivity and secretion of insulin to decrease the blood glucose level to physiological state which may explain the anti-hyperglycemic effect of *C. tougourensis*. Interestingly, in our previous investigation (15), we revealed the hypoglycemic effect of this plant using oral glucose tolerance test (OGTT) which is a good preliminary test to detect the possible antidiabetic effect of a specific plant species.

It's well known that the risk to develop type 2 diabetes is dramatically linked to an abnormal elevation in blood concentration of creatinine, urea, ALAT and ASAT (39, 40), considered the principal markers of renal and liver dysfunction but also responsible of pancreatitis which in result increase insulin resistance and suppress its secretion leading to an abnormal hyperglycemia. Several studies showed that the actual biocompounds found in plant can significantly lower urea and creatinine synthesis by preventing protein metabolism since amino acids like methionine, arginine, glycine, alanine citrulline and glutamate (41, 42) are the basic element of their production. It seems that these phytocompounds possess also a remarkable diuretic effect, which significantly contributes to the elimination of excessive amount of urea and creatinine from the body (43). Flavonoids participate also actively in the regulation of key hepatic enzymes activity such as glucose-6-phosphate dehydrogenase, ALAT and ASAT by regulating hepatic gluconeogenesis pathway (44).

Some researchers found that there is a link between antidiabetic and anti-inflammatory responses (45, 46), since the antidiabetic effect of secondary metabolites can significantly reduce bleeding process and microvascular complications related to chronic inflammation by decreasing significantly local cytokines release previously cited and even the secretion of a key protein involved in inflammatory process called C-reactive protein (CRP) (47, 48).

Another studies (49, 50) showed that anthocyanin, could play an important role in the treatment of type 2 diabetes and obesity, by enhancing insulin sensitivity and the expression of GLUT4 and GLUT2 genes. In fact, these transporters play an important role in carbohydrate metabolism, by stimulating glucose uptake into hepatocytes, adipocytes and muscle cells. It was suggested that the polyphenols generate this phenomenon *via* a mechanism called phosporylation by glucokinase to facilitate the diffusion of glucose across the cell membranes of these cells, which will decrease the glucose level in bloodstream and thus the hyperglycemia (51, 52).

It's well known that during fever; there is an important release of prostaglandin (PGs) which is a key pro-inflammatory cytokines, and the important decrease in rectal temperature recorded in this study, could be explained by the fact that *C. tougourensis* compounds may inhibit the production of this cytokine. A study made on two Centaurea species namely; *C. depressa* and *C. solstitialis* revealed that the high amount of terpenes in these species especially in sesquiterpene lactones is responsible of their antipyretic activities by acting on central nervous system (CNS) (53), which is in perfect accordance with the obtained results since *C. tougourensis* contains considerable amount of terpenes.

Bioactive compounds present in plants can also inhibit the pyrogenic effect of many microbes, and recent clinical tests have even shown that some classes of compounds, such as saponins and flavonoids may decrease the abnormal rise in temperature due to transplant rejection or tissue damage (54, 55). This antipyretic effect, could also be the result of a strong inhibition exerted by polyphenols on the activity of immune cells, to prevent the production of PGE_2, interferon-γ, TNF-α and IL-1β, considered the principal pro-inflammatory mediators during fever (56). Researchers supposed that the blockage of MAPK/NF-κB signaling pathway could prevent the onset of inflammatory response related to pyrogens (57). These scientific progresses will certainly contribute to the future elaboration of more effective anti-inflammatory drugs, instead of using the actual drugs present on the market, including antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) (3).

In this study, we used silymarin as reference drug; indeed, the hepatoprotective effect of this flavolignan compound is well known by scientific community. Several researches already reported the anti-inflammatory effect of silymarin on both animals and human hepatic stellate cells, but also to preserve liver functions and histological integrity (58, 59). For almost all biochemical parameters tested in this study, a very similar results

were observed in the groups treated with industrial silymarin or natural silymarin, except for ASAT and PAL parameters, which were better in the group treated by industrial silymarin. This difference could be explained by the fact that industrial silymarin was more active on liver, especially on hepatic mitochondria considered as main production site of these enzymes (60). Indeed, the active portion of silymarin called silybin, may actually limited the production of ASAT, ALAT, PAL and other pro-oxydant markers *via* a strong downregulation process exerted on cyclooxygenase cycle, leukotrienes, and the production of free radicals in other specialized cells localized in liver such as Kupffer cells in mice (61, 62). Silymarin may also reduced markers of hepatic fibrosis such as alpha smooth muscle actin, collagen α 1(I) (αSMA) (63), which explain in part the normal hepatocytes aspect observed in the group of mice which received industrial silymarin. The hepatoprotective potential of polyphenols is well known especially those of flavonoids and terpenes and researchers explained it by the fact that these biocompounds may actually slow down and even prevent necrosis process of liver cells (64), and this has been demonstrated in several studies using paracetamol or tetrachloride (CCl4) as hepatotoxic agents (65, 66). There is an important relationship between antioxidant and hepatoprotective process since we know that the liver is the principal center of detoxification. In oxidative stress situation, polyphenols are able to activate an important pathway called Nrf-2 signaling pathway which in return considerably increase the expression of key enzymes namely; heme oxygenase-1 (HO-1) and phosphoramidite adenine dinucleotide quinone oxidoreductase-1 (NQO-1) to significantly decrease oxidative stress damage in liver tissues by enhancing free radical elimination process (67). Another study suggested that phytocompounds may actually regulate membrane channel exchange process especially those of hepatocytes which contribute to an optimal exchange of fluids across the membrane (68).

The hepatoprotective activity of phenolic acids has also been reported, these compounds can inhibit in a dose-dependent manner the activity of 5-lipoxygenase, since the activity of this enzyme is associated with several liver disorders, such as hepatic steatosis and other hepatic inflammatory injuries linked to macrophage infiltration and monocyte chemoattractant protein-1 (MCP-1) expression (79-71). Phenolic acids could also increase the activity of several antioxidant enzymes such as glutathione reductase and glutathione transferase (72), but also inhibited lipid peroxidation (73) and thus decrease the oxidative stress exerted on hepatocytes.

A study carried out by Sikora-Wiorkowska *et al.* (74) revealed that the co-administration of rosiglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (PGDJ2), considered as agonists of peroxisome-proliferator-activated receptors PPARγ, can significantly reduce the hepatotoxic effect exerted by cyclosporine A (CsA), and this protection was reported on the biochemical and histopathological levels.

Another study made by Atta *et al.* (75) underlined the possible link between the high proportions of alkaloids, flavonoids and phenolic acids compounds detected in their plants and the preservation of the histological structure and integrity of rat's liver and pancreas. These researchers suggested that these biocompounds may have reduced the oxidative stress exerted on these organs, by stabilizing liver enzymes functions and other parameters. Another compound named ellagic acid demonstrated a non-negligible antidiabetic effect by preserving cardiomyocyte structure, *via* the activation of a key protein named silent information regulator 1 (SIRT1), but also by preventing the acetylation of key factors such as nuclear factor erythroid-derived 2-like 2 (Nrf2) and NF-κB, which in return will considerably attenuate cardiomyocyte injury from oxidative stress (76). This information is very important, since cardiovascular complications are frequent in both type 1 and type 2 diabetes. Noting that a similar approach was used by these researchers to determine the antidiabetic effect of their plants, which is in accordance with the results of this study.

In conclusion, the results of this study indicated that the n-BuOH extract of *C. tougourensis* may offer a good protection against diabetes induced by streptozotocin, hepatotoxicity induced by paracetamol, ear edema induced by croton oil, and hyperthermia induced by yeast. These pharmacological properties could be explained by the richness of this plant in various phytocompounds that may acted with a synergistic way. Further investigations are required for better understanding of the chemical composition, to identify the bioactive compounds responsible of the pharmacological properties of *Centaurea tougourensis*.

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REFERENCES

1. Tan CL, Knight ZA. Regulation of body temperature by the nervous system. *Neuron* 2018; 98: 31-48.
2. Relja B, Land WG. Damage-associated molecular patterns in trauma. *Eur J Trauma Emerg Surg* 2020; 46: 751-775.
3. Davis A, Robson J. The dangers of NSAIDs: look both ways. *Br J Gen Pract* 2016; 66: 172-173.
4. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* 2014; 5: 491. doi: 10.3389/fimmu.2014.00491
5. Tsalamandris S, Antonopoulos AS, Oikonomou E, *et al.* The role of inflammation in diabetes: current concepts and future perspectives. *Eur Cardiol* 2019; 14: 50-59.
6. Sozzani S, Abbracchio MP, Annese V, *et al.* Chronic inflammatory diseases: do immunological patterns drive the choice of biotechnology drugs? A critical review. *Autoimmunity* 2014; 47: 287-306.
7. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. *Biochem Pharmacol* 2020; 180: 114147. doi: 10.1016/j.bcp.2020.114147
8. Atanasov AG, Zotchev SB, Dirsch VM *et al.* Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov* 2021; 20: 200-216.
9. Rui L. Energy metabolism in the liver. *Compr Physiol* 2014; 4: 177-197.
10. Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. *Compr Physiol* 2017; 8: 1-8. doi: 10.1002/cphy.c170012
11. Hodges RE, Minich DM. Modulation of metabolic detoxification pathways using foods and food-derived components: a scientific review with clinical application. *J Nutr Metab* 2015; 2015: 760689. doi: 10.1155/2015/760689
12. Halban PA, Polonsky KS, Bowden DW, *et al.* β-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *J Clin Endocrinol Metab* 2014; 99: 1983-1992.
13. International Diabetes Federation. (2021). (https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html, accessed 25 May 2021).

14. Bensaad MS, Dassamiour S, Hambaba L, C Bensouici, Haba H. In vitro assessment of antioxidant, anti-inflammatory, neuroprotective and antimicrobial activities of Centaurea tougourensis Boiss. & Reut. *J Pharm Pharmacogn Res* 2021; 9: 790-802.
15. Bensaad MS, Dassamiour S, Hambaba L. Antihyperglycemic activity of Centaurea tougourensis. *Intern J Hum Settl* 2021; 5: 93-98.
16. Celikezen FC, Hayta S, Ozdemir O, Turkez H. Cytotoxic and antioxidant properties of essential oil of Centaurea behen L. in vitro. *Cytotechnology* 2019; 71: 345-350.
17. Kose YB, Iscan G, Goger F, Akalin G, Demirci B, Baser KH. Chemical composition and biological activity of Centaurea baseri: new species from Turkey. *Chem Biodivers* 2016; 13: 1369-1379.
18. Russo A, Cardile V, Graziano AC, *et al.* Effect of three Centaurea species collected from central Anatolia region of Turkey on human melanoma cells. *Nat Prod Commun* 2016; 11: 275-278.
19. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, National Academies Press, 2010.
20. Manga HM, Brkic D, Marie DE, Quetin-Leclercq J. In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae). *J Ethnopharmacol* 2004; 92: 209-214.
21. Kim JH, Pan JH, Cho HT, Kim YJ. Black ginseng extract counteracts streptozotocin-induced diabetes in mice. *PLoS One* 2016; 11: e0146843. doi: 10.1371/journal.pone.0146843
22. Hussain L, Ikram J, Rehman K, Tariq M, Ibrahim M, Akash MS. Hepatoprotective effects of Malva sylvestris L. against paracetamol-induced hepatotoxicity. *Turk J Biol* 2014; 38: 396-402.
23. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. *BMC Complement Altern Med* 2012; 12: 59. doi: 10.1186/1472-6882-12-59
24. Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. *Adv Biochem Eng Biotechnol*. 2015; 147: 59-110.
25. Mortimer JC. Plant synthetic biology could drive a revolution in biofuels and medicine. *Exp Biol Med* 2019; 244: 323-331.
26. Bensaad MS, Dassamiour S, Hambaba L, Kahoul MA, Benhoula M. Evidence of anti-inflammatory and anti-ulcer properties of aerial parts of Centaurea tougourensis Boiss and Reut. *Trop J Pharm Res* 2021; 20: 1647-1654.
27. Panaro MA, Carofiglio V, Acquafredda A, Cavallo P, Cianciulli A. Anti-inflammatory effects of resveratrol occur via inhibition of lipopolysaccharide-induced NF-κB activation in Caco-2 and SW480 human colon cancer cells *Br J Nutr* 2012; 108: 1623-1632.
28. Goettert M, Schattel V, Koch P, Merfort I, Laufer S. Biological evaluation and structural determinants of p38α mitogen-activated-protein kinase and c-Jun-N-terminal kinase 3 inhibition by flavonoids. *Chembiochem* 2010; 11: 2579-2588.
29. Catarino MD, Alves-Silva JM, Pereira OR, Cardoso SM. Mediterranean diet: a precious tool for fighting inflammatory diseases In: Polyphenols: Food Sources, Bioactive Properties and Antioxidant Effects. Cobb D.T. (ed). New York, Nova Science Publishers, 2014, 87-112.
30. Lv J, Liu F. The role of serotonin beyond the central nervous system during embryogenesis. *Front Cell Neurosci* 2017; 11: 74. doi: 10.3389/fncel.2017.00074
31. Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP. Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients* 2016; 8: 56. doi: 10.3390/nu8010056
32. Wu H, Denna TH, Storkersen JN, Gerriets VA. Beyond a neurotransmitter: the role of serotonin in inflammation and immunity. *Pharmacol Res* 2019; 140: 100-114.
33. Hassan HS, Sule MI, Musa AM, Musa KY, Abubakar MS, Hassan AS. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. *Afr J Tradit Complement Altern Med* 2011; 9: 250-255.
34. Boudoukha C, Bouriche H, Ortega E, Senator A. Immunomodulatory effects of Santolina chamaecyparissus leaf extracts on human neutrophil functions. *Pharm Biol* 2016; 54: 667-673.
35. Yang Y, Oh JM, Heo P, *et al.* Polyphenols differentially inhibit degranulation of distinct subsets of vesicles in mast cells by specific interaction with granule-type-dependent SNARE complexes. *Biochem J* 2013; 450: 537-546.
36. Kubacey TM, Haggag EG, El-Toumy SA, Ahmed AA, El-Ashmawy IM, Youns MM. Biological activity and flavonoids from Centaurea alexanderina leaf extract. *J Pharm Res* 2012; 5: 3352-3361.
37. Rocha S, Ribeiro D, Fernandes E, Freitas M. A systematic review on anti-diabetic properties of chalcones. *Curr Med Chem* 2020; 27: 2257-2321.
38 Ota A, Ulrih NP. An overview of herbal products and secondary metabolites used for management of type two diabetes. *Front Pharmacol* 2017; 8: 436. doi: 10.3389/fphar.2017.00436
39. Xie Y, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z. Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. *Kidney Int* 2018; 93: 741-752.
40. Kanazawa I, Yamaguchi T, Sugimoto T. Relationship between bone biochemical markers versus glucose/lipid metabolism and atherosclerosis; a longitudinal study in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2011; 92: 393-399.
41. Cao YF, Li J, Zhang Z, *et al.* Plasma levels of amino acids related to urea cycle and risk of type 2 diabetes mellitus in Chinese adults. *Front Endocrinol* 2019; 10: 50. doi: 10.3389/fendo.2019.00050
42. da Silva RP, Nissim I, Brosnan ME, Brosnan JT. Creatine synthesis: hepatic metabolism of guanidinoacetate and creatine in the rat in vitro and in vivo. *Am J Physiol Endocrinol Metab* 2009; 296: E256-E261.
43. Vargas F, Romecin P, Garcia-Guillen AI, *et al.* Flavonoids in kidney health and disease. *Front Physiol* 2018; 9: 394. doi: 10.3389/fendo.2019.00050
44. Zareei S, Boojar MMA, Amanlou M. Inhibition of liver alanine aminotransferase and aspartate aminotransferase by hesperidin and its aglycone hesperetin: an in vitro and in silico study. *Life Sci* 2017; 178: 49-55.
45. Katsiki N, Ferrannini E. Anti-inflammatory properties of antidiabetic drugs: a promised land in the COVID-19 era? *J Diabetes Complicat* 2020; 34: 107723. doi: 10.1016/j.jdiacomp.2020.107723
46. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; 115: 1111-1119.
47. Kothari V, Galdo JA, Mathews ST. Hypoglycemic agents and potential anti-inflammatory activity. *J Inflamm Res* 2016; 9: 27-38.
48. Shi YC, Liao JW, Pan TM. Antihypertriglyceridemia and anti-inflammatory activities of monascus-fermented dioscorea in streptozotocin-induced diabetic rats. *Exp Diabetes Res* 2011; 2011: 710635. doi: 10.1155/2011/710635
49. Solverson P. Anthocyanin bioactivity in obesity and diabetes: the essential role of glucose transporters in the gut and periphery. *Cells* 2020; 9: 2515. doi: 10.3390/cells9112515

50. Rozanska D, Regulska-Ilow B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. *Adv Clin Exp Med* 2018; 27: 135-142.
51. Sun C, Zhao C, Guven EC, *et al.* Dietary polyphenols as antidiabetic agents: advances and opportunities. *Food Front* 2020; 1: 18-44.
52. Leighton B, Atkinson A, Coghlan MP. Small molecule glucokinase activators as novel anti-diabetic agents. *Biochem Soc Trans* 2005; 33: 371-374.
53. Akkol EK, Arif R, Ergun F, Yesilada E. Sesquiterpene lactones with antinociceptive and antipyretic activity from two Centaurea species. *J Ethnopharmacol* 2009; 122: 210-215.
54. Li K, Zhang R, Li SY, *et al.* Potential quality evaluation approach for the absolute growth years' wild and transplanted Astragali Radix based on anti-heart failure efficacy. *Chin J Nat Med* 2020; 18: 460-471.
55. Jan S, Khan MR. Antipyretic, analgesic and anti-inflammatory effects of Kickxia ramosissima. *J Ethnopharmacol* 2016; 182: 90-100.
56. Abbasi WM, Ahmad S, Perveen S, Rehman T. Preliminary phytochemical analysis and in vivo evaluation of antipyretic effects of hydro-methanolic extract of Cleome scaposa leaves. *J Tradit Complement Med* 2017; 8: 147-149.
57. Nguyen T, Chen X, Chai J, *et al.* Antipyretic, anti-inflammatory and analgesic activities of Periplaneta americana extract and underlying mechanisms. *Biomed Pharmacother* 2020; 123: 109753.
58. Freitag AF, Cardia GF, da Rocha BA, *et al.* Hepatoprotective effect of silymarin (Silybum marianum) on hepatotoxicity induced by acetaminophen in spontaneously hypertensive rats. *Evid Based Complement Alternat Med* 2015; 2015: 538317. doi: 10.1155/2015/538317
59. Trappoliere M, Caligiuri A, Schmid M, *et al.* Silybin, a component of sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J Hepatol* 2009; 50: 1102-1111.
60. Mirzaei E, Sabetian G, Masjedi M, *et al.* The effect of silymarin on liver enzymes and antioxidant status in trauma patients in the intensive care unit: a randomized double blinded placebo-controlled clinical trial. *Clin Exp Hepatol* 2021; 7: 149-155.
61. Federico A, Dallio M, Loguercio C. Silymarin/silybin and chronic liver disease: a marriage of many years. *Molecules* 2017; 22: 191. doi: 10.3390/molecules22020191
62. Vargas-Mendoza N, Madrigal-Santillan E, Morales-Gonzalez A, *et al.* Hepatoprotective effect of silymarin. *World J Hepatol* 2014; 6: 144-149.
63. Hellerbrand C, Schattenberg JM, Peterburs P, Lechner A, Brignoli R. The potential of silymarin for the treatment of hepatic disorders. *Clin Phytosci* 2016; 2: 1-14. doi: 10.1186/s40816-016-0019-2
64. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of Psidium guajava Linn. leaf extract. *Indian J Exp Biol* 2006; 44: 305-311.
65. Zhang X, Feng J, Su S, Huang L. Hepatoprotective effects of Camellia nitidissima aqueous ethanol extract against CCl4-induced acute liver injury in SD rats related to Nrf2 and NF-κB signalling. *Pharm Biol* 2020; 58: 239-246.
66. Rezende TP, do A Correa JO, Aarestrup BJ, Aarestrup FM, de Sousa OV, da Silva Filho AA. Protective effects of Baccharis dracunculifolia leaves extract against carbon tetrachloride- and acetaminophen-induced hepatotoxicity in experimental animals. *Molecules* 2014; 19: 9257-9272.
67. Hseu YC, Chou CW, Senthil Kumar KJ. Ellagic acid protects human keratinocyte (HaCaT) cells against UVA-induced oxidative stress and apoptosis through the upregulation of the HO-1 and Nrf-2 antioxidant genes. *Food Chem Toxicol* 2012; 50: 1245-1255.
68. Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: a membrane trafficking machine. *J Cell Biol* 2019; 218: 2096-2112.
69. Shah K, Verma RJ. Protection against butyl p-hydroxybenzoic acid induced oxidative stress by Ocimum sanctum extract in mice liver. *Acta Pol Pharm* 2012; 69: 865-870.
70. Martinez-Clemente M, Ferre N, González-Periz A, *et al.* 5-lipoxygenase deficiency reduces hepatic inflammation and tumor necrosis factor α-induced hepatocyte damage in hyperlipidemia-prone ApoE-null mice. *Hepatology* 2010; 51: 817-827.
71. Reyes MT, Mourelle M, Hong E, Muriel P. Caffeic acid prevents liver damage and ameliorates liver fibrosis induced by CCI4 in the rat. *Drug Dev Res* 1995; 36: 125-128.
72. Perez-Alvarez V, Bobadilla RA, Muriel P. Structure-hepatoprotective activity relationship of 3,4-dihydroxycinnamic acid (caffeic acid) derivatives. *J Appl Toxicol* 2001; 21: 527-531.
73. Hohmann MS, Cardoso RD, Pinho-Ribeiro FA, *et al.* 5-lipoxygenase deficiency reduces acetaminophen-induced hepatotoxicity and lethality. *Biomed Res Int* 2013; 2013: 627046. doi: 10.1155/2013/627046
74. Sikora-Wiorkowska A, Smolen A, Czechowska G, Wiorkowski K, Korolczuk A. The role of PPAR gamma agonists - rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 in experimental cyclosporine A hepatotoxicity. *J Physiol Pharmacol* 2019; 70: 885-893.
75. Atta AH, Saad SA, Atta SA, *et al.* Cucumis sativus and Cucurbita maxima extract attenuate diabetes-induced hepatic and pancreatic injury in a rat model. *J Physiol Pharmacol* 2020; 71: 507-518.
76. Altamimi JZ, Alfaris NA, Alshammari GM, *et al.* Ellagic acid protects against diabetic cardiomyopathy in rats by stimulating cardiac silent information regulator 1 signaling. *J Physiol Pharmacol* 2020; 71: 891-904.

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