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ANTIULCEROGENIC AND ANTIOXIDATIVE EFFECTS OF AQUEOUS EXTRACT OF *CHROMOLAENA ODORATA* L. (KING AND ROBINSON)

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ABSTRACT

This study aimed to evaluate the antiulcer and antioxidative effects of aqueous extract of *C.odorata*. The antiulcer effect was evaluated in the mice and rat by using HCl/ethanol mixture, ethanol (EtOH) and the indometacin like ulcerogenic agents. The antioxidative effect was evaluated by spectrophotometer by using the technique of reduction of 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical. The results obtained show that the aqueous extract (400 and 800 mg/kg) protects the gastric mucosa of experiment animals against the ulcers induced by the ulcerogenic agents. However, no effect was observed on the gastric pH. This action could pass by the increasing gastric mucosa production and not by an inhibition of acid secretion. Moreover, the aqueous extract presents an anti-oxidative effect by reduction of DPPH radical.

KEYWORDS: Antioxidative, *C.odorata*, ulcerogenic.

INTRODUCTION

Gastroduodenal ulcers are the affections which result from an imbalance in one or several points of the gastric or duodenal mucous membrane between the aggression and defense factors. This imbalance can be caused by the decrease of the mucous membrane resistance (anomaly of the mucosa, decrease of the mucous blood flow and the bicarbonates secretion, and prostaglandin biosynthesis) to the aggression agents.[1] Moreover, the environmental factors such as *Helicobacter pylori* and stress can also be at the origin of the arisen of gastroduodenal ulcers. Gastroduodenal ulcers represent one of the vastest domains of the digestive pathology both by their frequency and by their ubiquitous characters in the diverse populations of the world, or by the gravity of the complications which accompany them. In Africa, duodenal ulcer is answered in the tropical region with a higher frequency in urban areas where its incidence is increasing.[2] However, the accessibility to the modern drugs is a real problem; the population is often deprived of the financial or alive means in inaccessible sectors. That's why the majority of African population turn to healing plants stemming from the traditional medicine. Since the declaration of Alma-Ata,[3] all the recommendations of the WHO are in favor of the consideration of the resources of the traditional medicine in the primary health care. This is way the WHO of African region encourages the African countries to begin researches on healing plants and to promote their use in the systems of health care.[4] That's why in this study, we aimed investigated the antiulcerogenic and antioxidative effects of aqueous extract of leaves of *C.odorata* who is a Congolese medicinal plant used in wounds treatment.[5]

MATERIALS AND METHODS

Plant material

The leaves of *C. odorata* were used. Botanical identification of the plant material was done by Mousamboté, botanist systematist of Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the botanical laboratory of Research Institute in Exact and Natural Sciences (RIENS) in Brazzaville where the sample of *C.odorata* were compared with the reference sample of the herbarium to the number 1183,07/ 1965. After that, plant material were dried and pulverized with a mortar. The aqueous was prepared by decoction. 100 g of powder are mixed

with1000 mL of distilled water. The mixture was boiled for 15 min. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60 °C). The concentrate obtained was preserved to evaluate the antiulcer effect.

Animal material

Albino rats (200-250 g) and albino mice (25-30 g) of either sex obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. They were fed with a standard feed and water *ad libitum*. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of 27 ± 1 °C. The rules of ethics published by the International Association for the Study of Pain have been considered.[6]

Methods

HCl 0.3 M/ethanol 60% induced gastric ulcer

Method described by Astudillo et al, (2002)[7] was using. The animals were divided into groups of 5 rats each. Different doses of aqueous extracts of *C.odorata* (400 and 800 mg/kg), sucralfate (standard drug, 100 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups fifty min prior to the HCl/ethanol administration.[8] One hour after ulcer induced, the mice were sacrificed by dry blow. The stomach of each mouse was taken, opened according to the great curve by using a chisel. Each stomach was washed with the salt solution and fixed with formol 5% during 30 mn. The ulcers were observed macroscopically and by using an electric binocular magnifying glass (Leica Zoom 2000). The length of each lesion was measured by using a scale. The number and the gravity of the ulcers are given according to an arbitrary scale from 0 to 6 as reported by Germano et al, (1996).[9] The index of lesion (mm) for each stomach was expressed as the sum of length of all lesions.

Ethanol 90 % induced gastric ulcer in rat

The animals were divided into groups of 5 rats each. Different doses of aqueous extracts of *C.odorata* (400 and 800 mg/kg), sucralfate (standard drug, 100 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups one hour prior 0,5 mL of ethanol administration.[9] One hour after ulcer induced, the rats were sacrificed by dry blow. The stomach of each mouse was taken, opened according to the great curve by using a chisel and washed with the salt solution. The length of each lesion was measured by using a scale. The index of lesion (mm) for each stomach was expressed as the sum of length of all lesions.

Ethanol 50° + (indomethacin 20 mg/kg) induced ulcers in rat

The method put back by Galati et al, (1999)[10] slightly modified was used. Rats were preprocessed by the indomethacin (20 mg / kg, sc). Fifty min after, Different doses of aqueous extracts of *C.odorata* (400 and 800 mg/kg), misoprostol (standard drug, 0.5 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups one hour prior 1 mL of ethanol administration. One hour after ulcer induced, the rats were sacrificed by dry blow. The stomach of each mouse was taken, opened according to the great curve by using a chisel and washed with the salt solution. Mucosa was taken by means of a spatula and put in beforehand weighed tubes containing distilled water (1ml). The length of each lesion was measured by using a scale. The index of lesion (mm) for each stomach was expressed as the sum of length of all lesions.

Indomethacin induced gastric ulcers in rats

Method described by Sayanti et al, (2007)[11]was using. Different doses of aqueous extracts of *C.odorata* (400 and 800 mg/kg), ranitidine (standard drug, 50 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups one hour prior indomethacin (30 mg/kg, per os) administration. Four hours after indomethacin [12], rats were sacrificed by dry blow. The stomach of each rat was taken, opened according to the great curve by using a chisel. The pH was measured by using an electronic pH-meter (Hanna HI 8314). Buffer solutions pH7.01 (HI 7007) and pH4.01 (HI 7004) were used for the calibration of the device. The length of each lesion was measured by using a scale. The index of lesion (mm) for each stomach was expressed as the sum of length of all lesions.

Antioxidative activity

The antioxydative activity of the aqueous extract was estimated in the presence of the free radical DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals. The (DPPH) was prepared at the concentration of 10 mg/250 mL [12]. 10 mL of DPPH were mixed with 100 µl of methanol and also with methanolic solutions of the aqueous extract of *C.odorata* at the concentrations of 1.25; 2.5 and 5 mg/mL. The absorbance was given 30 min after in dark condition and at the ambient temperature at 517 nm [12]. Gallic acid at the concentrations of 0.3125; 0.625 and 1.25 mg/mL was used like a standard antioxidatve. Radical scavenging activity was calculated by the following equation:

$$DPPH radical\ scavenging\ activity\ (\%) = \frac{(Ac - At)}{Ac} \times 100$$

Ac=Absorbance of control; At=Absorbance of test sample

Statistical analyze

All values were expressed as mean ± ESM. Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p<0.05$

RESULTS

Effect of aqueous extract of *C.odorata* against gastric ulcer induced by HCl/ethanol

The observation of the gastric mucous of the animals shows that the HCl/ethanol mixture causes ulcers at all the experiments. However, the ulcers are pronounced

much in the animals which received distilled water (figure 1).Their gastric mucous are red and present the ulcers on all surface compared to the animals treated with the aqueous extract *C.odorata* (400 and 800 mg/kg) and sucralfate (figure 1). In addition, the aqueous extract and sucralfate significantly reduce ($p<0.001$) the index of the ulcers compared to the control group (table 1).These results also show that the most important percentage of protection is obtained with the dose of 800 mg/kg of the aqueous extract. The index of ulcers are of 22.52 ±0.62; 9.00 ±0.83 (60.03 % of percentage of protection);15.80 ±0.37 (29.84 %) and of 3.40 ±0.50 (84.90 %) respectively for distilled water (control group), the sucralfate and the aqueous extract of *C.odorata* (400 and 800 mg/kg).

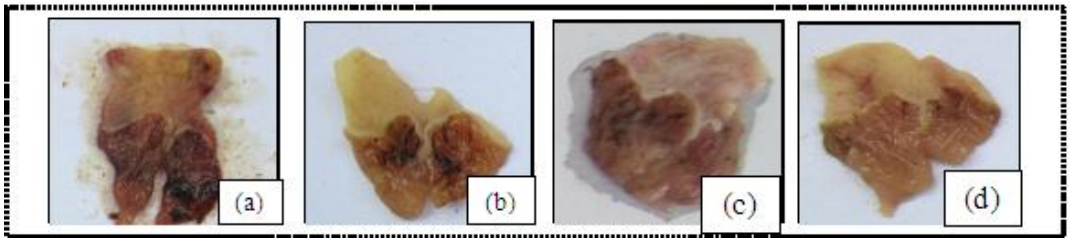

Figure 1: Illustrations of gastric mucous treated with distilled water (a), Sucralfate (b), aqueous extract of *C.odorata* at the dose of 400 mg/kg (c) and 800 mg/kg (d).

Table 1: Effect of aqueous extract of *C.odorata* against gastric ulcer induced by HCl/ethanol in mice.

Treatment	Doses	Index of ulcers (mm)	Protection (%)
Control group	0.5 mL/100g	22.52 ±0.62	/
Sucralfate	100 mg/kg	9.00 ±0.83***	60.03
C. odorata	400 mg/kg	15.80 ±0.37***	29.84
	800 mg/kg	3.40 ±0.50***	84.90

Each value represents the mean ± ESM of Index of ulcers (mm). ***P<0,001 significant different (Student t-test) versus control group

Effect of aqueous extract of *C.odorata* against gastric ulcer induced by ethanol 90% in rat

The results are presented by the table 2.They show that the aqueous extract of *C.odorata* (400 and 800 mg/kg) significantly decreases ($p<0.01$ and $p<0,001$) the index of the ulcers compared to distilled water. They show in addition that the most significant percentage of protection is obtained with the dose of 800 mg/kg of aqueous extract.

Table 2: Effect of aqueous extract of *C.odorata* against gastric ulcer induced by ethanol 90% in rat.

Treatment	Doses	Index of ulcers (mm)	protection (%)
Control group	0.5 mL/100g	11.60±0.67	/
Sucralfate	100 mg/kg	6.40±1.28***	44.82
C.odorata	400 mg/kg	6.20±0.96**	46.55
	800 mg/kg	1.20±0.73***	89.65

Each value represents the mean ± ESM of Index of ulcers (mm). **p<0.01; ***P<0.001 significant different (Student t-test) versus control group.

Effect of aqueous extract of *C.odorata* against gastric ulcer induced by ethanol 50° + (indomethacin 20 mg/kg) in rat

The results are presented by the table 3. They show that the misoprostol (standard drug) and the aqueous extract of *C.odorata* (400 and 800 mg/kg) significantly decrease ($p<0.01$ and $p<0.001$) the index of the ulcers compared to the control group (distilled water). It is also noted that the misoprostol and the aqueous extract of *C.odorata* (800 mg/kg) significantly increase ($p<0.001$) the production of gastric mucosa compared to the control group (distilled water). However, the dose of 400 mg/kg of the aqueous extract of *C.odorata* did not produce gastric mucus ($p>0.05$). The weight of mucus is of 0.95±0.28; 13.50±3.38 (92.96% percentage of mucosa production); 1.24±0.40 (23.38%) and 14.50±1.83 (93.44%) respectively of control group, the misoprostol and the aqueous extract of *C.odorata* (400 and 800 mg/kg).

Table 3: Effect of aqueous extract of *C.odorata* against gastric ulcer induced by ethanol 50° + (indomethacin 20 mg/kg) in rat.

Treatment	Doses	Index of ulcers (mm)	Weight of mucosa (10^{-2} mg)	Production of mucosa (%)
Control group	0.5 mL/100g	21.25 ±0.98	0.95 ±0.28	/
Misoprostol	100 mg/kg	3.50±0.94***	13.50 ±3.38**	92.96
C.odorata	400 mg/kg	13.30±1.04***	1.24±0.40 ns	23.38
	800 mg/kg	2.50±0.74***	14.50±1.83***	93.44

Each value represents the mean ± ESM of index of ulcers and the weight of mucosa. **p<0.01; ***P<0.001 signifiant different (Student t-test) versus control group. ns= p>0.05; no significant different (Student t-tes) versus control group.

Effect of aqueous extract of *C.odorata* against gastric ulcer induced by the indomethacin in rat

The results are given by table 4.They show that the aqueous extract (400 and 800 mg/kg) and ranitidine significantly decrease (p<0.05 and p<0.001) the index of the ulcers compared to the control group (distilled water).The percentages of protection of the mucous membrane are 81.04; 14.21 and of 84.07% respectively for the ranitidine and aqueous extract (400 and 800 mg/kg). In addition, the ranitidine (standard drug) significantly increased (p<0.001) the gastric pH compared to the control group (distilled water). However the aqueous extract at doses used is without effect on gastric pH (p>0.05). The gastric pH is of 2.11±0.21; 3.82±0.27; 2.41±0.07 and 2.43±0.16 respectively for control group, the ranitidine and the aqueous extract of *C.odorata* (400 and 800 mg/kg).

Table 4: Effect of aqueous extract of *C.odorata* against gastric ulcer induced by the indomethacin in rat.

Treatment	Doses	Index of ulcers (mm)	pH	Protection (%)
Control group	0.5 mL/100g	6.42 ± 0.38	2.11±0.21	/
Ranitidine	50 mg/kg	3.30±0.20***	3.82±0.27***	81.04
C.odorata	400 mg/kg	4.95±0.32*	2.41±0.07 ns	14.21
	800 mg/kg	0.83±0.16***	2.43±0.16 ns	87.07

Each value represents the mean ± ESM of index of ulcers and of the gastric pH. *p<0.05; ***P<0.001 significant different (Student t-test) versus control group. ns=p>0.05; no significant different (Student t-tes) versus control group.

Antioxidative effect of aqueous extract of *C.odorata*

Figures 2, 3 and 4 below represent the inhibition of the oxidative activity of the DPPH and the inhibiting concentration 50 (IC50).They show that the aqueous extract and the gallic acid inhibit the oxidative activity of the DPPH according to the concentrations used (figures 2 and 3).The inhibiting concentration 50 (IC50) is of 3.52 mg/mL for the aqueous extract and of 0.17 mg/mL for the gallic acid (figure 4).

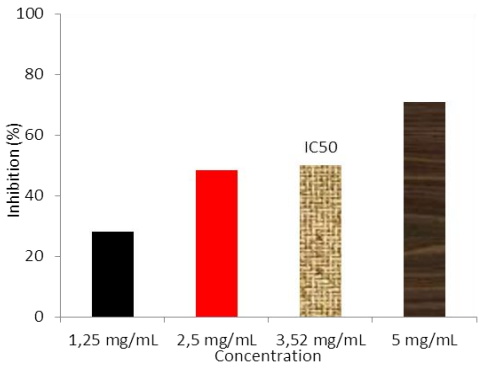

Figure 2: Inhibition of DPPH oxidative activity by aqueous extract of *C.odorata.*

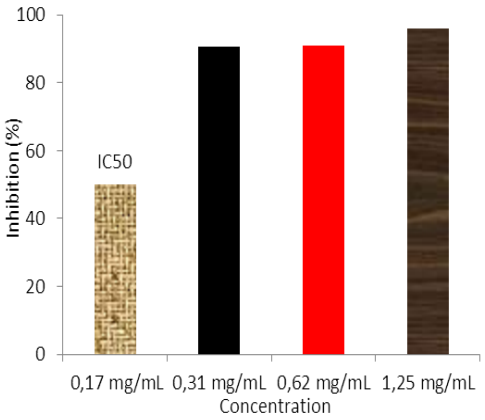

Figure 3: Inhibition of DPPH oxidative activity by gallic acid.

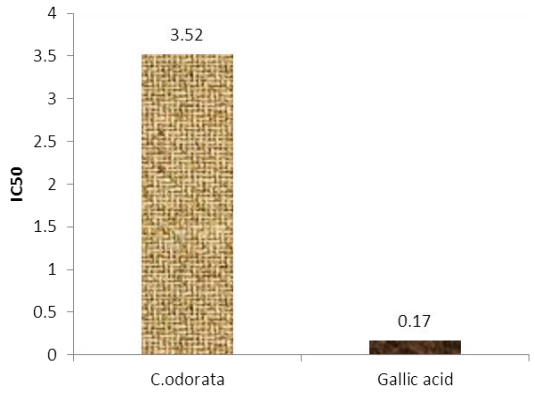

Figure 4: Inhibiting concentration 50 of aqueous extract of *C.odorata* and gallic acid

DISCUSSION

The aqueous extract of *C.odorata* reduced the index of ulcer in the animals whose ulcers were induced by the Hcl/ethanol mixture and ethanol only. Indeed, the ethanol induces the ulcers by deterioration of the permeability of mucosa to the electrolytes as well as a production of the free radicals or reactive oxygen species [1,8]. Apart from their beneficial effects in the normal biological processes like biosynthesis of the lipids bioactifs (prostaglandins and of the leucotrienes) and of the hormones (steroids),[14] the free radicals can react with the many compounds in the processes often nonspecific and cause damage of the ADN, the oxidation of proteins and lipids. It can result from it some pathology like cardiovascular, neurodegenerative, gastroduodenal diseases, metabolic dysfonction of the vital bodies, cancers and premature ageing [15].The fact that the aqueous extract of *C.odorata* (400 and 800 mg/kg) decreases the index of ulcer suggests that it could interfere with the mechanism of induction of the ulcers by ethanol. To appreciate the effect of the aqueous extract of *C.odorata* (400 and 800 mg/kg), it was necessary in this study to induce the ulcers with ethanol in the presence of indomethacin and of indomethacin only. The results obtained showed that the aqueous extract of *C.odorata* increases the production of mucus, but it is without effect on the gastric pH contrary to the ranitidine (Rani-Denk*) used like a antisecretory standard drug. Indeed, the indomethacin is a non-steroidal anti-inflammatory drugs (NSAIDs) whose mode of action passes by an inhibition of the cyclo-oxygenase, key enzyme of the synthesis of endogenous prostaglandins, which occurs without taking account of the mode of administration of the AINS, and which has as an essential consequence an endogenous prostaglandin deficit within the gastric mucous [1]. However these endogenous prostaglandins have a true mucoprotective effect by increasing the production and gastric secretion of mucus, secretion of bicarbonates and surfactant and by stimulating blood flow**.** In addition, they interfere on intrinsic protection by increasing the cellular renewal. By this fact, they prevent the gastric lesions due to agents of aggression such as the hydrochloric acid or soda with strong concentration, ebullient water, pure alcohol, the hypertonic solutions and the aspirin.[1] The fact that the aqueous extract of *C.odorata* (400 and 800 mg/kg) increases the production of gastric mucosa without increasing the pH suggest that the aqueous extract of *C.odorata* (400 and 800 mg/kg) could have mucoprotective and not antisecretory properties. In this study, the antioxidative effect of the aqueous extract of *C.odorata* (400 and 800 mg/kg) was shown in the presence of the DPPH. The inhibiting concentration 50 (IC50) of free radicals of the aqueous extract was 3.2 mg/ml and 0.17 mg/ml for the gallic acid. The antioxidative activity observed with the aqueous extract could take part in the antiulcerous activity.

CONCLUSION

The aim of this work was to evaluate the antiulcerous and antioxidative effects of aqueous extract of *C.odorata*. It comes out from this study that the aqueous extract of *C.odorata* could have a mucoprotective and not antisecretory effect. In addition, this extract has also antioxidative properties which could explain the antiulcerous effect observed.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

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