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Original Research Article

Antioxidant, anti-inflammatory and antiulcer effects of Moroccan *Ceratonia siliqua* pulp in animal models

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Abstract

Purpose: *To investigate the potential antioxidant, anti-inflammatory and antiulcer effects of methanol extract of Ceratonia siliqua (Fabaceae).*

Methods: *The methanol extract of Ceratonia siliqua pulp was obtained using Soxhlet apparatus. Xylene (20 µL) induced ear inflammation administered intraperitoneally and HCl/ethanol solution (1 mL) induced gastric ulceration administered intra-gastrically were used to induce inflammation and gastric ulcer, respectively. Twenty-four mice were randomly divided into 4 groups (n = 6). Furthermore, eighteen rats (Sprague Dawley) were divided into 4 groups. Control group received (10 mL/kg of saline), Ceratonia siliqua group received (250 and 500 mg/kg), and other groups received diclofenac sodium (10 mg/kg; p,o), and omeprazole (30 mg/kg; p,o). Phytochemical screening was carried out by following standard procedures. The percentage of inhibition, pH, and ulceration index were measured using pH metric titration and degree of ulceration. The antioxidant capacity was determined in vitro using radical DPPH scavenging and FRAP assays.*

Results: *Phytochemical screening reveals the presence of flavonoids, tannins, quinones and sterols. Acute toxicity assessment showed that methanolic extract of Ceratonia siliqua has a DL_{50} higher than 5 g/kg. Oral administration of methanolic extract of Ceratonia siliqua (500 mg/kg) reduced significantly ($p < 0.001$) the percentage inflammation and ulceration index in both assays in comparison to control groups, these effects were comparable to those observed in reference drugs. The extract possessed a strong antioxidant capacity with low IC_{50}.*

Conclusion: *Ceratonia siliqua extract has antioxidant, anti-inflammatory and anti-ulcer properties. Standardization of Ceratonia siliqua extract will be required for the development of lead molecules against ulcers and inflammation.*

Keywords: *Ceratonia siliqua, Anti-inflammatory effect, Antioxidant activity, Antiulcer effect*

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INTRODUCTION

Inflammatory process which may be either acute or chronic is a very complex biological response of vascular tissues to harmful stimuli including damaged cells, pathogens or irritants (chemical/physical). It acts as a mechanism of defense to eliminate harmful stimuli and initiate the healing process of tissue [1]. A host of degenerative disorders including cancer, rheumatoid arthritis, polymyalgia, rheumatic shoulder, gouty arthritis, asthma, tendonitis heart disease and inflammatory bowel disease are frequently associated with inflammatory processes [2].

More than 30 million people consume nonsteroidal anti-inflammatory drugs (NSAIDs) daily. It has risen dramatically because of increased usage of over-the-counter and prescription NSAIDs, low-dose aspirin and claims of their anti-neoplastic properties [3]. The effectiveness of NSAIDs as anti-inflammatory, and analgesic is undeniable, but their side effect is alarming. These mostly include altered renal, hepatic and cardiovascular activities. Although, cardiovascular adverse events are currently receiving a lot of attention, that cannot be said about the frequency and severity of gastrointestinal injury [3]. As a result, gastroduodenal ulcer incidence varies from 5 to 80 % in short-term endoscopic investigations and from 15 to 40 % in long-term users [4]. These drugs harm the small intestine when used frequently, and 30 % of users develop ulcers and erosions. Gastric and small bowel injury was linked to a variety of management issues and, in some cases, life-threatening complications including strictures, perforation and bleeding [5].

To surmount the toxicity of NSAIDs, the discovery of novel anti-inflammatory drugs from natural sources such as medicinal plants is important. Carob (*Ceratonia siliqua* L.) is an evergreen plant from the Fabaceae family that originated in the Mediterranean circuit. Pods and seeds have numerous uses in the culinary, cosmetic and medicinal sectors. Furthermore, carob bean gum (E410) is a widely used food ingredient. The fruits from this tree are extremely nutritious and have a variety of traditional therapeutic uses, including purgative, laxative, diuretic and antidiabetic. Recently, carob has attracted great interest due to its abundance of bioactive compounds, which include polyphenols, dietary fibers, polyphenols and tannins [5].

This study aimed to validate the ethno-medical usage of *Ceratonia siliqua* pulps by investigating their effect on gastric ulcers and acute inflammatory animal models.

EXPERIMENTAL

Plant material

Pulps of *Ceratonia silliqua* were first collected in the region of Aghafala Azilal in 2021. The sample was identified by Professor Ouhammou of the Faculty of Sciences Semlalia, Cadi Ayyad University and deposited at Mark herbarium under a specimen (CS 21). The pulps were shade-dried at 25 ^{o}C and then transformed to fine powder. *Ceratonia silliqua* powdered pulps (150 g) were extracted with methanol (750 mL) using a soxhlet apparatus for 75 h at 65 ^{o}C. Then, the solvent was evaporated at 45 °C using Rota-vapor apparatus.

Animals

Wistar rats and Swiss mice weighing between 150 - 200 g; and 27 - 35 g, respectively were used in this study. The animals were acclimatized before proceeding and kept under standard conditions with a stable temperature of 23 ± 2 ^{o}C, about 24 – 50 % relative humidity and a dark/light cycle. The animals were provided by Faculty of Sciences, Semlalia Facility, Cadi Ayyad University, Marrakech, Morocco, and handled based on approved institutional procedures and in accordance with the provisions for animal care and use described in Scientific Procedures on Living Animals ACT [6]. All efforts were made to minimize the suffering of animals and to reduce the number of animals used in all experiments. The acute toxicity assessment followed the standard procedure described in OECD guidelines 425 [7].

Animal grouping

Mice were separated into 4 groups (n = 6), rats were divided into two groups (n = 6) and two groups were considered as control, and six groups were treated with saline and/or methanol extract of *Ceratonia siliqua* according to the following design.

Development of anti-inflammatory model

Group 1: Negative control of inflammatory assay (sodium chloride {NaCl} 0.9 %, i.p)

Group 2: xylene induced ear edema + *Ceratonia siliqua* (250 mg/kg)

Group 3: xylene induced ear edema + *Ceratonia siliqua* (500 mg/kg)

Group 4: xylene induced ear edema + diclofenac (10 mg/kg)

Development of ulcer model

Group 1: Negative control of anti-ulcer activity (NaCl 0.9 %)

Group 2: gastric ulcer induced by Hydrochloric acid (HCL)/Ethanol mixed + *Ceratonia siliqua* (250 mg/kg)

Group 3: gastric ulcer induced by HCL/Ethanol mixed + *Ceratonia silliqua* (500 mg/kg)

Group 4: gastric ulcer induced by HCL/ethanol mixed + omeprazole (30 mg/kg)

Phytochemical screening

The following secondary metabolites were determined for qualitative and quantitative content: flavonoids, coumarins, steroids, terpenes, anthocyanin, tannins, saponins, alkaloids and quinines [9].

Acute toxicity assay

Acute toxicity of *Ceratonia siliqua* extract was evaluated in mice. They were divided into three groups containing six mice each and fasted for 12 h before experiments. Doses of methanol extract of *Ceratonia siliqua* pulp (2 and 5 g/kg) were administered to the first 2 groups, respectively through the oral route. While, mice of negative control received saline (10 mL/kg; p,o.). The symptoms of acute toxicity were recorded for 48 h post-treatment and then once a day for 14 days [8].

Determination of antioxidant activity

DPPH assay

The capacity of *Ceratonia siliqua* (*C. siliqua*) methanol extract to trap Diphenylpicryl hydrazyl radical (DPPH) was assessed according to the protocol described [10]. Two milliliters of DPPH methanolic solution (60 µM) was mixed with 50 µL of several sample concentrations. The mixture was stirred and maintained for 20 min at ambient temperature. The absorbance at 517 nm of different concentrations of extract was recorded by spectrophotometry using methanol as a blank. Ascorbic acid and Butylhydroxytoluene (BHT) were used as positive control.

Reducing FRAP assay

The extract reductive ability was assessed based on the method described [10]. Potassium (2.5 mL, 1 %) and Phosphate buffer (2.5 mL, 200 mM, pH 6.6) were combined with 1 mL of different concentrations of samples. The mixture was incorporated at 50 ^{o}C for 20 min. Then, a volume of 2.5 mL of 10 % trichloroacetic was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer solution (2.5 mL) was added to 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride. Quercetin and BHT were positive control.

Pharmacological investigations

Xylene test

The possible anti-inflammatory activity of plant extract was evaluated using xylene assay. The assay was done based on the method described previously [11]. Four groups of 5 mice each were administered as described; group 1 received intraperitoneal injection of NaCl (10 mL/kg), groups 2 and 3 got methanolic pulp extract of *C. siliqua* (250 and 500 mg/kg, p.o. respectively), group four received an intraperitoneal injection of Diclofenac (10 mg/kg i.p.). Inflammation was induced by intradermal injection of xylene solution (20 µL) into the right ear's dorsal and frontal surfaces. All treatments were administrated 45 min before the xylene application. After one hour of xylene injection, mice were sacrificed by cervical dislocation and their ears were cut off [11]. Circular pieces (7 mm) of the left (untreated) and right (treated) ear were prepared and weighed. The degree of inflammation was determined as the difference in weight between both the left and right ears.

Antiulcer activity

Acute gastric ulcers were induced by ethanol/hydrochloric acid (EtOH/HCL) using an intra-gastric route according to a previous report [12]. After 24 h of fasting, rats (n = 6/each group) received methanolic extract doses of *C. siliqua* pulp (250 and 500 mg/kg). Groups 1 and 2, the vehicle (normal saline 10 mL/kg) in sham; group 3 and Omeprazole (30 mg/kg p.o.) as a positive control; group 4, 1 h before receiving 1 mL of 150 Mm HCL in 60 % ethanol (p.o.). After one hour of EtOH/HCL administration, rats were sacrificed by an excessive dose of chloral hydrate (6 %) intraperitoneally. Gastric pH was determined. The stomachs were removed rinsed thoroughly with saline and fixed in 10 % formalin for 10 min. The macroscopic examination was performed after opening along the greater curvature. After measuring the gastric lesions, ulceration index

and the percentage of inhibition of lesion formation were calculated [13].

Statistical analysis

The graph-pad (version 9) software was used to analyze all statistical differences and data were expressed as mean ± standard error of the mean (SEM) using ANOVA followed by Tukey and Kruskal Wallis post hoc analysis.

RESULTS

Acute toxicity

There were no deaths or symptoms indicating toxicity such as convulsion, ataxia, diarrhea or increased diuresis, and no behavioral abnormalities during the observation period. Consequently, the oral lethal dose 50 is greater than 5 g/kg in mice.

Phytochemicals

Qualitative phytochemical screening of *C. siliqua* methanol extract revealed an abundance of tannins, flavonoids, sterols and quinones. Thus, the absence of coumarins, terpenes, saponins and alkaloids was confirmed.

Antioxidant activity

The antioxidant capacity was measured in vitro utilizing two complementary assays: DPPH and FRAP. The outcomes are shown in (Table 1). The antioxidant activity of the extract was compared with ascorbic acid, butylated hydroxytoluene (BHT) and quercetin. Lower IC_{50} values indicate stronger antioxidant activity. *C. siliqua* extract exhibited insignificant antioxidant activity, with the lowest IC_{50} obtained with DPPH (IC_{50} = 15.233 ± 0.340 µg/mL). This action was less powerful than those of the reference standard antioxidants.

Table 1: IC_{50} (µg/mL) values of *Ceratonia siliqua*

Sample	DPPH (µg/mL)	Reducing power (µg/mL)
BHT	4.04±0.01	2.31±0.01
Quercetin	1.91±0.02	2.66±0.05
Ascorbic acid	1.81±0.01	4.05±0.01
Methanol extract	15.23±0.34	23.45±3.12

Note: Values are presented as mean ± SEM for each replicate (number of replicates equals 3)

Anti-inflammatory activity

Figure 1 shows the effect of *C. siliqua* pulp methanol extract on the xylene-induced ear edema. After xylene application, a significant increase in edema was observed in negative control. However, the methanol extract at all pharmacological doses (250 and 500 mg/kg) significantly reduced the ear edema induced by xylene in comparison to negative group, in a dose-dependent manner. However, the observed effect mentioned previously was less than that of the reference anti-inflammatory drug (diclofenac).

Table 2: Phytochemical screening of methanol extract of *Ceratonia siliqua*

Phytochemical	Inferences
Flavonoids	+++
Tannins	+++
Quinones	+++
Sterols	+++
Saponins	-
Coumarins	-
Terpenes	-
Alkaloids	-

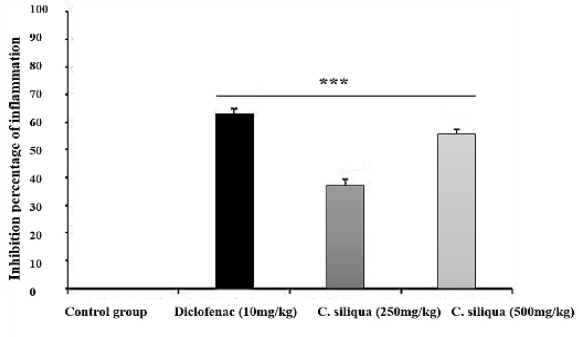

Figure 1: Anti-inflammatory effect of C. siliqua methanol extract in xylene test. Data are provided as mean ± SEM. ***P < 0.001, significant difference between treated groups and negative control

Antiulcer activity

Concerning antiulcer capacity, results demonstrated that the control group treated with (HCL/ethanol) solution presented has a greater degree of ulceration. On the other hand, groups treated with omeprazole and the methanol extract of *C. siliqua* pulp (250 and 500 mg/kg) recorded a marked significant decrease (p < 0.001) in the ulceration index compared to the negative group (Figure 2).

Inhibitory effect

Concerning inhibitory effect of *C. siliqua* pulp the methanol extract, we noted that all doses (250 and 500 mg/kg) presented a higher inhibitory effect in comparison with the negative group. This suggests a strong inhibitory and protective effect of the Moroccan pulp of *C. siliqua* (Table 3).

Gastric lesions

As shown in Figure 3, animals pretreated with methanolic extract present minor lesions in their stomachs. However, there was a change in coloration in negative control indicating inflammation as well as major lesions (Figure 3).

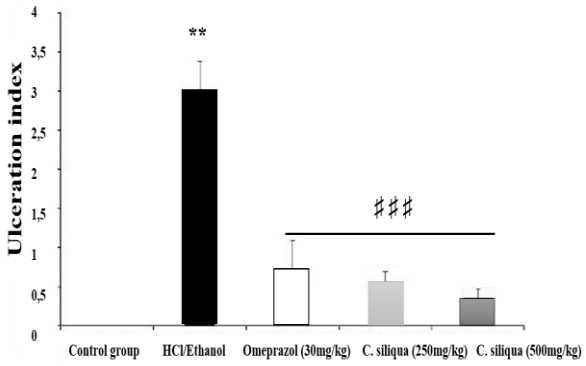

Figure 2: Effect of *C. siliqua* methanol extract on ulceration index in ulcers model induced by HCl/Ethanol. ***Note:*** Data are presented as mean ± SEM. *Difference between HCl/Ethanol group and control group, #indicates difference between treated groups and HCl/Ethanol group. Statistical analysis were done using ANOVA one-way followed by Kruskal Wallis post hoc test

Table 3: Inhibitory activity of *Ceratonia siliqua* L. pulp methanol extract on ulcers induced by HCl/Ethanol

Treated group	Inhibition percentage (%)
Group treated with HCl/Ethanol	0±0
Group treated with Omeprazole (30 mg/kg)	86.51±3.50***
Group treated with *C. siliqua* (250 mg/kg)	90.66±3.48***
Group treated with *C. siliqua* (500 mg/kg)	92.66±3.71***

Note: percentage of inhibition was calculated using formula that was mentioned previously, where *$p < 0.001$ indicates significant difference between control and treated groups

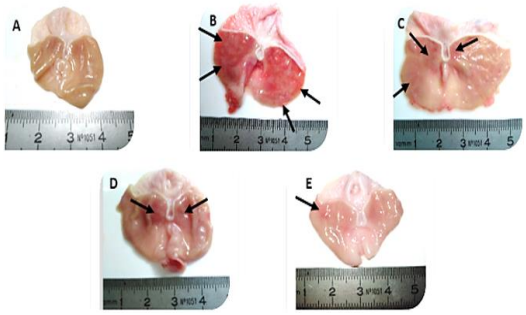

Figure 3: Illustrations of gastritis caused by ethanol-HCl solution in rat stomach. (A), normal stomachs; (B), control treated with HCL/Ethanol; (C), 30 mg/kg Omeprazole (D), 250 mg/kg of *Ceratonia siliqua* extract and (E) 500 mg/kg *Ceratonia siliqua* extract

Effect of *C. siliqua* methanol extract on the pH

As mentioned in Figure 4, results show a low decrease in gastric pH in the group treated with omeprazole compared with the negative group. Concerning both groups of rats treated with 250 and 500 mg/kg of methanol extract of *C. siliqua,* approximately the same gastric ph values were noted, but no significant statistical difference.

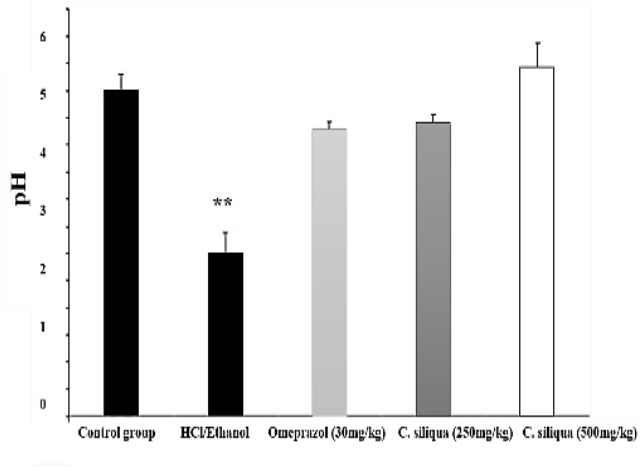

Figure 4: Effect of C. siliqua methanol extract on pH in HCl/Ethanol ulcers model. Data are presented as mean ± SEM. * Difference between HCl/Ethanol group and other groups

DISCUSSION

This study is an approach that aims to validate the interesting effect of a medicinal plant in the prevention of a common symptom that mars the majority of diseases. *C. siliqua* is widely used in traditional medicine in the Mediterranean region as a diuretic, purgative, anti-diabetic, anti-inflammatory and laxative. During the past few years, *C. siliqua* has garnered attention due to its rich bioactive compounds and exceptional antioxidant ability [14].

Studying the biological effects of extracts of any plant starts by testing its toxicological profile, preliminary assessment of *C. siliqua* methanolic extract's acute toxicity leads to the conclusion that the plant is safe based on the absence of toxicity symptoms in different doses including the highest one (5 g/kg). Investigation into the effects of treatment with methanolic extract of *C. siliqua* on xylene-induced acute inflammation was conducted in this study, building upon the classical method of utilizing xylene-induced ear inflammation in mice to assess the efficacy of anti-inflammatory agents.

Following topical application of xylene, sharp rises in weight of the ear were determined, pointing towards an acute inflammatory response. These changes in ear weight serve as reliable indicators of the anti-inflammatory potential. Results showed an important dose-dependent inhibition exerted by pretreatment with both doses of the extract as well as Diclofenac (reference drug). This inhibition provides direct evidence suggesting that the extract exhibits a beneficial effect in reducing acute inflammatory responses. While the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) in alleviating inflammation and pain is well-established, their associated side effects pose significant concerns. The use of this medication has been linked to gastric and small intestinal injuries. Given the toxicity associated with NSAIDs, there is an imperative need to develop new anti-inflammatory drugs and biologics that offer the potential to treat inflammation with minimal or no adverse effects [15].

The cyclooxygenase (COX) pathway plays a crucial role in the production and release of inflammatory prostaglandins. Several reports confirm the immunomodulatory activity exerted by flavonoids, which make plants rich in these metabolites great anti-inflammatory agents [16].

On this basis, we resorted to the use of *Ceratonia siliqua* fruit to treat inflammation and prevention of gastric ulcer. The potential anti-ulcer effects of the methanolic extract derived from the pulp of *Ceratonia siliqua* were investigated through the evaluation of three indices: the ulceration index, the percentage of gastric ulcer inhibition, and gastric pH levels. The HCL/ethanol gastric ulcer is a classic model designed to evaluate the anti-ulcer effect of some herbal drugs on gastric ulcers and severe damage to the lining stomach [16]. Alcohol and hydrochloric acid are two chemicals that disrupt the balance between the aggressive and protective factors of the gastric mucosa.

There is evidence from these results that HCL/ethanol administration induced visible macroscopic lesions, which included hemorrhage and hyperemia, in addition to histopathological changes such as erosive lesions in rats. However, pretreatment with *Ceratonia siliqua* methanolic extract significantly reduced macroscopic gastric mucosal damage, with both doses acting to decrease gastric pH in a manner dependent on the dose. The potential anti-inflammatory and antiulcer effects of *Ceratonia siliqua* extract might be associated with its phytochemical components such as phenolic compounds, mainly flavonoids that possess several pharmacological properties. The gastro-protective effect is linked to the presence of antioxidant components. Besides their anti-inflammatory action, flavonoids like rutin, quercitin, P-coumaric acid and catechin possess significant anti-ulcer activity [17].

This study investigated the protective capacity of *Ceratonia siliqua* methanol extract against oxidative stress employing assays for DPPH free radical scavenging activity and ferric reducing power. The outcomes reveal an interesting antioxidant activity with a low IC_{50}, consistent with prior studies as earlier reported [18]. Many reports suggest that antioxidants mitigate ulcers and inflammation induced by chemical stimulators [19]. The antioxidant capacity of plant extract could be related to their phenolic compounds. Regardless, the antioxidant properties of these plants might be due to various mechanisms during gastrointestinal route and inflammation [19].

CONCLUSION

The therapeutic properties of *Ceratonia siliqua* are attributed to the abundance of polyphenols and sterols, which play a significant role in their anti-inflammatory and antiulcer effects. *Ceratonia siliqua's* anti-inflammatory and anti-ulcer properties make it important for further investigations as a source of active pharmaceutical ingredients.

DECLARATIONS

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Ethical approval

This study received institutional approval from Faculty of Sciences, Semlalia Facility, Cadi Ayyad University, Marrakech, Morocco.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Souad Moubtakir wrote the original draft, did the review and editing of the final manuscript. Chafik, Terrafe, Majda, Badaoui, Khadija oubella, Mehdi Aitlaaradia carried out all investigations. Data analysis was done by Fatima Zahra and Agouram while Rachida, Aboufatima, Loubna, Yazouli, and My Ahmed did the validation and review. Methodology, validation, writing the original draft, editing and supervision, were carried out by Chait, and Abderrahman. All authors read and approved the manuscript for publication.

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