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RESEARCH ARTICLE

Anti-inflammatory and Antioxidant Effects of *Lotus corniculatus* on Paracetamol-induced Hepatitis in Rats

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Abstract: ***Background*:** Herbal medicines have been used in the treatment of liver diseases for a long time. The current study was elaborated to evaluate *in vitro* and *in vivo* antioxidant and anti-inflammatory effects of *Lotus corniculatus* (*L. corniculatus*) butanolic extract.

***Methods*:** The *in vitro* antioxidant and anti-inflammatory properties of *L. corniculatus* were investigated by employing DPPH radical scavenging, H_2O_2 scavenging and BSA denaturation assays. I*n vivo* antioxidant and anti-inflammatory effects of *L. corniculatus* were evaluated against paracetamol (APAP)-induced hepatitis in rats. *L.corniculatus* at doses of 100 and 200 mg/kg was administered orally once daily for seven days. Serum transaminases (AST and ALT) and lactate dehydrogenase (LDH), total bilirubin levels, liver malondialdehyde (MDA), reduced glutathione (GSH), glutathione S- transferase (GST) and superoxide dismutase (SOD) levels and inflammatory markers, such as serum C-reactive protein (CRP), circulating and liver myeloperoxidase (MPO) levels were investigated. Further histopathological analysis of the liver sections was performed to support the effectiveness of *L. corniculatus*.

***Results*:** *L. corniculatus* exhibited strong antioxidant and anti-inflammatory effects *in vitro*. In the *in vivo* study, our findings demonstrate that *L. corniculatus* (100 and 200 mg/kg) administration led to an amelioration of APAP effects on liver histology, liver functions parameters (AST, ALT, LDH, and total bilirubin levels) and liver oxidative stress markers (MDA, GSH, GST and SOD levels). Furthermore, serum CRP, circulating MPO and liver MPO levels were declined by both doses of *L. corniculatus* extract. The best benefits were observed with 200 mg/kg of *L. corniculatus* extract.

***Conclusion*:** Antioxidant and anti-inflammatory effects of *L. corniculatus* extract may be due to the presence of active components.

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1. INTRODUCTION

Drug-induced hepatotoxicity contributes to more than half of the cases of fulminant hepatitis, with APAP being the principal offending agent in western countries [1]. APAP is a common analgesic and antipyretic drug that can cause acute liver damages at a very larger dose [2]. Recent findings on the pathogenesis of APAP induced acute liver injury revealed that the outcome of the insult does not only depend on the direct hepatotoxic effects of the noxious metabolite N-acetyl parabenzo-

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quineimine, but it is critically dependent on the complex interplay of the different immune cells, constituting inflammatory responses to liver injury [1, 3]. Nowadays, people are becoming increasingly interested in botanical drugs for their low toxicity and good therapeutic performance. Lotus corniculatus (Fabaceae) commonly known as Bird's foot trefoil belongs to a genus that contains many dozens of species distributed worldwide *e.g*. *Lotus aboriginus*, *Lotus angustissimus* and *Lotus argophyllus* [4]. *L. corniculatus* is in the legume family and produces stem up to two feet in length. Leaves are alternate and compound with five ovals to linear leaflets. The plant flowers from May to August, when clusters of yellow, pea-like flowers develop. The fruits are slender and straight pods. *L. corniculatus* is utilized to increase the milk yield of dairy cows [1, 5]. In relation to phytochemical studies of Lotus species, there are many reports showing many constituents including flavonoids, phenolic acids and saponins [6, 7]. Several studies have demonstrated that *L. corniculatus* has therapeutic properties, such as anti-inflammatory effects [8, 9], anti-proliferative activities [7], anti-helminth parasites [10] and antioxidant effects [11]. The review of this plant revealed very little information about the use of the plant to counteract drugs induced hepatitis. Therefore, this study aims at evaluating the anti-inflammatory and hepatoprotective properties of *L. corniculatus* growing in Algeria in the rat model, following APAP induced hepatitis.

2. MATERIALS AND METHODS

2.1. Chemicals

DPPH°: 2,2-diphényl-1-picrylhydrazyle radical, Thiobarbituric acid (TBA), Folin- Ciocalteus's phenol reagent, gallic acid, quercetin, hexadecyl trimethylammonium bromide, and O-dianisidine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents and/or reagents were of analytical grade.

2.2. Plant Material and Extract Preparation

L. corniculatus L. was collected in March 2011 from Djbele El- louahch near Constantine City, Algeria. The plant was identified by Pr. M. Bouhroum. The voucher specimen (number: LC/20/04/11) was deposited in the Herbarium of VARENBIOMOL Research Unit, University of Mentouri Brothers, Constantine. Air dried aerial parts of *L. corniculatus* (500 g) were macerated four times with 70% MeOH solution by replacing the solution every day with fresh solvent. The hydro-alcoholic solutions were concentrated under reduced pressure to dryness and the residue was dissolved in water (200 ml) and kept in cold overnight. After filtration, the aqueous solution was successively extracted with $CHCl_3$, EtOAc and n-BuOH three times for each solvent, then the $CHCl_3$ (1.7 g), EtOAc (2.5 g) and n-BuOH (24.6 g) extracts were concentrated to dryness (20). The n-BuOH extract was subjected to the present investigation.

2.3. Phytochemical Screening and TP and TF Content

Chemical tests for the screening of certain phytochemical compounds were performed using standard qualitative procedures [12, 13]. Total phenolic (TP) content was determined by Folin-Ciocalteu assay [14]. The assay contained 100 µl of *L. corniculatus* extract, 250 µl of Folin- Ciocalteu reagent and 1250 µl of 20 % sodium carbonate solution. After 2 h of incubation at room temperature, the absorbance of the reaction was measured at 760 nm. Total flavonoids (TF) content was determined using a colorimetric method [15]. The mixture contained 0.5 ml of *L. corniculatus* extract, 2 ml of water, 0.15 ml of 5 % Na_2CO_3, 0.15 ml of 10% $AlCl_3$ and 2 ml of 4 % NaOH and measured at 510 nm. The TP and TF contents were expressed as Gallic acid equivalent and Quercetin equivalents /g extract (mg GAE/g extract and mg QE/g extract) respectively.

2.4. *In vitro* Antioxidant Activity Assays

2.4.1. DPPH° Radical Scavenging Activity

DPPH° radical scavenging activity was based on the method of Blois *et al*. [16]. Briefly, 1.25ml of methanolic DPPH° solution (0.1mM) was incubated with varying concentrations of *L. corniculatus* or ascorbic acid (standard). After 30min of incubation at room temperature, the absorbance of the reaction was measured at 517 nm. The percent inhibition of DPPH° was calculated as follows:

% Inhibition= [(Abs C- Abs S) /Abs C] x100 (1)

where Abs C was the absorbance of the control and Abs S was the absorbance of the extract or standard.

2.4.2. Hydrogen Peroxide (H_2O_2) Scavenging Activity

H_2O_2 scavenging ability of *L. corniculatus* was determined according to the method of Gülçin *et al*. [17]. A solution of H_2O_2 (20 mM) was prepared in phosphate buffer solution (PBS, pH 7.4). Various concentrations of 1 ml of *L. corniculatus* or trolox (standard) were added to 2 ml of H_2O_2 solutions in PBS. The absorbance of H_2O_2 was measured at 230 nm after 10 min against a blank solution that contained phosphate buffer without hydrogen peroxide. The percentage of H_2O_2 scavenging of *L. corniculatus* and trolox according to Eq. (1).

2.5. *In vitro* Anti-inflammatory Activity (BSA Denaturation Assay)

The anti-inflammatory activity of *L. corniculatus* was studied by using the inhibition of BSA (bovine serum albumin) denaturation technique [18]. To 1.8 ml of 1% of BSA solution, 0.2 ml of *L.corncculatus* or sodium diclofenac at various concentrations was added. The pH was adjusted to 6.5 using 1N HCl. This solution was incubated at 37°C for 20 minutes and then heated to 57°C for 15 minutes. After cooling, turbidity was measured at 660 nm. The percentage inhibition of BSA denaturation was calculated by using Eq. (1).

2.6. Animal Experimentation

Male Albino Wistar rats (200-210 g) used for this study were purchased from Pasteur Institute, Algiers (Algeria). All animals were kept in a temperature-controlled room with a 12/12 hours dark/light cycle. All animals had free access to regular rat food and tap water. All animals were acclimatized for at least 1 week prior to the experiments. The assay was conducted in accordance with the European Communities Council Directive (2010/63/EU) for animal experiments and the protocol employed was approved by the biology institute (Mentouri Brothers University Constantine1) under inscription number 06/SNL/14. The animals were divided into five groups (n=6). Control group animals received 5 ml of saline solution (0.9%) for seven days. APAP-treated group animals received 750 mg/kg of APAP as a single dose on day 7 [18]. *L.corniculatus* pre-treated groups animals received 100 or 200mg/kg of *L. corniculatus* extract for seven days. N-acetylcysteine pre-treated group animals received 200 mg/kg of N-acetylcysteine (Standard) for seven days [19].

On the 7th day, all groups except control groups received 750 mg/kg of APAP as a single dose by oral route after the administration of test drugs. All drug administration was done orally.

2.6.1. Serum Hepato-specific Markers Levels

18 hours after APAP administration, all animals were sacrificed to collect blood samples. After centrifugation at 1000 g for 10 minutes, obtained serum was used for estimation of biochemical parameters such as alanine aminotransferase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH), total bilirubin and C-reactive protein (CRP) using diagnostic kits and automatic analyzer. Livers were then excised, washed in ice-cold saline and weighed. A section from the median lobe was used for histological analysis. The remaining liver was stored at 80°C for further analysis [20].

2.6.2. Liver Oxidative Stress Markers Levels

Liver samples were homogenized in ice-cold 0.15M Tris-HCl (pH7.4) using Ultra Turax homogenizer and processed for the measurement of MDA levels as an index of lipid peroxidation [21]. A part of the homogenate was treated with 10% metaphosphoric acid for the estimation of reduced glutathione (GSH) using Ellman's reagent [22]. The remaining homogenate was centrifuged at 1000g for 10 min and the obtained supernatant was subsequently used for glutathione S-transferase (GST) and superoxide dismutase (SOD) according to Habig *et al*. [23] and Winterbourn *et al*. [24] respectively. The specific activity of GST and SOD was expressed as a unit per mg of protein (U/mg protein). The protein content was determined by the method of Lowry *et al*. [25].

2.6.3. Serum and Liver MPO Levels

Serum and liver myeloperoxidase (MPO) levels, a markers liver cells inflammation, were

measured with an O-dianisidine-H_2O_2 assay [26]. Livers samples were homogenized in 50 mM potassium phosphate buffer (pH 6.0) and centrifuged at 41,400 g for 10 min at 4°C. The pellets were then suspended in 50 mM phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide. After freeze-thawing 3 times, the samples were centrifuged at 41,400 g for 10 min at 4°C and the resulting supernatant was assayed spectrophotometrically for MPO activity. In brief, 0.3ml of the serum or liver (resulting supernatant) was mixed with 2.3 ml of the reaction mixture containing 50 mM PB, O-dianisidine, and 20 mM H_2O_2 solution. One unit (U) of enzyme activity was defined as the amount of MPO present, which caused a change in absorbance measured at 460 nm for 2 min. Serum and liver MPO levels were expressed as U/L and respectively U/mg protein. The protein content was determined by the method of Lowry *et al*. [25].

2.6.4. Histological Study

The median lobe of the liver from each group was fixed in 10% formalin and embedded in the paraffin. The sections of 5µm thickness were stained with hematoxylin and eosin (H&E) for liver injury detection.

2.7. Statistical Analysis

Results are expressed as a mean value ± standard deviation (Mean ± SD). Statistical comparisons were made by Student's t-test. The *in vitro* assays were repeated 3 times.

3. RESULTS

3.1. Phytochemical Screening, TP and TF Contents

The results of the phytochemical characteristics, total phenolic and flavonoid contents of *L. corniculatus* extract are summarized in Table **1**. The phytoscreening results reveal the presence of flavonoids, phenols, saponins, and terpenoids in *L. corniculatus* extract, while alkaloids were completely absent. The total phenolic and flavonoid contents of *L. corniculatus* were found to be 87.1 ± 14.5 mg GAE/g extract and 36.5 ± 2.1 mg QE/g extract respectively.

Table 1. Phytoscreening tests and TP and TF content of *L. corniculatus*.

Phytochemicals/Test	*L. corniculatus* Extract
Alkaloids (Wagner's reagent test)	-
Flavonoids (Alkaline reagent test)	+
Phenols (Ferric chloride test)	+
Saponins (Foam test)	+
Terpenoids (Salkowki's test)	+
TPC (mg GAE/g extract)	87.1 ± 14.5
TFC (mg QE/g extract)	39.5 ± 2.1

(+): Present; (-): Absent; TPC: Total Phenolic Content; TFC: Total Flavonoid Content.

3.2. *In vitro* Antioxidant and Anti-inflammatory Activities

The results of *in vitro* antioxidant and anti-inflammatory activities of *L.corniculatus* are summarized in Table **2**. For *in vitro* antioxidant assay, the extract was capable of scavenging DPPH radical in a concentration-dependent manner. At 300 µg/ml, the high scavenging activity of DPPH radical for *L. corniculatus* and ascorbic acid was 59.7 and 91.8% respectively. As observed in DPPH radical scavenging assay, the percentage of H_2O_2 scavenging ability was determined to be increased with the increase in the *L. corniculatus* concentration. At 300 µg/ml, the strong scavenging activity of H_2O_2 scavenging ability for *L. corniculatus* and trolox was 79.7 and 81.8% respectively. In *in vitro* anti-inflammatory assay, the inhibition of BSA denaturation by heat was increased on increasing *L. corniculatus* concentration from 75 to 300 µg/ml. At 300µg/ml, the high percentage of BSA denaturation inhibition was 68.8% and 89.1% for *L. corniculatus* and sodium diclofenac respectively.

3.3. Serum Biochemical Parameters

Serum biochemical parameters in experimental groups are depicted in Table **3**. Oral administration of APAP to animals caused a significant rise in serum ALT, AST and LDH levels, compared to control animals. Conversely, *L. corniculatus* pretreatment markedly decreased serum ALT, AST

Table 2. *In vitro* antioxidant and anti-inflammatory activities of *L. corniculatus.*

-	DPPH° Assay (% of Inhibition)		H_2O_2 Scavenging Assay (% of Inhibition		BSA Denaturation Assay (% of Inhibition)	
Concentration (µg/ml)	*L. corniculatus*	Ascorbic acid	*L. corniculatus*	Trolox	*L. corniculatus*	Diclofenac sodium
75	10.5 ± 6.7	57.2 ± 5.7	26.9 ± 1.3	31.5 ± 4.1	18.43 ± 4.9	39.75 ± 6.9
150	27.2 ± 6.1	70.9 ± 5.6	41.3 ± 5.1	60.1 ± 8.2	38.46 ± 3.1	63.17 ± 2.1
300	59.7 ± 2.2	91.8 ± 9.8	79.7 ± 6.7	81.8.5 ± 2.7	68.87 ± 12.8	89.15 ± 10.9

DPPH°: 2,2-diphényl-1-picrylhydrazyle radical, H_2O_2 :hydrogen peroxide, BSA: Serum Bovine Albumin, n=3.

Table 3. Effect of *L. corniculatus* extract on serum hepato-specific markers in APAP- intoxicated animals.

Treatments	ALT (U/L)	AST (U/L)	LDH (U/L)	TB (mg/dL)	CRP (mg/L)	MPO (U/L)
Control	67.6 ± 7.2	85.8 ± 8.5	131.8 ± 38.2	0.5 ± 0.1	0.93 ± 0.2	7.7 ± 0.6
APAP (750 mg/kg)	$148.4 \pm 26.7^{**}$	$247.4 \pm 26.2^{***}$	$315.6 \pm 56.5^{***}$	$1.4 \pm 0.2^{**}$	$1.95 \pm 0.3^{***}$	$18.5 \pm 1.1^{***}$
L. corniculatus 100 + APAP	$120 \pm 4.9^{\#}$	$196 \pm 28.9^{\#}$	$222.66 \pm 39^{\#}$	$0.7 \pm 0.1^{\#}$	$1.16 \pm 0.2^{\#}$	$14.9 \pm 1.2^{\#\#}$
L. corniculatus extract (200 mg/kg) +APAP	$91.5 \pm 9.9^{\#\#}$	$148.5 \pm 21.7^{\#\#\#}$	$184.3 \pm 35.4^{\#\#}$	$0.6 \pm 0.1^{\#\#}$	$0.89 \pm 0.3^{\#\#}$	$9.33 \pm 1.4^{\#\#\#}$
N-acetylcysteine (200 mg/kg)+APAP	$82.1 \pm 12.9^{\#\#}$	$129 \pm 12.9^{\#\#\#}$	$161.7 \pm 39.4^{\#}$	$0.5 \pm 0.1^{\#\#}$	$0.68 \pm 0.1^{\#\#\#}$	$8.3 \pm 0.9^{\#\#\#}$

Vales are Mean ± SD (n=6). $^{**}p \leq 0.01$;$^{***}p \leq 0.001$ compared to control group. $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$ compared to APAP- treated group. AST and ALT: aspartate and alanine aminotransferase respectively; LDH: lactate dehydrogenase; TB: Total Bilirubin; CRP: C-Reactive Protein; MPO: myeloperoxidase; APAP: paracetamol.

and LDH levels in a dose dependent manner compared to the APAP- treated group. Serum total bilirubin, CRP and MPO levels were significantly increased in APAP- treated animals. *L. corniculatus* extract at 100 and 200 mg/kg considerably reduced serum total bilirubin (44 and 53% respectively), CRP (40 and 54% respectively) and MPO levels (19 and 49% respectively). The N-acetylcysteine pre-treated animals also showed the significant decrease in serum AST, ALT, LDH, total bilirubin, CRP and MPO levels when compared to animals exclusively treated with APAP.

3.4. Liver MDA, GSH, GST, SOD and MPO Levels

The effects of pre-treatment with *L. corniculatus* extract on liver MDA, GSH, GST, SOD, and MPO levels are represented in Table **4**. Liver MDA levels, as a diagnostic index of the extent of lipid peroxidation, were increased after treatment with APAP compared to control animals. However, the pre-treatment of rats with 100 and 200 mg/kg extract obtained from *L. corniculatus* reduced liver MDA levels by 33 and 46%, respectively. The APAP treatment significantly reduced the level of liver GSH, GST and SOD levels compared to control animals. Pre-treatment with 100 and 200mg/kg of *L. corniculatus* significantly enhanced the liver GSH and enzymes antioxidants levels in a dose-dependent manner as follow: GSH (13.9 and 54.1% respectively), SOD (14.8 and 33.8 % respectively) and GST (18.1 and 34.8% respectively). APAP- treatment significantly increases the activity of liver MPO compared to the control animals, while pre-treatment with 100 and 200mg/kg of *L. corni-culatus* reversed these levels by 28% and 47% respectively. The standard drug (N-acetylcysteine) pre-treatment significantly pre-

Table 4. Effect of *L. corniculatus* on liver oxidative stress and inflammatory markers in APAP-intoxicated animals.

Treatments	MDA (nM/g Liver)	GSH (µM/g Liver)	GST (U/mg Protein)	SOD (U/mg Protein)	MPO (U/mg Protein)
Control	5.6 ± 1.1	14.5 ± 1.1	30.4 ± 1.6	14.0 ± 1.9	11.35 ± 1.1
APAP (750 mg/kg)	12.6 ± 1.9**	8.4 ± 1.5***	18.3 ± 1.2***	8.1 ± 0.4***	30.06 ± 5.4***
L. corniculatus (100mg/kg) +APAP	8.5 ± 1.9$^{\#}$	9.6 ± 0.6$^{\#}$	21.5 ± 2.2$^{\#}$	9.3 ± 1.4$^{\#\#}$	21.13 ± 1.4$^{\#}$
L. corniculatus (200mg/kg) +APAP	6.8 ± 1$^{\#\#}$	12.9 ± 0.8$^{\#\#}$	24.7 ± 2.3$^{\#\#}$	10.8 ± 0.5$^{\#\#\#}$	15.8 ± 4.6$^{\#\#}$
N-acetylcysteine (200mg/kg)+APAP	6.8 ± 1.9$^{\#\#}$	13.9 ± 1.1$^{\#\#\#}$	28.9 ± 1.7$^{\#\#\#}$	12.7 ± 0.5$^{\#\#\#}$	12.1 ± 1.4$^{\#\#\#}$

Vales are Mean ± SD (n=6). $^{**}p \leq 0.01$ compared to control group. $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$ compared APAP- treated group. APAP: paracetamol; MDA: malondialdehyde; GSH: reduced glutathione; MPO: myeloperoxidase.

served the decline in the levels of the above-mentioned markers of liver oxidative stress and inflammation compared to APAP- treated animals.

3.5. Histology Observations

Results of the liver histology analysis for different experimental groups are shown in Fig. (**1**). Liver sections from control animals showed no histological abnormalities (Fig. **1A**), while in the sections from APAP- treated animals, a massive inflammation with cells undergoing necrosis around the peri-venular area was observed (Fig. **1B**). The preparations from *L. corniculatus* extract (100 mg/kg) pre-treated animals showed a minimal hepatic change, which was only accompanied by a mild focal inflammation (Fig. **1C**). Liver specimens from *L. corniculatus* extract (200 mg/kg) pre-treated animals showed a partial recovery in the structure of hepatic tissue (Fig. **1D**). Liver section from N-acetylcysteine pre-treated animals showed the normal lobular architecture of the liver with hepatocyte arranged in the single cord (Fig. **1E**).

4. DISCUSSION

Herbal medicines have been used in the treatment of liver diseases for a long time. Solvent extraction is the most frequently used technique for the isolation of medicinal plant antioxidant compounds [27]. In the current study, *L. corniculatus* butanolic extract was chosen because it is known that n-BuOH is a suitable solvent for polyphenols mainly flavonoids, phenolic acids, tannins extraction from plants materials [28]. Besides, it was demonstrated that n-BuOH of medicinal plants is rich in polyphenols that mitigate oxidative stress and inflammatory processes in the liver following APAP toxicity [20]. Phytochemical screening of *L. corniculatus* extract under study revealed the presence of polyphenols, flavonoids, terpenoids, and saponins. These components are reported to have antioxidant and curative properties against various liver disorders.

Preliminary antioxidant and anti-inflammatory screening of *L. corniculatus* extract is considered to be necessary. We evaluated their antioxidant and anti-inflammatory abilities by employing *in vitro* methods. The DPPH method is fast, easy and reliable for antioxidant activity screening and does not require a special reaction and device. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components [16]. In the current study, *L. corniculatus* show a noticeable effect on scavenging DPPH radical and act as a primary antioxidant. These extract may be rich in radical scavengers, such as flavonoids, phenolic acids derivatives known as antioxidants. Measurement of antioxidant activity of medicinally important plant extracts, anti-inflammatory drugs has been widely reported for a long time because of the wider

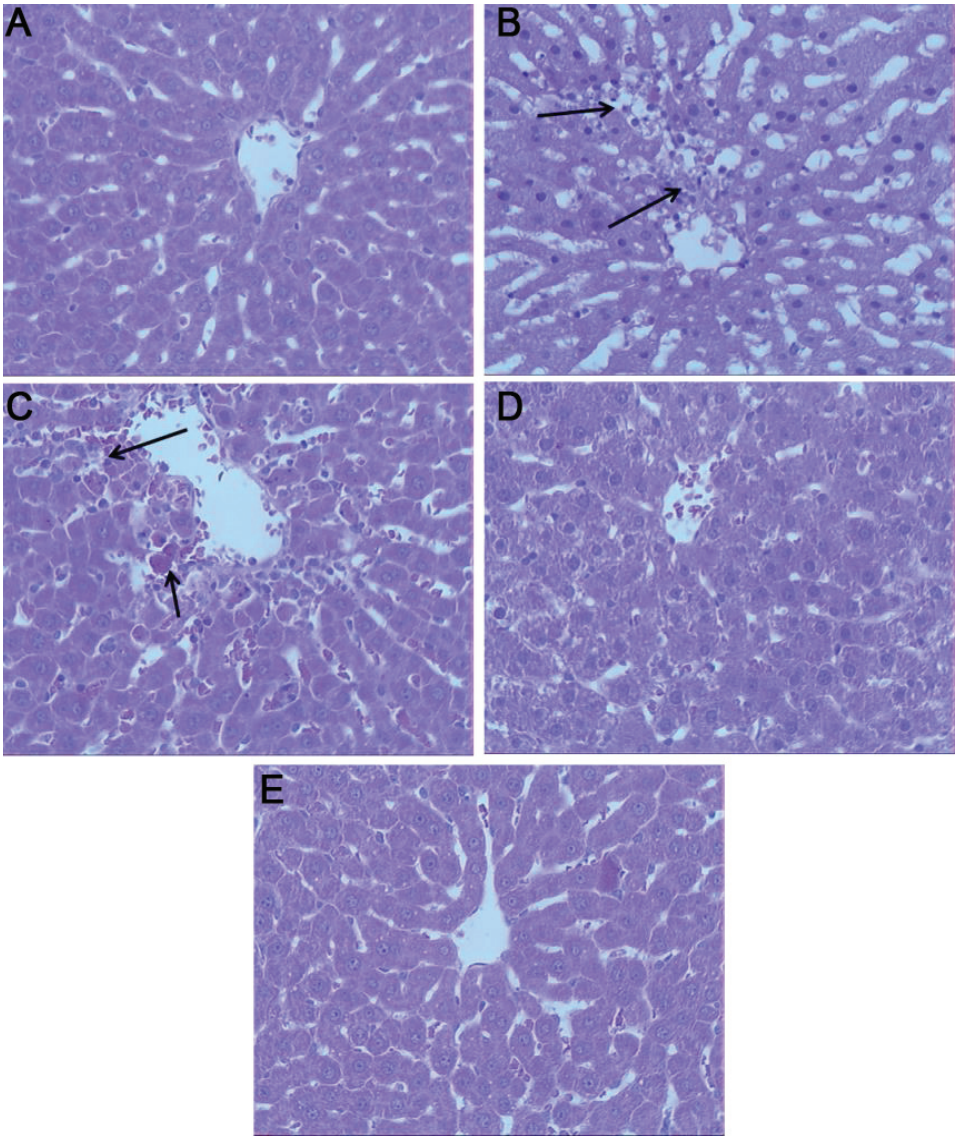

Fig. (1). Effect of *L. corniculatus* extract on liver tissue architecture (H&E, 200X). **A**: Control; **B**: APAP alone; **C**: *L. corniculatus* extract (100 mg/kg)+ APAP; **D**: *L. corniculatus* extract (200 mg/kg)+ APAP; **E**: N-acetylcysteine+ APAP. A massive to mild inflammatory cell infiltration and cells undergoing necrosis around the perivenular area (Arrows).

applications of antioxidants in pharmaceutical, food and cosmetics industries. This is because reactive oxygen species (ROS) is proved and proposed to be one of the most important factors in the pathogenesis of diverse diseases [29]. Amongst the ROS, H_2O_2 is an important molecule as although it is not toxic by itself, but can be converted to other even more toxic radicals such as hydroxyl radical by Fenton reaction or hypochlorous acid by the enzyme MPO. The generation of H_2O_2 by activated phagocytes is known to play an important role as bactericidal and antifungal since it also acts as mediators of inflammation by activation of signal transduction pathways [30]. Although H_2O_2 itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important to combat oxidative stress and MPO-dependent ROS [17, 29]. *L. corniculatus* extract was capable of scavenging H_2O_2 in a concentration-dependent manner. Scavenging of H_2O_2 by extracts is attributed to their phenolics, which could donate electrons to H_2O_2. The extract might be used to provide a good H_2O_2 scavenger for human health and foods food systems. Furthermore, the inhibition of protein denaturation is the fundamental

mechanism of NSAIDs action before the discovery of their inhibitory ability on cyclooxygenase [31]. Therefore, the ability of *L. corniculatus* to prevent protein denaturation was assessed as part of their anti-inflammatory effect. *L. corniculatus* inhibited the denaturation of BSA in a dose-dependent manner. It is previously reported that many plants extract and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activity of many plants [31-33]. These finding of *in vitro* models suggested that *L.corniculatus* extract has potent antioxidant and anti-inflammatory potential. Results indicate also, that these beneficial activities may be due to the occurrence of bioactive compounds, such as polyphenols and flavonoids in this plant. The observed *in vitro* activities suggest that the investigated plant extract could exert protective effects also *in vivo* against oxidative and inflammatory process occurring in the different pathological condition.

In *in vivo* study, the administration of APAP to rats increased the levels of marker enzymes like ALT, AST, and LDH, which are normally stored in the liver cells. The increase in the levels of these marker enzymes in serum indicates damage in the liver cells [34]. Serum total bilirubin is considered as one of the reliable tests to assess the liver function as well as reflecting the ability of the liver to take up and process bilirubin into bile [35]. High levels of total bilirubin in the APAP- treated rats may be due to drug toxicity. The lowering of AST, AST, LDH, and total bilirubin are the definite indication of the hepatoprotective action of *L. corniculatus* extract. The histopathological observations supported the biochemical evidence of hepatoprotection. APAP hepatotoxicity occurs through formation of the noxious NAPQI metabolite, which is present in excessive quantities, as augmented by features of GSH depletion, oxidative stress, and mitochondrial dysfunction, leading to depletion in adenosine triphosphate stores [36]. Earlier studies argued for cytochrome P450-generated reactive oxygen species during APAP metabolism, which resulted in massive lipid peroxidation and subsequent liver injury [28]. In this study, APAP alone increased liver MDA levels, which were paralleled by the concomitant decrease of liver GSH, GST and SOD levels. These indicating enhancements of lipid peroxidation leading to liver damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. The enzymatic and non- enzymatic antioxidant defense system is the natural protector against lipid peroxidation. GSH plays an essential role in detoxification of NAPQI and prevention of APAP induced liver injury. Because NAPQI is directly detoxified by GSH *via* GST, the fall in hepatic GSH levels probably reflects its intensive use in this process. In addition, the simultaneous decrease in GST and SOD activity also significantly contributed to the reduction in antioxidative capacity inducing a stronger degradation of the cell status following the APAP challenge [20]. Pretreatment with *L. corniculatus* significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by *L. corniculatus* is due to its antioxidant effect. The ability of medicinal plants to prevent APAP induced hepatotoxicity has been previously reported [20, 37].

Inflammation and oxidative stress are considered critical factors in the progression of drugs induced liver disease. There is evidence that inflammatory cells may be involved in the pathophysiology of APAP [18, 36]. CRP is an exquisitely sensitive systemic marker of inflammation and tissue damage [38]. Furthermore, myeloperoxidase (MPO) is a key component of the oxygen-dependent microbial activity of phagocytes but it also been linked to tissue damage in acute or chronic inflammation [39]. MPO is involved in the generation of ROS and nitric oxide-derived oxidants [40]. Thus, MPO promotes inflammation and oxidative stress [41]. Estimation of MPO activity is a reliable indicator of inflammation, being more sensitive than histopathological examination of tissue and as good as the measurement of IL-6 concentrations [42]. However, the association of circulating (plasma or serum) and /or liver MPO levels in outcomes of experimental inflammatory diseases has been reported [42-45]. In agreement with these data, APAP administration significantly increased serum CRP level and serum and liver MPO levels, suggesting the occurrence of an inflammatory response. These triggered by the release of cellular contents from necrotic hepato-

cytes into the systemic circulation, initiate the recruitment of neutrophils into the liver [19]. Thus, the enhanced level of MPO activity is one of the best diagnostic tools of inflammatory and oxidative stress biomarkers among these commonly occurring diseases. Even though a strong correlation has been found between inflammatory diseases, ROS formation, and MPO release, little work has been done to inhibit MPO to suppress these diseases. Several naturally occurring anti-inflammatory and antioxidant compounds possess inhibitory activities against MPO [46]. These compounds include nonsteroidal anti-inflammatory drugs (Flufenamic acid) and resveratrol [47, 48]. In the present study, neutrophil migration was measured indirectly by MPO level and *L. corniculatus* pretreatment decreased the MPO level compared to the APAP- treated animals, suggesting a decrease of neutrophil infiltration and anti-inflammatory effectiveness of *L. corniculatus*. We believe that the possible mechanism of anti-inflammatory action offered by *L. corniculatus* extract is due to their bioactive components antioxidant ability to inhibit the peroxidasic activity of the MPO *via* inhibition of the chlorination, oxidation, and nitration activities of MPO [49]. Besides, the observed inhibitory effect of the *L. corniculatus* bioactive compounds on circulating and liver MPO levels can be related to their scavenging activity or inhibition of H_2O_2 consumption by MPO and consequently inhibition of hypochlorous acid (HOCl) production by the MPO-H_2O_2-chloride system [29]. The HOCl is implicated as a key event in the progression of a number of inflammatory diseases [50]. Thus, the removing ability of H_2O_2 is very important for antioxidant defense and the inhibitory effect of *L. corniculatus* on MPO activity and /or MPO-specific ROS. Inhibition of BSA denaturation may further confirm an important role in the anti-inflammatory activity of *L. corniculatus*. Thus, it is possible to demonstrate that polyphenols from *L. corniculatus* may be a promising candidate for anti-inflammation treatment. Previous research has reported that polyphenols from *L. corniculatus* possess antioxidant and anti-inflammatory activity; however, there are few literature reports on its anti-inflammatory mechanism [7-9, 11].

CONCLUSION

These results showed for the first time that *L. corniculatus* extract enriched with flavonoids and polyphenolic compounds possesses significant hepatoprotective and anti-inflammatory activity manifested by combating the oxidative stress and modulating the levels of the inflammatory biomarkers involved in APAP induced hepatitis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol employed was approved by the Institute of Biology, Mentouri Brothers University Constantine, Algeria, (under inscription number 06/SNL/14).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the animal procedures were performed in accordance with the guidelines of European Communities Council Directive (2010/63/EU) for animal experiments.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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