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Synergistic antiulcer activity of methanolic extract of a mixture of *Cinnamon cassia* Bark and *Annona squamosa* twigs in indomethacin-induced ulcer

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ABSTRACT

Background: *Cinnamon cassia* bark and *Annona squamosa* twigs have been individually described to possess antiulcer activity. In an effort to develop a pharmaceutical product with gastroprotective potential, the synergistic antiulcer effect of methanolic extract of a mixture of *C. cassia* bark and *A. squamosa* twigs at 50:50 ratios was assessed in experimental animals. **Aim:** Antiulcer activity of methanolic extracts of *C. cassia* (MECC) bark and *A. squamosa* twigs was studied in rats, in which gastric ulcers were induced by indomethacin (20 mg/kg, orally) followed by pylorus ligation method. **Method:** The methanolic extract of *A. squamosa* twigs (MEAS) (100 mg/kg); MECC bark (100 mg/kg); mixture of a (MEAS) twigs and MECC bark (50 mg/kg:50 mg/kg, respectively) was administered orally to the rats for 6 consecutive days followed by pylorus ligations of a stomach on the 7th day of experiment. After 4 h of ligation, the animals were subjected for evaluation of ulcer index and gastric acid content. **Result:** The reduction of ulcer index and gastric acid content in mixture of a MEAS and MECC-treated animals was seen to be statistically significant with respect to control group of rats. **Conclusion:** The mixture of a MEAS and MECC exhibited better ulcer protection effect as compared to individual effect of MEAS and MECC. Misoprostol was used as a standard drug for ulcer protection.

Key words: Annona squamosa, antiulcer, Cinnamon cassia, indomethacin, pylorus ligation

INTRODUCTION

Peptic ulcer is one of the well-known diseases which had affected around 9–10% of the world population.[1] Of these numbers, 5% suffers from gastric ulcers. Various aspects including the usage of steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, a rough lifestyle, drugs which stimulate secretion of gastric acid and pepsin, infections cause by *Helicobacter pylori*, as well as smoking contribute to the pathogenesis of gastric ulcers.[2] An imbalance among the aggressive elements such as secretion of acid and pepsin, *Helicobacter pylori*, deliverance of leukotrienes, reactive oxygen species and refluxed bile, and mucosal protective factors that include mucus-bicarbonate barrier, bicarbonate secretion, prostaglandins (PGs), mucosal blood circulation, surface-active phospholipids, cell renewal as well as relocation, enzymatic and non-enzymatic antioxidants, and certain growth factors leads to gastric damages.[3]

Existing gastroprotective medicines are generally based on the proton-pump inhibitors and antimuscarinics, inhibition of gastric acid secretion by histamine H2-antagonists, as well as on acid-independent therapy provided by sucralfate and bismuth cholinergic.[4] Though numbers of anti ulcer drugs are available in the market but most of them are frequently related with side effects. In this context, the usage of medicinal plants has gained attention of several investigators.[5] The natural products field is in incessant expansion globally and became an attractive source of novel drug for the treatment as well as prevention of numerous illnesses.[6] Plants have a number of antioxidant compounds such as carotenoids, phenols, Vitamin C, and flavonoids.[7] *Cinnamon cassia* bark has already reported for antioxidant, gastroprotective,[8] antibacterial, and hepatoprotective activities.[9] Chinese *Cinnamon* has antiulcer property.[10] *Annona squamosa* also broadly used traditionally as an antioxidant,[11] anticancer, antiulcer, and hepatoprotective.[11,12] Several studies have shown that *C. cassia and A. squamosa* have rich sources for numerous types of active ingredients such as flavonoids, phenols, and tannins, which have medicinal uses due to its antioxidant, anti-inflammatory, and curative properties.[13] Its various parts also are used as remedial agents in traditional therapies as well as in modern era also.[14] Hence, we have hypothesized the study to investigate the synergistic antiulcer potential of mixture of *C. cassia and A. squamosa* and its comparison with individual effect of *C. cassia* and *A. squamosa.*

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MATERIALS AND METHODS

Plant Material and Extract Preparation

The *A. squamosa* twigs and *C. cassia* barks were collected from the Anantapur regions, Andhra Pradesh, India. The both herbs were identified and authenticated by Dr. J. Raveendra Reddy, JNTUA, Anantapur, where the voucher specimen was deposited for further reference. The twigs and barks were dried in shade and powdered in a blender. The powder extracted with methanol using a Soxhlet extractor.

Animal Models

A preclinical pharmacological screening for antiulcer activity was performed in Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Anantapur, from February 20 to March 05, 2018. The protocol was approved by IAEC.

A total of 36 male Wistar albino rats, weighing 170–200 g were obtained from the Raghavendra Enterprises, Bengaluru, Karnataka, India. The rats were maintained in RIPER, Anantapur. In each cage, three rats were placed with standard diet and water. The animals were acclimatized to laboratory atmosphere for a minimum of week before the study.

Animal Grouping and Treatment Schedule for Antiulcer Study

For the experiment, animals were divided into six groups with six animals in each group. Animals were kept without food for 24 h before the study but allowed for free access to water. Group I treated as normal with distilled water; Group II negative control; III and IV treated as treatment groups 100 mg/kg each extract; V group received with combined methanolic extract of *A. squamosa* (MEAS) and methanolic extracts of *C. cassia* (MECC) at the dose of 50 mg/kg each, respectively; and Group VI as standard group received misoprostol 100 mcg/kg.

Gastric Lesions Induced by Indomethacin and Pylorus Ligation Model

Gastric lesions remained brought to all groups of animals by administration of indomethacin (20 mg/kg suspension in 1% carboxymethyl cellulose [CMC]) orally for up to ulcer induce (6 successive days) except Group I followed by pylorus ligation under pentobarbitone (45 mg/kg i.p.) anesthesia on the 7th day. The standard drug misoprostol (100 mcg/kg i.p.) was administered after 1 h of oral indomethacin administration for 6 successive days. In the same manner, 100 mg/kg of MEAS and 100 mg/kg of MECC were administered orally in Group III and IV. In Group V, mixture of methanolic extract at 50 mg/kg dose of each extract was administered orally after 1 h of indomethacin administration for 6 successive days.[15]

Ulcer Index Test

To determine the ulcer index after 4 h of pylorus ligation, the abdomen of the rats was opened by making an incision. Gastric contents were collected to determine total acid output and the abdomen was completely rinsed with water. Ulcer index was calculated using ulcer score scale.

The ulcers were scored as below:
Normal stomach without any red coloration=0;
Red coloration=0.5;
Ulcer spot=1;
Hemorrhagic streaks=1.5;
3–5 ulcer spots=2;
More than five ulcer spots=3.
Mean ulcer score for each group was calculated and recorded as ulcer index.[16]

Determination of Total Gastric Output

Gastric contents of the treated animals were collected and centrifuged at 1000 rpm for 10 min. Supernatant was pipetted out. 1 ml of this fluid was diluted with 9 ml of distilled water and titration was carried out against 0.01 N prestandardized sodium hydroxide using Topfer's reagent (dimethyl-amino-azo-benzene with phenolphthalein) being indicator. End point noted the titer value (pink to orange color) which was expressed as free acid. Titration carried until the pink color was reappeared, and the titer value was noted again which was expressed as total acid.[17]

Histopathology

At the end of the study, the abdomens of treated animals were well preserved in a 10% buffered formalin solution and treated for following embedment. The flesh samples remained cut into sections of 2.5 µm. Two slides remained stain with hematoxylin-eosin (HE) and Periodic acid–Schiff. The slides were observed under compound microscopy (×10) and photomicrograph was taken.[18]

Statistical Analysis

The results were expressed as mean ± SEM. Statistical differences were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. Results were measured to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The MEAS, MECC, and mixture of both extract showed a significant reduction in the ulcer index, free acidity, and total acidity as compared to negative control ($P < 0.01$). The results obtained from MEAS + MECC at the dose of 50 mg/kg each, were set up near to the standard drug, and the results demonstrated that the MEAS + MECC at the dose of 50 mg/kg was seen to be more effective as compared to methanolic extract of individual plant [Table 1]. In addition, histopathological examination showed minimal damage to the gastric mucosa of a group treated with MEAS + MECC at

Table 1: Effect of MEAS and MECC and its mixture on ulcer index, free acidity, and total acidity

Treatment	Ulcer index	Free acidity	Total acidity
Negative control (vehicle)	4.01±0.37	47.3±0.74	51.81±0.32
MEAS: 100 mg/kg, p.o	1.93±0.18**	25.16±0.31**	29.5±0.39**
MECC: 100 mg/kg, p.o	1.67±0.18**	21.83±0.30**	26.5±0.41**
MEAS and MECC: 50 mg/kg each, p.o	0.38±0.17**	15.16±0.31**	21.5±0.39**
Standard (misoprostol: 100 mcg/kg i.p)	0.19±0.12**	10.15±0.45**	18.5±0.41**

Values are expressed as mean±SEM, n=6, **P<0.01 when compared with control group. Statistically analyzed by one-way ANOVA followed by Dunnett's *t*-test. Methanolic extract of *Annona squamosa*, MECC: Methanolic extracts of *Cinnamon cassia*, SEM: Standard error mean

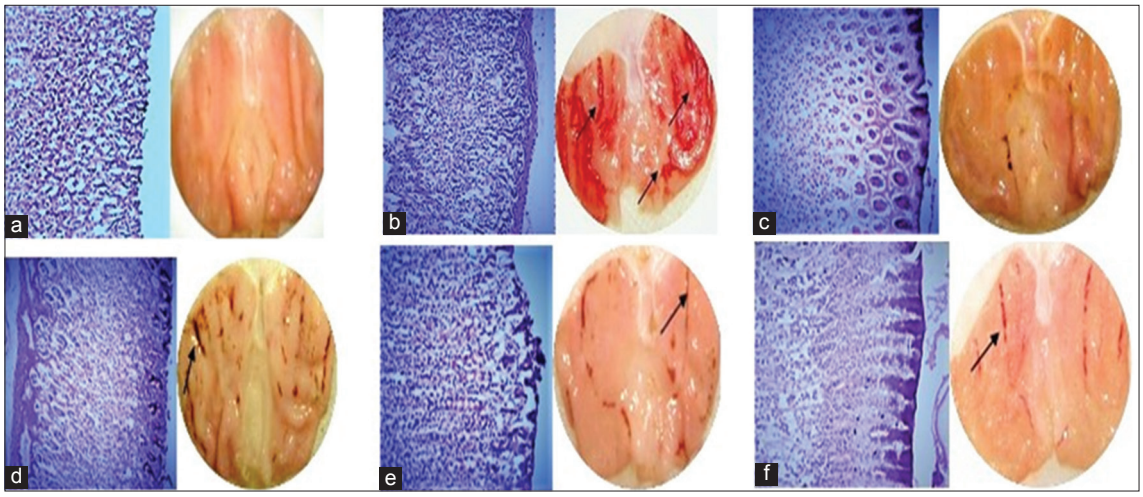

Figure 1: Macroscopic and histological images of a gastric mucosa of rats treated with methanolic extract of *Annona squamosa* (MEAS) and methanolic extracts of *Cinnamon cassia* (MECC) and its mixture. (a) Normal control treated with distilled water; (b) negative control treated with indomethacin (20 mg/kg suspension in 1% CMC) orally; (c) standard control treated with indomethacin (20 mg/kg suspension in 1% CMC) orally and misoprostol (100 mcg/kg i.p.); (d) treated with indomethacin (20 mg/kg suspension in 1% CMC) orally and 100 mg/kg MEAS orally; (e) treated with indomethacin (20 mg/kg suspension in 1% CMC) orally and 100 mg/kg MECC orally; and (f) treated with indomethacin (20 mg/kg suspension in 1% CMC) orally and 50 mg/kg MEAS + 50 mg/kg MECC

the dose of 50 mg/kg each [Figure 1].

Various reported literatures on medicinal plants have shown them to be alternative source for new compounds with potential pharmacological activity. In this present study, antiulcer effect of MEAS + MECC was investigated against indomethacin-induced ulcer in experimental animals. Indomethacin is an NSAID which was known to cause ulcer by inhibiting synthesis of PG through cyclooxygenase enzyme inhibition, thus induces gastric damage.[19,20] The antiulcer effects of MEAS + MECC can be due to the presence of flavonoids, tannins, and antioxidant activity in the extracts. MEAS + MECC might be stimulating the biosynthesis of PG. PG synthesis protects the stomach from the irritation and injuries by stimulating the secretion of protective substance such as mucus and bicarbonates.[21,22] Further, detailed study on the separation and categorization of the compounds responsible for the antiulcer activity in methanolic extract of the mixture of *A. sqamosa* and *C. cassia* needs to be carried out.

CONCLUSION

The results lead to the conclusion that methanolic extract of the mixture of *A. squamosa* and *C. cassia* possesses significant antiulcer activity. The significant antiulcer activity of this methanolic mixture of plant extracts might be because of the presence of compounds such as flavonoids, phenols, and tannins as well as due to its antioxidant property. Further, an isolation and characterization of a specific compound is required which is responsible for antiulcer property.

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REFERENCES

1. Herszenyi L, Juhasz M, Mihaly E, Tulassay Z. Peptic ulcer disease and stress. Orv Hetil 2015;156:1426-9.
2. Aase S. Disturbances in the balance between aggressive and protective factors in the gastric and duodenal mucosa. Scand J Gastroenterol Suppl 1989;163:17-23.
3. Kaunitz JD. Barrier function of gastric mucus. Keio J Med 1999;48:63-8.
4. Mejia A, Kraft WK. Acid peptic diseases: Pharmacological approach to treatment. Expert Rev Clin Pharm 2009;2:295-314.
5. Yamagishi H, Koike T, Ohara S, Horii T, Kikuchi R, Kobayashi S, et al. Stronger inhibition of gastric acid secretion by lafutidine, a novel H(2) receptor antagonist, than by the proton pump inhibitor lansoprazole. World J Gastroenterol 2008;14:2406-10.
6. Myles IA. Fast food fever: Reviewing the impacts of the western diet on immunity. Nutr J 2014;13:61.
7. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 2015;11:982-91.
8. Shen Y, Jia LN, Honma N, Hosono T, Ariga T, Seki T. Beneficial effects of cinnamon on the metabolic syndrome, inflammation, and pain, and mechanisms underlying these effects - A review. J Tradit Complement Med 2012;2:27-32.
9. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evid Based Complement Alternat Med 2014;2014:642942.
10. Bandara T, Uluwaduge I, Jansz ER. Bioactivity of cinnamon with special emphasis on diabetes mellitus: A review. Int J Food Sci Nutr 2012;63:380-6.
11. Yadav DK, Singh N, Dev K, Sharma R, Sahai M, Palit G, et al. Anti-ulcer constituents of *Annona squamosa* twigs. Fitoterapia 2011;82:666-75.
12. Vikas B, Akhil BS, PR, Sujathan K. Free radical scavenging properties of annona squamosa. Asian P J Cancer Prev 2017;18:2725-31.

13. Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. A potential of some medicinal plants as an antiulcer agents. Pharmacogn Rev 2010;4:136-46.
14. Goswami HK, Ram HK. Ancient food habits dictate that food can be medicine but medicine cannot be "food"!!: Food is the substance digested by an individualto suppress the basic vital instinct of hunger(H.K.G). Medicines 2017;4:82.
15. Patil AN, Advani MG, Mali SN, Pawar S, Raut SB. Evaluation of anti-ulcer effect of amlodipine in gastric ulcer models in rats. Indian J Pharm 2012;44:387-9.
16. Khare S, Asad M, Dhamanigi SS, Prasad VS. Antiulcer activity of cod liver oil in rats. Indian J Pharm 2008;40:209-14.
17. Batista LM, de Almeida AB, de Pietro Magri L, Toma W, Calvo TR, Vilegas W, et al. Gastric antiulcer activity of syngonanthus arthrotrichus silveira. Biol Pharm Bulletin 2004;27:328-32.
18. Asmari AA, Omani SA, Otaibi MA, Abdulaaly AA, Elfaki I, Yahya KA, et al. Gastroprotective effect of minocycline in experimentally induced gastric ulcers in rats. Int J Clin Exp Med 2014;7:586-96.
19. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. J Clin Biochem Nutr 2011;48:107-11.
20. Takeuchi K. Pathogenesis of NSAID-induced gastric damage: Importance of cyclooxygenase inhibition and gastric hypermotility. World J Gastroenterol 2012;18:2147-60.
21. Cohen MM. Role of endogenous prostaglandins in gastric secretion and mucosal defense. Clin Invest Med Med 1987;10:226-31.
22. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: Why doesn't the stomach digest itself? Physiol Rev 2008;88:1547-65.

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