

Ulcer Healing and Gastroprotective Activity of Methanolic Extracts of *Hyoscyamus Albus* and *Umbilicus Rupestris* Leaves against Gastric Injury Caused by Ethanol in Rats

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Abstract

The present study was performed to evaluate the anti-ulcer activity of methanolic extract of *Hyoscyamus albus* and *Umbilicus rupestris* against ethanol-induced gastric ulcer. Experimental groups were orally pre-treated with and 100, and 200 mg/kg b.w of methanolic extracts HAMEOH and URMeOH. Ulcer control group were pre-treated just with absolute ethanol and reference group was orally pre-treated with omeprazole (20 mg/kg b.w). After one hour of pretreatment, all rats were received the absolute ethanol to generate gastric mucosal injury. And after one hour, our rats were anesthetized and sacrificed and the ulcer areas of the gastric walls were determined. Grossing evaluation has revealed that the negative control rats exhibited severe mucosal injury, whereas, pre-treatment with *U. rupestris* leaf extract and *H. albus* has resulted significantly anti gastric mucosal injury and flattening of the mucosal folds. Histological studies of the gastric wall that the pre-treated with URMeOH and HAMEOH leaf extract was pronounced as gastric protection along followed by reduction or inhibition of leucocytes in the infiltration of the submucosa.

Keywords: Antiulcer activity; *Hyoscyamus albus*; *Umbilicus rupestris*; Ethanol; Methanolic extract

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Introduction

Umbilicus rupestris (Salisb.) Dandy is a perennial plant, belongs to the family of *Crassulaceae*. It presents on the rocks, the old walls and in mountains; only in North Africa. Leaves of this plant are used in traditional medicine against the ignitions of skin, wounds, burns, disinfectant, parasitocidal. Infused (leaves) of this plant is used like an ophthalmic disinfectant [1]. *H. albus* is a plant which belongs for *Solanaceae* family, it used in traditional medicine as a nervous sedative and para sympatholytic [2]. Natural products are very important in pharmaceutical industry and also in search of new potential sources of bioactive molecules [3].

Omeprazole is drug highly selective for the proton pump and catalyzed conversion into active form within the acid forming space [4]. We used in our study; omeprazole was used as the reference anti-ulcer drug.

The ulcer can cause by a lot of caused non stroidiens inflammatory,

stress or alcohol. They researchers found that ethanol is the simple method that the method of absolute ethanol is the most simple used to induce gastric ulcer. Ethanol induces the liberation of free radical species and Nordmann [5].

Therefore, the present study was to evaluate the antiulcerogenic property of the methanolic extract of *Hyoscyamus albus* and *Umbilicus rupestris* against ethanol-induced gastric mucosal injury in experimental rats.

Materials and Methods

Collection of the plant

The leaves of *Hyoscyamus albus* and *Umbilicus rupestris* were harvested in March 2015 from the Wilaya of Batna region Tibhirine Algeria. Botanical species identification was realised by Dr. B. OUDJHIH, Botanical Laboratory, Department of agronomy,

University of EL-Hadj Lakhdar – Batna. 40 days in a dry and aerial place for the extraction of active compounds

Extraction by organic solvents

1 Kg of powdered leaves was extracted with 5 L of petroleum ether for three times. Then, the marc was dried and extracted with 5 L of chloroform for three times and with 5 L of methanol for three times and the supernatants were filtered separately using cotton and Whatman filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature (30°C) using a vacuum rotary evaporator (Buchi Rotavapor).

Phytochemical screening

The phytochemical screening of HAMEOH and URMeOH was realised by using the method of 8 Phytochemical constituents which is: phenolic compounds, terpenoids, saponins, alkaloids, steroids and tannins were analysed qualitatively.

Animals

Wistar rats weighted (140-170 g) provided by the Pasteur Institute – Algiers. These rats were allowed at favorable conditions before and during the experiment: Temperature (23 ± 2)°C, relative humidity 50-55% with 12 h light/12 h night cycle respectively. The food and water were given *ad libitum*.

Evaluation of Antiulcer activity

The study of this activity, we have uses the method of Garg et al. The rats males was divided into six groups of six rats. These rates were deprived 48 h before the experiment but with accesses of water two hours before the experiment and received by gavage the following treatments: Group I: received distilled water with (5 mL/kg p c) and is useful like a witness, Group II: received distilled water (5 mL/kg b.w.) and served as a control, Groups III and IV: received HAMEOH with the two concentrations (100 and 200 mg/Kg b.w.) respectively, Groups V and VI: received URMeOH (100 and 200 mg/Kg b.w.) respectively with a volume of (10 ml/Kg PC), Group VII: received the omeprazole (20 mg/kg PC) (5 mL/kg b.w.) as standard. 30 min after pretraitement, the ulcer was induced by oral administration of absolute ethanol (5 mL/kg b.w.) except control group. The rats were dessicated 60 min after ethanol administration and each stomach was open along the great curve, and were washed with water physiological [6].

Evaluation of the gastric lesions

Ulcers found in the gastric mucous membrane appearing in the form of bands ellongate of hemorrhagic lesions parallel to the long axis of the stomach. Surface d'ulcere gastric was calculated according to the method of Kauffman and Grossman with some modification [7]. The length (mm) and the width (mm) of the ulcer on the mucous membrane gastric were measured by the method of squaring under a binocular magnifying glass (of x1.8). The surface of each lesion of ulcer was measured by counting the number of small squares, 1 X 1 mm, covering the length and the width of each band of the ulcer. The sum of surfaces of all the lesions for each stomach was applied for calculation surface

of ulcer (UA) when the sum of small squares is multiplied with magnification 1,8.

Measure of gastric volume of juice

The gastric volume of juice has ete measurement has l'aide d'une syringe.

Percentage inhibition

The inhibition percentage of the ulcers was determined according to the formulate below: [8,9].

Inhibition (%)=[(UA controls UA traited)/UA control] x 100 UA controls: ulcerous surface of the group controls. Treated

UA: ulcerous surface of the treated group.

Measure of total acidity

1 mL of gastric juice was diluted 10 times with distilled water and subjected a titration has by NaOH has 0.01 NR with adding few drops of phenolphthalein drops until pink color.

Total acidity is calculated according to there following formula.

Total acidity=(volume of NaOH × Normality × 100 mEq/L/100 g)/0.1

Histopathology

Stomach Samples have fixed in phormol 10% for histopathological study with using hematoxyline-eosine.

Results

The HAMEOH extract reduced the surface of ulcer in a significant way (P<0.05) with an inhibition of 63% and 95.90% for (100 and 200 mg/kg b.w.) respectively. Still URMeOH could inhibit in a significant way (P<0.05) the ulcer with an inhibition (48.98% and 65.94%) for the concentrations (100 and 200 mg/kg b.w.) respectively.

The omeprazole which was used like a standard of reference educed in a significant way (P ≤ 0.05) the ulcer with an effect (60.23%) (Figure 1).

In the group controls, of the serious wounds were observed on the level of the gastric mucous membrane. The ethanol produced one necrosis hemorrhagic visible gastric mucous membrane. It caused disturbances of the epithelium of surface and necrotic lesions which penetrated deeply in the mucous membrane and a papilloma keratosis (Figure 2). However, in animals treated with the extracts, the whole of the signs observed at control group were gone in a significant way especially with the extract HAMEOH 200 mg/Kg b.w. (Figures 3-6).

According to results of measurement of the gastric juice volume, we noted that there is a significant reduction (P ≤ 0.05) in the gastric volume of juice in the rats treated by HAMEOH (200 mg/kg b.w.) and by the omeprazole compared to control and any difference between the rats treated with URMeOH compared to control (Figures 7 and 8). Moreover, we noted that there is a highly significant increase (P<0.0001) in the value of the pH in the rats treated with HAMEOH (100 and 200 mg/kg b.w.) with values

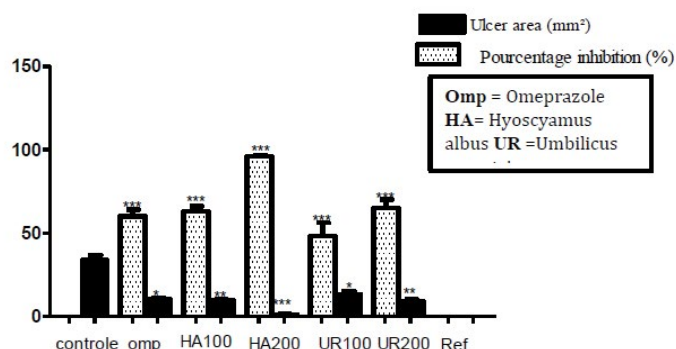


Figure 1 Effects of HAMEOH and URMeOH on surface of ulcer (mm²) and percentage of inhibition. The results are compared to control *P ≤ 0.05, **P ≤ 0.001, ***P ≤ 0.0001.

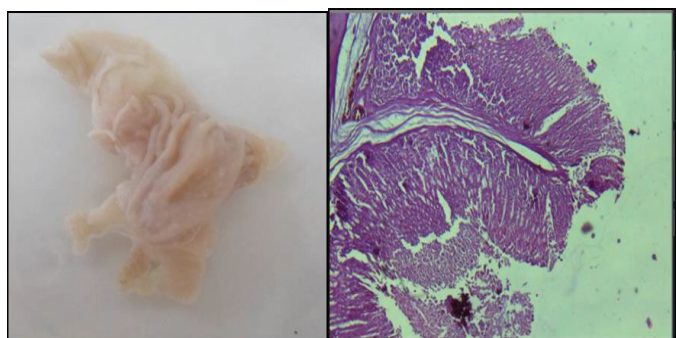


Figure 2 Macroscopic aspect and the histological study fixed by hematoxyline-eosine of the gastric mucous membrane in the pretreated rats with distilled water.

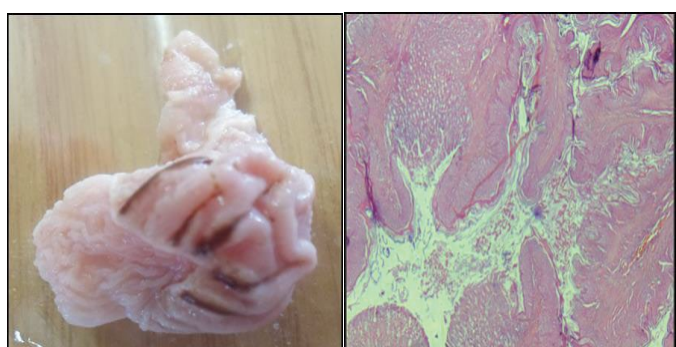


Figure 3 Macroscopic aspect and the histological study fixed by hematoxyline -eosine of the gastric mucous membrane in the pretreated rats with the ethanol (control), presence of keratotic papiollome.

of (5.035 ± 0.035) and (5.240 ± 0.240) respectively compared to the control which presents a pH (3.525 ± 0.1768). Still a highly significant increase (P<0.0001) in the values of pH at the groups treated with URMeOH (100, 200 mg/kg b.w.) and the omeprazole compared to control with values of (5.355 ± 0.417), (5.475 ± 0.332) and (6.94 ± 0.332), respectively, was noted (**Figure 9**).

The results are compared to control *P ≤ 0.05, ** P ≤ 0.001, ***P ≤ 0.0001.

For total acidity, the omeprazole showed a significant reduction (P ≤ 0.05) compared to control. And this reduction was noticed in a significant way (P ≤ 0.05) also at the group treated with HAMEOH 100 mg/Kg and 200 mg/Kg (**Figure 10**).

The gastrin is the only hormone able to control the secretion of acid per the increase in the mass of the parietal cells and stimulation of the form of the acid pump H⁺ K⁺ ATPase. Although the parietal cells express the protein H⁺ K⁺ ATPase.

Thus, it appears that the mode of action of the HAMEOH extract is similar to that of the omeprazole, which bind very specifically to only one sub-unit of H⁺K⁺ ATPase on the secretory surface of

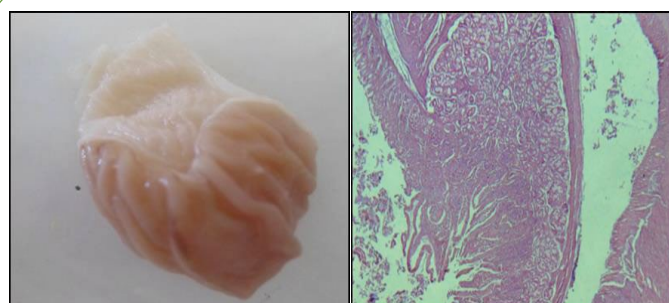


Figure 4 Macroscopic aspect and the histological study fixed by hematoxyline-eosine of the gastric mucous membrane in the pretreated rats with omeprazole.

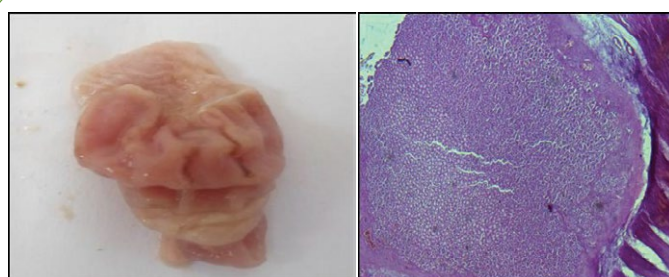


Figure 5 Macroscopic aspect and the histological study fixed by hematoxyline-eosine of the gastric mucous membrane in the pretreated rats with HAMEOH (100mg/kg b.w.).

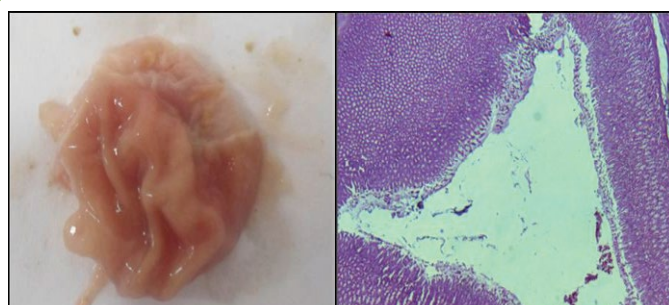


Figure 6 Macroscopic aspect and the histological study fixed by hematoxyline -eosine of the gastric mucous membrane in the pretreated rats with HAMEOH (200mg/kg b.w.).

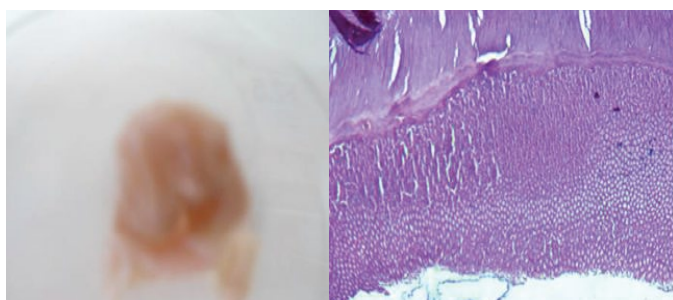


Figure 7 Macroscopic aspect and the histological study of the gastric mucous membrane in the pretreated rats with URMeOH (200mg/kg b.w.).

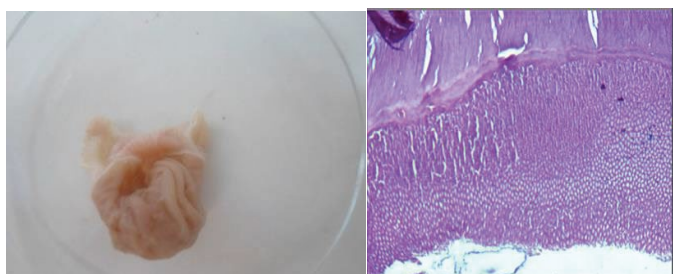


Figure 8 Macroscopic aspect and the histological study fixed by hematoxyline-eosine of the gastric mucous membrane in the pretreated rats with URMeOH (100 mg/kg b.w.).

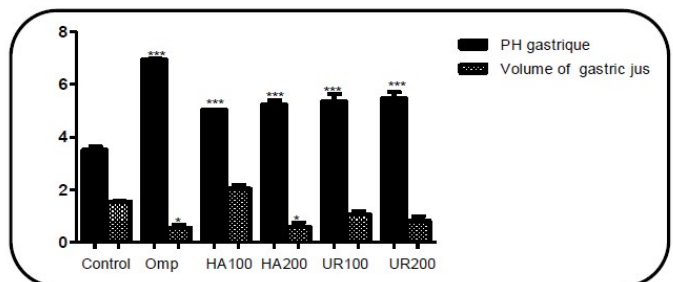


Figure 9 Effect of HAMEOH et URMeOH on pH gastric and the juice gastric volume.

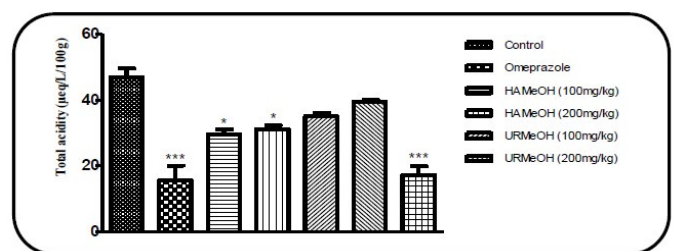


Figure 10 Effect of HAMEOH et URMeOH on totale acidity (μ eq/L/100g).

the parietal cell and the inactive one, and it makes it possible to reduce the secretion of acid whatever the source of stimulation of secretion. The omeprazole is effective in the treatment of UGD and the backward flow gastro-oesophagian after its use is with length or short-term [10].

Discussion

Gastroduodenal ulcer (UGD) described a state in which there is a discontinuity in all the thickness of gastric and the mucous membrane which persists because of the acid and pepsin in the gastric juice [11]. The gastroduodenal ulcers are caused by an imbalance between the defensive mechanisms (defensive mucosal barrier, secretion of bicarbonate, PG) and the aggressive mechanisms (acid, pepsin and *Helicobacter pylori* of the mucous membrane [12,13]. The ethanol produces necrotic lesions by its direct action on the stomach what reduces the factors of defense like secretion of bicarbonate and the production of mucus [12]. The ethanol can reach the mucous membrane by the rupture of the mucus-bicarbonate barrier and the rupture of the cells in the wall of the blood vessels. These effects are probably due to the some biological phenomena's like the peroxidation of the membranes, the formation of the free radicals, intracellular effort oxidizing, the change of the permeability and depolarization of the mitochondrial membrane before cellular death [14].

Many drugs including the inhibitors of the pump with protons, the prostaglandin analogues, histamine antagonists and the cyto-protective agents are available for the treatment of the gastro-duodenal ulcer. But the majority of these drugs produces several adverse effects including toxicity and can even deteriorate the biochemical mechanisms of the body especially after the chronic use [15]. Currently, the natural products are generally used to avoid the chronic use of the drugs. Several natural products were announced like having an anti-ulcer genic activity by their effect of defense of mucous membrane [16].

The anti-ulcer effect of the methanolic extracts of *H. albus* and *U. rupestris* is with their wealth's of secondary metabolites such as: flavonoids, saponins and the tannins which were announced known by their anti-ulcerogenic and anti-gastric activity [17]. Many work reported that the flavonoids and the tannins play a significant role in the reduction of the ulcer and the protection of the gastric cells [18]. The flavonoids are antioxidants and powerful trappers of the free radicals which prevent ulceration and the cellular oxidative lesions. In fact major, the compounds are known by their antiulcer effects by inhibition of the lesions of the gastric mucous membrane [18]. It was also reported that the flavonoids ensures a protection against gastric cancer [19,20].

According to Havsteen, the antiulcer effect of the flavonoids is due to the inhibition of the regulation of phosphorylation of proteins. The inhibition of protein phosphatase which reverses the action of the protein p kinase.

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