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Comparative Anti-Ulcers Effect of Hydroalcoholic Extract of Leaves and Root of *Calotropis procera* Ait (Asclepiadaceae)

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ABSTRACT

Calotropis procera leaves latex and root are commonly used in traditional Togolese medicine to treat various disease like gastric inflammation and ulcer. The anti-ulcerogenic potential of hydro-ethanolic extract of leaves (HELE) and root (HERE) were investigated using ethanol induced gastric lesion method in experimental Sprague Dawley rats (150 - 200 g). Administration of HERE to the rats by oral route (125-500 mg/kg) dose-dependently prevented the formation of acute gastric lesions while HELE inhibits non dose-dependently gastric lesions induced by ethanol. The dose-dependent reduction of lesion formation by HERE was accompanied by significant increases in gastric mucus production and significant decreases of gastric acidity. HELE not modified significantly gastric mucus nor gastric acidity. Anti-ulcerogenic effect of HERE and HELE were demonstrated and confirmed *C. procera* root used in Togolese folk medicine. However the exact mechanisms of action are still unknow.

Keys words: Gastric ulcer, *Calotropis procera,* Mucus, Acid secretion, Ethanol.

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INTRODUCTION

Calotropis procera (Ait.) (Asclepiadaceae) is a xerophytic, erect shrub growing widely troughout the tropics of Africa and Asia, commonly known as *gboloti* or *aflagbe* in Togo. In Togolese folk medecine, different parts of this plant have been used. For example, *C. procera* leaves are used in the manufacture of "Wagashi", a local cheese, to coagulate milk. *C. procera* is also used by traditional healers to treat asthma, whooping cough, tuberculosis, threatened abortion, ulcer, leprosy, epilepsy, diabetus [1,2]. Various studies have been conducted to confirm medicinal properties of this plant. *C. procera* leaves, flowers, root sterm bark have antipaludic effect and inhibite chloroquine –sensitive and chloriquine resistant *Plasmodium falciparum* schizont [3]. The anti-inflammatory properties of root bark have been demonstrated and the barks are used for the preparation of a traditional medicine product FACA indicated for the treatment of sickle cell disease in Burkina Faso [4]. Basu et al. (1997) [5] have demonstrated the anti-ulcer activity of chloroform extract of the root. Experimentally, the latex of this plant has been shown to possess potent anti-inflammatory, analgesic, antipyretic, anti-ulcers, anticonvulsivant, anti-diarrhoeal, hepatoprotective, cardioprotective properties [6-10]. Despite, a lot of pharmacological properties of latex, some studies show that it is toxic. C. procera latex would cause irritation, corneal edema and induced permanent loss of endothelial cells [11]. It has been shown to possess edematogenic property in several models of acute and chronic inflammation [12]. Administration of crude latex induced cardiotoxic and hepatotoxic effect on rat [13]. Worries about preservation of C*. procera* plant by using it root in the treatment of ulcers and to avoid toxic adverse effect of latex, we purposed to compare anti-ulcers effect of hydroalcoholic extract of the dried root (HERE) and leaves (HELE).

MATERIALS AND METHODS

Animals

Sprague dawley rats, of both sexes weighing 150-200 g were the subject of testing. They were placed in an Animal house within the Faculty of Science's Laboratory of Physiology/Pharmacology. The animals were housed in large cages in environmentally controlled room (25 ± 2◦C, 12-hlight/12-h dark cycles) with free access to standard laboratory food and water. The animals were randomly distributed into different experimental groups. Each control and experimental group consists of six rats each. The animals were deprived of food but not water 24 h before the experiment.

Plant material

C. procera leaves and root were collected in April 2010 at Tsévié, located at 35 km north of Lomé. They were then subsequently analyzed and verified by the Laboratory of Botany. A reference sample was deposited in the Laboratory of Botany and Plant Ecology's Herbarium, part of the Faculty of Science, "University of Lomé". The voucher specimen number is N° 23. The selected leaves and root were washed, then dried under air-conditioning and finally ground to attain a powder. The powder was subsequently extracted with a mixture of water: ethanol (1:1, v/v) for 72 h and after that filtered. The filtrate was

evaporated and a hydro-alcohol extract of leaves and root (respectively yield: 26.39 and 7.58%) were obtained.

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out by using standard procedures. Tests were carried out to identify some components of aqueous-ethanol (v:v) extracts of *C. procera*. The extracts were screened for alkaloids, saponins, tannins, flavonoids

Gastric ulcers induction

Gastric ulcers were induced by ethanol according method described by Metowogo et al. (2011) [14]. Briefly ulcers were induced by orally administration of 1 mL/100 g body weight of ethanol 95°. Rats were subjected to fasting for 24 h preceding their ulcer induction. The control group (Group I) received distilled water and Group II to IV received respectively 125; 250; 500 mg/kg of *C. procera* leaves extract (HELE) while Group V to VII received 125; 250; 500 mg/kg of *C. procera* root extract (HERE) 30 min prior to ulcer induction. Sucralfate 200 mg/kg was used as a reference drug and was administrated to group VIII. Two hours following ulcer induction the subjected rats were then sacrificed under ether anaesthesia. Their stomachs were removed and opened along the greater curvature. Gastric contents juices were gently collected. Following this, stomach was immersed in 10% formaldehyde for ten minutes and the dimension of the ulcer was evaluated by planimetry, using 0.25 mm^2 ulcer area by unit. The percentage of linhibition was then calculated using the following formula:

$$\frac{\text{Dimension of the control rat's ulcer} - \text{Dimension of the treated rat's ulcer}}{\text{Dimension of the control rat's}} \text{ X } 100$$

Evaluation of gastric mucus production

Gastric mucus production was measured according to the method used by Metowogo et al. (2011) [14]. Gastric mucus production was measured in the rats that were subjected to ethanol - induced lesions. After estimating the degree of lesion formation, the gastric mucosa of each rat was gently scraped using a glass slide and the mucus obtained was weighed using a precision electronic balance.

Measurement of gastric secretion and acidity

Gastric contents collected in the rats that were subjected to ethanol-induced lesions were centrifuged for 10 min at 3.500 rpm. The supernatant was collected and used for the estimation of volume of gastric juice, and gastric juice acidity. Gastric juice acidity was determinate by titrating the juice with 0.1N NaOH.

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STATISTICAL ANALYSIS

Data obtained from the animal experiments was expressed as mean ± SEM statistical tests including one-way analysis of variance (ANOVA) followed by bonferroni's significant difference test were used to analyze any differences between the groups that were subjected to testing. A p-value of less than 0.05 was considered as being statistically significant.

RESULTS

Qualitative phytochemistry screening show that HELE contents alkaloids, tannins, flavonoids, saponins while HERE contents tannins, flavonoids and saponins (Table 1).

Table 1: Phytochemical screening result of hydro-ethanolic extracts of leaves (HELE) and root (HERE) of *C. procera*

Phytochemical compound	HELE	HERE
ALCALOIDS	+++	-
FLAVONOIDS	+	++
SAPONINS	+++	++
TANNINS	+++	+++

+++ : Very abundant ++: Abundant +: trace - : Absent

The characteristic striated lesions, which result from the oral intake of ethanol solution, were obtained in the glandular portion of the rat stomachs. Oral administration of 1 ml/100 g (b.w) ethanol 95° induced gastric ulceration. The macromorphologically characterization of ulceration is an abrasion of the gastric epithelium, erosion associated with one necroses ischemic with blood exudates colorized on black after immersion in formol 10% (fig 1). Ulceration dimension was evaluated by planimetry and gastric epithelial lesion on control rat was estimated at 21 ± 1.37 units. HERE or HELE, administrated orally one hour before gastric ulcers induction, inhibit significantly ($p \leq 0.01$) gastric ulcers induced by ethanol. Root extract inhibit ulceration at 46.67 and 51.43% at dose of 250 and 500 mg/kg while leaves extract on the same dose inhibit respectively ulceration at 60.95 and 67.61% (fig 2).

Gastric mucus production was evaluated by measure gastric mucus weight. Gastric mucus weight in control rat is 0.41 ± 0.09 g. Root extract significantly ($p \leq 0.05$) increased gastric mucus weight while leaves extract have not significantly increased it. At 250 and 500 mg/kg mucus weight are respectively 0.91 ± 0.07, 1.25 ± 0.21 g for root extract while it is 0.69 ± 0.17, 0.74 ± 0.11 g for leaves extract (fig 3).

Gastric juice secretion and acidity are one of factors involved in gastric ulcer advent. We then measured gastric juice and his acidity. The two extract have not stimulated gastric juice secretion significantly at variable doses. HERE only decreased significantly at 500 mg/kg b.w. gastric acidity (Table 2). At this dose gastric acidity is 12.18±2.07 mmol/L when for control group is 21.10 ± 3.06 mmol/L.

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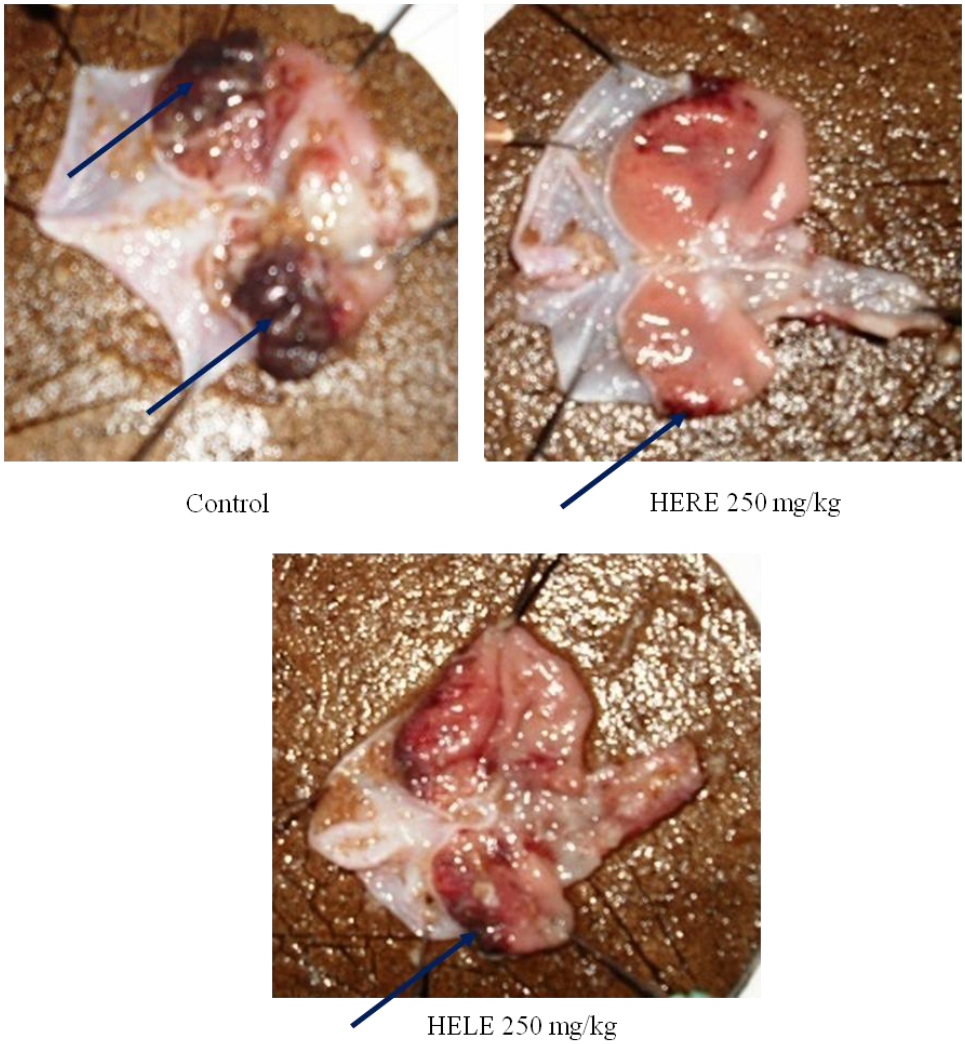
Table 2: Effects of hydro-ethanolic extracts of leaves (HELE) and root (HERE) of *C. procera* administered orally (p.o.) on gastric juice parameters in ethanol-induced gastric lesions in rat.

Group	Dose	Gastric juice volume (ml)	Gastric acidity (mmol/l)
Control	-	2,540 ±0,753	24,105 ±3,604
HELE	125 mg/kg	1,420 ±0,605	25,128 ±3,879
	250 mg/kg	1,600 ±0,254	18,690 ±3,725
	500 mg/kg	2,200 ±0,641	20,029 ±3,277
HERE	125 mg/kg	2,240 ±0,495	22,944 ±3,152
	250 mg/kg	2,380 ±0,684	18,464 ±3,939
	500 mg/kg	3,540 ±0,669	**12,186 ±2,075***
Sucralfate	250 mg/kg	**0,440 ±0,097***	**37,500 ±5,590***

Values are the mean ± ESM, n = 6, analysis of variance (ANOVA) followed by Bonferroni's multiple range test. Means bearing same superscripts do not differ significantly. (P ≤ 0.05).

Figure 1: Effects of hydro-ethanolic extracts of leaves (HELE) and root (HERE) of *C. procera* on ethanol induced gastric lesions in rat.



Macroscopic view showing ulcer formation and inhibition effect of extracts at 250 mg/kg

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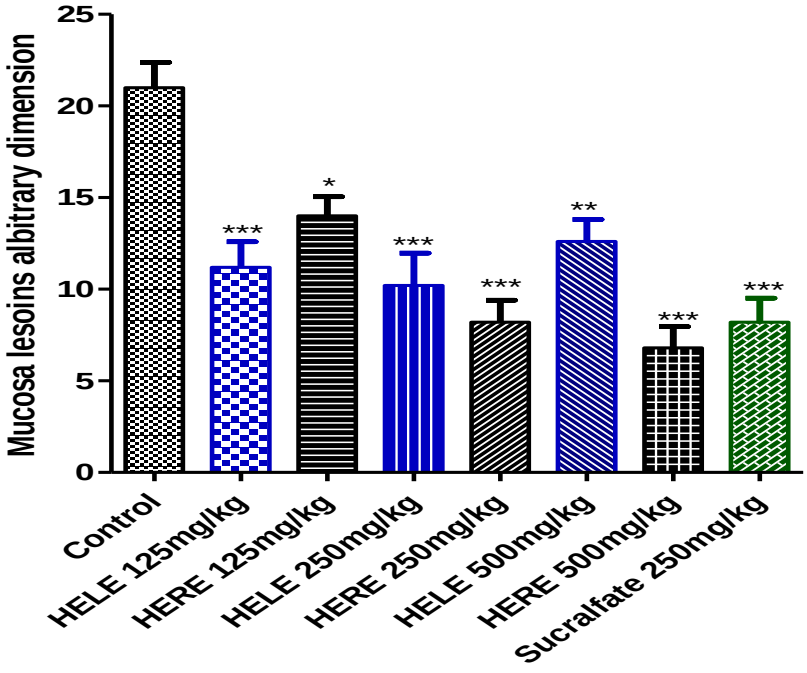
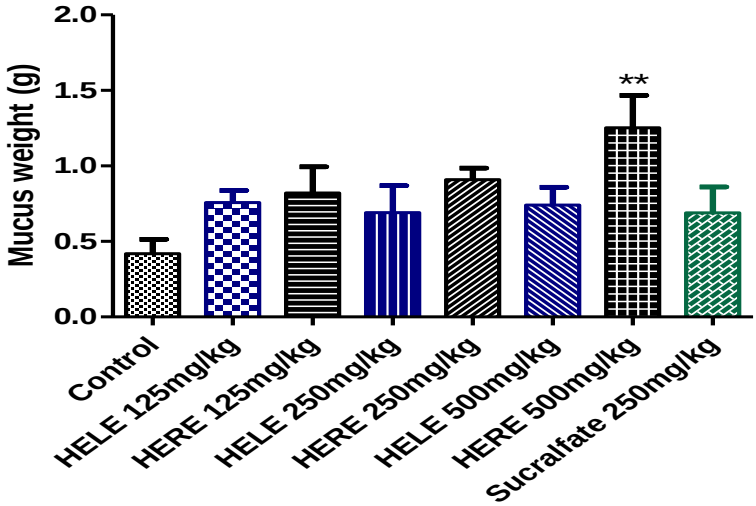
Figure 2: Effects of sucrafalte and the hydro-ethanolic extract of leaves (HELE) and root (HERE) of *C. procera* on ethanol induced gastric lesions in rat.



Extracts were administered by gavage 30 min prior ulcer induction. Two hours after ulcer induction rats were killed by cervical dislocation before ether anesthesia. Gastric mucosa lesions were evaluated by planimetry. Values are means ±SEM, n = 6 for each point. Pair wise differences were analyzed by Bonferroni's test after ANOVA. The differences between control and treated groups are statistically significant when * P≤ 0.05 , ** P≤ 0.01, ***P≤ 0.001.

Figure 3: Effect of the hydro-ethanolic extract of leaves (HELE) and root (HERE) of *C. procera* on gastric mucus production.



Extracts were administered by gavage 30 min prior ulcer induction. Two hours after ulcer induction rats were killed by cervical dislocation before ether anesthesia. Their stomachs were opened and mucus was removed by scraping.Values are means ±SEM, n = 6 for each point. Pair wise differences were analyzed by Bonferroni's test after ANOVA. The differences between control and treated groups are statistically significant when * P≤ 0.05.

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DISCUSSION

The present study was carried out to evaluate the ulcer protecting effect of *C. procera* leaves (HELE) and root (HERE) extracts in rats where gastric lesions were induced by oral administration of ethanol. The aim of this study is to compare gastric protective effect of the two extract. Gastric ulcers result from an imbalance between gastric protection and aggressive factors [15]. It began when gastric juice acid alters gastric mucus which covered stomach or duodenal wall. Several ulcerogenics agents are known like substances (chlorhydric acid, acetic acid, ethanol) or drugs (indomethacin, aspirin...). Ethanol is well known to induce gastric ulcers via multi-factorial mechanisms such as the impairment of gastric defensive factors like mucus dissolution [16] or by increasing offensive factors such as acid secretion or gastrin release [17]. Ethanol then disturbs gastric secretory activity, alter cell permeability and deplete gastric mucus [18]. In this study, HELE and HERE extract inhibit significantly gastric mucosa lesions induced by oral administration of ethanol. This result is comparable as those obtained by Barthi et al. (2010) [10] with C. procera dried or methanolic latex extract on gastric ulcer induced by oral administration of ethanol. In this study root extract protects gastric wall more significantly than leaves extract in the same condition.

The presence of acid in gastric wall is still considered as a factor in the development of acute and chronic gastric mucosa lesions in the occurrence of the disease. As a result, suppression of gastric acid by surgical and a variety of pharmacological means [19] provides effective and rapid healing of ulcer [20]. We evaluate the effect of the extract on gastric gastric juice secretions and acidity. In the model used, leaves or root extracts did not modified significantly gastric juice secretion or acidity. However root extract at 500 mg/kg increases significantly gastric decreased gastric acidity. In other words, gastric mucus helps to maintain integrity of gastric mucosa. According to Hiruma-Lima *et al*. (2006) [21] gastric mucus is a viscous, elastic, adherent and transparent gel formed by water and glycoproteins covering the entire gastrointestinal mucosa. These authors reported that the protective properties of the mucus barrier depends not only on its gel-like structure but are also related to the amount or thickness of the layer covering the mucosal surface. Mucus protects the gastric mucosa against irritants such as ethanol, HCl and acetyl acid. Our results demonstrated that only HERE at 500 mg/kg increased significantly gastric mucus. Finally real antiulcer mechanism of C. procera leaves and root remains to elucidate.

CONCLUSION

C. procera root and extract could be used to prevent and treat gastric ulcer. This study confirmed the use of the root in togolese traditional medicine. To preserve *C. procera* plant we suggest leaves utilization in the place of root.

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