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Gastroprotective activity of Patoladi decoction in rats

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Abstract

In one of the Ayurvedic treatise 'Patoladi' decoction (PD) consisting of five ingredients (*Terminalia chebula Retz, Terminalia belerica Roxb, Phyllenthus emblica Linn. Trichosanthes cucumarina Linn, Azadirachta indica A, Juss.*) is recommended for the treatment of 'Amlapitta' (dyspepsia/gastritis). This indicates that PD may posses gastroprotective action. The aim of this study was to investigate this possibility in rats using ethanol-induced gastric lesions. Different concentrations of freshly prepared PD was either orally [30% (n=6), 15% (n=6), 7.5% (n=6) of 0% (n=6)] or% IP [30%(n=6), 7.5% (n=6), or (n=6)] administered to fasted rats. 30 min later, 1mL of absolute ethanol was orally administered to these rats and 1 h later the number and length of gastric linear haemorrhagic lesions were determined. The results show that both treatments of PD markedly and significantly impaired the number and the length of gastric lesions. The PD did not significantly alter the volume, pH, free and total acidity and peptic activity of the gastric content. But, it caused significant increase in carbohydrate and mucus content of the gastric mucus layer. This may be the main mechanism of action of PD. We conclude that PD has potent gastroprotective action and may be therapeutically useful in the treatment of 'Amlapitta' in Ayurvedic Medicine in Sri Lanka.

Keywords : Patoladi decoction, dyspepsia, gastritis, gastric lesions, gastroprotection, Ayurveda, Sri Lanka.

1. Introduction

In an ancient Indian Ayurvedic authentic treatise 'Patoladi' decoction (PD) is recommended for the treatment of 'Amlapitta' (dyspepsia/gastritis) (1). This indicates that PD may have gastroprotective action. This decoction contains five ingredients: pericarp of dry fruits of *Terminalia chebula Retz. (Family; Combretaceae, "aralu" in Sinhala "kadukkay" in Tamil), Terminalia belerica Roxb.* (Family, Combretaceae, "bulu" in Sinhala "akkam" in Tamil), *Pyllenthus emblica Linn.* (Family; Euphorbiaceae, "nelli" in Sinhala "nellika" in Tamil), dry whole plant of *Trichosanthes cucumerina Linn.* (Family; Cucurbitaceae, "dummella" in Sinhala "pudal" in Tamil) and dry bark of *Azadirachta indica A. Juss.* (Family; Meliaceae, "kohomba" in Sinhala and "veppu" in Tamil). However, the validity of this claim is neither scientifically proven or refuted. The number of patients seeking Ayurvedic treatment for gastric ulcers at the Colombo Ayurvedic Hospital has increased over the last few years. Therefore, it was appropriate to investigate whether PD possesses gastroprotective activity. If gastroprotective activity is found then it could be used therapeutically at the Ayurvedic Hospital as an alternative clinical treatment for gastritis.

The aim of this study was to investigate whether PD possesses gastroprotective activity and if present to investigate the possible mode of action. This was done in rats using the ethanol-induced gastric lesion technique (2). This technique is frequently and widely used in evaluating gastroprotective action of drugs.

2. Materials and Methods

Dry fruits of *Terminalia chebula, Terminalia belerica, Phyllenthus emblica,* and whole dry plant of *Trichosanthes cucumerina,* were purchased from a Ayurvedic drug outlet in Colombo, Sri Lanka. Dry bark of *Azadiracthta indica,* was obtained from a tree in the garden in the Ayurvedic Teaching Hospital, Borella, Sri Lanka. Identification of these ingredients were authenticated by Dr. D. K. Vithanage, Department of Meteria Medica, University of Colombo, Colombo, Sri Lanka.

The PD was made as described in the Ayurvedic treatise (1). Briefly 12 g of each ingredient in dried form was introduced into a clay pot and 1920 ml of tap water was added. This was boiled down to 240 ml using a low flame. This was considered as the 100% PD. The PD was used within 6 h of preparation.

Healthy adult male cross bred albino rats (175-225g) from the Department of Zoology, University of Colombo, Sri Lanka were used. In this study they were housed individually in raised mesh bottomed cages (to pre-

vent coprophagia) under standardised animal house conditions (temperature : 28°-30°C, photoperiod: about 12 h light and 12 h dark, relative humidity: 50-55%) with free access to pelleted food (Master Feed Ltd. Colombo, Sri Lanka) and tap water *ad-libidum*.

Male rats (n=54) were randomly divided into seven groups. Group 1 (n=18) was orally treated with 1mL distilled water (DW), group 2 (n=6) 1mL of 30% PD, group 3 (n=6)lmL of 15% PD, group 4 (n=6) lmL of 7.5% PD, group 5 (n=6) was treated intraperitonially (IP) with 1 mL of DW, group 6 (n=6) (IP) with 1mL of 30% PD, and group 7 (n=6) IP with 1mL of 7.5% PD.

Food but not water was withheld 36 h prior to the experiment and 1mL of either vehical or PD was administered IP or orally as specified above. 30 min later, lmL of absolute ethanol (Fluka Chemical Co., Buchs, Switzerland), was given orally to induce gastric lesions (2). 1 h following ethanol treatment the animals were killed by an over dose of ether (B. D. H. Chemicals Ltd., Poole, UK). The stomachs were removed immediately and were instilled with 5mL of 10% formal saline (v/v) and left immersed in 10% formal saline in a beaker for 6-10 min. The stomachs were slit opened along the greater curvature and were examined macrosopically for linear haemorrhagic lesions in the mucosa of the glandular portion. The number of linear haemorrhagic lesions were recorded and the length (mm) of the liner haemorrhagic lesions were measured with a vernier caliper (Fisions Scientific Equipment, Loughborough, UK) and each length was summed per stomach.

Concentrations of the extract when given orally required for 50% inhibition of lesions (EC_{50}) were calcuted with 95% confidence limits using probit analysis. The dose dependency of the gastroprotective activity of the extract was determined using linear regression analysis.

Twelve rats were selected and were fasted for 12 h. Either 1mL of 30% PD (n=6) or lmL of DW (n=6) was orally administered. 1h later, these rats were anaesthetized and their abdomens were slit opened (2-3 cm midline incision just below the xiphoid process) using aseptic precaution. The pylorus was ligated using 5/0 silk ligature without interfering with the blood supply to the gastrointestinal tract. The abdomen was closed. An antibiotic cream (Polymycin $^{(R)}$, Astron Ltd., Ratmalana, Sri Lanka) was applied and the animals were allowed to regain consciousness. These rats were kept fasted for 4h and were killed using an over dose of ether. The gross external appearance of the stomach was noted. The gastric content was aspirated

(using a plastic syringe with a 21 guage needl) and the volume recorded. This was centrifuged (Eliex of Sweden Ltd., Bradford, UK) at 3200 r.p.m for 15 min and the supernatant was separated. Its pH was determined using a pH meter (TOA Electronics, Tokyo, Japan.)

The free acidity and the total acidity of the supernatant were determined titrometrically using methyl orange and phenol red, respectively, as indicators (3).

The pepsin content of the supernatants collected from the gastric juice of rats orally treated with 30% PD (n=6) and DW (n=6) were determined using the method described by Sanyal and Mitra (4). Briefly, to 1mL of each supernatant diluted with 0.5mL of 0.01 N HC1, 2.5 mL of 2% haemoglobin solution (in 0.06N HC1) was taken in separate test tubes and incubated in a water bath at 37°C for 10 min. The supernatant was added to the haemoglobin solution and incubated for 20 min. 3.5 mL of trichloroacetic acid was then added and the resulting solution was kept at 4°C for 1.5min. This was centrifuged at 3200 rpm for 10 min and the supernatant removed. To 0.5mL of this supernatant 5.0mL of 0.5N NaOH and 0.5mL of phenol were added. 10min later, the absorbence of this solution together with a substrate blank was measured spectrophotometrically at 610 nm.

The total carbohydrate content of the supernatants obtained from the above experiment [control (n=6), 30% PD (n=6)] were individually estimated as descrbed by Nair (5). Briefly, to 0.1mL of supernatant 0.9mL of DW was added. To this, 1mL of 5% phenol and 5mL concentrated H_2SO_4 were added. This resulting solution was kept at 4°C for 10min and then at 37°C for 20 min in a water bath. The absorbance of each of these samples and a standard solution of glucose (100mg/dl) were measured at 400 nm using a spectrophotometer (Jasco Corporation, Tokyo, Japan).

Quantitative estimation of mucus adheared to the stomachs were made in 12 rats deprived of food but not water for 36 h as described by Fernandopulle *et al (6)*. Briefly, rats were IP administered with either 1mL of 30% PD(n=6) or 1mL of DW (n=6). 1 h later, these animals were killed using an overdose of ether. The stomachs were excised, slit open along the lesser curvature and rinsed in 0.25M sucrose. Each stomach was then incubated in a 10mL aliquot of 0.1% Alcian blue soltion (w/v) containing 0.15M sucrose and 0.05M sodium acetata (pH=5.8) for 2 h at 30°C. The stomachs

were then washed twice in 0.25M sucrose solution and each was immersed in 10mL of 0.5M magnesium chloride for 2 h at 30^{0}C to elute the Alcian blue bound to the mucus. The solution was thoroughly shaken with 10mL of ether and the absorbance measured at 605 nm using a spectrophotometer. The result were expressed as mg Alcian blue/stomach.

The results were expressed as means ± SEM. Statistical analysis was made by Mann Whitney U-test and Kruskal Wallis test. The level of significance was set at $P<0.05$.

3. Results

In the vehicle treated rats prominent haemorrhagic lesions were evident in the glandular region of the stomach. The number and the length of these lesions are depicted in Table1. Further, in two rats treated orally with 15% PD erythromatous patches were also evident in between the lesions. The PD significantly ($P<0.05$) inhibited both the number (43%-90%) and length (46%-95%) of the lesions in both treatment regimens. (See Table 1) THe EC_{50} value for the inhibition of the unmber of lesions and the length of lesions were respectively 12% and 11% with oral treatment. Further, there was a significant inverse relationship between the concentration of PD and the number ($r^2=0.92$, $P<0.05$) and the length ($r^2=0.89$, $P<0.05$) of gastric lesions. 30% PD failed to significantly alter the volume (control vs treatment:3.73±0.72 vs 3.22±0.30 mL) the PH (Control us treatment:3.20±0.25 us 2.95±0.18), free acidity (control us treatment:0.057±0.006us 0.067±0.009 mol^{-h}) or total acidity (control vs treatment: 0.123±0.027 vs 0.120±0.015 mol^{-h}) of the gastric content. 30% PD caused a slight (16%) but non-significant reduction in peptic activity of the gastric contents (control vs treatment: 785.8±107.1 vs 660.0± 130.5 ml/kg). In contrast, 30% PD significantly increased both the mucus content [by 45%,$P<0.05$, (control vs treatment: 33.8±6.4 (range: 13.5-56.0) vs 61.6±0.43 (range: 50.5-74) mg/stomach)] and the carbohydrate content of the gastric juice [by 45% $P<0.05$, (control vs treatment: 0.17±0.03 (range:97.6-262.2) vs 0.31±0.46 (range:89.6-392.8) mg/dl)].

4. Discussion

The results of this study demonstrate that PD possesses potent gastroprotective activity (both in terms of number and length of haemorrhagic gastric lesions) when given orally or IP to rats. This gastroprotective action of the PD was dose-dependent and superior to some botanicals such as *Murraya kenigii* (7), *Jania sp.* (8), tested in rats using the ethanol-induced gastric lesion technique. This is a novel and an important finding. Firstly,

it provides scientific evidence justifying the recommendation of PD in the Ayurvedic system of medicine for the treatment of gastric ulcers. After all over 70% of the worlds (9) and about 90% of the Sri Lankan rural population (10) rely on traditional, predominately, herbal medicine for their primary health care. Secondly, the results show the potential of developing a pharmaceutical as a gastroprotective agent from PD: there is great demand for potent gastroprotective agents with lesser-side-effects for the treatment of gastric ulcers (11).

A gastroprotective agent can mediate its action via several potential mechanisms. Mild local irritant activity is one such mechanism as evident with chemical such as HCI, NaCI of NaOH (12). Such a mechanism seems unlikely to be operative here as PD was capable of inducing gastroprotection even when given IP. Further, the ability of PD to elicit gastroprotection both by oral and IP treatment suggest that gastric prostaglandins are not involved in the mediation of its action: mid irritants are usually gastroprotective via indigenous gastric prostaglandins(13).

An increase in gastric secretoary volume has been implicated with gastroprotection (14), possibly through dilution effects. However, in this study PD did not increase the quantity of gastric content in pyloric ligated rats. This mode of action in mediating gastroprotection therefore seems unlikely.

Some drugs such as cimetidine offer gastroprotection by inhibiting gastric acid secretion (15). However, in this study, PD failed to change the pH, basal acid secretion or total acidity of the gastric contents. Thus, this mechanism too is unlikely to be operative in offering gastroprotection.

An enhancement of peptic activity causes gastric lesions (6). The PD did not impair peptic activity. The gastroprotective action of PD therefore cannot be attributed to this candidate mechanism.

The gastric mucus coat is claimed to play an important role both in preventing damage to the gastric epithelium and in facilitating its repair (16). Drugs like sucralfate and carbenoxolone provide gastroprotection by stimulating the mucus production (16,17). PD caused a marked increase in gastric mucus (as revealed by Alcian blue technique) and carbohydrare (as judged by glucose estimation) contents. The gastroprotective action of PD can be attributed to these two mechanisms. Furthermore, the possibility also exists that PD may enhance the physical integrity of the gastric mucus layer by altering the sialyated moeities of molecules thereby providing an auxiliary mechanism for gastroprotection.

There are other putative mechanisms through which PD can initiate gastroprotection. These include, enhancement of gastric blood flow, alkaline secretion (see 15 and references there in), gastric nitric oxide production (18) and impairment of gastric histamine synthesis (14). Currently, we have no evidence which argues in favour or against these potential mechanisms.

Usually, Ayurvedic decoctions have several ingredients and PD contains five. However, it is not known whether the gastroprotective action is induced through a synergistic effect of all the five components or by a single component in PD: one preliminary study has shown that *Terminalia chebula, Terminalia belerica* and *Phyllenthus emblica*, collectively posses some gastroprotective action (19).

In conclusion, this study shows that PD has potent gastroprotective action as is claimed in one of the Ayurvedic treaties (1). This action is mediated through alterations in the thickness of the gastric mucus layer and its carbohydrate composition. Further, the results indicate that it may be worthwhile to initiate a clinical trial at the main Ayurvedic Hospital in Sri Lanka to investigate the therapeutic efficacy of PD for the treatment of gastric ulcers.

Table 1 : Effect of 1ml of different concentrations of Patoladi decoction (PD) on the number and length of ethanol-induced gastric lesions in rats (means±SEM: ranges in parenthesis)

Route of Administration	Treatment	n	Number of gastric lesions	Length of gastric lesions(mm)
	Vehical (DW)	6	9.0±0.792 (5-16)	81.0±7.21 (50-145)
Oral	30% (PD)	6	0.6±0.34*** (1-2)	3.8±2.6*** (8-15)
	15%(PD)	6	0.3±1.34*** (2-7)	22.8±10.7*** (5-64)
	7.5% (PD)	6	51.6±8.3** (3-9)	42.5±9.0** (24-72)
Intra Peritoneal	Vehical (DW)	6	40 (2-5)	34.0±3.2 (22-42)
	30% (PD)	6	0±0***	0±0***
	7.5% (PD)	6	2.0 (1-4)	8.5±3.7** (2-15)

As compared with control, treatment, P<0.01, P<0.001*****

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