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CHEMICAL COMPOSITION, ANTI-ULCER AND ANTI-INFLAMMATORY EFFECTS OF CAROB PODS (*CERATONIA SILIQUA L.*) POLYPHENOLS FROM AIN TEMOUCHENT

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ABSTRACT

This research was aimed to evaluate the chemical composition by HPLC/UV, anti-inflammatory effect *in vitro* (hyaluronidase assay) and anti-ulcer activity *in vivo* against gastric ulcer. This anti-ulcer activity was explored *in vivo* on Wistar rats exposed to a gastric ulcer provoked with oral administration of HCl /ethanol (150 mM /60%) for one day after five days of oral treatment with 250 mg/kg of ethanolic extract of carob pods (EECp), to restore stomach ulceration. Several compounds of carob have been identified by HPLC/UV. This identification reveals the existence of phenolic compounds; flavonoids (chrysin, galangin, pinocembrin, quercetin, genistein, kaempferol, etc.) and phenolic acids (rosmarinic acid, caffeic acids, ferulic acid, etc.). Our extract showed strong inhibitory activity of hyaluronidase with (IC_{50} = 20.7 mg / ml). The results obtained indicated that administration of HCl /ethanol alone produced stomach ulceration in rats with very extensive and bleeding gastric lesions with 44.09% of percentage lesion and 362.26 mm^2 of lesions area in the stomach mucosa. However, pretreatement of rats with EECp revealed a spectacular inhibition of gastric mucosal damage (7.46%), a significant reduction in malondialdehyde (4.86 nmol/mL) and an increase in enzymes of oxidative status (superoxide dismutase, catalase and glutathione peroxidase). The present study results can attribute to carob an antioxidant, anti-inflammatory and anti-ulcer effects.

Keywords: Carob, HPLC/UV, hyaluronidase activity, gastroprotective effect, oxidative stress

INTRODUCTION

The most common gastrointestinal disorder in the world is considered the peptic ulcer; this disease is accompanied by a high rate of mortality and morbidity **(Daniel et al., 2017)**. It is a gastrointestinal tract disease, which embraces both ulcers (gastric and duodenal). Imbalances between destructive and defensive factors characterize this disease **(Mekonnen et al., 2020)**. HCl, pepsin, biliary reflux, lipid peroxidation, and the formation of reactive oxygen species (ROS) represent the endogenous destructive factors in the stomach, on the other hand are excessive use of ethanol, indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAID), stress, smoking, and infection byHelicobacter pyloribacteria represent the and the exogenous factors. The defensive factors include mucus-bicarbonate barrier, mucin secretion, surface phospholipids, prostaglandins (PGs), nitric oxide (NO), mucosal bloodflow, cell renewal, growth factors, and antioxidant enzymes **(Brito et al., 2018)**.
The process of Inflammation is implicated in the pathologic process and progression of multiple illnesses. This process is also considered as a physiological reaction that defends our body from microorganisms or damaged tissue. The reaction of inflammation reestablishes the tissue that are touched with infection or injury. The response of inflammatory is controlled by multiple mechanisms and acts as a body defense tool. Several pathological disorders can be caused by exacerbation of inflammation **(Ribeiro et al., 2018).** In the event that a marked inflammatory reaction occurs, the use of anti-inflammatory medicaments wish suppress or regulate inflammation will be necessary. These medications commonly have undesirable side impacts, which make it essentially to look for alternative substitutes **(Ghasemian et al., 2016).**
The production of free radicals is the inflammation consequence. While the production of free radicals can be curb by the natural antioxidant defenses of the body's organism. When inflammation is chronic or too intense, free radicals multiply, submerge antioxidant defenses and engender unsafe chain reactions. Though, chemical anti- inflammatory or antioxidant synthetic substances uses are ever accompanied by side impacts, while phytochemicals utilization is helpful without side impacts **(El Hilah et al., 2015; Moulai- Hacene et al., 2020)**.

Carob (*Ceratonia siliqua L.*) is a slow growing green tree cultured for years in Mediterranean countries. Carob bean (fruit) 10-25 cm long; contain several bioactive substances like dietary fiber, sweet carbohydrate, polyphenols and tannins **(Papagiannopoulos et al., 2004)**. Different studies have shown various biological activities and pharmacological characteristics of *Ceratonia siliqua* such as antioxidant **(Boufadi et al., 2015)** antibacterial activities **(Ouis and Hariri, 2018)** antidiabetic and antimicrobial activity **(Darwish et al., 2021)** anticancer activity **(Gregoriou et al., 2021)** gastroprotective activity **(Rtibi et al., 2015)** and anti-inflammatory activity **(Rtibi et al., 2017)**. Apparently, these properties are linked to the phenolic compounds that they include **(Vekiari et al., 2011)**.
Hence, the objectives of the present work were focused on the anti-inflammatory activity, gastroprotective effect and chemical composition of ethanolic pod extract of *Ceratonia siliqua L.* by HPLC/UV.

MATERIALS AND METHODS

Plant materials

The pods of ripe carob (*Ceratonia siliqua L.*) were collected in August 2020 from Ain Témouchent district, Algeria (Latitude: 35.3687, Longitude: 1.23556). They were wiped off with a sterile pad and dry.
The plant specimen was identification by one of the co-authors (K.B.) and voucher specimens (#CS 12) are kept at the Laboratory of Biotoxicology (Faculty of Nature and Life Sciences, University of Djillali Liabes, Sidi Bel abbes, Algeria).
The identification and authentication of this plant was made by Dr. K. M. Chetty, Sri Venkateswara University, Tirupathi, where the voucher specimen was deposited for more reference.

Plant extraction

The pods were shelled and then they were crushed to a fine powder. The carob extract was prepared (10 g of powder in 100 ml of ethanol 70%), in a tightly closed bottle in the shade at room temperature for 24 h **(Boufadi et al., 2015)**.

Then, the suspension was filtered with filter paper (Whatman Nº1) and after the solvent was evaporated and dried at 50 °C under vacuum. The filtrate presents the ethanolic extract of carob pods (EECp).

HPLC/UV analysis of phenolic compounds from carob extract

Chromatographic analysis of ethanolic extract of carob pods (EECp) was conducted by high performance liquid chromatography (Agilent 1100). Separation was carried out on an Agilent poroshell 120EC column (100 mm × 2.1 mm, 2.7 m), using mobile phases: water/TFA/formic acid (99:0.25:0.75) (A) and acetonitrile (B). The elution was effected at a flow rate of 0.6 ml/min with a 10 µL aliquot and at 55°C. Using a gradient method as noted below (t/min,% B): (0,0), (1,10), (2,12.5), (3,15), (9,80), (10,100), (11,100), (14,0) with post 5 min. Chromatograms were enregistred at 270 and 320 nm **(Chaa et al., 2019)**.
The sample was prepared by diluting EECp with methanol 1:100 (v/v). Identification of carob constituents were realized by comparing their retention times and their UV spectra with different commercially phenolic standards (trans-cinnamic, gallic acid, benzoic acid, ferulic acid, m-coumaric acid, caffeic acid, rosmarinic acid, and ellagic acid), flavonoids (catechin, hesperidin, thymol, luteolin, benzyl cafféate, galangin, genistein, tectochrysin, pinocembrin, acacetin, rutin, methylated quercentin, chrysin, apigenin, myricetin, resveratrol, kaempferol, and quercetin) and others constituents (ascorbic acid, menthol). The standards were dissolved in methanol to give stock solutions at 1 mg/ml. The quantification of components was determined using standard curves expressed in mg per 1 g of crude carob.

Anti-inflammatory activity hyaluronidase assay *in vitro*

This assay was performed by the method explained by **Boufadi et al. (2021)** with minor modifications.
50 µL of EECp (10, 20, 50, 100 and 200 mg/ml) was mixed with 50 µL (350 units) of hyaluronidase enzyme and incubated at 37°C for 20 min. Later, 1.25 µL of calcium chloride was added to activate the enzyme. After incubation, the reaction was place medium at 37°C for 20 minutes, and next we added 0.5 ml of hyaluronic acid sodium salt.
After incubation at 37°C for 40 min, 0.1 ml of potassium tetraborate was added and the mixture was incubated in a boiling water bath for 3 min. The mixture was placed at +6°C for stopping reaction, and 3ml of p-dimethylaminobenzaldehyde was added. The incubation was performed at 37°C for 20 min. Lastly, the absorbance was measured at 585 nm. All tests were executed three times.

Treatments of animals

Twenty five male Wistar rats (115-120 g) were obtained from Pasteur institute (Algiers, Algeria). housed at 22±2oC, with relative humidity of 40% and a 12:12 h light and dark cycle and given food and water ad libitum. After 2 weeks of acclimatization, we started the experiment. The animals fasted for 12 h before the experiment, with free access to water.
the experiments was conducted as per the council instructions about the protection of living animals used in scientific investigation issued from the council of European Communities.

Antiulcer activity

Effect of EECp on HCL /ethanol-induced ulcer and inflammation

We elicited induction of ulcers by the administration the HCl /éthanol to rats by orally, as done by **Martins et al. (2014)**. Animals were weighed and randomized in 4 groups. Groups 1 and 2 (G1, G2) was treated with 1 ml of Tween80. Groups 3 and 4 (G3, G4) was treated with 1 ml of 250 mg/kg of EECp. At day 5, and 1 hour after administration of treatment EECp, the rats of G2 and G4 were administrated orally with 1 ml with HCl/Ethanol solution (150 mM /60%) for inducing a gastric ulcer. And 1 h later, animals were euthanized. To prevent any risk of changes in biochemical parameters by administration of a general anaesthetic, they were maintained below light chloroform anaesthesia just before blood taking. Blood was collected by cardiac puncture in anticoagulant tubes.
Stomachs, esophagi, spleens, livers and intestines were removed. The stomach removed and opened along the greater curvature.

Analysis of serum parameters

Analysis of serum parameters (CBC, glycemia, albumin, total protein and fibrinogen) was performed by enzymatic and/or colorimetric methods (kits bioMérieux SA, France) using an auto-analyzer (Technicon RA, Opera systems N°ref. T01-2801-56).

Assessment of inflammation markers

Preparation of peritoneal fluid

Grind the peritoneum with PBS, centrifuge at 300 g / min for 15 min. The supernatant represents the peritoneal fluid (in order to assay the PGE2) **(Boufadi et al., 2021)**.

PGE2 measurement

Quantification of levels of PGE2 (prostaglandin E2) at the peritoneal fluid was performed using the Prostaglandin E2 ELISA kit (Abcam Discover More, UK) as per the instructions of manufacturer.

Oxidative stress analysis

Superoxide dismutase was performed in accordance by the technique of **Elstner et al. (1983)** and enzymatic activity of catalase level in the plasma with the protocol of **Lück (1965)** and **Aebi (1974)**. The activity of GPx in plasma was determinate in accordance with the protocol of **Paglia and Valentine (1967)**. Regarding malondialdehyde (MDA) measurement, we utilized serum in accordance by the method outlined by **Yagi (1976)**.

Histopathological analysis

With the aim of study the lesions affecting the stomachs and verify existence of any changes in the structure of the organs removed. A physiological solution was used to wash these organs to remove gastric contents and blood clots and fixed in 10% formaldehyde. Then, they were set in paraffin and cut on microtome to have 5 microns with a rotating micro tome (Leica, Germany) which was posed on clean blades and deparaffinized successively by two baths of 10 min of xylene. The slides were coloured with hematoxylin and eosin, and the slides were observed by optical microscopy **(Drury & Wallington, 1980)**.

Macroscopic evaluation of stomachs

Lesion areas (mm^2), percentage of lesion and inhibition rate compared to total area of stomach (%) have been defined **(De Andrade et al., 2007)**.
The determination of percentage of ulcer inhibition and ulcer index was performed such:
Ulcer Indice (UI) %= (total lesion area / total surface area of stomach)*100
Inhibition %= $(IU_{control} - IU_{traited}) / IU_{control})*100$

Statistical analysis

All tests were realized three times. Results were presented as the Mean ± Standard Error (SEM) and compared on the basis of XLSTAT. The values were considered significant when $p < 0.05$.

RESULTS

EECp chemical composition by HPLC/UV

The ethanolic extract of carob pods (EECp) from Ain Temouchent were subjected to HPLC analysis and the chromatograms are achieved at 270 and 320 nm (Fig 1 A and B, accordingly).

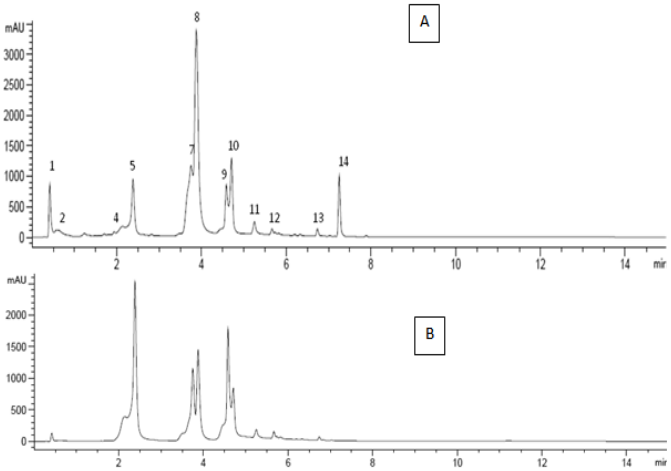

Figure 1 Chromatograms by HPLC/UV analysis of EECp at a wavelength of 270 (A) and 320 nm (B): 1. Chrysin; 2. Tectochrysin; 3. Acacetin; 4. Pinobanksin-3-acetate; 5. Quercetin; 6. Bis methylated quercentin; 7. Genistein; 8. Kaempferol; 9. Myricetin; 10. Galangin; 11. Catechin; 12. Pinocembrin; 13. Menthol; 14. Apigenin; 15. Luteolin; 16. Benzyl cafféate; 17. Pinobanksin-3-acetate; 18.

Resveratrol; 19. Ascorbic acid; 20. Trans cinnamic; 21. Ferulic acid; 22. Ellagic acid; 23. Rosmarinic acid; 24. Galic acid; 25. Cafeic acid.

Table 1 shows the levels of phenolic compounds (phenolic acid and flavonoids) in EECp. Ferulic acid (7.4 mg/g at 2.38 min) was found to be the main phenolic acid, while kaempferol is the principal flavonoid (3.12 mg/g at 3.87 min).

Table 1 Composition of ethanolic extract of carob pods (EECp) from Ain Temouchent by HPLC/UV (mg/g).

Peak number	Compound	Amount (mg/g EECp)	Retention time (min)
1	Chrysin	0,36	0,41
2	Tectochrysin	0,09	0,58
3	Acacetin	0,08	1,23
4	Pinobanksin-3-acetate	0,11	2,13
5	Quercetin	0,37	2,38
6	Bis methylated quercentin	0,06	2,61
7	Genistein	0,56	3,75
8	Kaempferol	3,12	3,87
9	Myricetin	0,31	4,58
10	Galangin	0,86	4,7
11	Catechin	0,22	5,25
12	Pinocembrin	0,19	5,66
13	Menthol	0,21	6,73
14	Apigenin	0,55	7,25
15	Luteolin	0,04	7,88
16	ascorbic acid	0,28	0,41
17	Trans cinnamic	0,39	2,13
18	Ferulic acid	7,4	2,38
19	Ellagic acid	5,3	3,74
20	rosmarinic acid	6,90	3,87
21	Galic acid	7,19	4,58
22	cafeic acid	4,21	4,7
23	Benzyl caffeate	0,31	5,25
24	Pinobanksin-3-acetate	0,29	5,63
25	Resveratrol	0,22	6,73

Hyaluronidase inhibition

From the results presented in Fig 2 it is deduced that increase in inhibitory activity of hyaluronidase is proportional to that of ethanolic extract of carob pods (EECp) concentrations (IC_{50} value of 20.7 ± 1.63 mg/ml). The inhibition percentage was 93.12 % at an EECp concentration of 200 mg/ml.

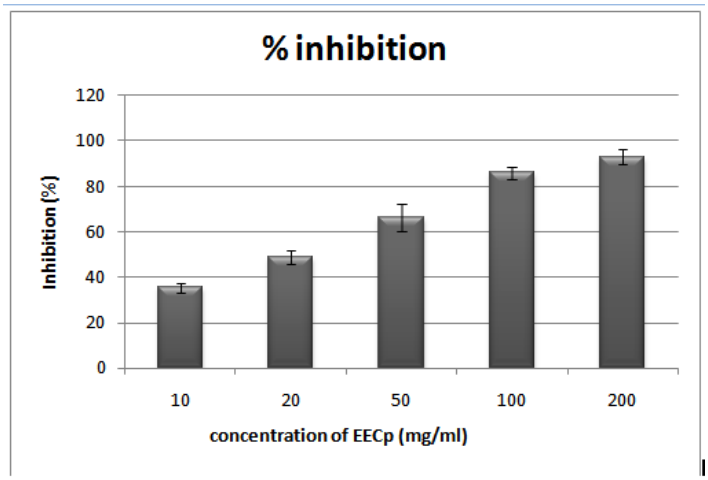

Figure 2 Percentage of inhibition of hyaluronidase activity by ethanolic extract of carob pods (EECp) for each concentration. The values are exhibited as mean ± SD.

Analyses of serum parameters

From our results, we notice that the G3 which received only the EECp showed an agreement toward the control group**.**
Table 2 revealed that glycemia level of G2 (1.84 g/l) significantly increased (P <0.05) compared to G1 (0, 78 g/l). The increase in glucose levels in this group shows a metabolic imbalance typical of diabetic syndrome with hyperglycemia**.** The pretreatment of animals with EECp (G4) significantly reduced blood glucose levels.

Table 2 Serum parameters values of the treated rats, G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days).

Groups	Glucose (g/L)	Total Protein (g/L)	Albumin (g/L)	Fibrinogen (g/L)
G1	0,78±0,52	75,52±0,64	45,11±0,73	2,76±0,45
G2	1,84±0,06	49,7±1,14	29,39±2,04	6±0,15
G3	0,86±0,02	79,52±0,88	40,61±0,91	3,17±0,21
G4	1,28±0,07	54,08±1,49	33,68±1,12	3,98±0,1

The results of the complete blood count (CBC) showed that oral administration of HCL/ethanol after tween 80 (G2) decreased significantly hematological parameters: red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), hemoglobin (Hb), and platelets (PLA), comparatively to the control group and G3 treated uniquely via EECp as indicated in Table 3. Pretreatment of animals (G2) with 250 mg/kg of Ego increases these hematological parameters compared to G1.

Table 3 Complete blood count (CBC) values of the treated rats, G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days).

Groups	G.B ($\times 10^3$)	G.R ($\times 10^6$)	H.B (g/dL)	HCT (%)	PLA ($\times 10^3$)
G1	5,75±1,34	6,18±0,95	13,35±1,14	51,65±0,86	391±0,79
G2	4,67±0,26	4,68±0,13	9,87±0,08	31,04±0,89	749±4,05
G3	9,04±0,08	8,9±0,1	13,37±0,34	47,87±1,15	252±3,77
G4	9,38±0,21	8,85±0,1	13,9±0,06	39,85±0,1	127±0,41

From the results obtained in (Table 2), we see that there is a drop in the albumin and total protein level (29.39 g/l and 49.7 g/l respectively) in G2 compared to G1 group. In contrast, oral administration of 250 mg/kg of at G4 showed an increase of (33.68 g/l and 54.08g/l) by contribution to G2.
The results illustrate a significant decrease of (4.29g/l) in fibrinogen rate of G4 (pretreated with 250 mg/kg of EECp before administration of HCL/ethanol) relative to G2. While the G2 showed an increase in fibrinogen (6g /l) compared to G1 (Table 2).

Prostaglandin E2 quantification

The prostaglandin (PGE2) concentration was measured in peritoneal exudates of rats from all groups (Fig 3). Administration of HCL/ethanol to G2 significantly decreased the level of PGE2 (122.33 pg/ml) compared to G1 (524.68 pg/ml). The pretreatment of G4 with 250 mg/kg of EECp reverse this decrease which revealed a significantly high level of PGE2 (420.33 pg/ml) compared to G2. Whereas, PGE2 significantly increases in G3 treated solely by EECp at a concentration of 553.33 pg/ml comparatively to G2.

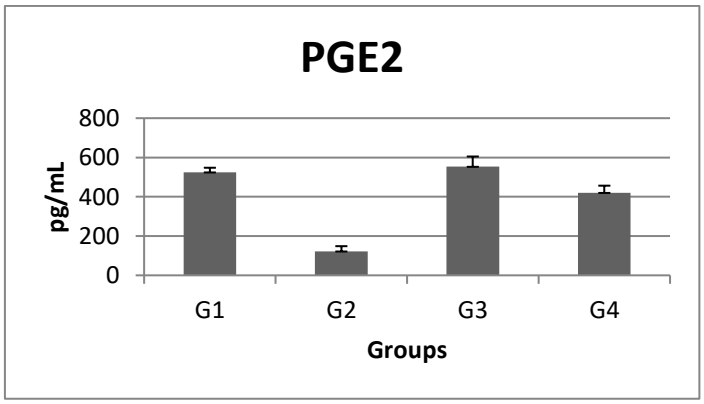

Figure 3 Prostaglandin E2 Concentrations (pg/ml) in peritoneal exudates of rats for the tested groups. The values are expressed as mean ± SD (n = 5). G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days).

Effects of ethanolic extract of carob pods (EECp) on antioxidant markers of HCL/ethanol-stressed rats

With regard to the impact of HCL/ethanol and EECp on oxidative stress condition, effects of ethanolic extract of carob pods (EECp) on the main plasma or serum of antioxidant parameters (malonaldehyde "MDA", superoxide dismutase "SOD", catalase "CAT" and glutathion peroxidase "GSH-PX") of HCL/ethanol-stressed rats was studied, the results are illustrated in Table 4.

Table 4 Activities of anti-oxidant enzymes in the serum and plasma of the experimental groups of rats, G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days).

Groups	MDA (nmol/ml)	SOD (U/cg Hb)	CAT (U/mg Hb)	GSH-PX (U/g Hb)
G1	8,34±0,92	20,59±1,9	70,10±0,44	27,65±1,9
G2	16,75±1,89	6,05±0,83	19,33±1,91	37,32±1,9
G3	2,81±0,95	23,69±2,17	81,83±3,23	56,21±1,97
G4	4,86±0,86	15,53±2,06	60,90±2,35	52,29±1,94

Oral administration of HCL/ethanol to G2 drastically increased the MDA and GSH-PX levels compared to G1. The increase of lipoperoxidation (MDA) and glutathion peroxidase (GSH-PX) induced by administration of HCL/ethanol was significantly inverted by EECp pre-treatment. G3 oral treatment with only EECp decreased the rate of MDA and increased that of GSH-PX.
Comparatively to G1 treated only with tween 80, EECp, increased levels of antioxidant enzymes activities (superoxide dismutase SOD and catalase CAT) at G3; whereas the necrotizing agent (HCL/ethanol) lowered them significantly in G2 and EECp treatment of the HCL/ethanol-stressed rats (G4) induced decrease of this parameters.

Antiulcer activity

Histopathology

The histopathological stomachs study from (G2) who's treated by HCL/ethanol, we observed a gastric ulcer with loss of substance taking all thickness of mucosa (epithelium area with chorion and glands), fibrino-leukocyte coating and hemorrhagic areas (Fig 4).

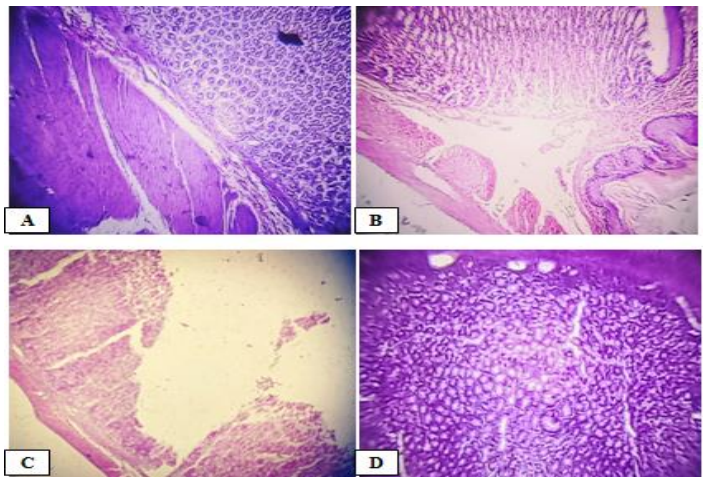

Figure 4 Effect of ethanolic extract of caob pods (EECp) and vehicle on histological changes caused by HCL/ethanol in rats. G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days). A: G1; B: G3; C: G2; D: G4.

The administration of 250 mg/kg of EECp to rats (G4) significantly reduced the histopathological changes such as superficial erosion with detachment of epithelium, presence of leukocyte fibrinoid coating and few congested blood vessels. Moreover, G1 and G3 treated with EECp shows normal structure of the stomach without lesions.
HCL/ethanol administration caused inflammation of the esophagus and hepatitis in the liver. Treatment with EECp before HCL/ethanol to G4 prevent onset of inflammation of the organs mentioned above.
These data are histologically pertinent, as because of its absorptive and secretory functions, the gastric mucosa is considered one of the the principal tissues **(Schubert and Peura, 2008)**. Exposition of Gastric mucosa to ulcerogenic necrotizing products like indomethacin and alcohol or to ischemia develops ultrastructural and functional alterations and histopathological characteristics leading to injuries **(De Carvalho et al., 2011)**.

Macroscopic evaluation of stomachs

HCL/ethanol administration provoked immense stomach damage.
The pretreatment with EECp of animals (G4) significantly reduced gastric lesions caused by HCL/ethanol (48.74 mm^2), decreased ulcer index 7.46% and increased ulcer inhibition compared to "G2" (Table 5). The G3 and G1treated only by EECp and tween 80 respectively are similar. This investigation showed that EECp is a potent antiulcer extract.

Table 5 Ethanolic extract of carob pods (EECp) effect on macroscopic quantitative modifications caused by HCL/ethanol in rats, G1: tween 80 (2%); G2: tween 80 (2%) then HCL/ethanol (5 days); G3: only 250 mg/Kg of EECp (5 days); G4: 250 mg/Kg of EECp then HCL/ethanol (5 days).

Groups	Lesion areas (mm^2)	Ulcer Index (%)	Ulcer Inhibition (%)
G1	0,96	0,03	99,10
G2	362,26	44,09	0,00
G3	0,42	0,02	99,33
G4	48,74	7,46	48,18

DISCUSSION

The stomach is a sensible digestive organ principally exhibited to exogenous pathogens from the diet **(Hidekazu et al., 2012)**. The HCl/ethanol was used to induce gastric ulcer as it is substantially identical to that followed in acid hypersecretion cases in humans **(Mekonnen et al., 2020)**. The direct toxic effect on the epithelium caused by HCl/ethanol, leads to the formation of characteristic necrotic lesions due to a reduction in the mucus production and bicarbonate secretion **(Massignani et al., 2009)**. Injures in the vascular endothelial cells of the gastric mucosa, microcirculatory disturbance and hypoxia, linking to the overproduction of oxygen radicals are induced by ethanol **(Park et al., 2015)**. Acceleration of the ulcerogenesis process, intensification of injuries, and reduction of the mucosal protection against chemicals agents are caused by the addition of HCl **(Ramesh et al., 2011)**. The formation of ulcer can be assured by acidification of ethanol (**Mekonnen et al., 2020)**. Thus, in order to explore the cytoprotector effect of medicinal plants the experimental model of HCl/ethanol-induced gastric damage in rats has vastly been used.
Many medicinal plants contain, flavonoids, triterpenoids and tannins, which protect the stomach mucosa by induction of gastroprotective mechanisms or acting as natural antioxidants **(Gonzalez et al., 2001; Kahraman et al., 2003)**.
The composition of carob by HPLC/UV has shown the presence of many chemical components that have biological activity, including phenolic compounds as well as many types of flavonoids. According to **Gregoriou et al. (2021)** gallic acid is the main constituent of phenolic acids in carob. **Jambi (2015)** observed quercetin as the most abundant flavonoids and Gallic as the most phenolics compounds in carob. In the other hand, **Rico (2019)** found that hydroxybenzoic acid as the most abundant phenolics acid in carob pod. Furthermore, **Darwish et al. (2021)** showed that carob contains a great level of gallic acid, catechin and protocatechuic acid, respectively, and gallic acid is the main constituent. Comparison of the phenolic composition study of carob with other investigation rest difficult, given that the articles submitted on the subject employed different extraction conditions and plant material leading to different phenolic profiles **(Bernardo-Gil et al., 2011; Benchikh et al., 2016)**.
Hyaluronan is the main constituent of the extracellular matrix hydrolyzed by Hyaluronidase which is a family of enzymes. It increases granulation tissue, decreases the volume of edema, and a regulates the inflammatory response by provoking production of pro- and anti- inflammatory cytokines, growth factors, and mediators of eicosanoids **(Monzón et al., 2008)**.
In vivo, in the first place we showed that HCL/ethanol administration caused a gastric ulcer by increase in ulcer index and area lesion and also histopatological changes like hemorrhagic injuries and pretreatement with EECp revealed the gastroprotective activity of carob.
The EECp results achieved reinforce the gastroprotective activities of *Ceratonia siliqua L*. and its use for treatment of gastric disorders. The results of this work are in agreement as those of **Rtibi et al. (2015)** who demonstrated the gastro-protective power of carob against ethanol-prompted oxidative stress in rats. Moreover, **Bakhtaoui et al. (2014)** showed significant gastro-protective power of carob against HCL/ethanol-provoked ulcer model in rats. This suggests that the components present in EECp must be suppressing gastric damage. However, carob is a medicinal plant, which exhibits important pharmacological properties in the digestive tract; these activities include anti-ulcer actions **(Darwish et al., 2021)**. The antiulcer effect of phenolic components and flavonoids is due to their antisecretory, cytoprotective, antioxidant, anti-inflammatory, and anti-H. pylori activities **(Mekonnen et al., 2020)**.
In this investigation, HCL/ethanol caused increase in oxidative stress markers in gastric mucosa (lipid peroxidation) and decrease in endogenous antioxidative agents level like GSH-PX, catalase and superoxide dismutase activity. This oxidative stress in gastric mucosa has been successfully reversed by Carob pod.

Certainly, this antioxidant property is owed to the existence of phenolic acids and flavonoids in EECp, as did their capacity to ambush free radicals.

The process of inflammation is part of the immune response to damage or an infection **(Boufadi, et al., 2021)**. In certain circumstances, the inflammation process induces tissues injuries, a problem that justifies the utilization of anti-inflammatory agents. For the avoidance of injurious effects, in the final stage of inflammation, a few constituents are formed and liberated to solve the inflammatory response decreasing the gravity of inflammation **(Napimoga et al., 2007, 2012)**. Albumin and total proteins tend to reduce in plasma while the inflammation and in oxidative stress.

Administration of HCL/ethanol to cause inflammation results in decreased total protein and albumin levels and increased fibrinogen. This can be owed to a deficit of their synthesis as a consequence of liver which explains the hepatitis previously observed in the liver.

The lipid peroxidation affects biological membranes receptors and increases in permeability **(Nehru and Anand, 2005)**. **Newairy et al. (2009)** have indicated that the consequence of decreased superoxide dismutase (SOD) and catalase (CAT) activity is a consequence of increased lipoperoxydation.

EECp inhibited PGE2 production during HCL/ethanol-provoked toxicity. This may explicate the anti-inflammatory activities of carob pod extract, and also the inhibition of neutrophil mobilization in the peritoneal cavity; and the cytoprotective activity of carob pod. Inhibition of prostaglandin synthesis diminishes mucus secretion and therefore increases the exposure of the gastric mucosa to acid attacks causing gastric injury. Consequently, plants with antiulcer activity as carob enhance the defensive factors, by increasing the mucin synthesis through activating prostaglandin production **(Bakhtaoui et al., 2014)**. Furthermore, carob pods exert anti-inflammatory activity **(Darwish et al., 2021)**.

CONCLUSION

Finally, our results indicated that EECp have a protective power against HCL/ethanol-caused ulcer in the rat gastric mucosa, anti-inflammatory activities and it can preserve the body from the damages resulted by oxidative stress, these carob properties were straightaway linked to phenolic constituents detected in the extracts.

REFERENCES

Aebi H (1974). Catalase. In *H.U.*, Methods of enzymatic analysis, Bergmayer (ed). Verlag Chemie 2nd ed., (2); 673–684.

Bakhtaoui FZ, Lakmichi H, Chait A and Gadhi CA (2014). *In vivo* Gastro-Protective Effects of Five Moroccan Medicinal Plants against Gastric Ulcer. *American Journal of Phytomedicine and Clinical Therapeutics*, 2 (11), 1262-1276. https://doi.org/10.7324/JAPS.2014.40115

Ben Hsouna A, Trigui M, Ben Mansour R, Mezghani Jarraya R, Damak M and Jaoua S (2011). Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential oil with preservative effects against Listeria inoculated in minced beef meat. *Journal of Food Microbiology*, 148, 66-72. https://doi.org/10.1016/j.ijfoodmicro.2011.04.028

Benchikh Y, Louaileche H, George B, Merlin A (2014). Changes in bio-active phytochemical content and *in vitro* antioxidant activity of carob (*Ceratonia siliqua L.*) as influenced by fruit ripening. *Ind Crops Prod*, 60, 298–303. https://doi.org/10.1080/12538078.2014.909325

Bernardo-Gil MG, Roque R, Roseiro LB, Duarte LC, Gírio F and Esteves P (2011). Supercritical extraction of carob kibbles (*Ceratonia siliqua L.*). *The Journal of Supercritical Fluids*, 59, 36-42. https://doi.org/10.1016/j.supflu.2011.08.007

Boufadi YM, Elaoufi M, Tabet F, Benali M and Riazi A (2015). Chemical composition and antioxidant activity of Carob pulp (*Ceratonia Siliqua L.*) extracts. *South Asian J Exp Biol*; 5 (2): 76-83.

Boufadi MY, Soubhye J and Antwerpen PV (2021). Anti- inflammatory, antioxidant effects, and bioaccessibility of Tigzirt propolis. *J Food Biochem*., 00, 13663. https://doi.org/10.1111/jfbc.13663.

Brito S.V, Ferreira de Almeida C.L Italo de Santana T., Oliveira A.R.S., Figueiredo J.C.B.N., Souza I.T., de Almeida L.L., Vanusa da Silva M., Borges A.S., de Medeiros J.W., Neto J.C.S., Gonçalves R.C.R., Kitagawa R.R., Sant'Ana A.E.G., Rolim L.A., de Menezes I.R.A., da Silva T.G., Caldas G.F.R. and Wanderley A.G. (2018). Antiulcer Activity and Potential Mechanism of Action of the Leaves of *Spondias mombin L.* Hindawi Oxidative Medicine and Cellular Longevity, Volume 2018, Article ID 1731459,20. https://doi.org/10.1155/2018/1731459

Chaa S, Boufadi MY, Keddari S, Benchaib AH, Soubhye J, Van Antwerpen P, Riazi A (2019). Chemical composition of propolis extract and its effects on epirubicin-induced hepatotoxicity in rats. Revista Brasileira de Farmacognosia, 29: 294-300. https://doi.org/10.1016/j.bjp.2019.01.005

Daniel V. T., Wiseman J. T., Flahive J. and Santry H. P. (2017). Predictors of mortality in the elderly after open repair for perforated peptic ulcer disease. Journal of Surgical Research, vol. 215, pp. 108–113. https://doi.org/10.1016/j.jss.2017.03.052

Darwish WS , Khadr AE , Kamel MA , Abd Eldaim MA, El Sayed IET , Abdel-Bary HM , Ullah S and Ahmed Ghareeb D. (2021). Phytochemical Characterization and Evaluation of Biological Activities of Egyptian Carob Pods (*Ceratonia siliqua L.*) Aqueous Extract: In Vitro Study. *Plants*, 10, 2626. https://doi.org/10.3390/plants10122626

De Andrade SF, Lemos M, Comunello E, Noldin VF, Filho VC, Niero R (2007). Evaluation of the antiulcerogenic activity of *Maytenus robusta* (*Celastraceae*) in different experimental ulcer models. *Journal of Ethnopharmacology*, 113, 252-257. https://doi.org/10.1016/j.jep.2007.06.002

De Carvalho CA, Fernandes KM, Matta SLP, da Silva MB, de Oliveira LL and Fonseca CC (2011).Evaluation of anti-ulcerogenic activity of aqueous extract of *Brassica oleracea var. capitata* (cabbage) on Wistar rat gastric ulceration. *Arquivos de Gastroenterologia*, 48 (4), 276–282. https://doi.org/10.1590/S0004-28032011000400011

Drury RAB and Wallington EA (1980). Bone and decalcification. In *H. M. Carleton's histological techniques*, Carleton (Ed), Oxford: Oxford University Press ; 5th ed., 199–220.

El Hilah F., Ben Akka F., Dahmani J., Belahbib N. and Zidane L. (2015). Étude ethnobotanique des plantes médicinales utilisées dans le traitement des infections du système respiratoire dans le plateau central marocain. *Journal of Animal & Plant Sciences*, 25(2), 3886–3897.

Elstner E., Youngman RJ and Obwald W (1983). *Superoxyde dismutase in methods of enzymatic analysis* (3nd ed.). H. B Bergmeyer.

Ghasemian M, Owlia S, Owlia MB (2016). Review of anti-inflammatory herbal medicines. *Adv Pharmacol Sci*. 2016:9130979.

Gonzalez FG, Portela TY, Stipp EJ, Di Stasi LC (2001). Antiulcerogenic and analgesic effects of *Maytenus aquifolium*, *Sorocea bomplandii* and *Zolernia ilicifolia*. *J Ethnopharmacol.*, 77, 41–7. https://doi.org/10.1155/2016/9130979

Gregoriou G., Neophytou C.M., Vasincu A., Gregoriou Y. , Hadjipakkou H. et al. (2021). Anti-Cancer Activity and Phenolic Content of Extracts Derived from Cypriot Carob (*Ceratonia siliqua L.*) Pods Using Different Solvents. ***Molecules,*** 26 (16) : 5017. https://doi.org/**10.3390/molecules26165017**

Hidekazu S, Toshihiro N, Hitoshi T, Sachiko M, Toshifumi H (2012). Roles of oxidative stress in stomach disorders. *J Clin Biochem Nutr.*, 50, 35–9. https://doi.org/10.3164/jcbn.11-115SR

Jambi HA (2015). Effect of Rosting Process on Polyphenols Content of Carob Powder. *Life Sci J.*, 12 (12), 1-5. (ISSN: 1097-8135). doi: https://doi.org/10.7537/marslsj121215.01.

Kahraman A, Erkasap N, Koken T, Serteeser M, Aktepe F, Erkasap S (2003). The antioxidative and antihistaminic properties of quercetin in ethanol induced gastric lesions. *Toxicol.*, 183, 133–42. https://doi.org/10.1016/s0300-483x(02)00514-0

Luck H (1965). Catalase. In *H.U. Methods of enzymatic analysis*, Bergmeyer (ed). *Academic Press*; 895–897.

Martins JLR, Rodrigues ORL, Moreira da Silva D, Galdino PM, Realino de Paula J, Romão W, Barcellos da Costa H, Vaz BG, Ghedini PC and Costa EA (2014). Mechanisms involved in the gastroprotective activity of *Celtis iguanaea* (Jacq.) Sargent on gastric lesions in mice. *Journal of Ethnopharmacology*, 155, 1616–1624. https://doi.org/10.1016/j.jep.2014.08.006

Massignani JJ, Lemos M, Maistro EL, Schaphauser HP, Jorge RF, Sousa JPB, Bastos JK and Andrade SF (2009). Antiulcerogenic activity of the essential oil of *Baccharis dracunculifolia* on different experimental models in rats. *Phytotherapy Research*, 23, 1355–1360. https://doi.org/10.1002/ptr.2624

Mekonnen AN, Atnafie SA, and Atta MAW (2020). Evaluation of Antiulcer Activity of 80% Methanol Extract and Solvent Fractions of the Root of *Croton macrostachyus* Hocsht: Ex Del. (Euphorbiaceae) in Rodents. *Evidence-Based Complementary and Alternative Medicine*, 11. https://doi.org/10.1155/2020/2809270

Monzón M E, Manzanares D, Schmid N, Casalino- Matsuda S M and Forteza RM (2008). Hyaluronidase expression and activity is regulated by pro- inflammatory cytokines in human airway epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, 39,289–295. https://doi.org/10.1165/rcmb.2007-0361OC

Moulai-Hacene F., Boufadi M.Y. Keddari S. Homrani A. (2020). Chemical composition and antimicrobial properties of *Elettaria cardamomum* extract. *Journal of Pharmacognosy*, 12, 5. https://doi.org/10.5530/pj.2020.12.149

Napimoga MH, Cavada BS, Alencar NMN, Mota ML, Bittencourt FS, Alves JC, Grespan R, Goncalves RB, Clemente-Napimoga JT, de Freitas A, Parada CA, Ferreira SH and Cunha FQ (2007). *Lonchocarpus sericeus lectin* decreases leukocyte migration and mechanical hypernociception by inhibiting cytokine and chemokines production. *International Immunopharmacology*, 7, 824–835. https://doi.org/10.1016/j.intimp.2007.02.001

Napimoga MH, da Silva CAT, Carregaro V, Farnesi-de-Assuncao TS, Duarte PM, de Melo NFS and Fraceto LF (2012). Exogenous administration of 15d- PGJ(2)-loaded nanocapsules inhibits bone resorption in a mouse periodontitis model. *The Journal of Immunology*, 189, 1043–1052. https://doi.org/10.4049/jimmunol.1200730

Nehru B, Anand P (2005). Oxidative damage following chronic aluminium exposure in adult and pup rat brains. *Journal of Trace Elements in Medicine and Biology*, 19, 203–208. https://doi.org/10.1016/j.jtemb

Newairy ASA., Salama AF, Hussien HM and Yousef MI (2009). Propolis alleviates aluminium- induced lipid peroxidation and bio-chemical parameters in male rats. *Food Chemistry Toxicology*, 47, 1093–1098. https://doi.org/10.1016/j.fct.2009.01.032

Ouis N., Hariri A. (2018). Antioxidant and antibacterial activities of the essential oils of *Ceratonia siliqua*. *Banat's Journal of Biotechnology*, 9 (17); 13-23. https://doi.org/10.7904/2068-4738-IX(17)-13

Paglia DE and Valentine W N (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxydase. *Journal of Laboratory and Clinical Medicine*, 70, 158–169.

Papagiannopoulos M, Wollseifen HR, Mellenthin A, Haber B and Galensa R (2004). Identification and quantification of polyphenols in carob fruits (*Ceratonia siliqua L.*) and derived products by HPLC-UV-ESI/MS. *J. Agric. Food. Chem,*. 52, 3784–379. https://doi.org/10.1021/jf030660y

Park JH, Jang KJ, Kim CH, Kim JH, Kim YK, Yoon HM (2015). *Ganoderma Lucidum* Pharmacopuncture for Teating Ethanol-induced Chronic Gastric Ulcers in Rats. *J. Pharmacopuncture*., 18, 72-8. https://doi.org/10.3831/KPI.2015.18.008

Paulino N, Abreu SRL, Uto Y, Koyama D, Nagasawa H, Hori H, Dirsch VM, Vollmar AM, Scremin A and Bretz WA (2008). Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. *European Journal of Pharmacology*, 587, 296– 301. https://doi.org/10.1016/j.ejphar.2008.02.067

Ramesh A, Alekhya N, and Iohitha I (2011). Antiulcer activity of *Eugenia jambolana* leaves against ethanol induced gastric ulcer in albino rats. *International Journal of Pharmaceutical Research and Development*, 3, 106–112. https://doi.org/10.1155/2020/2809270

Ribeiro V.P., Arruda C., Abd El-Salam M and Bastos J.K. (2018). Brazilian medicinal plants with corroborated anti-inflammatory activities: a review, *Pharmaceutical Biology*, 56:1, 253-268. https://doi.org/10.1080/13880209.2018.1454480

Rico D, Martín-Diana AB, Martínez-Villaluenga C, Aguirre L, Silvan JM, Duenas M, De Luis DA, Lasa A (2019). *In vitro* approach for evaluation of carob by-products as source bioactive ingredients with potential to attenuate metabolic syndrome (MetS). *Heliyon*, 5, 2405-8440. https://doi.org/10.1016/j.heliyon.2019.e01175

Rtibi K, Jabri MA, Selmi S, Souli A, Sebai H, El-Benna J, Amri M and Marzouki L (2015). Gastroprotective effect of carob (*Ceratonia siliqua L.*) against ethanol-induced oxidative stress in rat. *BMC Complementary and Alternative Medicine*, 15, 292. https://doi.org/10.1186/s12906-015-0819-9

Rtibi K, Selmi S, Grami D, Amri M, Eto B, El-benna J, Sebai H and Marzouki L (2017). Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua L.*) on the gastrointestinal tract: A review. *Biomedicine & Pharmacotherapy*, 93, 522–528. https://doi.org/10.1016/j.biopha.2017.06.088

Schubert ML and Peura DA (2008). "Control of gastric acid secretion in health and disease". *Gastroenterology*, 134 (7), 1842–1860. https://doi.org/10.1053/j.gastro.2008.05.021

Vekiari SA, Ouzounidou G, Ozturk M and Görk G (2011). Variation of quality characteristics in Greek and Turkish carob pods during fruit development. *Procedia-Social and Behavioral Sciences*, 19, 750-755. https://doi.org/10.1016/j.sbspro.2011.05.194

Yagi K (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical Medicine*, 15, 212–216. https://doi.org/10.1016/0006-2944(76)90049-1.