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Cytoprotective effects of the leaves aqueous extract of *Combretum glutinosum* (Combretaceae) on gastric ulcers in mice

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Abstract

This work was designed to verify the cytoprotective property of the leaves aqueous extract of Combretum glutinosum (LAECG) on gastric ulcers (GU) in mice. The cytoprotective property of LAECG was evaluated by using three experimental methods of GU in mice: HCl/EtOH model, HCl/EtOH with indomethacin pretreatment model and indomethacin model. For each method, male mice were divided into 4 groups of 5 mice and treated respectively with distilled water (negative control), sucralfate (positive control) and extract (200 and 400 mg/kg). A qualitative phytochemical analysis was realized to detect some classes of compounds present in extract like alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, and coumarins. C. glutinosum extract (400 mg/kg), showed his best activity in all the methods. Administration of LAECG (400 mg/kg) by oral route inhibited the formation of the GU of 27.84, 36.11 and 100%, respectively for the HCl/EtOH model, HCl/EtOH with indomethacin pretreatment model and indomethacin model. These percentages of inhibition of extract at 400 mg/kg were accompanied by the increase of mucus secretion of 97.50, 163.18 and 111.63%, respectively. Flavonoids, tannins and phenolic compounds were found to be present in LAECG. LAECG would protect the gastric mucosa membrane by stimulating mucus secretion through a mechanism which would not involve synthesis of endogenous prostaglandins, but rather by a direct effect on mucus secreting cells.

Keywords: *Combretum glutinosum*; phytoconstituents; cytoprotective activity; gastric ulcers; mice.

Introduction

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are symptomatic drugs used for their anti-inflammatory effects, but also as antalgic and antipyretic. $NSAID_S$ are the most used therapeutic class, by the population in the world, whether in the context of medical prescription or self-medication[1]. In USA, $NSAID_S$ represent approximately 70 million prescriptions each year while in Europe, they represent more than 7.7% of all the prescriptions[2]. However, $NSAID_S$ are responsible of gastrointestinal side effects, due to the inhibition of the constitutive cyclooxygenase 1 or the inducible cyclooxygenase 2 by selective $NSAID_S$[3].

Other non-selective $NSAID_S$, as indomethacin which is one of the most prescribed $NSAID_S$, inhibit both isoforms of cyclooxygenase. They induce the reduction of intrinsic PGE_2 production in the gastric mucosal membrane, which leads to a deterioration of gastric protection by reduction of mucus and bicarbonate production and the reduction of blood flow of mucosa[2]. This alteration provokes the appearance of lesions in gastric mucosa with risk of ulceration[4] which can lead to complications such as digestive hemorrhage, perforation, stenose and cancer[5-6].

The prevalence of digestive hemorrhages and perforations related to $NSAID_S$ intake is about 20% and 10%, respectively[6]. The treatment used to heal or prevent the gastro-duodenal ulcers related to $NSAID_S$ intake aims to restore the rate of endogenous prostaglandins. When $NSAID_S$ intake can be stopped, gastric or duodenal ulcers must be treated by misoprostol. Misoprostol is effective for the treatment of ulcers induced by NSAIDS, however, it can lead to adverse effects such as diarrheas, giddinesses, headaches, vomiting, nauseas, constipation, abdominal pains and skin rashes[7].

This multitude of side effects associated to the use of misoprostol underlines the need for research of alternative ways for the treatment of this type of ulcers. More than 100 medicinal Cameroonian plants were quoted for the treatment of the ulcerous disease[8] as examples: *Combretum glutinosum, Eremomastax speciosa*[9].

Combretum glutinosum is a plant belonging to Combretaceae family and used for many pharmacological properties[10] such as: antibacterial[11], anti-malarial[12], and antischistosomial[13]. It is also used in ethnomedicine to treat gastric pains and wounds[14]. Thus, this work was designed to verify the cytoprotective activity of LAECG on GU induced in mice.

Materials and methods

Animal material: Male mice (Swiss strain, 10-14 weeks old and 20-25 grams) were used. These animals were obtained from the animal house of the University of Maroua, Cameroon. Mice received a standard diet composed as follows: corn flour (50%), soya flour (20%), fish flour (15%), bone flour (4%), complex vitamin (0,1%), cotton oil cake (10%), palm oil (0,1%), cooking salt (0,8 %), with access to tap water.

Plant material: The leaves of *C. glutinosum* were harvested in May in the locality of Mokolo, Far-North Region of Cameroun. The plant was identified by Vounserbo Emmanuel of the wildlife School of Garoua by comparison with the existing specimen (number HEFG/734). After the harvest of *C glutinosum* leaves, they were shade dried during three weeks. These leaves were reduced into powder which served to prepare the extract.

Procedure of extract preparation: Powder (300g) was macerated in distilled water (3l) during 24 hours. This solution was filtered (Whatman paper No 3) and the filtrate evaporated (50°C) with a ventilated oven during 24 hours. The solid mass obtained (11g), representing our extract (3.6% yield), was kept at 4°C.

Qualitative phytochemical Screening: The phytochemical screening of LAECG was realized according to the protocol of Harborne[15], in order to search for the presence of followings: phenolic compounds, alkaloids, flavonoids, terpenoids, tannins and coumarins.

Induction of ulcers: HCl/ethanol induction: Induction of ulcers in mice with HCl/ethanol (HCl/EtOH) was performed as per Hara and Okabe[16] protocol. Twenty-five (25) mice were deprived for food, with water *ad libitum*, during 12 hours and divided in 5 groups of 5 mice each, namely: two control groups negative and positive treated respectively with distilled water (0.5ml/30g) and sucralfate (50mg/kg) and three tests groups extract-treated (100, 200 and 400mg/kg). One hour following the treatment, all the groups received HCl/ethanol solution (150 mmol/60 %), orally. After one hour, mice were sacrificed, their stomachs removed and 5 ml of 2% formalin were injected into. Mucus production, length and width of GU and their scores were evaluated as reported by Tan *et al*.[17].

HCl/ethanol with indomethacin pretreatment induction: The method of Sun *et al.*[18] was employed to induce gastric ulcers in mice. After 24 hours fasting, 20 mice were divided in four groups. They were given indomethacin intraperitoneally (20 mg/kg). After thirty minutes, animals received distilled water (negative control), sucralfate (positive control) and extract (200 and 400mg/kg). After 1 h, all animals were given HCl/ethanol solution (0.5ml/30mg) *per os*. After 1h, the mice were sacrificed and the stomachs examined in the same way as for the HCl/ethanol model.

Indomethacin induction: Gastric lesions were experimentally induced as reported by Pillai and Santhakumari[19]. Repartition and treatment of animals were similar to the previous method, except that: indomethacin (50mg/kg) was orally given and animals were sacrificed 5 h after ulcer induction.

Results and discussion

Phytochemical analysis: The classes of compounds present in LAECG are depicted in Table-1.

Table-1: Phytochemical screening of LAECG

Compounds classes	Observation
Alkaloids	-
Phenolic compounds (phenols)	+
Flavonoids	+
Terpenoids	-
Tannins	+
Coumarins	-

(+) = Presence; (-) = Absence

Effects of *C. glutinosum* on GU induced with HCl/EtOH: HCl/EtOH solution led to the formation of GU, present as dark red color bands, on the glandular part of stomachs (Figure-1). These bands were numerous and larger in negative control (Photographs 1A). Their number and size dropped in positive control as well as in extract-treated mice (100mg/kg) (Photographs B & C). This reduction of lesions size was more emphasized in extract-treated (200 and 400mg/kg) mice (Photographs D & E).

Table-2 shows the macroscopic aspect of stomach after treatment with LAECG, following gastric ulcers induction with HCl/EtOH. LAECG (100, 200 and 400mg/kg) reduced the ulcerated surface in dose-dependent manner, with percentages of inhibition of 28.99; 30.43 and 31.26%, respectively. In animals treated with sucralfate, it was also observed a reduction of ulcerated surface, with a percentage of inhibition of 21.95 %. In extract-treated (100, 200 and 400mg/kg) animals, it was equally observed an increase of mucus secretion (15.50; 29.25 and 97.50%, respectively), likewise for the positive group (24.00%).

Cytoprotective effects of LAECG on GU induced with HCl/EtOH and indomethacin pretreatment: Lesions provoked with HCl/EtOH and indomethacin pretreatment, were located on the glandular part of stomachs and appeared like dark red bands (Figure-2). These bands were larger and more abundant in negative and positive controls (Photo A and B) just like in extract-treated (200mg/kg) mice (Photo C). Their sizes and numbers decreased in animals treated with LAECG (400 mg/kg) (Photo D).

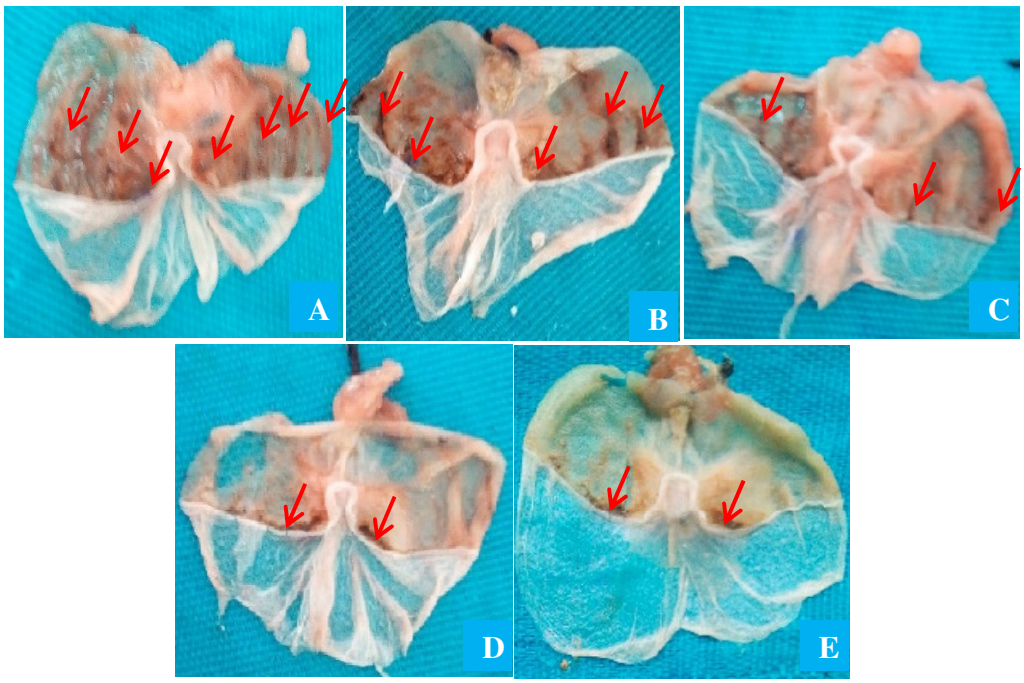

Figure-1: Photographs of stomachs subjected to gastric ulcers induced with HCl/EtOH solution. A = negative control; B = positive control (50mg/kg of sucralfate); C = LAECG (100mg/kg); D = *C. glutinosum* (200mg/kg); E = LAECG (400mg/kg); → = indications of gastric lesions.

Table-2: Cytoprotective effects of LAECG on GU induced with HCl/EtOH.

Treatments	Dose (mg/kg)	Ulcerated surface (mm^2)	Ulcerated surface (%)	Ulcer index	Inhibition (%)	Mucus mass (mg)	Mucus' increase (%)
Negative control	-	42.40 ± 4.28	13.25	4.83 ± 0.41	-	8.00 ± 1.93	-
LAECG	100	27.80 ± 4.94**	8.69	3.43 ± 0.14***	28.99	9.24 ± 1.51	15.50
LAECG	200	20.80 ± 2.71***	6.50	3.36 ± 0.11***	30.43	10.34 ± 2.12	29.25
LAECG	400	18.20 ± 4.25***	5.69	3.32 ± 0.13***	31.26	15.80 ± 1.31**	97.50
Sucralfate	50	24.60 ± 3.47**	8.31	3.77 ± 0.21***	21.95	6.08 ± 0.78	24.00

Values are expressed as Mean ± Standard Error on Mean. ** p<0.01, *** p<0.001= significant differences by comparison to the negative control.

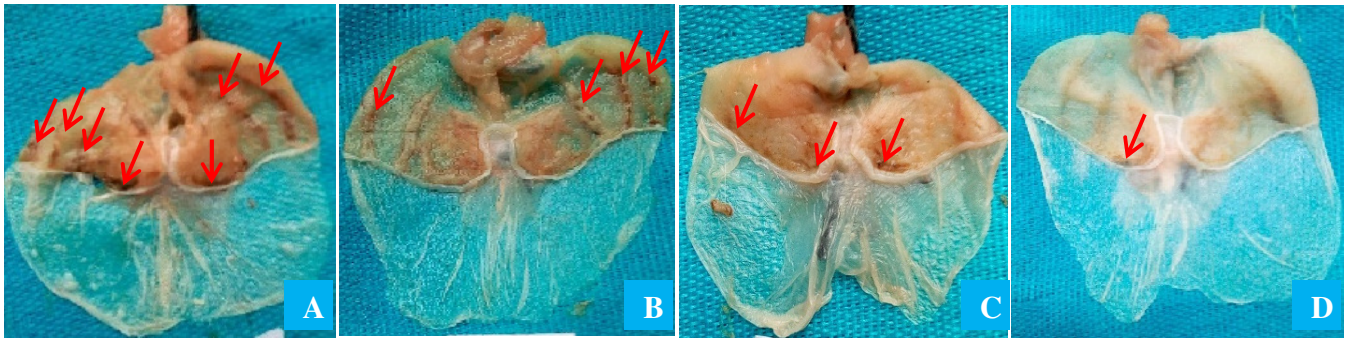

Figure-2: Photographs of stomachs subjected to GU induced with HCl/EtOH and indomethacin pretreatment. A = negative control; B = positive control (50 mg/kg of sucralfate); C = *C. glutinosum* (200 mg/kg); D = *C. glutinosum* (400 mg/kg); → = indications of gastric lesions.

The macroscopic effects of LAECG on GU provoked by HCl/EtOH with indomethacin pretreatment are presented in Table-3. LAECG (200 and 400mg/kg) reduced the ulcerated surface (7.06 and 4.32%, respectively), compared to negative control (7.38%). This was accompanied by an increase of the percentage of inhibition of ulcers (0.78 and 36.53%, respectively). In positive control, the percentage of ulcerated surface was 9.00 corresponding to 10.36% of inhibition. The reduction of ulcerated surface (200 and 400mg/kg) was correlated to an increase of mucus secretion (79.49 and 163.17 %, respectively).

Cytoprotective effects of LAECG on GU induced with indomethacin: Gastric lesions obtained after induction by indomethacin are illustrated by Figure-3. The lesions, appearing as small red bands, were more visible in negative control (Photographs 3A). Their size and number decreased in positive control as well as in extract-treated (200mg/kg) mice (Photographs 3B and 3C). *C. glutinosum* (400mg/kg) prevented totally the occurrence of lesions (Photographs 3D).

The macroscopic effects of *C. glutinosum* on GU provoked with indomethacin are consigned in Table-4. Extract (200 and 400 mg/kg) induced a decrease of percentage of ulcerated surface (0.75 and 0.00%, respectively), corresponding to 58.46 and 100.00% of inhibition, respectively. Those above were 46.59% at the positive control. This reduction of ulcerated surface (200 and 400mg/kg) was correlated to an increase of mucus secretion (55.27 and 111.63%, respectively). The percentage of increase mucus secretion was 13.81% in sucralfate-treated group.

Table-3: Cytoprotective effects of LAECG on GU induced with HCl/EtOH and indomethacin pretreatment**.**

Treatments	Dose (mg/kg)	Ulcerated surface (mm^2)	Ulcerated surface (%)	Ulcer index	Inhibition (%)	Mucus mass (mg)	Mucus' increase (%)
Negative control	-	23.60 ± 3.17	7.38	3.86 ± 0.18	-	4.78 ± 0.68	-
LAECG	200	22.60 ± 2.77	7.06	3. 83 ± 0.42	0.78	8.58 ± 1.11	79.50
LAECG	400	13.83 ± 3.43***	4.32	2.45 ± 0.62*	36.53	12.58 ± 2.22*	163.18
Sucralfate	50	28.80 ± 3.80	9.00	3.46 ± 0.13	10.36	9.20 ± 1.18	92.47

Values are expressed as Mean ± Standard Error on Mean.* p<0.05, *** p<0.001= significant differences by comparison to the negative control.

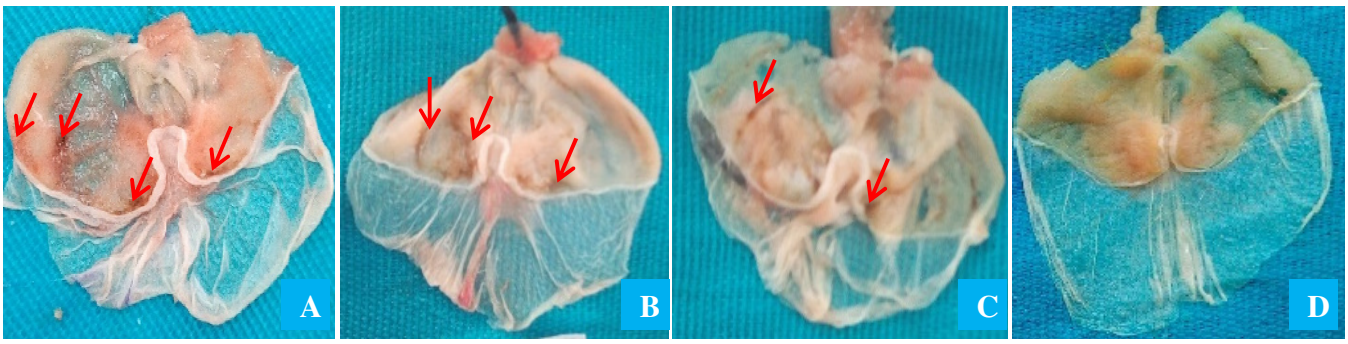

Figure-3: Photographs of stomachs subjected to GU induced with indomethacin**.** A = negative control; B = positive control (50mg/kg of sucralfate); C = LAECG (200mg/kg); D = LAECG (400mg/kg); ⟶ = indications of gastric lesions.

Table-4: Gastroprotective effects of LAECG on GU induced with indomethacin.

Treatments	Dose (mg/kg)	Ulcerated surface (mm^2)	Ulcerated surface (%)	Ulcer index	Inhibition (%)	Mucus mass (mg)	Mucus' increase (%)
Negative control	-	11.40 ± 3.37	3.56	3.37 ± 0.30	-	5.50 ± 0.53	-
LAECG	200	2.40 ± 1.75**	0.75	1.40 ± 0.87*	58.46	8.54 ± 0.82	55.27
LAECG	400	0.00 ± 0.00***	0.00	0.00 ± 0.00**	100.00	11.64 ± 0.93***	111.63
Sucralfate	50	3.00 ± 1.38**	0.94	1.80 ± 0.73	46.59	6.26 ± 0.79	13.81

Values are expressed as Mean ± Standard Error on Mean. *p<0.05, **p<0.01, ***p<0.001= significant differences by comparison to the negative control.

Discussion: Cytoprotective effects of LAECG were evaluated in male albino mice by using three experimental methods, namely: HCl/EtOH, HCl/EtOH pretreated with indomethacin and indomethacin models. HCl/EtOH model was used for the cytoprotective screening of LAECG against irritating agents. The HCl/EtOH (pretreated with indomethacin) model and indomethacin model were used to determine the gastroprotective mechanism of the extract. From the results, it appeared that *C. glutinosum* prevented the formation of GU whatever the model used.

HCl/EtOH model is a well-established method for evaluation of gastroprotective effects of medicines. Indeed, the mixture HCl/EtOH exerts a direct deleterious effect on gastric mucosa, leading to the formation of necrotic lesions. This direct irritation of the gastric mucosa induces a solubilization of mucus components which in turn leads to a reduction of the mucosa resistance and a rupture of the mucous barrier[20]. LAECG (100, 200 and 400mg/kg) reduced ulcerated surface to 27.80; 20.80 and 18.20mm^2, respectively, by comparison with the negative control 42.40mm^2. LAECG protected the gastric epithelium against HCl/EtOH mixture, with inhibition percentages of 28.99; 30.43 and 31.26 observed (100, 200 and 400mg/kg, respectively). This inhibition was less significant at the positive control with a percentage of inhibition of 21.95. These findings are in line with Mezui *et al.*[21] results obtained when evaluating gastroprotective effects of *Anthocleista shweinfurthii*. Mucus represents the first line of defense against lesions as well as retrodiffusion of hydrogen ions[22]. The benefic action of this mucus depends of its quantity and its thickness on mucosa surface[23]. Administration of LAECG, in HCl/EtOH model, could protect the gastric mucosa through an increase of mucus secretion, preventing thus the formation of lesions.

However, HCl/EtOH model does not permit to explain the mechanism by which extract increase mucus secretion. The pretreatment with indomethacin exposes the gastric mucosa to damages caused by HCl/EtOH given that indomethacin inhibits the synthesis of endogenous prostaglandins through inhibition of cyclooxygenases. The gastroprotective effects of endogenous prostaglandins on gastric mucosa are well-known[24]. Indeed, prostaglandins are very important in protecting gastric epithelium against lesions by: increasing mucus and bicarbonate production, enhancing the microcirculation in stomach, promoting the repair of gastric mucosa[25]. Thus, inhibition of prostaglandins predisposes the gastric mucosa to damages while its stimulation can be protective. In this study, *C. glutinosum* provoked a dose dependent decrease of ulcerated surfaces compared to negative control. LAECG (400mg/kg) prevented gastric ulcers occurrence similarly to result found in HCl/EtOH model (% I = 31.2; M = 15.80mg and % US = 5.69) and with HCl/EtOH with pretreatment with the indomethacin (% I = 36.56; M = 12.58mg and % US = 4.32). This suggests that the extract would not act by the way of endogenous prostaglandins but could act by a direct action on mucus secreting cells.

Indomethacin (*per os*) model was used to investigate the mechanism by which extract promotes mucus secretion. These lesions were completely inhibited at 400mg/kg. This inhibition was associated to an augmentation of mucus secretion (11.64 mg) near to that observed for induction with HCl/EtOH with pretreatment with the indomethacin (12.58mg). These results are in line with those obtained by Mezui *et al.*[26] during evaluation of cytoprotective activity of *Cassia arereh* in rats. Authors suggested that this increase of mucus secretion was related to a direct action of the extract on mucus secreting cells because of some phytoconstituents present in the extract. Flavonoids and tannins found in this extract are known to possess benefic effects for the management of GU. Flavonoids reinforce the defense of gastric mucosa by direct stimulation of gastric mucus secretion as well as by inhibition of histidine decarboxylase synthesis[27]. Tannins prevent the development of ulcers and cause the precipitation of proteins to the site of ulcers. This precipitation of proteins forms an impermeable layer on the site of ulcer, which protects the gastric mucosa against the irritating substances[28].

Conclusion

The aqueous extract of *C. glutinosum* leaves would protect the gastric mucosa by stimulating mucus secretion through a mechanism which would not involve synthesis of endogenous prostaglandins, but rather by a direct effect on mucus secreting cells.

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