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Research Article

Acute Toxicity and Anti-Ulcer Activity of Mixture Leaves-Flowers Extracts of *Cytisus Triflorus L*.

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ABSTRACT

The extract of leaves-flowers mixture of *Cytisus triflorus L* was selected for their anti-ulcer effect and oral acute toxicity investigation. The acute toxicity method described in European guideline 425, is a method of administering a specified oral dose of 2 g/kg of the extract, to exclude possible toxicity due to extract, a histological study of the liver and kidneys was performed. The results showed the absence of liver and renal toxicity following the administration of the 2 g / kg dose of the crude leaves and flower mixture extract of *Cytisus triflorus L*. Concerning gastric antiulcer activity, it is necessary to check the protective action of the crude extract at doses 200 mg/kg and 400 mg/kg, against the ulcer caused in animals by administration of an ulcerogenic agent such as ethanol. The evaluation of the gastroprotective effect of the extract was carried out by macroscopic and microscopic analysis which will look at the observable external and internal lesions, as well as an estimate of the percentage of ulceration. The results show a very significant reduction in the areas injured in the Ranitidine treated groups and the crude extract at doses of 200 or 400 mg / kg, with percentages of 0.75 ± 0.16%, 2.71 ± 0.13. % and 1.49 ± 0.14% respectively. The gastroprotective effect of the crude extract at 400 mg/kg is confirmed by the restoration of the normal architecture of the rats' stomachs. These results confirm the validity of the use of this plant in traditional medicine and offer hope for the development of effective anti-ulcer therapy without side effects.

INTRODUCTION

Gastric ulcer is one of the most common gastrointestinal disorders and affects approximately the world's population, particularly in non-industrialized countries [1]. Gastric ulcer is a lesion of the gastric mucosa, characterized by necrosis, neutrophil infiltration, reduced blood flow, induction of oxidative stress, and secretion of inflammatory mediators [2]. This imbalance is often due to either exogenous factors that include *Helicobacter pylori* infection, stress, alcohol, ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs), or smoking, either to endogenous factors such as hydrochloric acid, pepsin, and reactive oxygen species [3].

Synthetic drugs such as proton pump inhibitors, H2 antihistamines, cytoprotective agents, anticholinergics, antacids and prostaglandin analogues are used for the treatment of ulceration but these drugs produce several side effects. The occurrence of the latter medicinal plants is considered as substitutes with fewer side effects, and as the main source of new drugs [1].

Our choice fell on *Cytisus triflorus L.,* a plant that belongs to the family Fabaceae (Leguminosae), it is counted in Algeria among the medicinal plants representing a large arsenal therapeutic, because of its richness in phenolic components. The aim of this study is to evaluate in vivo the acute toxicity and gastroprotective potency of the crude leaf and flower extract extract of Cytisus triflorus L. against ethanol-induced gastric ulcer, to provide a scientific basis for the traditional use of this plant.

MATERIALS AND METHODS

Plant Material

The plant *Cytisus triflorus* was harvested in April 2014 from the region of Chemini (Bejaia). The

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identification was made by Prof. Oudjhih Bachir, Elhadj Lakhdar University, Batna. The leaf and flower mixture is cleaned, dried in the shade and at room temperature and stored in the dark until use.

Animals

The in vivo study was carried out on male rats, Albino Wistar whose weight varies between 200g and 250g, provided to the Pasteur Institute of Algiers. These rats are used after a 7-day adaptation period during which they have free access to water and the standard feed provided by the Bejaia National Livestock Feed Office (ONAB).

Methodology

Preparation of the Raw Extract

The leaf and flower mixture of *Cytisus triflorus* is macerated in methanol for seven nights at room temperature [4]. The macerate is filtered, evaporated by a rotavapor (Buchi) and then completely dried in an oven (40 ° C) for 24 hours. The extract obtained is considered to be the crude extract (E.Br) of the mixture of *Cytisus triflorus.*

Acute Toxicity Study of *Cytisus triflorus*

The toxicity study was conducted using the "dose adjustment" method of OECD line 425 [5]. It consists in testing the extract of *C. triflorus* at a dose of 2000 mg / kg. The trial was conducted on 6 male rats and their behavior and number of deaths were observed over a 14-day period.

After 15 h of fasting, the rats were distributed as follows: control group consisting of 6 rats receiving distilled water at a rate of 10 ml / kg; experimental batch consisting of 6 rats receiving the crude extract at a rate of 2000 mg / kg. A behavioral observation was performed 3 h after the administration of the substances. Then, feeding of the rats was carried out daily for 14 days. During this period, signs of toxicity including change in coat, motility, tremors, respiration, sensitivity to noise after metal shock, stool appearance, mobility and death were noted. At the end of the treatment, the rats were fasted for 4 hours, then a blood sample was done followed by a dissection were performed, and the organs (both kidneys) were removed. Part of the liver and kidneys are stored in 10% formalin solution for histopathology study. The blood recovered in heparinized tubes is immediately centrifuged at 4000 rpm for 10 min. The sera are recovered and stored at -4 ° C until their use for biochemical assays.

Gastroprotective Effect Evaluation

The method followed is that described by Germano et al. [6]. It consists in checking the protective action of E.Br at 200 and 400 mg / kg against the ulcer caused in animals by administration of ethanol. A total of four batches of seven rats were set up for the test, one batch for each extract dose (200 and 400 mg / Kg), one batch for the control (distilled water) and one batch for Ranitidine (5 mg / kg) which is used as a positive control. The rats were fasted for 24 hours with free access to the glycosylated water, they are placed separately in individual cages before experimentation.

At time T = 0, the rats received intracellularly the crude extract (0.5 ml / 200 g) at doses of 200 and 400 mg / kg. The control group received only distilled water. One hour later, each rat received 0.5mL of 70% ethanol intragastrically. Half an hour after administration of the ethanol, the rats were sacrificed. The stomach of each rat was removed, opened with great curvature using a chisel, washed with physiological solution and then spread well and fixed on a tablet to better observe the ulcers formed with the naked eye. The stomachs were photographed for better vision. Histological analyzes of the glandular gastric mucosa were performed to determine the severity of the ulcers. The total area of the lesions and the total area of the stomach was measured using AUTOCAD-2018 software. The percentage of ulceration is calculated according to the following formula:

$$\% \text{ ulceration} = (\text{total lesion area} / \text{total stomach area}) * 100$$

The percent inhibition of the ulcer was calculated for each group treated according to this formula:

$$\textbf{\%} \text{ Inhibition} = \frac{(SUc - SUt) * 100}{SUc}$$

Where:

SUc: ulcer surface of the control.

SUt: ulcer surface of the tested extract.

Histological Sections

The histological sections were made at the pathology anatomy laboratory (CHU) of Sétif. After having fixed the liver and kidneys in formalin (10%) for a week, they were cut into small pieces. These samples are dehydrated by passage through three successive ethanol baths of 30 min (70 °, 90 ° and 100 ° C). Then they are thinned in two baths of 20 minutes of toluene and included in the paraffin (two baths of 2

hours each). The operation is automated using an automaton (TISSUE-TEK). The final inclusion is then carried out in metal molds. The paraffin blocks obtained are then cut with a microtome and the 5 µm thick sections are spread on slides with a 2% gelatin gel and then dried in an oven set at a temperature of 35-42 ° C., rehydrated and dried, stained with hematoxylin-eosin.

Statistical Analysis

The comparison of the mean percentages of increase and inhibition was made with the Student t-test. A significant difference is represented by a $p < 0.05$; $n = 6$, represents the number of experiments per group

RESULTS AND DISCUSSION

Acute Toxicity of *Cytisus triflorus*

During the entire experimental period, after oral administration of a single dose of 2000 mg / kg of the crude extract of *C. triflorus*; no deaths were observed in the rats treated throughout the study and no sign of toxicity was observed during the 4 hours following the administration of the extract, in particular the decrease in sensitivity to the stimulus (pain and noise). According to Dragstedt and Lang, any animal that survived a given dose would have survived any dose below that dose.

Weekly weighing of the rats showed that the difference between the averages obtained from the two batches (witness and treated with the extract) was not significant. The average experimental batch weight evolved from 230 ± 8 g to 229 ± 7 g by the end of the second week, implying that the crude *C. triflorus* extract did not interfere with rat growth.

In general, the biochemical parameters for hepatic and renal assessment assessed that there is no statistically significant difference between control and crude extract of *C. triflorus* (Fig.1). These results were confirmed by histological sections of the kidney and liver, which showed no abnormality on microscopic examination (Fig.2).

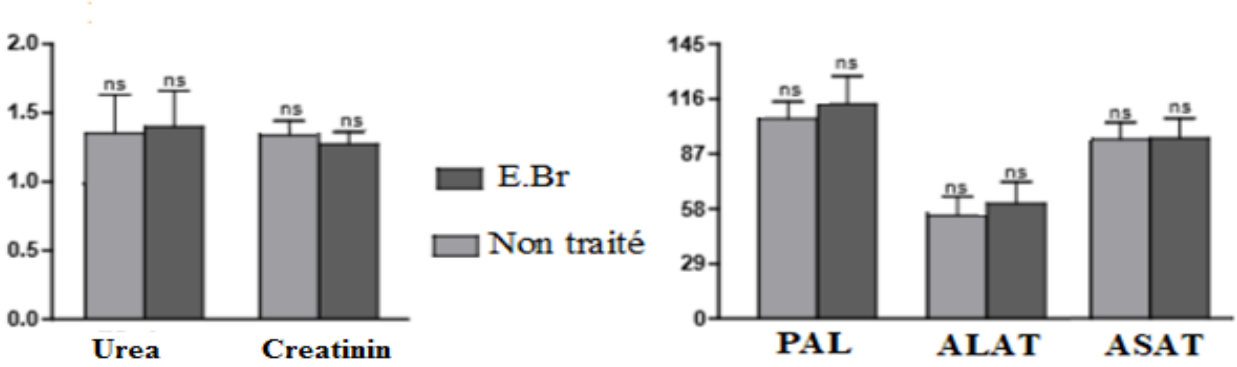

Figure 1: Biochemical parameters of the control and rats treated with crude extract of *C. triflorus*, measured during acute toxicity. E.Br: the crude extract, ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase, PAL: alkaline phosphatase and ns: not significant. Each value represents the mean ± SEM (n = 7)

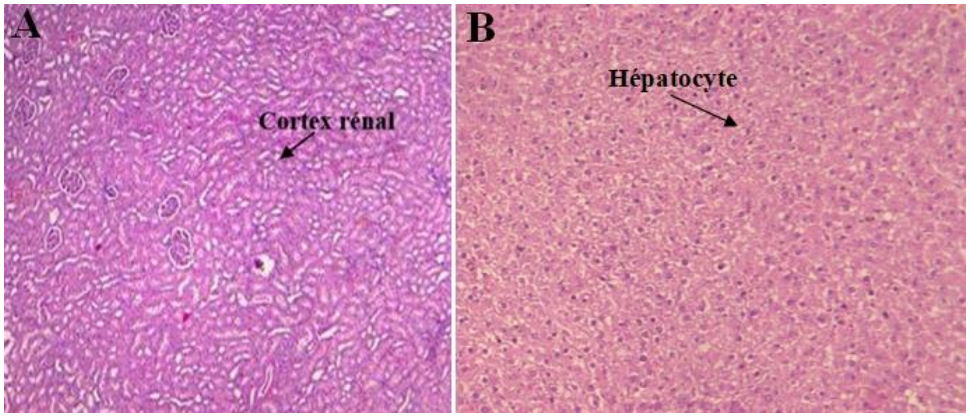

Figure 2: Histological section of liver and kidney tissue in rats treated with extract of *C .triflorus* in acute oral toxicity. Magnification (X 200), A: renal Tisus and B: hepatic tisus.

Gastroprotective Effect Evaluation
Macroscopic Evaluation of Lesions
Observations with the eye revealed massive production of characteristic gastric lesions in the stomach in 70% ethanol-fed rats, as well as severe hemorrhagic lesions and dilatation (edema) in the glandular part of the stomach. (stomach shown in Fig. 3-A). In contrast to the healthy stomachs in the control group, estomas, which were treated with E.Br 400 mg / kg and Ranitidine (5 mg / kg), had none of these characteristics (Fig. 3). The crude extract at 200 mg / kg significantly reduced the ulceration, so as to restore the normal appearance of the stomach; lesions were superficial (Fig. 3-E).

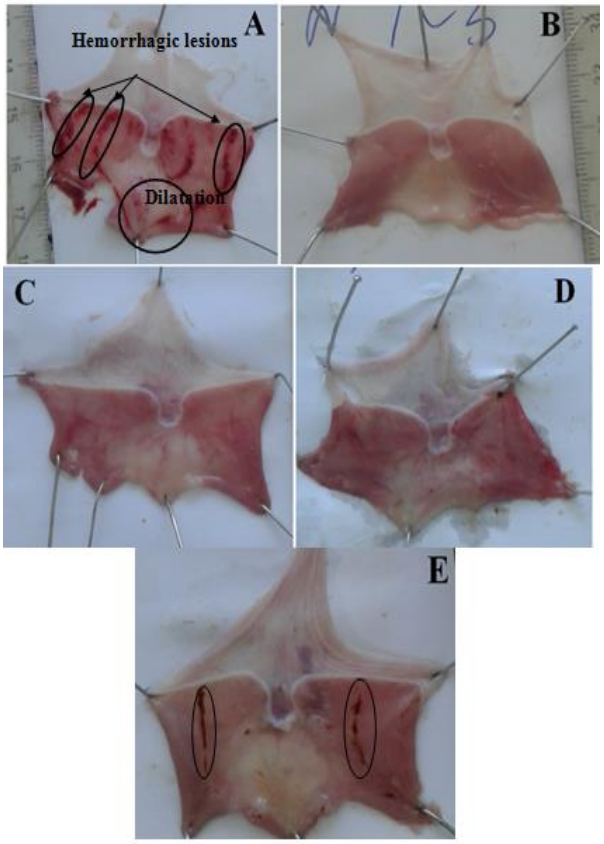

Figure 3: Macroscopic observations of the stomachs of untreated rats, ethanol-poisoned rats, and rats treated with raw C. triflorus extract. A: the effect of 70% ethanol, B: negative control, C: the effect of Ranitidine (5 mg / kg), D: the effect of the crude extract at 400 mg / kg and E: the effect of the crude extract at 200 mg / Kg.

Evaluation of the Degree of Ulceration by the Calculation of the Surfaces
The approximation of the total injured areas, using the AUTOCAD-2018 software, made it possible to evaluate the gastro-protective effect of E.Br against lesions induced by ethanol. Fig. 4, illustrates the percentages of ulceration of the different groups (control and tests) compared to the group that received only ethanol. The maximum percentage of ulceration corresponds to the ethanol group with 23.15 ± 1.19%. On the other hand, a very significant reduction of lesion areas was observed in the groups treated with Ranitidine and E.Br of *C. triflorus*, at doses of 200 or 400 mg / kg, with percentages of 0.75 ± 0.16. %, 2.71 ± 0.13% and 1.49 ± 0.14% respectively.

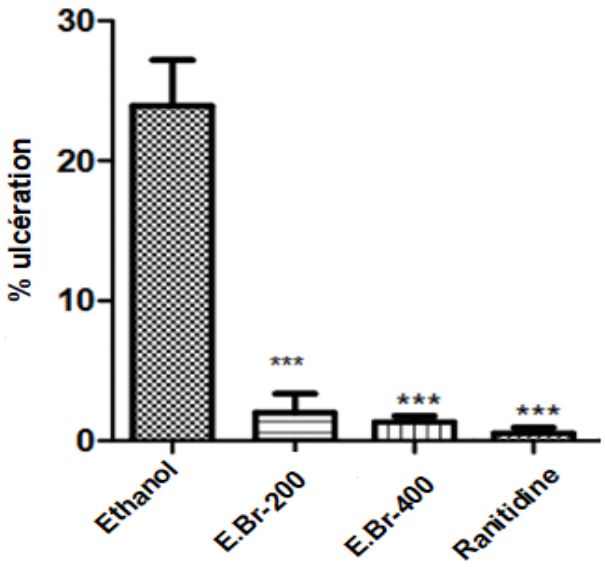

Figure 4: Percentage of ethanol-induced ulceration (total area of lesions). The values are expressed as mean ± SEM (n = 7). *** p <0.001 very highly significantly.

Microscopic Observations
The macroscopic results were confirmed by the study of histological sections made from the stomachs. Indeed, the observation of histological sections confirms that the rats that received neither the ulcerogenic product nor any other product, have a normal gastritis mucosa (Fig. 5), whereas the rats that received the ethanol presented an organization follicular in the context of acute inflammatory gastritis (Fig. 5), it is a gastritis with a mixture of lymphoplasmocytic and polymorphonuclear inflammatory elements.

Ethanol causes overproduction of reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical, which promote lipid peroxidation and hemorrhagic ulceration, resulting in triggering of the inflammatory reaction by releasing pro-inflammatory mediators (histamine), thus aggravating the lesions [7].

Pretreatment of the rats with the crude extract at 200 mg / kg improved ethanol injury to a certain

degree (Fig. 5), the histopathological analysis demonstrated discrete inflammatory gastritis, punctuated with a few lymphocytes. Animals receiving E.Br 400 mg / kg and Ranitidine were completely protected against the action of ethanol, retaining all histological aspects compared to the group of animals ulcerogenic by ethanol (Fig. 5). Their walls are regular, having a mucous layer, Muscular-mucous membrane and chorion loose descretely congestive.

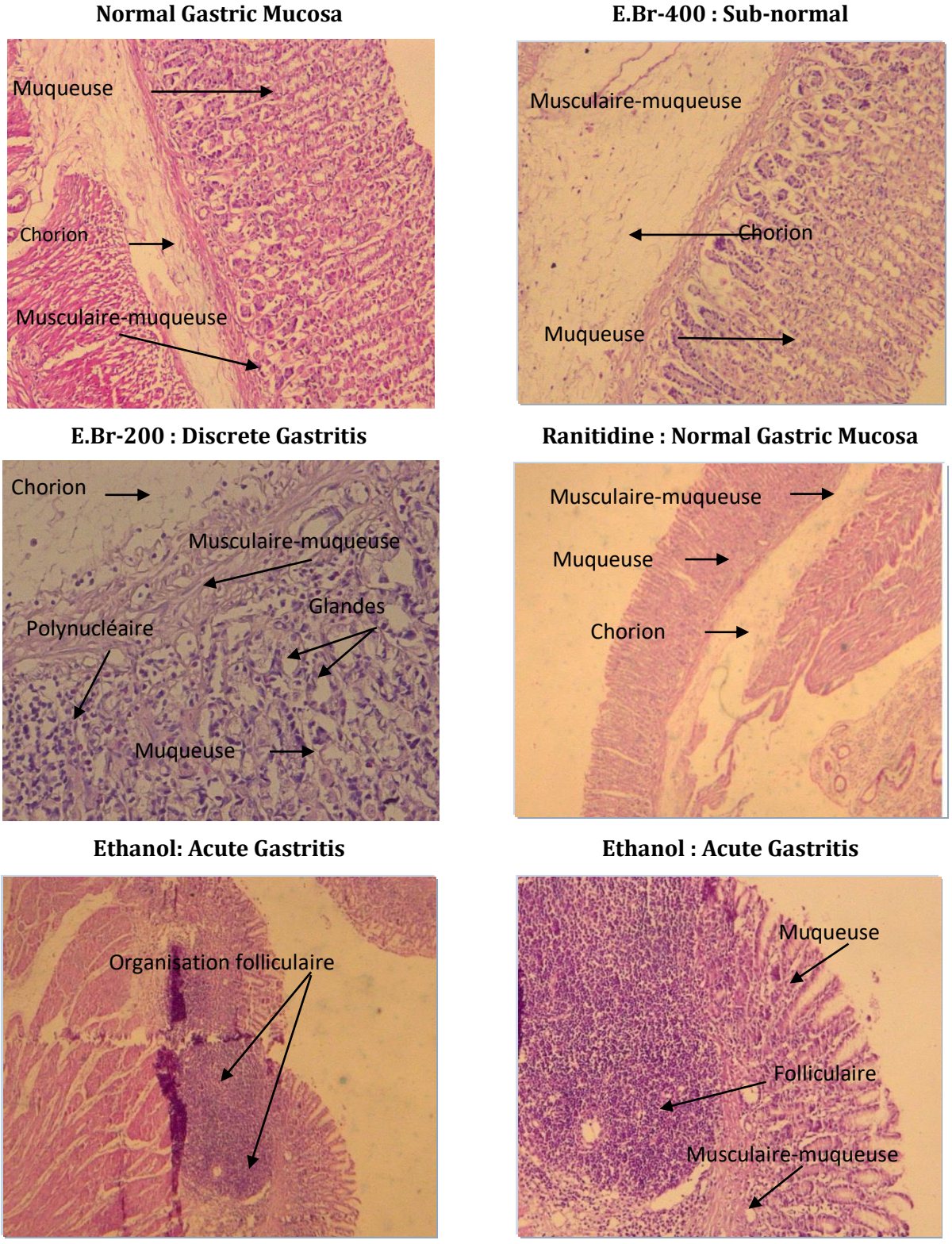

Figure 5: Histological sections of the stomach of untreated rats and of rats poisoned by ethanol and treated with the crude extract of *C. triflorus*.

Based on these results, our extract is considered to be a potent gastrointestinal, and the richness of C. triflorus in phenolics and flavonoids may be responsible for the observed gastro-protective activity. Bioactive chemical constituents including flavonoids, phenols and tannins have been shown to have gastroprotective potential. These components form complexes with cell wall proteins, stimulating the contraction of the wound [7]. Flavonoids have antioxidant properties that in addition to strengthening the mucosal defense system by stimulating gastric secretion of mucus, can trap reactive oxygen species (superoxide anions) and free radicals that play an important role in ulcerative and erosive lesions of the gastrointestinal tract [8, 9]. Several mechanisms have been proposed to explain the gastroprotective effect of flavonoids; these include increased mucosal prostaglandin content and decreased mast cell secretion by mast cell inhibition by histidine decarboxylase [7]. It is known that many flavonoids have antisecretory and cytoprotective properties in different experimental models of gastric ulcer [8, 9]. Comparing our results with those obtained with other plants, we found that the extract C.triflorus crude has gastro-protective effects similar to those of Glycurrhiza globra.L (Fabaceae) [10].

CONCLUSION

The knowledge and use of medicinal plants constitute a true heritage of the human being, populations rely a lot on traditional medicine, because of the side effects of conventional drugs. Scientific research in this area aims to validate herbal therapeutics. Our interest is focused on gastric ulcer, a chronic and widespread disease that can lead to gastric descancers. C. triflorus revealed antioxidant and anti-inflammatory powers related to its richness in phenolic acids and flavonoids.

The experimental approach that has been adopted has made it possible to test animal models of ulceration induced by ethanol. Our results revealed that the methanolic extract of *C. triflorus*. could act in a similar way to the drug Ranitidine thus validating its traditional use to fight inflammatory diseases such as ulcers.

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