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Research Article

Gastroprotective Effects of DAS-77 (a Phytomedicine) in Ulcer Models in Rats

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Abstract

Purpose: DAS-77 is a phytomedicine that contains the dried bark of *Mangifera indica* and root of *Carica papaya*. This study investigated the antiulcer effects of DAS-77 in rats.
Methods: DAS-77 was administered orally twice daily for five consecutive days at doses of 50 - 400 mg/kg. Ulcer was induced in rats with ethanol, indomethacin, pylorus ligation (PL) and cold restraint stress (CRS). Ulcer scores were recorded based on examination of excised stomachs. Estimations of gastric content volume, pH and titratable acidity in the PL model and determination of the levels of antioxidants and malondialdehyde (MDA) in gastric tissues in the CRS model were also done.
Results: In all the models, DAS-77 produced significant dose-dependent reductions in ulcer score. Peak effects were produced at the dose of 400 mg/kg with ulcer inhibition values of 98.57, 76.23, 99.28 and 96.70 % compared to 100.00, 93.79, 98.92 and 96.79 % for misoprostol/cimetidine, respectively, for the ethanol, indomethacin, PL and CRS models. In the PL model, DAS-77 caused a significant increase in pH of gastric content but a reduction in volume and titratable acidity. At doses of 50 and 100 mg/kg in the CRS model, DAS-77 significantly increased the level of reduced glutathione (GSH) and diminished MDA.
Conclusion: The results obtained in this study suggest that DAS-77 possesses gastroprotective activity possibly due to reduced gastric secretion and acidity, and antioxidant activity.

Keywords: DAS-77, Phytomedicine, *Mangifera indica*, *Carica papaya*, Gastroprotective effects, Ulcer.

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INTRODUCTION

Peptic ulcers are lesions of the mucous membrane localized in the stomach, duodenum or other parts of the gastrointestinal tract (GIT) [1]. Common causes include drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and severe emotional or physical stress [2]. Other potential causes include Zollinger-Ellison syndrome, cancer chemotherapy, radiation, vascular insufficiency [2], diet and lifestyle (smoking and alcohol) [1]. Ulceration of the GIT is the net result of a lack of homeostasis between factors responsible for the breakdown of food in the GIT (e.g. gastric acid and pepsin) and factors that promote epithelial defense and repair (e.g. bicarbonate, mucus secretion and prostaglandins) [2]. The goals of pharmacotherapy of peptic ulcer disease (PUD) include the eradication of infection, reduction of gastric acidity and/or enhancing mucosal protection [1,3]. The incidence of adverse effects, poor compliance, high relapse rate and contraindications necessitate the search for more efficacious, safer and better tolerated medicines for the treatment of PUD.

DAS-77 is a Nigerian herbal preparation that contains the milled dried callous bark of *Mangifera indica* Linn. (Anacardiaceae) and root of *Carica papaya* Linn. (Caricaceae) in powdered form. The phytomedicine is used to treat diverse ailments and claimed to be effective in the treatment of PUD. This study was designed to investigate the gastroprotective effects of DAS-77 in ulcer models in rats.

EXPERIMENTAL

The herbal product

The phytomedicine DAS-77 is a product of Doynik Ventures, Ijoko-Lemode, via Sango Ota, Ogun State, Nigeria. It contains the young callous bark of mango (*Mangifera indica*) and dried root of pawpaw tree (*Carica papaya*) in powdered form (1:1). The product is a light brown, coarse powder with a pungent smell, and solution pH 8.5.

Drugs and chemicals

Cimetidine, indomethacin and misoprostol were obtained from ZIM Laboratories Ltd, India, Yangzhou Pharmaceutical Co. Ltd, China, and Pharmacia Laboratories Ltd, India, respectively. The drugs were constituted in distilled water before administration to the experimental animals. Absolute ethanol, NaOH, urethane and chloralose were obtained from Sigma Chemical Co, St Louis, USA, Merck, Germany, Sigma Laboratories, Germany, and British Drug House, London, respectively. All other chemicals used were of analytical grade.

Animals

Healthy albino mice (15-25 g) and rats (100-200 g) of both sexes used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. Experimental animals were kept in a well ventilated room and were maintained under standard environmental conditions (23-25 ^{0}C, 12 h/12 h light/dark cycle). The animals had free access to standard rodent diet (Livestock Feeds Plc, Lagos, Nigeria) and water. All the animals were acclimatized for one week prior to experiments. The experiments were performed between 9.00 am and 6.00 pm on the assigned days. The procedures were carried out in compliance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals [4] and the requirements of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria.

Acute toxicity test

The mice were fasted for 12 h before the commencement of the experiment. A group

of mice received DAS-77 orally at a dose of 10 g/kg. Other groups of 30 albino mice, 5 animals per group, were assigned as control and 5 DAS-77 treatment groups. The control group received distilled water 10 ml/kg intraperitoneally while DAS-77 was administered to the other groups of mice at doses of 250, 500, 1000, 2000, and 3000 mg/kg. Animals were observed for behavioural changes and other signs of toxicity (including sedation, hyperactivity, diarrhoea, writhing, piloerection, and restlessness) for 2 h post-treatment. Mortality within 24 h was recorded and LD_{50} was estimated using the log-probit analysis method [5].

Phytochemical analysis

Qualitative and quantitative phytochemical screening of DAS-77 was done in accordance with the methods of Edeoga *et al* [6].

Ethanol-induced gastric ulcer model

Rats were divided into 6 groups of 6 animals each. Group 1 served as control and received distilled water (10 ml/kg, *p.o.*). Groups 2, 3, 4, and 5 were treated orally with 50, 100, 200 and 400 mg/kg of DAS-77 by oral intubation, respectively. Group 6 was treated with misoprostol (50 µg/kg) which served as the standard drug. The rats in each group were treated twice daily with these doses for 5 days. The rats were fasted for 24 h into the 6th day. Gastric ulcer was induced in the rats by administration of absolute ethanol (1 ml/200 g) [7]. After 1 h, the animals were sacrificed by cervical dislocation and the stomach was isolated and incised along the greater curvature, and examined for ulcers. Ulcer score was determined using the Magistreni scoring scale [8]:

0 = no lesion; 0.5 = haemorrhage; 1 = 1-3 small lesions (10 mm length); 2 = 1-3 large lesions (10 mm length); 3 = 1-3 thickened lesions; 4 = more than 3 small lesions; 5 = more than 3 large lesions; and 6 = more than 3 thickened lesions.

Indomethacin-induced gastric ulcer model

Another set of rats were allotted into groups, treated for 5 days, and then fasted for 24 h. Indomethacin (50 mg/kg) was administered to each animal [9]. Rats were sacrificed by cervical dislocation 6 h after indomethacin treatment. The stomachs were removed and they were cut open along the greater curvature. The gastric lumen was rinsed with normal saline and examined. The ulcer score was determined using Magistreni scoring scale [8].

Cold restraint stress-induced gastric ulcer model

The experimental rats in each group were treated as explained previously and fasted for 24 h into the 6th day. On day 6, the animals were immobilized by strapping the fore and hind limbs on a flat packaging foam, and kept in the refrigerator (4-6^{o}C) for 2 h [10]. Two hours later, each rat was sacrificed under chloroform anaesthesia, and the stomach was incised along the greater curvature. The lumen was rinsed with normal saline and examined. The ulcer score was determined according to the Magistreni scoring scale [8]. After ulcer scoring, the fundic part of the stomach was homogenized (5 %) and centrifuged [10]. The derived supernatant was subsequently used for assay of antioxidant enzymes activity.

Pylorus ligation-induced gastric ulcer model

Drugs were administered for a period of 5 days as described earlier and the rats were fasted for 24 h into the 6th day. On day 6, the animals were anaesthetized using 25% urethane and 1% chloralose at a dose of 1 ml of mixture/200 g weight of rat. Tracheotomy was done to remove bronchial secretion. The abdomen was opened and pylorus ligation was done without causing any damage to its

blood supply. The stomach was replaced carefully and the abdominal wall was closed in two layers with a moist swab of normal saline. After 4 h, each stomach was dissected out and cut open along the greater curvature. Ulcer score was determined using the Magistreni scoring scale [8].

Evaluation of ulcer index

In each of the models, the mean ulcer score for each group was calculated as in Eq 1 and expressed as the ulcer index (UI) [11]

UI = ADU (%RU/100) …….. (1)

where ADU = average degree of ulceration for each group (mean ulcer score); % RU = percentage of rats with ulceration.
Percent inhibition was determined according to the method of Okabe *et al* [12] as in Eq 2.

Inhibition (%) = {(UIc - UIt)/UIc} × 100 …. (2)

where UIc is the ulcer index for the control group and UIt the ulcer index for the treatment group.

The volume of gastric juice collected from each rat was determined by the use of measuring cylinder. The centrifuged gastric juice from each pylorus-ligated rat was titrated against 0.01N NaOH and pH was determined using a research ionalyzer (Orion, Beverly, MA, USA). All measurements were done in triplicate. Titratable acidity was determined and expressed as mEq/L [13,14].

Antioxidant enzymes and malondi-aldehyde assay

Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and malondialdehyde (MDA) levels were determined by the method of Soon and Tan [15]. Total protein was determined by the Biuret method [16].

Statistical analysis

The results are expressed as mean ± S.E.M (standard error of mean). Data were analyzed using One-way ANOVA followed by Dunnett's and Tukey's multiple post hoc tests using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Values were considered significant at $p < 0.05$.

RESULTS

Acute toxicity test

No mortality and visible signs of toxicity were observed with the oral administration of DAS-77 up to 10 g/kg. However, DAS-77 given *i.p.* caused 0% mortality at the dose of 250 mg/kg and 100 % mortality at the dose of 3000 mg/kg. The LD_{50} for the *i.p.* route was estimated to be 1122.0 mg/kg. Behavioural manifestations observed with the *i.p.* route include writhing, grooming, increased locomotor activity and convulsion although these were not quantified.

Phytochemical analysis

Phytochemical analysis of DAS-77 showed the presence of tannins, saponins, phenols, flavonoids, and alkaloids, with the crude yield of the phytoconstituents being 3.26, 2.32, 1.31, 0.54, and 0.04 %, respectively.

Ethanol-induced gastric ulcer model

As shown in Table 1, oral administration of DAS-77 produced significant ($p < 0.001$) dose-dependent reduction in ulcer score and index. Peak effect was produced at the dose of 400 mg/kg (98.57% inhibition). This effect was comparable to that produced by misoprostol (100.00 % inhibition).

Indomethacin-induced gastric ulcer model

DAS-77 produced significant ($p < 0.05$) reduction in ulcer score and index (Table 1). Misoprostol elicited 93.79 % inhibition of ulcer development. This effect was not

Table 1: Effect of DAS-77 on ulcer score and index in the ethanol and indomethacin ulcer models in rats

Group	Dose (mg/kg)	Ethanol model			Indomethacin model		
		US	*UI*	*Inhibition (%)*	*US*	*UI*	*Inhibition (%)*
Control	10 ml/kg	7.00±0.41	7.00	-	4.67±1.15	4.67	-
DAS-77	50	3.25±0.59***	3.25	53.57	2.17±0.67	2.17	53.53
DAS-77	100	1.92±0.30***	1.92	72.57	1.50±0.41*	1.50	67.88
DAS-77	200	1.67±0.36***	1.67	76.14	1.58±0.33*	1.58	66.17
DAS-77	400	0.58±0.49^{***,c}	0.10	98.57	1.67±0.80*	1.11	76.23
Misoprostol	(50 µg/kg)	0.17±0.11$^{***,c,•}$	0.00	100.00	0.58±0.20**	0.29	93.79

Values are mean ± S.E.M (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control; $^{c}p < 0.001$ vs. DAS-77 50 mg/kg; $^{•}p < 0.05$ vs. DAS-77 100 mg/kg (one-way ANOVA followed by Dunnett's and Tukey's multiple comparison test).

Table 2: Effect of DAS-77 on ulcer score and index in cold restraint stress and pylorus ligation ulcer models in rats

Group	Dose (mg/kg)	Cold restraint stress model			Pylorus ligation model		
		US	*UI*	*Inhibition (%)*	*US*	*UI*	*Inhibition (%)*
Control	10 ml/kg	5.58±0.52	5.58	-	3.33±0.51	3.33	-
DAS-77	50	3.00±0.68**	3.00	46.24	1.75±0.11***	1.75	47.45
DAS-77	100	1.83±0.21***	1.83	67.20	1.00±0.13***	0.83	75.08
DAS-77	200	2.42±0.68***	2.42	56.63	1.00±0.13***	0.83	75.08
DAS-77	400	0.25±0.17^{***,b,α}	0.04	99.28	0.17±0.11^{***,c}	0.11	96.70
Misoprostol/ Cimetidine	(50 µg/kg)/ 100	0.33±0.17^{***,b,α}	0.06	98.92	0.17±0.11^{***,c}	0.11	96.70

Values are mean ± S.E.M (n = 6). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control; $^{b}p < 0.01$, $^{c}p < 0.001$ vs. DAS-77 50 mg/kg; $^{α}p < 0.05$ vs. DAS-77 200 mg/kg (One-way ANOVA followed by Dunnett's and Tukey's multiple comparison test). Misoprostol was used as standard drug in the cold restraint stress model while cimetidine was used in the pylorus ligation model.

significantly different from that produced by DAS-77 at 400 mg/kg (76.23 % inhibition).

Cold restraint stress-induced gastric ulcer model

The significant ($p < 0.01$, $p < 0.001$) inhibition of ulcer development produced by DAS-77 relative to control in this model was not dose-dependent. Peak effect was produced at the dose of 400 mg/kg (99.28 % inhibition). This effect was comparable to that elicited by misoprostol (98.92 %; Table 2).

Pylorus ligation-induced gastric ulcer model

DAS-77 produced significant ($p < 0.001$) reduction of incidence of ulcer with peak effect produced at the dose of 400 mg/kg (96.70 % inhibition). This effect was equal to that produced by cimetidine (Table 2). As shown in Table 3, the phytomedicine also elicited significant ($p < 0.05$, 0.001) dose-dependent reduction in the volume of gastric content relative to control with peak effect produced at the dose of 400 mg/kg (46.51 %). This effect was comparable to that produced by cimetidine (51.16 %). Also, DAS

Table 3: Effect of DAS-77 on gastric content volume, pH and titratable acidity in the pylorus ligation-induced ulcer model in rats

Group	Dose (mg/kg)	Gastric content volume (mL/4h)	Reduction in volume (%)	pH	Titratable acidity (mEq/mL)
Distilled water	(10 ml/kg)	1.29 ± 0.07	-	3.44 ± 0.12	1.11 ± 0.08
DAS-77	50	1.05 ± 0.06*	18.60	3.68 ± 0.13	0.98 ± 0.07
DAS-77	100	0.84 ± 0.04^{***,a}	34.88	4.15 ± 0.10**	0.83 ± 0.08*
DAS-77	200	0.79 ± 0.02^{***,b}	38.76	4.30 ± 0.09^{***,b}	0.67 ± 0.02^{***,b}
DAS-77	400	0.69 ± 0.03^{***,c}	46.51	6.00 ± 0.08$^{***,c,•••,γ}$	0.64 ± 0.02^{***,b}
Cimetidine	100	0.63 ± 0.03^{***,c}	51.16	6.22 ± 0.14$^{***,c,•••,γ}$	0.63 ± 0.02^{***,b}

Values are mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. DAS-77 50 mg/kg; $^{•••}p < 0.001$ vs. DAS-77 100 mg/kg; $^{γ}p < 0.001$ vs. DAS-77 200 mg/kg (One-way ANOVA followed by Dunnett's and Tukey's multiple comparison test).

Table 4: Effect of DAS-77 on gastric tissue antioxidant enzymes and MDA level in cold restraint stress-induced gastric ulcer model in rats

Group	Dose (mg/kg)	GSH (units/mg protein)	SOD (units/mg protein)	CAT (units/mg protein)	Glutathione peroxidase (units/mg protein)	MDA (units/mg protein)	Total protein (mg)
Control	(10 ml/kg)	4.73 ± 1.53	2.88 ± 1.68	11.23 ± 5.93	0.91 ± 0.53	0.66 ± 0.11	27.84 ± 7.18
DAS 77	50	13.5 ± 2.09***	6.62 ± 2.57	30.53 ± 11.83	2.08 ± 0.80	0.18 ± 0.04**	6.92 ± 0.98*
DAS 77	100	2.08 ± 0.32^{c}	1.19 ± 0.30	5.52 ± 1.38^{a}	0.37 ± 0.09	0.31 ± 0.05*	46.22 ± 4.84^{c}
DAS 77	200	2.06 ± 0.15^{c}	1.27 ± 0.11	5.88 ± 0.49^{a}	0.39 ± 0.03	0.43 ± 0.12	41.87 ± 4.12^{c}
DAS 77	400	2.20 ±0.09^{c}	1.03 ± 0.32^{a}	4.75 ± 1.49^{a}	0.32 ± 0.10^{a}	0.42 ± 0.07	42.22 ± 3.52^{c}
Misoprostol	(50 µg/kg)	5.80 ± 2.02^{b}	1.55 ± 0.15	6.78 ± 0.79^{a}	0.48 ± 0.05	0.25 ± 0.05*	27.95 ± 7.26

Values are mean ± S.E.M (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. DAS-77 50 mg/kg (One-way ANOVA followed by Dunnett's and Tukey's multiple comparison test).

-77 caused significant (p < 0.05, 0.01, 0.001) dose-dependent increase in pH and reduction in titratable acidity relative to control. The effects produced at the dose of 400 mg/kg were comparable to those of cimetidine (Table 3).

Effect of DAS-77 on gastric antioxidant enzymes and MDA

DAS-77 at the dose of 50 mg/kg significantly (p < 0.05, 0.01, 0.001) increased GSH and reduced MDA and total protein levels compared with control. The level of MDA was also significantly (p < 0.05) reduced by DAS-77 at 100 mg/kg and misoprostol relative to control (Table 4).

DISCUSSION

DAS-77 showed significant antiulcerogenic activity, with peak effects produced at the dose of 400 mg/kg, in all the models used in this study. The effects of the phytomedicine were generally comparable to those of misoprostol (50 µg/kg) and cimetidine (100 mg/kg) except in ethanol and indomethacin models in which the positive controls were superior. Incidence of ethanol-induced ulcer is predominant in the glandular part of the

stomach and it has been associated with the formation of leukotriene C_4 (LTC_4), mast cell secretory products and reactive oxygen species [17]. Hence, the effect of DAS-77 may be mediated by the inhibition of the generation of these principles. Non-steroidal anti-inflammatory drugs induce gastric mucosal injuries as side-effect [18] due to the inhibition of cyclooxygenase enzyme. This leads to the suppression of prostaglandins production and disruption of gastric mucosal protective system [19]. According to Wallace [20], inflammation due to neutrophil–endothelial cell interaction is involved in the progression of gastric mucosal injuries due to NSAIDs.

Stress-induced ulcer involves damage by reactive oxygen species (ROS), apart from acid and pepsin related factors. There is an increase in generation of ROS during stress leading to oxidative damage. Oxidative stress is considered to cause gastric mucosal injuries [18] and it has been reported that vitamins, polyphenols and flavonoids ameliorate gastric mucosal injuries due to their antioxidant effects [21]. Polyphenols also suppress the neutrophil-endothelial cell system in addition to their antioxidant activity [18]. The increase in the level of GSH and corresponding reduction in MDA produced by DAS-77 in this study suggest that its antiulcer activity may be partly associated with enhancement of free radical scavenging activity and possibly suppression of the neutrophil-endothelial cell system. Oxidative stress is also thought to play a major role in NSAIDs-induced gastric mucosal injuries due to reactive oxygen species generated by the administration of NSAIDs [18]. It has been reported that gastric mucosal injuries due to NSAIDs can be prevented by reactive oxygen scavengers such as superoxide dismutase and catalase and administration of antioxidant agents such as polyphenols [18, 22].

Pylorus ligation-induced ulcers are due to auto-digestion of the gastric mucosa and breakdown of the gastric mucosa barrier [23]. DAS-77 probably enhances the effectiveness of gastric mucosa barrier and defensive mechanism against gastric ulceration as it caused reductions in gastric content volume and titratable acidity and increased pH. These effects suggest direct inhibition of gastric secretion and possibly neutralization of acid secreted in the stomach.

In this study, phytochemical analysis of DAS-77 showed the presence of phenols, tannins, alkaloids, saponins and flavonoids. Tannins and flavonoids are strong natural antioxidants which reduce the level of peroxidation, hence exhibit antiulcerogenic effect [24]. The effects of DAS-77 in this study may be due to the presence of one or a combination of phytoconstituents.

Considering the individual plant components of DAS-77, Severi et al. [25] reported the gastroprotective effect of the aqueous decoction of *Mangifera indica* leaves in several ulcer models in rats. Phenolic compounds consequently isolated from the extract include mangiferin and c-glucosylbenzophenone. In a study by Bafna and Balaraman [26], DHC-1 herbal formulation, which contains the methanol extracts of various plants including the bark of *Mangifera indica*, was found to possess antiulcer and antioxidant activities in pylorus ligation and ethanol-induced gastric mucosal injury in rats. In respect of *Carica papaya*, Ezike et al. [27] reported the antiulcer potentials of aqueous and methanol extracts of its whole unripe fruit in ethanol and indomethacin-induced gastric ulcers. The antiulcer effect of the alcohol extract of dried fruits of *Carica papaya* in pylorus ligation and aspirin-induced gastric ulcer models in rats was reported by Rajkapoor et al. [28].

No mortality and visible signs of toxicity were observed following acute oral administration of 10 g/kg DAS-77. The phytomedicine administered orally can therefore be said to be relatively non-toxic based on the assertion of Clark and Clarke [29] that a substance that does not produce lethality at a dose of 10

g/kg orally is relatively non-toxic. DAS-77 may therefore be safe for human use.

CONCLUSION

The results obtained in this study suggest that the phytomedicine DAS-77 possesses gastroprotective activity in ulcer models in rats possibly due to reduction in gastric secretion and acidity, and antioxidant activity. The observed antiulcer properties may be due to the presence of one or a combination of its phytoconstituents. DAS-77 may be relatively safe for human consumption as no lethality was produced in mice at doses of 10 g/kg.

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COMPETING INTERESTS

The authors declare no conflicts of interest

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