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Gastro-protective effect of methanol extract of *Ficus asperifolia* bark on indomethacin-induced gastric ulcer in rats

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Summary: The gastro-protective and antioxidant effects of methanol extract of *Ficus asperifolia* bark on indomethacin induced gastric ulcer were investigated in male rats. Thirty two male rats divided into 4 equal groups and were treated as follows: group1 (control), 0.5ml of 5% tween 80 (vehicle for the extract), groups 2 and 3, 100 and 500mg/kg of *Ficus asperifolia* extract respectively and group 4, cimetidine (100mg/kg). After two weeks of daily oral administration of vehicle, extract or cimetidine, gastric ulcer was induced in all rats with indomethacin (40 mg/kg, p.o). Gastric juice pH, gastric acid concentration, gastric ulcer score, percentage gastric ulcer inhibition, activity levels of superoxide dismutase (SOD), catalase and malondiadehyde (MDA) were determined. *Ficus asperifolia* extract significantly increased gastric pH ($p<0.05$) but decreased ($p<0.01$) gastric acid secretion in dose dependent manner when compared with the control. Inhibition of gastric ulcer in extract and cimetidine treated rats was similar. Activities of SOD and catalase were significantly increased ($p<0.05$) while MDA was significantly decreased ($p< 0.05$) in extract treated rats when compared with the control. The results suggest that *Ficus asperifolia* possesses gastro-protective and antioxidant properties against gastric ulcer induced by indomethacin.

Keywords*: Ficus asperifolia*, Gastric ulcer, Gastro-protection, Antioxidant, Rat

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INTRODUCTION

Peptic ulcers are among the leading causes of morbidity and mortality in Nigeria and many developing countries (Agbakwuru, et al., 2006). The occurrence of peptic ulcer is higher among low-income groups in Nigeria than their counterparts in Europe (Amure, 1967). Within Nigeria, the frequency is higher among the Southern indigenes than their Northern counterparts (Amure, 1967). Furthermore, the incidence of gastric ulcer is more common in elderly people of 40 years of age and above in Nigeria (Agbakwuru, et al., 2006).

Gastric acid is one of the most important factors influencing gastric ulcer development (Alphin and Wards, 1967). Protection of gastric mucosa involves factors such as acid-pepsin secretion, parietal cell activity, mucosal barrier, mucus secretion, blood flow, cell regeneration, and the release of endogenous protective agents especially prostaglandins and epidermal growth factors (Berglindh, 1977).

Several plants and herbs are used in folk medicine to treat gastrointestinal disorders, including gastric ulcers. Recently, there has been a growing interest in identifying and characterizing new anti-ulcer agents from plant sources. *Ficus asperifolia* plant belongs to the family of Moraceae. It is a small size tree, terrestrial or epiphyte which can reach 4-8 metre in height. It is found in Senegal, Uganda, Tanzania, Madagascar, Sudan, Zaire and Nigeria (Adjanohoun et al., 1996). Watcho, et al., (2010) found that this plant induced uterotonic effect by stimulating prostaglandin secretion. Watcho, et al., (2009) also reported the reproductive effects of *Ficus asperifolia* in female rats, the results of which added credence to the scientific value of its popular use for women with sterility and infertility problems. The antibacterial, antioxidant, fibroblast growth and wound healing properties of *Ficus asperifolia* have been reported (Annan and Houghton, 2008). Stem bark infusion of the plant is used in Nigeria and Ghana by traditional healers to treat sores and ulcer. There is no information on the effect of this medicinal plant extract on gastric ulcer. The present study was therefore designed to investigate the gastro-protective and antioxidant effects of methanol extract of *Ficus*

asperifolia bark on indomethacin induced gastric ulcers in rats.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh barks of *Ficus asperifolia* were collected from the Botanical Garden, University of Ibadan, Ibadan Nigeria. Authentication of the plant material was carried out in the herbarium of the department of Botany, University of Ibadan, and a voucher specimen of the plant was identified under the herbarium number FHI 106054 at the Forestry Research Institute of Nigeria (FRIN) Ibadan Nigeria. The plant material was then air dried and pulverized into powder. Methanol extract was prepared as described by Watcho et al (2009). Briefly, 1kg of *Ficus asperifolia* powder was soaked in 5L of methanol for 24h. The extract was filtered and the filtrate was evaporated to dryness at low temperature under reduced pressure in a rotary evaporator. Approximately 50g of dried methanol extract was obtained giving an extraction yield of 5%. For bioactivity investigations, the methanol extract was suspended in 5%Tween 80.

Animals

Thirty two Wistar male rats (150-250g) obtained from the central animal house, College of Medicine, University of Ibadan, Nigeria were used for the study. The animals were housed under standard laboratory conditions with 12 hours light and dark cycle and fed with standard food and water *ad libitum*. Ethic regulations were followed in accordance with National and Institutional Guidelines for the Protection of Animal Welfare during the experiments.

Experimental design

The experiment was carried out on 32 rats that were divided into 4 groups of 8 rats each. Group 1 served as the control and was given 0.5ml of 5% Tween 80 (vehicle for the extract). Groups 2 and 3 were given 100 and 500mg/kg respectively, of *Ficus asperifolia* extract while group 4 was treated with 100mg/kg of cimetidine. The doses of *Ficus asperifolia* extract used in this study had been used by others (Watcho et al, 2009). Vehicle, extract and drug were administered daily via oral route for 2 weeks. After 2 weeks of the experiment, all rats were fasted for 24 hr; gastric ulceration was then induced by administration of 40mg/kg indomethacin orally. Eight hours after indomethacin administration all rats were killed by cervical dislocation and the abdomen of each rat was opened to remove the stomach. Gastric secretion, gastric ulcer score, percent ulcer inhibition, superoxide dismutase activity, catalase and lipid peroxidation (measured by malondialdehyde concentration) were determined.

Gastric acid secretion assay

Gastric acidity was performed as earlier described (Gehan et al, 2009). Eight hours after the induction of gastric ulcer, the rats were killed by cervical dislocation; the abdomen was opened to remove the stomach. The stomach was opened along the greater curvature and gastric content was drained into a centrifuge tube. Five ml of distilled water was added and the resultant solution was centrifuged at 3,000rpm for 10 minutes. The pH of gastric juice was determined using a pH meter. Gastric acid output was determined in the supernatant by titration with 0.0025N NaOH.

Gastric ulcer scoring

Eight hours after the induction of gastric ulcer, the stomachs were opened along greater curvature and washed with normal saline. Gastric ulcer scoring was carried out as earlier described (Alphin and Wards, 1967; Elegbe and Bamgbose, 1976; Raji et al, 2004). The gastric lesions formed were scored and the mean ulcer index and percentage inhibition of ulceration were calculated as earlier described (Raji et al 2004), thus:

$$\text{Ulcer index (U.I)} = \text{Mean degree of ulceration} \times \frac{\%\text{ of group of ulceration}}{100}$$

$$\%\text{ Inhibition} = \frac{\text{U.I in control- U.I in test}}{\text{U.I in control}} \times 100$$

Superoxide dismutase (SOD) activity

The levels of SOD activity was determined by the method of Misra and Fridovich (1972). This involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in a UV vial spectrophotometer. For the determination of specific activity of SOD in homogenate sample of gastric tissue, the rate of autoxidation of epinephrine was noted at 30 seconds intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

Catalase assay

Homogenized sample of gastric tissue (0.5 ml) was mixed with equal volume of 30M of hydrogen peroxide, 1ml of 6M H_2SO_4 and 7ml of 0.01M of potassium permanganate. Absorbance was read at 480nm within 30 to 60 seconds against distilled water. The result was expressed in µmol/mg protein.

Malondialdehyde assay-Lipid peroxidation

Lipid peroxidation was estimated in terms of thiobarbituric acid (TBA), using malondialdehyde (MDA) concentration. Two ml of TBA reagent and 1ml of trichloroacetic acid (TCA) were mixed with 2ml of homogenate of gastric tissue. The mixture was heated at 60^0C for 20 minutes. It was then cooled and

centrifuged at 400rpm for 10 minutes. The absorbance of the supernatant was read at a wavelength 540nm.

Statistical analysis

Data were expressed as mean ± SEM. Statistical significant between the groups was determined by analysis of variance (ANOVA). P <0.05 was considered statistically significant.

RESULTS

Effects on gastric ulcer scoring

As shown in table 1, *Ficus asperifolia* extract significantly reduced (p< 0.05) ulcer scores in the treated rats when compared with the control (table 1). The percentage ulcer inhibition in the extract treated rats was dose-dependent and similar to that of the standard drug (cimetidine) used in this study (table 1).

Effects on gastric pH and gastric acid secretion

There was a significant increase in gastric pH of rats that were treated with the extract of *Ficus asperifolia* (groups 2 and 3) when compared with the vehicle treated (group 1) and cimetidine treated rats (group 4) (fig 1a). Similarly, gastric acid concentration of extract treated rats was significantly reduced when compared with the vehicle treated rats (fig b).

Effects on antioxidant enzymes

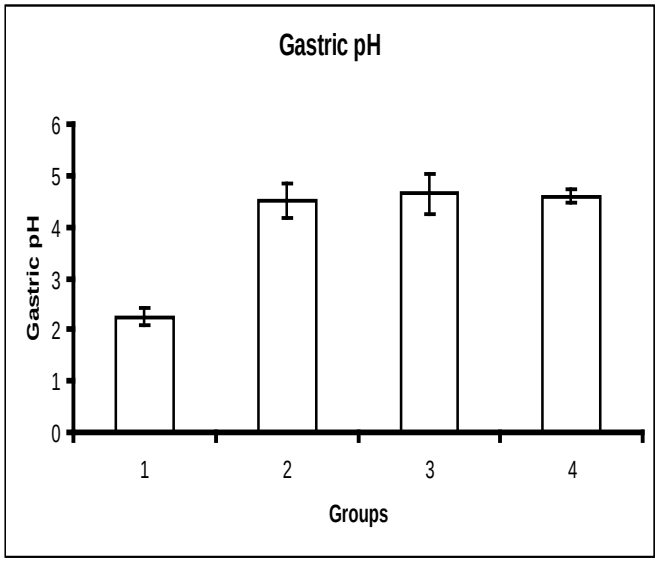
As shown in Fig. 2 there was a significant increase in SOD (a) and catalase (b) activities in extract and cimetidine treated rats when compared with control. Similarly, *Ficus asperifolia* significantly reduced (p< 0.05) MDA (c) concentration

Table 1:

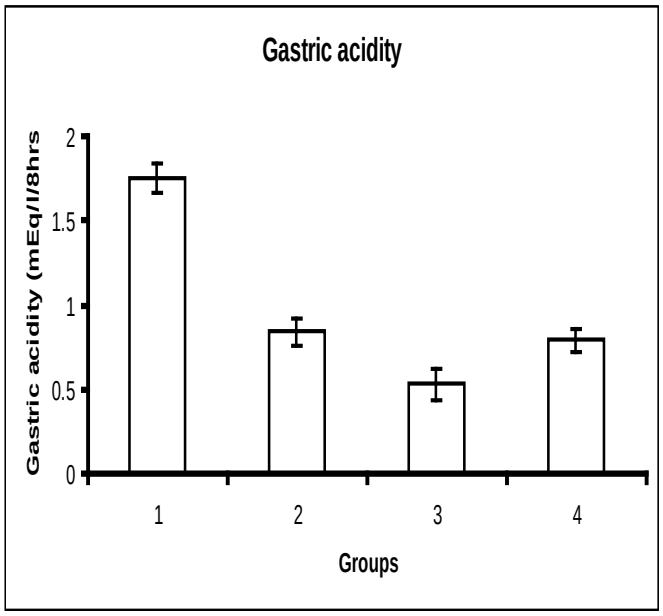
Effect of methanol extract of *Ficus asperifolia* bark on indomethacin induced gastric ulcer in male rats

Groups	Ulcer score (units)	Ulcer index	% Ulcer inhibition
1. Tween 80 (Control)	0.89 ± 0.31	0.71	-
2. *F.asperifolia* (100mg/kg)	0.69 ± 0.25*	0.55	22.5
3. *F.asperifolia* (500mg/kg)	0.54 ± 0.19*	0.43	39.4
4. Cimetidine (100mg/kg)	0.66 ± 0.23	0.53	25.4

* Significant with respect to the control (p < 0.05)



(a)

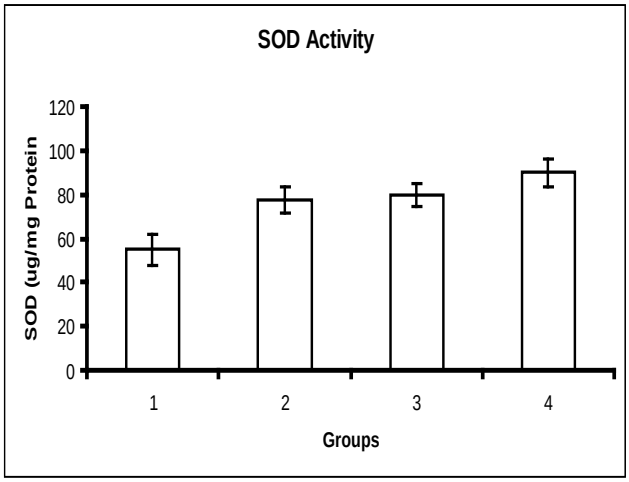


(b)

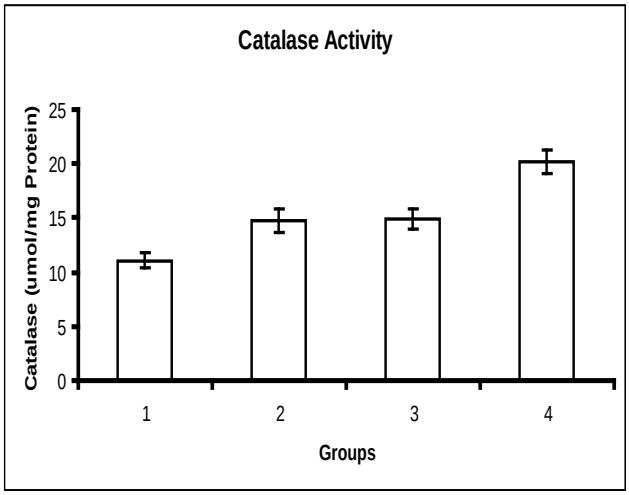
Figure 1:
Effect of methanol extract of *Ficus asperifolia* bark on gastric pH (a) and gastric acid concentration (b) in rats.

DISCUSSION

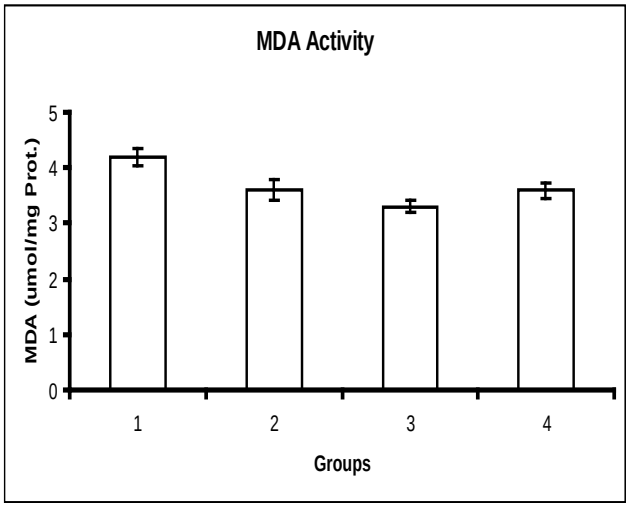
This study was carried out to investigate the gastro-protective and antioxidant effects of methanol extract of *Ficus asperifolia* bark on indomethacin induced gastric ulcer in rats. Ficus *asperifolia* had earlier been reported to have antioxidant properties (Annan and Houghton, 2008) and is known to promote prostaglandins secretion in smooth muscle (Watcho, *et al.*, 2010). Indomethacin has been reported in many studies to cause gastric ulcer through its inhibitory actions on prostaglandin synthesis. Prostaglandin inhibition causes elevation of gastric acid secretion, reduced mucosal blood flow, and bicarbonate secretion (Feldman et al 1992; Wallace, 2008).



(a)



(b)



(c)

Figure 2:
Effects of methanol extract of *Ficus asperifolia* bark on superoxide dismutase (SOD) (a), catalase (b) and malondialdehyde (MDA) (c) activities in rats. MDA was measured in μmol/mg Protein x 10^{-5}

The results of this study showed that *Ficus asperifolia* caused an increase in gastric pH and reduction total gastric acid concentration in rats which is similar to that produced by cimetidine. This suggests that *Ficus asperifolia* extract has inhibitory effect on gastric acid secretion which seems to mimic the inhibitory action of cimetidine (H_2 receptor blocker) on gastric acid secretion. The extract may act by blocking H_2 receptor leading to inhibition of histamine release whose stimulatory action on gastric acid secretion had been established (Berglindh 1977). It is established that inhibition of histamine through H_2 receptors, inhibit intracellular adenylate cyclase, Na^+-K^+ ATPase and inhibition of proton pump of parietal cells that eventually reduce the gastric acid secretion (Sasaki, et al., 2000; Ayada, et al., 2003).

The findings in the present study also showed that *Ficus asperifolia* extract has gastro-protective effect on the development of gastric ulcer. The mechanism through which *Ficus asperifolia* extract produces gastro protective effect on gastric ulcer by indomethacin might be due to its ability to inhibit gastric acid secretion. This might also be due to abolishment of indomethacin inhibitory effect on prostaglandins secretion as reported by Watcho, et al.,(2010), thereby increasing prostaglandin which is well known to increase gastric mucosal blood flow (Bruggeman, et al., 1979).

Phytochemical screening of methanol extract of Ficus *asperifolia* bark had previously been reported to contain alkaloids, saponins, sterols and triterpens (Watcho, et al., 2009, 2010). Nkafamiya, et al., (2010) also reported the present of oxalate, tannin, saponin, phytate, alkaloids, and HCN in the leaves of *Ficus asperifolia*. The reduction in gastric acid secretion seen in the group treated with *Ficus asperifolia* might also be due to action of oxalate which was reported to be present in the leaf extract of *Ficus asperifolia* (Nkafamiya, et al, 2010). Oxalate tends to render calcium unavailable by binding to calcium ion to form calcium oxalate complexes which may reduce intracellular Ca^{2+} that is necessary for gastric acid secretion (Hinkle, et al., 2003). Nkafamiya, et al., (2010) showed the presence of large amount of crude protein and all the essential amino acids in the leaf of *Ficus asperifolia*. Although, the protein and amino acid content of the bark of *Ficus asperifolia* is yet to be evaluated, high protein diet had been shown to be associated with low incidence of gastric ulcer (Oluwole and Bolarinwa, 1986), and was reported to have buffering effects on gastric acidity (Williams, et al., 1976).

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin have previously been reported to decrease antioxidant enzymes (SOD and catalase) activity in rat stomach thereby causing gastric ulceration (Halici, et al., 2005; Odabasoglu, et al., 2006). SOD activity was significantly increased in rats treated with *Ficus asperifolia* extract

indicating that SOD plays an important role in preventing gastric ulcer by catalyzing the breakdown of highly reactive radical superoxide (O_2^-) into oxygen and hydrogen peroxide (Zelko, et al., 2002; Gehan et al, 2009). The catalase activity was increased in rats treated with *Ficus asperifolia* extract. Catalase protective property from the action of indomethacin may be due to its antioxidant activity. Furthermore catalase had been shown to increase the synthesis of prostaglandin which might further contribute to the gastro-protection of the gastric mucosa (Chen, et al., 1998) by the *Ficus asperifolia* extract in the present study. Lipid peroxidation was estimated in terms of malondialdehyde (MDA). *Ficus asperifolia* extract and cimetidine produced significant reduction in MDA concentration suggesting that the antioxidant property of *Ficus asperifolia* extracts (Annan and Houghton, 2008) may be powerful enough to prevent gastric ulcer through lipid peroxidation of gastric mucosa. The findings in this study suggest that *Ficus asperifolia* extract possesses antiulcer activity and could be a potential source for isolation of new potent, safe and effective antiulcer agents. Future efforts should be directed at investigating the principles in this extract with this strong antiulcer effect.

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