

Antioxydant and gastric antisecretory properties of hydroalcoholic extract of *Aloe buettneri* A. Berger (Liliaceae).

Metowogo Kossi *, Eklugadegbeku Kwashie, Agbonon Amégnona, Aklikokou Kodjo A., Gbeassor Messanvi

*Corresponding author. Tél: (228)2255094 poste1337, BP : 1515 – Centre de Recherche et de Formation sur les Plantes Médicinales, Laboratoire de Physiologie Pharmacologie, Faculté des Sciences, Université de Lomé (Togo), E-mail: kossi.metowogo@gmail.com

Résumé

Intérêt ethnopharmacologique : Localement appelée ‘Adiadi’ *Aloe buettneri* est utilisée en médecine traditionnelle togolaise dans le traitement de l’inflammation, de l’ulcère gastrique et de la cicatrisation des plaies.

But : L’objectif de ce travail est d’évaluer les propriétés anti-sécrétoire gastrique, anti-ulcéreuse et antioxydante des feuilles d’*A. buettneri*.

Matériel et méthodes: Le modèle du pylore ligaturé et de lésion gastrique induite par l’acide acétique chez le rat est utilisé pour évaluer l’effet anti-sécrétoire gastrique, anti-ulcéreux de l’extrait hydro-alcoolique des feuilles d’*A. buettneri*. Le taux de sécrétion d’acide gastrique est évalué chez des rats ayant reçu une injection (i.v.) d’histamine. L’activité antioxydante est évaluée *in vitro* par le test de DPPH et le test d’AAPH induisant la lipoperoxydation membranaire des hématies.

Résultat : L’extrait hydro-alcoolique des feuilles d’*A. buettneri* montre dans le modèle du pylore ligaturé une réduction significative du volume de la sécrétion gastrique et d’acide gastrique. Le traitement préventif des rats protège la paroi gastrique contre la lésion de l’épithélium gastrique induit par l’acide acétique. Nos résultats montrent l’inhibition de

l'hypersécrétion acide induite par l'injection d'histamine. De plus l'extrait réduit *in vitro* le DPPH et inhibe la lipoperoxydation des membranes des hématies induite par l'AAPH.

Conclusion : La présente étude démontre les effets anti-sécrétoire et antiulcéreux de l'extrait hydro-alcoolique d'*A. buettneri* et par conséquent justifie son utilisation traditionnelle dans le traitement des ulcères peptiques et des maux de ventre.

Mots clés : *Aloe buettneri*, Lansoprazole, Cimetidine, acide gastrique, antioxydant, ulcère.

Abstract

Ethnopharmacological relevance: *Aloe buettneri* A. Berger (Liliaceae), locally called 'Adiadi' is used in Togolese folk medicine to treat inflammation, peptic ulcer, wound healing.

Aim of the study: To evaluate the gastric antisecretory, antiulcer and antioxidant activity of *A. buettneri* leaves.

Materials and methods: The hydroalcoholic extract of *A. buettneri* leaves was used to evaluate its gastric antisecretory and antiulcer effect in the pylorus-ligated rat model and gastric lesions induced by acetic acid respectively in rats. Gastric acid secretion rate was evaluated in rats which received histamine i.v. injection. Antioxidant activity was evaluated *in vitro* by two essays (DPPH essay and AAPH induced red blood cells membrane lipoperoxydation essay).

Results: The hydroalcoholic extract of *A. buettneri* leaves showed significant antisecretory activity as evidenced by decreased gastric juice volume and acid output in pylorus-ligated rats. Pretreatment with *A. buettneri* extract provided significant protection against the peptic ulceration caused by acetic acid administered individually. Our studies also revealed that *A. buettneri* extract inhibited hypersecretion induced by histamine injection. Further, the extract reduced to yellow *in vitro* DPPH solution and inhibits red blood cells membrane lipoperoxydation induced by AAPH.

Conclusion: The present findings demonstrate that *A. buettneri* hydroalcoholic extract has potent antisecretory and antiulcer effects and justify the traditional/ethnic usage of this herb to treat peptic ulcers and consequent stomach ache.

Key words: *Aloe buettneri*, gastric acid, antioxidant, ulcer.

INTRODUCTION

The genesis of peptic ulcer is complex and involves infection of the gastric mucosa with *Helicobacter pylori* plus other factors such as imbalance between mucosal damaging mechanism notably secretion of necrotizing agent such as acid and pepsin, and protecting mechanism (gastric mucus production, bicarbonate secretion, local prostaglandin synthesis, gastric mucosal blood flow, epithelial integrity). Epithelium integrity is an important factor in gastric protection. Drugs with multiple mechanisms of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases (Umamaheswari et al. 2007). It has been demonstrated that many drugs and formulations possess potent antioxidant action and are effective in healing experimentally induced gastric ulcers (Dhuley 1999).

Gastric acid plays an important physiological role however acid hyper secretion causes also problems such as peptic ulcer and reflux oesophagitis (Waldum *et al.* 1993). For this reason more of pharmacological agents which down regulate gastric acid secretion are most frequently used in gastric ulcer treatment.

Aloe buettneri A. Berger (Liliaceae) is a tropical cactus used in the traditional management of various illnesses like chronic skin ulcer, cough, dysmenorrhea, food poisoning, intestinal worms, difficult delivery, dysentery, general stomachaches, and lumbar pain (Tan *et al.* 2006). Other traditional oral report indicates that *A. buettneri* is used for antiseptic, purgative,

decoagulant, larvicidal, vermifuge, stomachic, tonic and stimulant activities. Telefo et al. (2002, 2004) have reported the beneficial effects of *A. buettneri* on ovarian steroidogenesis.

Yusuf et al. (2004) have shown that *A. vera* and other species of Aloe are used to treat inflammation, ulcer, chronic wound and others. Our previous investigations (Metowogo et al. 2008) showed that *A. buettneri*, similar species of *A. vera*, has anti-ulcer and anti-inflammatory properties. Similar result was obtained by Tan et al. (2006) through evaluation of anti-ulcer and toxicity profile of *A. buettneri* methanolic extract in mice and Wistar rat. Based on above information's, it is interesting to understand mechanisms of action of the extract in gastric wall protection. Our previous test proved that *A. buettneri* are gastroprotective effect: gastric pH and mucus secretion increasing properties (Metowogo et al 2011). The role of the extract on gastric acid secretion and its influence on experimental lesions in the gastric mucosa has been relatively unexplored. In the present work, we investigate the effect of the extract on gastric acid secretion and theirs antioxidants properties.

MATERIEL AND METHODS

Materiel

Plant material and extraction

Aloe buettneri leaves were collected in the botanical garden of the Faculty of Science of "Université de Lomé-Togo" in April 2006. Plants were identified by the Laboratory of Botany where a voucher specimen (UL-MET 001) was deposited in the Herbarium. The leaves were washed, dried under air-conditioning and reduced to powder. The powder was extracted in water/ethanol mixture (1:1; v/v) for 72 h. The solvent was evaporated to obtain a dark hydro-alcohol extract (yield: 24.25%). Phytochemical screening of this extract revealed the presence of tannins, flavonoids and alkaloids.

Animals

Wistar rats of either sex, weighing 150-200g were used. They were housed in the Animal house of the Faculty under conditions of ambient temperature, humidity and dark-light cycle (12h – 12h). The animals have free access of water.

Method

Acetic acid induced gastric ulcers

All the experiments were approved by the ethical committee of Université de Lomé.

Gastric ulcers were induced by luminal application of acetic acid as previously described by Cheng et al. (2004). Briefly, four groups of five rats were deprived of food for 24 h. The first group is the control while groups 2 and 3 are treated respectively by 250 and 500 mg / kg with *A. buettneri* hydro-acholic extract, the fourth group received the reference drug (Lansoprazole 30 mg/kg). Rats were treated by extract or lansoprazole or water 30 mn prior their stomachs exposed under ether anesthetization. The anterior and posterior walls of the stomach were clamped with a pair of metal rings of 11 mm internal diameter. 100% acetic acid (E. Merck, Darmstadt, Germany) solution (0.12 ml) was injected into the clamped portion and withdrawn into the syringe after 45 s. The abdomen was then closed and the rats were allowed to recover with free access to food and water. Height hours later the rats were sacrificed and stomach was removed and opened along the greater curvature and ulcer dimension was evaluated by planimetry.

Measurement of gastric secretion and acidity

Gastric secretion was measured in pylorus ligated or “Shay rat method”. Four groups of five rats each were constituted above. Thirty minute before pylorus ligation rats received extract or water orally. The abdomen was opened and the pylorus was ligated under urethane (1g/kg) anaesthesia. Four hours later, the animals were sacrificed, stomach was removed and then gastric contents were collected and 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 determined when gastric juice was titrated with 0.1N NaOH.

Gastric acid secretion rate

The methods described by Yusuf et al. (2004) were used. Four groups of rats (n= 5) were deprived of food 24 h before beginning of experiment. The animals were anaesthetized by single intraperitoneal injection of urethane at 1 g/ kg body weight. A trachea catheter was inserted to provide a clear airway, oesophagus was cannulated and attached to peristaltic pump. After a midline incision, stomach was exteriorized and pylorus cannulated with polyethylene tube. Abdomen was then sutured and closed to prevent desiccation and heat loss. For the duration of experiment, stomach was perfused from oesophageal end with isotonic saline at the rate of 1 ml/min. After 30 min of post surgery equilibration period, perfusates were collected from the distal cannula at 10 min interval for a total period of 120 min. Changes in the acid concentration of the perfusates were determined by titration with NaOH 0.1 N.

Control group received i.v. injection of 0.9 % NaCl and second group (treated group) received by the same route 500 mg/kg of hydro-alcoholic extract dissolved in 0.9% NaCl after two 10 min basal collections.

In another series of experiment, the third group received 500 mg/kg of hydro-alcoholic extract or 0.9% NaCl as described above 20 min before administration of 4 mg/kg histamine (i.v.). Perfusates were collected and treated as described previously. The fourth group was treated by cimetidine. The concentration of gastric acid secretion was calculated for each animal and expressed in mmol/10 min

Antioxidant activities

- DPPH assay

The free radical scavenging activity of *Aloe buettneri* was measured in vitro using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Kavegue 2007). About 100 mmol/l solution of DPPH in 100% methanol was prepared and 1.5 ml of this solution was added to 0.25 ml of the extract dissolved in methanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using spectrophotometer. Inhibition percentage [I (%)] was calculated as follow:

$I (\%) = (A_0 - A_t) / A_0 \times 100$, where A_0 is the absorbance of the control and A_t is the absorbance of the test compound. IC₅₀ value (concentration of sample which reduced 50% of DPPH) was determined from inhibition percentage curve according to the concentration. The IC₅₀ value of the extract was compared with that of quercetin, which was used as the standard.

- AAPH assay

The assay for hemolysis mediated by radicals followed the method previously reported (Miki et al., 1987; Sugiyama et al., 1993). Blood (5 ml/ rat) obtained from anesthetized four Wistar rats was collected into heparinized tubes and centrifuged at 1500 g for 10 min. After removing the supernatant, the pellet was washed three times with 5 volumes of PBS. During the last washing, the erythrocytes were centrifuged at 3000 g for 10 min. A suspension of erythrocytes was prepared by adding 5 volumes of PBS at pH 7.4. Test samples (0.5 ml of extract) at different concentrations and 0.5 ml of 400 mM AAPH were added separately to 0.5 ml of erythrocyte suspension. The reaction mixtures were incubated at 37°C for 3 h with gentle shaking. After incubation, the reaction mixture was centrifuged at 3000 g for 5 min. The absorbance of the supernatant (A) at 540 nm was read. Similarly, another aliquot of the

reaction mixture was diluted with distilled water to yield complete hemolysis and the absorbance of the supernatant (B) after centrifugation was measured at 540 nm. Inhibition percentage of hemolysis exhibited by each sample was calculated by the equation $(1-A/B) \times 100\%$. The IC₅₀ value of the extract was compared with that of ascorbic acid, which was used as the standard.

Statistical analysis

Results were presented as mean \pm S.E.M. Data were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. A P-value <0.05 was considered statistically significant.

RESULTS

Ulcer induced by acetic acid was significantly inhibited by *A. buettneri* hydro-alcoholic extract ($P < 0.001$). Ulcer dimension of control group was 13.60 ± 0.51 versus 3.40 ± 0.4 , 8.6 ± 0.4 in the groups treated respectively with 500 and 250 mg/kg (fig. 1).

A. buettneri extract inhibited gastric juice secretion and reduced gastric acidity (Table 1). Four hours after 'pylorus ligation' gastric juice volume was 0.56 ± 0.04 ml for control group but for group treated with *A. buettneri* extract at dose of 500 mg/kg gastric juice volume is 0.24 ± 0.04 ml ($P < 0.01$). Gastric juice acidity for treated group is 11.66 ± 0.33 μ mol but for control group is 22.50 ± 2.17 μ mol. Acid output is significantly ($P < 0.05$) reduced by the extract of *A. buettneri* on treated group.

Basal acid output did not fluctuate significantly over the course of 120 min (fig 2) in control and *A. buettneri* 500 mg/kg treated group. *A. buettneri* extract did not influence basal gastric acid output but inhibited significantly acid hyper secretion induced by injection of histamine. Inhibition of acid hyper secretion induced by histamine persists over the experiment times.

A. buettneri extract also reduces DPPH to a yellow coloured product in a concentration dependent manner. The IC₅₀ value of the extract was compared with quercetin, which was

used as standard. It is 10 mg/ml for the extract and 20µg/ml for quercetin. *A. buettneri* extract could also dose-dependently scavenge superoxide radicals, inhibit lipid peroxidation and prevent AAPH-induced blood red cells membranes hemolysis. The extract presented an IC50 value of 1 mg/ml whereas ascorbic acid was 50µg/ml (Table 2). *A. buettneri* hydroalcoholic extract have potent antioxidant activity *in vitro* and *ex-vivo*.

DISCUSSION

Gastric acid hyper secretion is an important factor in gastric ulcer occurrence. The effect of the extract is then evaluated on gastric acid secretion. The present study demonstrates that *A. buettneri* hydro-alcoholic extract inhibited gastric ulcer induced by acetic acid and have suppressive effect on gastric acid secretion. The same extract inhibit gastric lesion induced by ethanol in our previous studies. For Okabe et al. (1972) and Jainu et al. (2006), acetic acid induced gastric ulcer by stimulating gastric acid hyper secretion. Gastric ulcer produced by acetic acid is due to the release of histamine, which increases the capillary permeability and back diffusion of HCl (Umamaheswari et al. 2007). Gastric lesion induced by acetic acid inhibition effect of hydro-alcoholic extract of *A. buettneri* is due to suppressive effect of acid release. This result is confirmed by the test of pylorus ligation ("shay rat" method). Pylorus ligation induced ulcers occur because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion (Goel and Bhattacharya, 1991). The extract of *A. buettneri* inhibits gastric secretion volume and gastric acidity in shay rat's method. Furthermore administration of the extract inhibits hyperchlorhydria induced by injection of histamine but not influences gastric basal acidity. Histamine plays a pivotal role on gastric acid hyper secretion. For Brzozowski et al. (1999), secretion of gastric acid is stimulated by Hormonal (gastrin, histamine) and neural pathway (binding of acetylcholine release to its receptor muscarinic also located on the cell parietal). According to Barocelli and

Ballabeni (2003), Schubert and Peura (2008), the binding of histamine on their receptors H_2 located on parietal cells induced acid release by these cells. The ethanolic extract of *Dombeya buettneri* (Sterculiaceae) leaves inhibits acid secretion by opposition to the action of histamine and stimulation mucus secretion (Okwari et al. 2000). Suvitayavat et al. (2004) show that a preparation containing 80% of a gel of *A. vera* inhibits gastric acid secretion and stimulates gastric mucus production. Yusuf et al. (2004) demonstrated that ethanol extract of *A. vera* inhibits ulcer induced by acid solution, inhibits acid hypersecretion induced by histamine (i.p.) injection. We think that *A. buettneri* hydro-alcoholic extract reduces gastric lesion and inhibits gastric acid secretion by the same mechanism. Anti-ulcer effect of the extract of *A. buettneri* is due simultaneous by inhibition of gastric acid secretion and stimulation by mucus production.

In general, the balance of aggressive and defensive factors plays a pivotal role in gastric hemorrhage and ulcer formation. The aggressive factors may include gastric acid back-diffusion and oxyradical generation (Hung et al. 2002) while defensive factors are mucus production, mucosal blood flow, prostaglandin synthesis and gastric epithelial cells integrity. In the disease state, oxidative stress of the stomach may occur, resulting in an elevation of mucosal lipid peroxides that are generated from the reaction of oxyradicals and cellular polyunsaturated fatty acids. Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. We earlier demonstrated that *A. buettneri* hydroalcoholic extract showed gastroprotective properties and inhibits gastric ulcer induced by ethanol. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa (Pihan et al., 1987) and scavenging these free radicals can play an appreciable role in healing these ulcers. In this paper we have showed antioxydante activity '*in vitro*' of hydroalcoholic extract of *A.*

buettneri. This extract reduced DPPH to a yellow and inhibits membrane lipoperoxidation induced by AAPH. The role of free radicals is reported in the induction of ulcers. Oxygen derived free radicals cause lipid peroxidation, which leads to membrane fluidity and increases the influx of Ca²⁺ ions, resulting in reduced membrane integrity of surface epithelial cells, thereby causing gastric ulcers (Devi et al. 2007). Antioxydante activities of the extract had beneficial effect on the use of *A. buettneri* in the gastric ulcer treatment.

CONCLUSION

The results of our study prove that *A. buettneri* hydroalcohol extract possess antiulcer activity against experimentally induced gastric ulcer models. Hence, it can be suggested that the antiulcer activity of the extract may be attributed to its antisecretory and antioxidant activities and justify the traditional/ethnic usage of this herb to treat peptic ulcers and consequent stomach ache.

Références bibliographiques

- Barocelli E., Ballabeni, V., 2003. Histamine in the control of gastric acid secretion: a topic review. *Pharmacological Research* 47, 299–304
- Brzozowski T., Kontureka P.C., Kontureka, S.J., Pajdo R., Drozdowicz D., Kwiecien S., Hahn E.G, 1999. Acceleration of ulcer healing by cholecystokinin (CCK): role of CCK-A receptors, somatostatin, nitric oxide and sensory nerves. *Regulatory Peptides* 82, 19–33
- Cheng C.L, Guo J.S., Luk J., Koo M.W.L., 2004. The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. *Life Sciences* 74, 2237–2249
- Devi R. S., Narayan S., Vani G., Srinivasulu C., Devi S., 2007. Gastroprotective effect of *Terminalia arjuna* bark on diclofenac sodium induced gastric ulcer. *Chemico-Biological Interactions* 167, 71–83
- Dhuley J.N., 1999. Protective effect of Rhinax, a herbal formulation against physical and chemical factors induced gastric and duodenal ulcers in rats. *Indian Journal of Pharmacology* 31, 128–132.
- Goel R.K., Sairam K., 2002. Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum*, *Tamrabhasma*. *Asparagus racemosus* and *Zingiber officinale*. *Indian Journal of Pharmacology* 34, 100–110.
- Hung CR., Wang P.S., 2002. Role of acid back-diffusion, glutathione, oxyradical, and histamine in antral hemorrhagic ulcer in rats: The protective effect of lysozyme chloride and antioxidants. *Journal of Laboratory and Clinical Medicine* 140, 142-151
- Jainu M., Srinivasulu C., Devi S., 2006. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcermodels: Possible mechanism for the inhibition of acid formation. *Journal of Ethnopharmacology* 104, 156–163
- Kavegue A., 2007. Activité antioxydante de quelques plantes de la flore togolaise. Thèse de pharmacie.

- Miki M., Tamai H., Mino M., Yamamoto Y., Niki E., 1987. Free radical chain oxidation of rat red blood cells by molecular oxygen and its inhibition by α -tocopherol. Archives of Biochemistry and Biophysics 258, 373- 380.
- Metowogo K., Agbonon A., Eklugadegbeku K., Aklikokou A.K. , Gbeassor M. 2008. Anti-ulcer and anti-inflammatory effects of hydro-alcohol extract of *Aloe buettneri* A. Berger (lilliaceae). Tropical Journal of Pharmaceutical Research 7, 907-910.
- Metowogo K., Eklugadegbeku K., Agbonon A., Aklikokou A.K., Gbeassor M., 2011. Gastroprotective Effect of Hydroalcoholic Extract of *Aloe buettneri*. Iranian Journal of Pharmaceutical Research, 10, 69-74
- Okabe S., Pfeifler C.J., 1972. Chronicity of Acetic Acid Ulcer in the Rat Stomach. Digestions Diseases 17, 619
- Okwari O.O., Ettarh R.R., Akpogomeh B.A., Eteng M.U., 2000. Gastric anti-secretory and anti-ulcerogenic effects of *Dombeya buettneri* in rats. Journal of Ethnopharmacology 71, 315–319
- Pihan G., Regillo C., Szabo S., 1987. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosa injury. Digestive Diseases and Sciences 32, 1395–1401.
- Schubert M.L., Peura D.A., 2008. Control of Gastric Acid Secretion in Health and Disease. Gastroenterology 134, 1842–1860
- Sugiyama H., Fung K.P., Wu T.W., 1993. Purpurogallin as an antioxidant protector of human erythrocytes against lysis by peroxy radicals. Life Sciences 53, PL39- 43.
- Suvitayavat W., Sumrongkit C., Thirawarapan S.S., Bunyaphatsara N., 2004. Effects of Aloe preparation on the histamine-induced gastric secretion in rats Journal of Ethnopharmacology 90, 239-247
- Tan P.V., Enow-Orock G.E., Dimo T., Nyasse B., Kimbu S.F., 2006. Evaluation of the anti-ulcer and toxicity profile of *aloe buettneri* in laboratory animals. African Journal of Traditional, Complementary and Alternative Medicine 3, 8 - 20.

Telefo P.B., Moundipa P.F., Tchouanguép F.M., 2002. Oestrogenicity and effect on hepatic metabolism of the aqueous extract of the leaf mixture of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis*. *Fitoterapia* 73, 472-478.

Telefo P.B., Moundipa, P.F., Tchouanguép, F.M., 2004. Inductive effect of the leaf mixture extracts of *Aloe buettneri*, *Justicia insularis*, *Dicliptera verticillata* and *Hibiscus macranthus* on in vitro production of estradiol. *Journal of Ethnopharmacology* 91, 225-230.

Umamaheswari M., Asokkumar, K., Rathidevi, R., Sivashanmugam, A.T., Subhadradevi, V., Ravi T.K., 2007. Antiulcer and *in vitro* antioxidant activities of *Jasminum grandiflorum* L. *Journal of Ethnopharmacology* 110, 464–470

Waldum H. L., Brenna, E., Kleveland, P. M., Sandvik, A. K., Syversen, U., 1993. Review article: the use of gastric acid-inhibitory drugs-physiological and pathophysiological considerations. *Alimentary Pharmacology and Therapeutics* 7, 589-596.

Yusuf S., Agunu, A., Diana, M., 2004. The effect of *Aloe vera* A. Berger (Liliaceae) on gastric acid secretion and acute gastric mucosal injury in rats. *Journal of Ethnopharmacology* 93, 33-37.

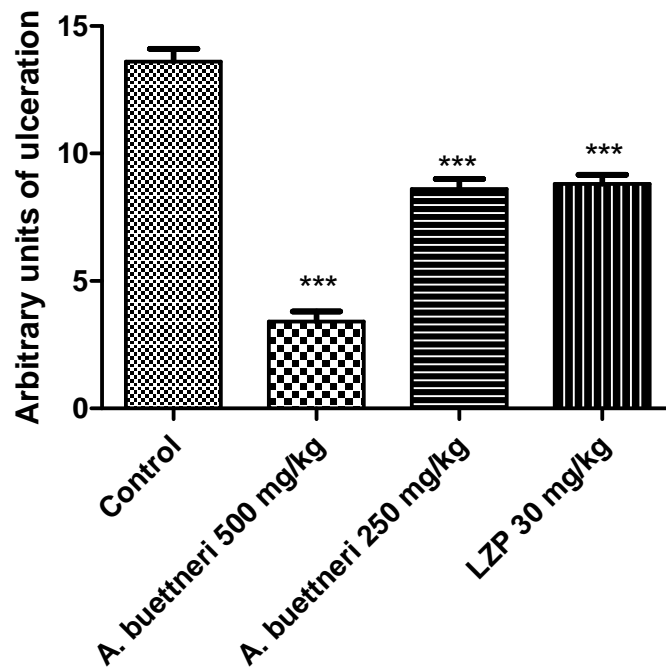


Figure 1: Effect of *A. buettneri* hydro-alcoholic extract on the gastric mucosal damage induced by 100% acetic acid.

Extract was administrated 30 minutes before ulcer induction by acetic acid. Results are mean \pm SEM for 5 rats. Results are significant if *** $P < 0.001$ (Control vs treated)

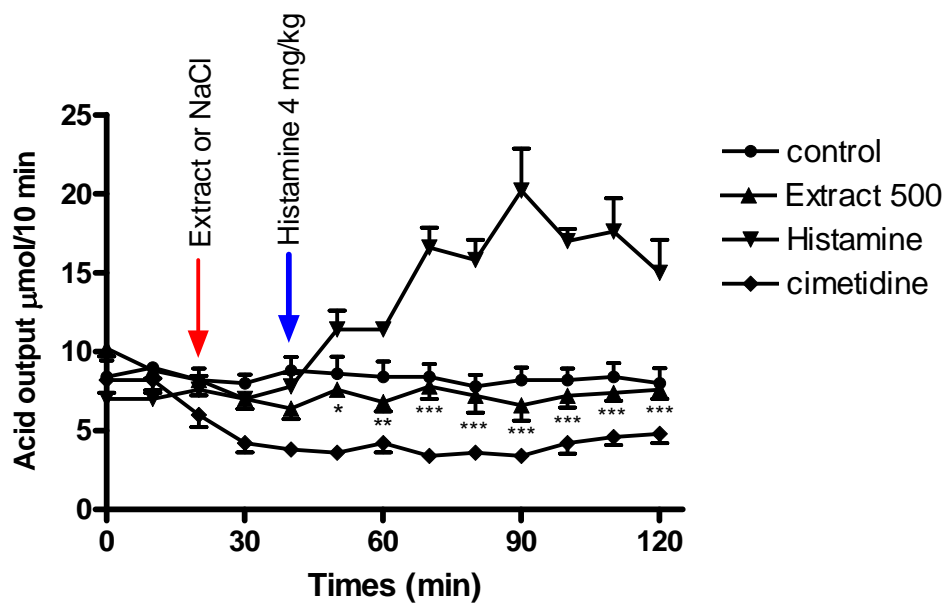


Figure 2: Inhibitory effect of *A. buettneri* hydro-alcoholic extract on histamine-stimulated gastric acid and secretion in rats.

Rats were prepared as described previously. After a 30 min equilibration period, hydro-alcoholic extract of *A. buettneri* or NaCl 9‰ solution were i.v. 20 min before histamine

Each point represents the mean \pm S.E.M. of five rats per group. Results are significant if * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ histamine treated group versus extract treated group.

Treatment	Gastric contents (ml)	Gastric acidity (μmol)
Control	0.56 ± 0.04	22.50 ± 2.17
Extract 500 mg/kg	$0.24 \pm 0.04^{**}$	$11.66 \pm 0.33^*$
Extract 250 mg/kg	0.35 ± 0.03	16.33 ± 2.40
Lanzoprazole 30 mg/kg	$0.31 \pm 0.08^*$	15.33 ± 0.88

Table 1: Effect of hydroalcoholic extract of *A. buettneri* administrated orally on gastric juice parameters in pylorus ligated rats

Results are mean \pm S.E.M. of five rats. Results are significant if * $P < 0.05$, ** $P < 0.01$ Treated versus control.

	DPPH	AAPH
IC₅₀ value of extract (mg/mL)	10	1
Quercetin (µg/mL)	20	-
Ascorbic acid (µg/mL)	-	50

Table 2: DPPH reducing power and anti-lipoperoxidation effect of *A. buettneri* hydro-alcoholic extract.

DPPH reducing assay *in vitro* was performed. AAPH induced red blood cells membrane lipoperoxidation *in vitro*. Extract IC₅₀ value was determinate using respectively quercetin and ascorbic acid as standard for DPPH and AAPH tests.