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Anti-Ulcer Property of Madecassoside a Triterpene Isolated from *Centella asiatica (Apiaceae)*

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ABSTRACT

The antiulcer activity of madecassoside, isolated from the leaves of *Centella asiatica*, was studied *in vivo* using Wistar rats, and *in vitro*, using *Helicobacter pylori*. This study investigated its effect on gastric ulcer induced by indomethacin orally administered at the dose of 30 mg/kg, acid secretion by stress, mucus secretion, and on *H. pylori*. The results showed that ulceration provoked by the indomethacin on the gastric mucosa surface was reduced from 2.76 ± 0.1 mm^2 in the control group to 1.95 ± 0.6, 1.48 ± 0.06 and 1.03 ± 0.05 mm^2 in the rats treated with madecassoside at doses 20, 40 and 80 mg/kg respectively ($p<0.05$). Madecassoside reduces ethanol-induced hyperhemia from 31.2 ± 3.22 mm^2 in the control group to 17.3 ± 1.32, 13.8 ± 2.15 and 7.41 ± 0.06 mm^2 for the animals treated with madecassoside at 20, 40 and 80 mg/kg respectively ($p<0.05$). Stress induces gastric lesion, and madecassoside reduces the lesion surface from 12.2 ± 0.5 mm^2 in the control group to 8.03 ± 0.9, 6.06 ± 0.7 and 4.38 ± 0.7 mm^2 in the animals treated with madecassoside at the doses of 20, 40 and 80 mg/kg respectively ($p<0.05$). The mucus secretion increases from 32.3 ± 2.26 mg in the control group to 46.94± 3.40, 52.40 ± 0.2 and 69.17 ± 1.50 mg in the groups treated with madecassoside at 40, 60 and 80 mg/kg respectively ($p<0.05$). Madecassoside inhibits the growth of *H. pylori* with Minimal Inhibitory Concentration (MIC) of 250µg/ml. These results show the antiulcer activity of madecassoside as it protects the gastric mucosa against damages induced by indomethacin and ethanol, decreases gastric acidity and increases mucus production. It also inhibits *H. pylori* growth.

INTRODUCTION

Gastroduodenal ulcer is a sore on gastric or duodenum lining which can be superficial or deep. It is one of major problems affecting the digestive tract (Jyoti G. *et al*., 2012). Before the discovery of *Helicobacter pylori*, a Gram negative bacteria, gastric ulcer was considered as the consequence of imbalance between the aggressive factors (hydrochloric acid, pepsin, bile salts, ethanol, non-steroid anti-inflammatory drugs) and the protective factors (prostaglandins, good mucosal blood flow, mucus-bicarbonate layer and cellular regeneration) of the gastric lining (Kang J.M. *et al*., 2012; Sunil K. *et al*., 2012). Acetylcholine, via M3 receptors, histamine through H2 receptors, ethanol and stress via pneumogastric nerve induce hydrochloric acid secretion. Even though hydrochloric acid is useful in activating pepsinogen, when it is in excess, it becomes harmful to the gastric lining. It is the same for pepsin, a protein breakdown enzyme, produced after pepsinogen activation. It can digest the gastric lining when it is in excess. Non-steroid anti-inflammatory drugs inhibit COX 1 and COX 2, enzymes responsible for the biosynthesis of prostaglandins (DivakarM.C. *et al*., 2011; Malfertheiner P. et al., 2009).

Prostaglandins are important for mucosal integrity, the good blood flow that they provoke increases mucus and bicarbonate production which coat and form the first line of defense of the stomach and duodenum. Prostaglandins also ensure cellular regeneration (Cata G., 1995; Salducci J., 2005).

Madecasssoside is a triterpene isolated from *Centella asiatica*. It is used for wound healing and added in cosmetics as anti-aging and moisturizer. It is also effective on Gram negative bacteria (Jagtap N.S. et al., 2009), and in Madagascar, the decoction of *C. asiatica* is used in the treatment of stomach ache (Quansah N. & Randrianavony P., 2012). Considering these activities of madecassoside and the traditional use of *C. asiatica*in Madagascar, we investigated the activity of madecassoside on induced gastric ulcer and on *H. pylori*.

MATERIALS AND METHODS

Experimental animals

Male rats of Wistar strain, aged 4 months and weighing around 250 g were used. They were bred in the animal house of the Pharmacology and Cosmetology Department of The Science Faculty, University of Antananarivo, Madagascar. The room temperature was around 22°C,

with the dark and light cycle fixed at 12 / 12 hours. The animals were fed with animal feed LFL 1420®, and had water *ad libitum*. They were fastened during 18 hours before tests but had free access to water (ShirishaB. *et al*., 2012).

All the experiments were accepted by the Committee of Animal Ethics at the Sciences Faculty of the University of Antananarivo, Madagascar.

1. Evaluation of madecassoside activity on indomethacin induced ulcer

Fastened rats were divided in 4 groups of 6 animals each: 1 control group, and 3 groups treated with madecassoside dissolved in water, at the doses of 20, 40 and 80 mg/kg, by oral route. Indomethacin was administered *per os*, once a day during 5 days, in the mornings, at a fixed hour at the dose of 30 mg/kg (Ode *et al*., 2011). After 30 minutes of indomethacin administration, the animals of control group received 10 ml/kg of distilled water, while the animals of the other groups received 20, 40 and 80 mg/kg of madecassoside in 10 ml/kg of distilled water (Dielh-Heinz K., 2010). On the sixth day, animals were anesthetized by intramuscular injection of barbiturate in a dose of 100 mg/kg and exsanguinated by carotid section. Laparotomy was practiced, and their stomachs were isolated, then cut open along the greater curvature and rinsed with physiological solution to remove their contents. The macroscopic lesion surface was measured by direct planimetry method using transparent millimeter paper (Ntsayo, 2011).

2. Evaluation of madecassoside activity on hyperhemia provoked by ethanol

This experiment was carried out on 4 groups of 6 rats per group, fastened during 18 hours prior the test. The first group was used as control and received 10 ml/kg of distilled water, and the remaining groups received madecassoside at the dose of 20, 40 and 80 mg/kg, by oral route (Dielh-Heinz K., 2010).

One hour after the administration of the products, 1ml/200g of absolute ethanol was administered by oral route (Hollander D. *et al*., 1985). One hour later, the animals were anesthetized by intramuscular injection of barbiturate at the dose of 100 mg/kg and exsanguinated by cutting the carotids. The stomachs were isolated then cut open along the greater curvature and rinsed with physiological solution to remove their contents. The mucus surface hyperhemia was measured by direct planimetry method using transparent millimeter paper (Manjusha K. *et al*., 2013).

3. Evaluation of madecassoside activity on induced acid secretion

The fastened animals were divided into 4 groups of 5 rats per group. The first group served as the control group, received distilled water, and the 3 groups received madecassoside at the dose of 20, 40 and 80 mg/kg, by oral route. Immediately after administration of these products, the animals were fixed in decubitus position by extending their 4 limbs during 18 hours (Gairard *et al*., 1967). After this period, the animals were euthanized by intramuscular injection of 100 mg/kg of barbiturate. Stomachs were isolated and opened by cutting the great curvature, then rinsed with physiological solution to remove their contents. The lesion surface on the gastric wall was measured by direct planimetry using transparent millimeter paper (Manjusha K. *et al*., 2013).

4. Evaluation of madecassosisde activity on mucus production

The animals were fastened during 12 hours before the test. They were divided into 4 groups of 6 rats per group. The first group served as control, the 3 groups received madecassoside at different doses. Every morning at the same time, during 10 days, the animals of the control group received 10 ml/kg of distilled water, and the treated groups received 20, 40 and 80 mg/kg of madecassoside, by oral route, in 10 ml/kg of distilled water. After 10 days of treatment, the animals were anesthetized with 100 mg/kg of barbiturate administered by i.m. and exsanguinated by cutting the carotids. Their stomachs were isolated and rinsed, and the mucus was grated and weighed (Randrianavony P. et al., 2015).

5. Evaluation of madecassoside activity on *Helicobacter pylori*

Helicobacter pylori used in this work was collected from biopsy samples of patients suffering stomach ache undergoing endoscopy at Gastro Enterology Department of Joseph Raseta Befelatanana Hospital, Antananarivo. Those patients didn't take any antibiotics during 10 days prior examination. Two samples were taken, one at the pyloric antrum and one on the fundus. Each sample was put in sterile polypropylene screw capped bottle (GOSSELIN TP 53-001®), containing 0.5 ml of NaCl (9‰). The bottle was put in a bag with microaerophile atmosphere generator (GENbag microaer 45 5325®) and transported to the lab within 4 hours, at ambient temperature. Biopsic samples were ground in Potter, and 2 precultures on solid medium made of selective gelose PYL® (BioMérieux), and 1 preculture on liquid medium (Mueller Hinton Broth, MHB®) were undertaken. The first one was to confirm the presence of *Helicobacter pylori* and to eliminate the cellular debris. It was carried out in Petri

dish in microaerophile atmosphere (5 % of O_2, 10 % of $C0_2$ and 85 % de N_2) at 37 °C during 4 days (Bury-Mone S. *et al*., 2006). Colony from the medium was observed under optic microscope at x1000 magnification. Once determined, the colonies were cultured in the second solid medium made of gelose PYL (BioMérieux®) to multiply the colonies, and incubated in the same conditions as the previous one, during 48 hours. After this period, 6 colonies were withdrew and put in Erlen Meyer containing MHBin amicroaerophile atmosphere, at 37°C during 4 days. Two milliliters of the medium were diluted in 18 ml of MHB, and the optic density (OD) of this medium was read with spectrophotometer for microdilution plate (Opsys MR, DYNEX Technologies ®) at 525 nm. This OD was used as reference of the culture broth turbidity.

Microdilution method was used to assess madecassoside activity against *H. pylori,* using *Mueller–Hinton broth (MHB)* as growth medium and 96-well microdilution plate.

Madecassoside and amoxicillin, used as reference, were dissolved in distilled water and filtrated with millipore 0.45µm. They were diluted with culture broth to a range of concentrations from 1000 to 4 µg/ml.

The wells of the first column were left empty, those of 4^{th} and 8^{th} rows were filled with madecassoside and amoxicillin diluted in culture broth, and used as blank control, while those of the 12^{th}column were filled with 100 µl of inoculum, used as growth control. The remaining wells were used as test wells and filled with 100 µl of *H. pylori* suspension, 5 µl of each dilution of madecassoside and amoxicillin were distributed in the test wells. All experiments were performed in triplicate and the microdilution plate was incubated at 37ºC for 18 h. *H. pylori* growth was detected by optical density (Opsys MR, DYNEX Technologies®) at 525 nm. The plate was reincubated in the same conditions, for 12 hours, and the optic density was read. (Rios J.L. et al., 1988).

7. Expression and Analysis of results

Results were expressed as mean value ± standard error of the mean (SEM) of optic density. The means were compared using the Student‘’t’’ test. A value of $p<0.05$ was considered significant.

RESULTS

1. Madecassoside activity on indomethacin induced ulcer

Administered at the dose of 30 mg/kg, once a day, for 5 days, indomethacin induces lesion on the gastric layer. The lesion surface in the control group is higher than that of the test group. It is equal to 2.76 ± 0.1 mm^2 in the control group, versus 1.95 ± 0.6, 1.48 ± 0.06 and 1.03 ± 0.05 mm^2 in the rats treated with madecassoside at doses 20, 40 and 80 mg/kg ($p<0.05$) (Figure 1).

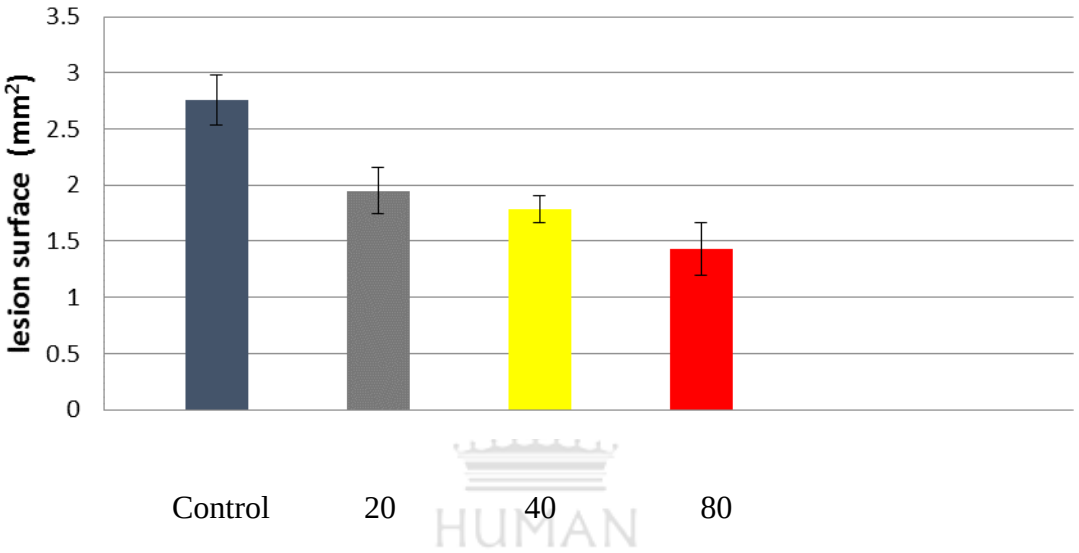

Figure 1.Surface of indomethacin induced lesions on the gastric layer, in control group, and animals treated with madecassoside at 20, 40 and 80 mg/kg by oral route ($\bar{m}$± e.s.m.; n=6; $p<0.05$).

2. Madecassoside activity on hyperhemia provoked by ethanol

Ethanol administration induces hyperhemia on gastric layer. Madecassoside reduces the hyperhemia surface from 31.2 ± 3.22 mm^2 in the control group to 17.3 ± 1.32, 13.8 ± 2.15 and 7.41 ± 0.06 mm^2, in the groups treated with madecassoside at the doses of 20, 40 and 80 mg/kg ($p<0.05$) (Figure 2).

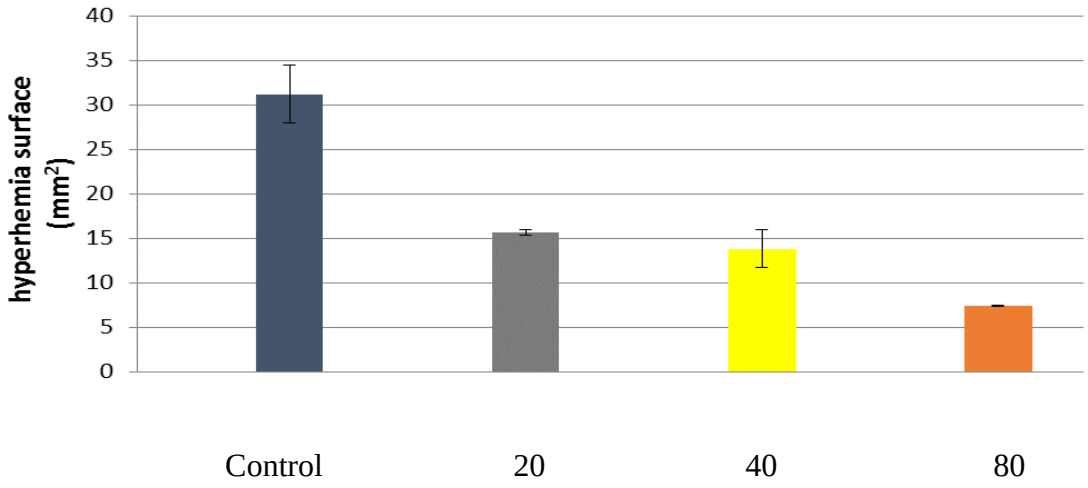

Figure 2. Variation of hyperhemia surface, on the gastric layer, 1 hour after ethanol administration *per os*, in the control group, and groups treated with madecassoside administered *per os* at doses 20, 40 and 80 mg/kg (m̄± e.s.m.; n=6; p<0.05).

3. Madecassoside activity on induced acid secretion

Stress induces increase of gastric acid secretion, which induces ulceration on the gastric layer. The ulceration surface is equal to 12.2 ± 0.5 mm^2 in the control group, versus 8.03 ± 0.9, 6.06 ± 0.7 and 4.38 ± 0.7 mm^2, in the animals treated with madecassoside at the doses of 20, 40 and 80 mg/kg ($p<0.05$) (Figure 3).

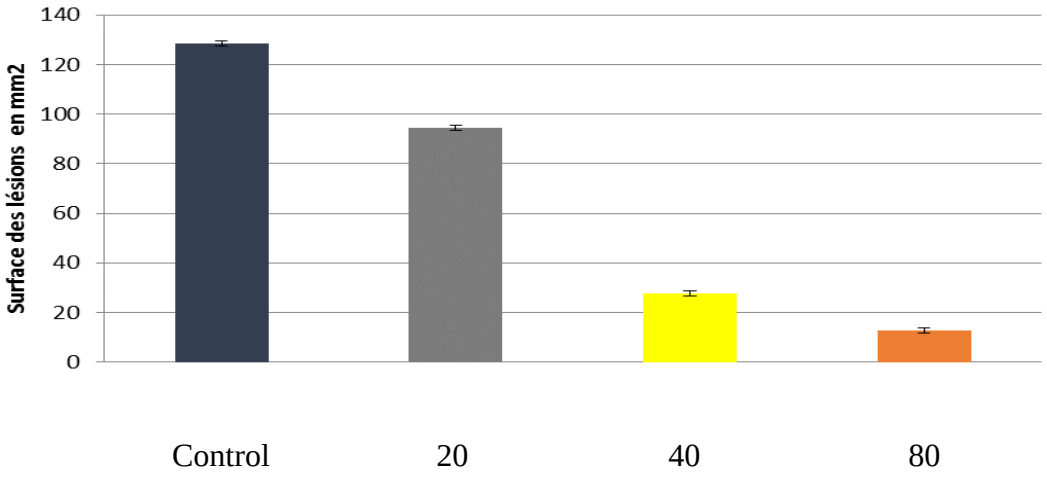

Figure 3.Stress induced lesion surface, in the control groupandin the animals treated with madecassoside at the doses of 20 40 and 80 mg/kg administered by oral route (m̄± e.s.m.; n=6; p<0.05).

4. Madecassosisde activity on mucus production

Administration of madecassoside *per os*, once a day, during 5 days increases the mucus production noted by the weight increase of mucus on the gastric wall. It is 32.3 ± 2.26 mg in the control group, versus 46.94 ± 3.40, 52.40 ± 0.2 and 69.17 ± 1.50 mg in the animals treated with madecassoside at 20, 40 and 80 mg/kg ($p<0.05$)(Figure 4).

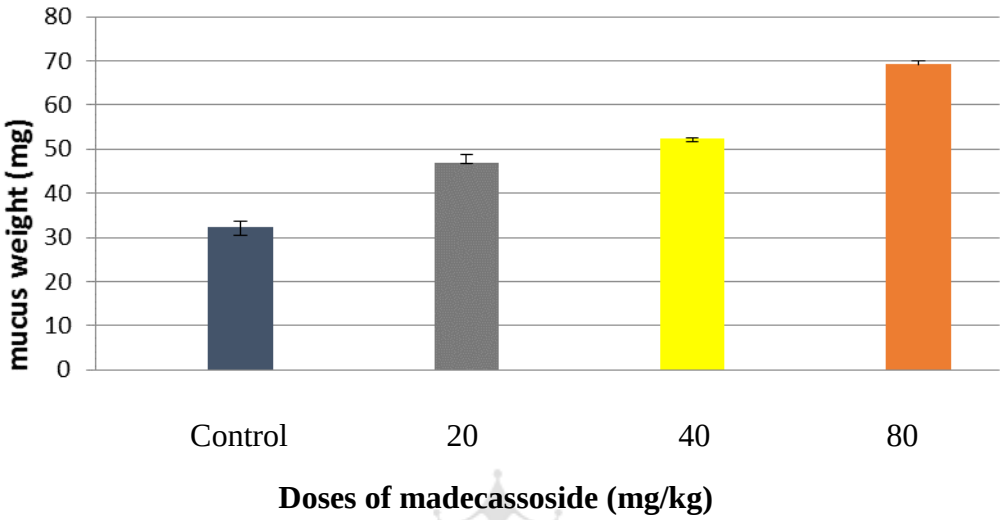

Figure 4.Gastric mucus weight after 10 days treatment with madecassoside at 20 , 40 et 80 mg/kg *pre os*, and in the control group (m± e.s.m.; n=6; p<0.05).

5. Madecassoside activity on *Helicobacter pylori*

The optical density of the 12th column, used as growth control is equal to 0.073±0.002; those of the 4th and 8th rows,used as blank control are 0.086 ± 0.0016 and 0.078±0.002 respectively, while the empty column is equal to 0.031±0.0023.

After 24 hours of incubation, the OD of the 4th and 8th rows remain unchanged. While the 12th column increases to 0.195 ± 0.004. In the presence of madecassoside the inoculum ODin the test wells is inferior to the ones of the growth control group. It is equal to 0.158 ± 0.004, 0.117± 0.005 and 0.109 ± 0.003 at concentrations of 2, 500 and 1000 µg/ml ($p<0.05$)(Figure 5). Graphic determination of MIC of madecassoside gives a value of 250 µg/ml, versus 180 µg/ml for amoxicillin.

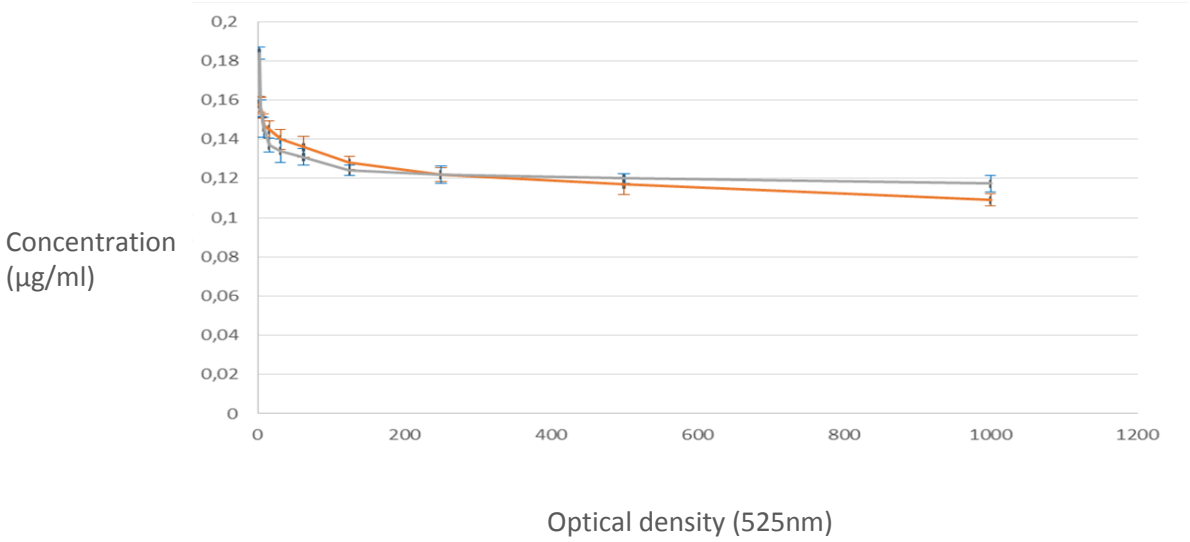

Figure 5. Variation of OD., of inoculum containing *H. pylori* in the presence of madecassoside ▬ and amoxicillin▬, at increased concentrations after 24 hours of incubation at 37°C, in microphile condition (m̄± e.s.m.; n=3; p<0.05).

After a second incubation, in the same conditions as the previous one, the OD of the growth control increases to0.252 ± 0.004, while the OD of the test wells remains the same at 0.157 ± 0.002, 0.113 ± 0.002 and 0.112 ± 0.002 with the concentration of 2, 500 and 1000 µg/ml respectively (p<0.05) (Table I).

Table I. Comparision of theOD of the inoculum after the first and second incubations in the presence of madecasoside (m̄± e.s.m.; n=3; p<0.05).

OD madecassoside (µg/ml)	First incubation	Second incubation	p
1000	0.109 ± 0.003	0.112 ± 0.003	NS
500	0.117 ± 0.005	0.113 ± 0.002	NS
2	0.158 ± 0.004	0.157 ± 0.002	NS
0	0.195 ± 0.004	0.252 ± 0.004	p<0.05

DISCUSSION

Gastric ulcer is characterized by substance loss in the gastric layer (Sunil *et al*., 2012) and is protected by the mucus and the bicarbonate, the secretion of which depends on prostaglandins (PGE2) (Moore *et al*., 2006).

Our results show that madecassoside protects the gastric layer against aggressive agents, such as indomethacin, ethanol, hydrochloride acid and *H. pylori*; it also increases the gastric wall protection by increasing the secretion of mucus.

Indomethacin, as a non-steroid anti-inflammatory agent, inhibits cyclooxygenase (COX 1 and COX 2) responsible for prostaglandins biosynthesis. This inhibition decreases bicarbonate and mucus secretion, two protective agents of gastric wall (Mac-Naughton *et al.*,1988), leading to its ulceration (Brodie & Knapp, 1996; Gerhard *et al*., 2002; Khare *et al*., 2008). The fact that madecassoside decreases the lesion surface induced by indomethacin means that it protects the gastric wall against indomethacin action. Administered by oral route, ethanol stimulates the G cells at the pyloric antrum, which secretes gastrin, one of the agents responsible for acid secretion by the parietal cells (Levitt *et al.*, 1997). Ethanol also stimulates the parasympathetic system and provokes vasodilation, observed as hyperhemia on gastric wall. It also reduces the blood flow, which decreases the intake of nutritious elements and oxygen necessary for the formation of barrier on the gastric wall, and the regeneration of epithelial cells (Robert *et al*., 1979). The reduction of the hyperhemia surface might be due to the amelioration of the local circulation, as a result of prostaglandin action (Ly, 1980). Blood flow amelioration increases mucus and bicarbonate secretion, two main protectors of the gastric layer (Goel & Sairam, 2002). Our results show that madecassoside increases the mucus weight. Stress stimulates the parasympathetic system, which releases acetylcholine which in turn stimulates the secretion of hydrochloride acid, one of the aggressive agents (Minaire & Lambert, 1976). Meanwhile, madecassoside reduces the stress induced lesion surface. This reduction might be due to the increase in mucus secretion, or the neutralization of acid secreted, due to the increase of bicarbonate secretion (Divakar &Lakshmi, 2011). These mechanisms of protection might occur via prostaglandins.

Microdilution method, using liquid medium (Mueller–Hinton broth), is suitable for determining MIC of pure substances, like the case of madecassoside. Optic density was used to determine the action of madecassoside on *H. pylori*, a Gram negative bacteria (Rios J.L. et al., 1988).Using this method, the OD of the growth control wells is higher than those of the test wells containing madecassoside, which supposes that this substance inhibits the growth of the bacteria. Also, after the second incubation, there is no significant difference between the OD of the test wells at the first and second incubation. These results mean that madecassoside possesses a bacteriostatic action (Rios J.L. et al., 1988). From our results, the

effect of madecassoside is inferior to the action of amoxicillin, a known bacteriostatic, however, this suggests that it would inhibit the synthesis of bacterial membrane (Wangbaoshou, 2009). Previous work have shown that madecassoside is active on Gram negative bacteria (Jagtap *et al.*, 2009), which supports our results on *H. pylori*, a gram negative bacteria.

Gastric ulcer is a multifactorial pathology, due to the lack of the stomach protective layer. When *H. pylori* are present, it aggravates the ulcer and provokes its recidivism. In that case, its eradication is necessary (Kamguia *et al*., 2011). Meanwhile, some antibiotics are sensitive to gastric acidity (Grima, 2005). That is why gastric ulcer treatment is tritherapy, using anti-secretory medication with one bacteriostatic and another bactericide (Lamarque *et al.*, 2017). Our results demonstrate that madecassoside reduces the gastric acidity, which gives an antibiotic optimum condition. It also stabilizes the growth of *H. pylori*, diminishing the aggravator effect of this bacteria. Considering these properties of madecassoside, it could be associated with a bactericide antibiotic in the treatment of gastric ulcer. This would change the treatment regime from tritherapy to bitherapy.

CONCLUSION

Based on the results obtained in our study, madecassoside ameliorates the gastric layer protection via prostaglandin, which increases secretion of mucus and bicarbonate. It also has a bacteriostatic activity against *H. pylori*. In conclusion, these properties make madecassoside effective against gastric ulcer.

REFERENCES

1. Brodie A, Knapp PG. The mechanism of the inhibition of gastric secretion produced by esophageal ligation in the pylorus-ligated rat. Gastroenterol, 1996; 50: 787-95.
2. Bury-Mone S, Kaakoush NO, Asencio C, Megraud F, Thibonnier M, De Reuse H et al. Is *Helicobacter pylori* a true microaerophile? Helicobacter, 2006; 11 (4): 296-03.
3. Cata G. Les antisécrétoires. Act. Pharm, 1995; 336: 41-52.
4. Dielh- Heinz K. A good practice guide to the administration of substances and removal of blood, including rat and volume. J Applied Toxicol, 2010; 21: 15-23.
5. Divakar MC, lakshmi DS. Anti-ulcer activity of *Wrightia tinctoria* (Roxb.) R.Br. Der Pharmacia Sinica, 2011; 2 (2):355-60.
6. Gairard A, Marnay-Gulat C, Raoul Y. Essai d'analyse de la contrainte ulcérogène du rat par l'étude de l'élimination urinaire de divers ions. C R SocBiol, 1967; 2132-36.
7. Gerhard HV, Wolfgang HV, Bernward A, Jürgen S, Günter M, Wolfgang FV. Drug discovery and evaluation: pharmacological assays, 2nd Ed. Johann Wolfgang Goethe Universität -Frankfurt (Germany), 2002, pp 869-870.

8. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with Emphasis on *Musa sapientum*, *Tamrabhasma, Asparagus racemosus* and *Zingiber officinale*. Indian J Pharmacol, 2002; 34: 100-10.
9. Grima M. Les medicaments des pathologies digestives : les inhibiteurs de la pompe à protons. Faculté de Médecine de Strasbourg, Module de Pharmacologie Clinique DCEM3 2005/2006, 2005, chap. 12 : 1-7.
10. Hollander D, Tarnawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. Macroscopic, histologic, ultrastructural, and functional time sequence analysis. Gastroenterol, 1985; 88 (1): 366–74.
11. Jagtap NS, Khadabadi SS, Ghorpade DS, Banarase NB, Naphade SS. Antimicrobial and antifungal activity of *Centella Asiatica* (L.) Urban, Umbeliferae. Research J Pharm Tech, 2009; 2 (2): 328-30.
12. Jyoti G, Dinesh K, Ankit G. Evaluation of gastric antiulcer activity of methanolic extract of *Cayratiatrifolia* in experimental animals. Asian Pac J Trop Dis, 2012; 2: 99-102.
13. Kamguia G, Fokunang C, Ngameni B, Njiki O, Tembe E. Effet cytoprotecteur de l'extrait aqueux des racines de *Dorsteniapsilurus*sur l'ulcère gastrique chez les rats mâles de la souche wistar. Health Sci Dis, 2011; 12: 10-11.
14. Kang JM., Seo PJ, Kim N, Lee, Kwon J, Lee DH et al. Analyse des coûts des soins médicaux directs de l'ulcère gastroduodénal dans un centre médical tertiaire coréenne. Scand J Gastroenterol, 2012; 47: 36-42.
15. Khare S, Asad M, Dhamanigi SS, Prasad VS. Antiulcéreux de l'huile de foie de morue chez le rat. J Ind Pharmacol, 2008; 40:209-14.
16. Lamarque D, Burucoa C, Courillon-Mallet A, De Korwin JD, Delchier JC, Heluwaert E et al. Recommendations for treatment of *Helicobacter pylori* infection in adults. Hépato-gastro etoncologie digestive,2017; 24 (8): 157-70.
17. Levitt MD, Li R, Demaster EG, Elson M, Furne J, Levitt DG (). Use of measurements of ethanol absorption from stomach and intestine to assess human ethanol metabolism. Am J Physiol, 1997; 273 (4): 951–57.
18. Ly C. Topical effects of 16, 16-dimethyl prostaglandin E2 on gastric blood flow in dogs. Am J Physiol, 1980; 238: 514-19.
19. Mac-Naughton WK, Williamson TE, Morris GP.HCl is truly adaptative cytoprotection by 0.25 "cytoprotective" and may not depend upon elevated levels of prostaglandin synthesis. J Physiol Pharmacol, 1988; 66: 1075-81.
20. Malfertheiner P, Chan FKL, McColl KEL. Peptic ulcer disease. Lancet, 2009; 374 (9699):1449-61.
21. Manjusha K, Vipin K, Surender S. Gastroprotective activity of methanol leaves extract of *Barleriaprionitis* Linn. on ethanol and indomethacin induced ulcer in rats. Br J Pharm Res, 2013; 3 (4): 817-29.
22. Minaire Y, Lambert R. Physiologie humaine. La digestion, Ed. Simep (Villeurbane, France), 1976,pp 114-135.
23. Moore N, Molimard M., Fourrier-Reglat A, Latry K, Haramburu F, Miremont-Salame G et al. Pharmacologie générale, Pharmacologie de la sécrétion gastrique, Ed. Jacques DANGOUAU (Bordeaux), 2006, chap.14, pp 290-293.
24. Ntsayo F. Effets antiulcérogeniques de l'extrait aqueux des écorces *d'Anthocleistaschweinfurthii* (LOGAGNIACEAE) sur les ulcères gastriques aigus induits chez le rat. Mémoire présenté en vue de l'obtention du Diplôme de Professeur de l'Enseignement Secondaire de grade II, 2011,pp 53-54.
25. Ode OJ, Oladele GM, Asuzu VM. The antiulcer activities of the methanol extract of *Cassia singuena* leaves using indomethacin induced gastric ulcer model in rats. J AdvSci Res, 2011; 2 (3): 66-69.
26. Quansah N., Randrianavony P.*Nature's Gift to Humanity: Natural Remedies for Selected Common Health Problems*. Author House, Bloomington, IN, USA, 2012,56pp.
27. Randrianavony P, Raharivelonina EA, Randrianjafitrimo N, Quansah Nat, Randimbivololona F. Anti-ulcer activity of hydro alcoholic extract of *Acridocarpus excelsus* a. Juss leavesin rat. Am J Pharm Tech Res,2015; 5(6): 78-86.
28. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmac, 1988; 23: 127–49.
29. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterol, 1979; 77: 433-43.

30. Salducci J, Desjeux A, Chamlian A. Ulcère gastrique et duodenal. Faculté de Médecine de Marseille, Module de « Hépato-Gastro-Entérologie, 2005; 290: 1-13.
31. Shirisha B., Subash V. Animal models in experimental gastric ulcer screening. Int J Pharmacol, 2012; 2 (2): 82-87.
32. Sunil K, Amandeep K, Robin S, Ramica S. Peptic ulcer: a review on etiology and pathogenis. Int Res J Pharm, 2012; 3 (6): 34-38.
33. Wangbaoshou. L' Amoxicillinecomprimés; 2009 http://fr.aliub.com/lamoxicilline-comprimes/, site consulted 02/07/2018.