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Prevention of Acute Gastric Mucosal Lesions by *R. hasseltii* in Rats

Noor, S.M., A.A. Mahmood, I. Salmah and K. Philip
Department of Molecular Medicine, Faculty of Medicine,
University Malaya 50603 Kuala Lumpur, Malaysia

Abstract: Cytoprotective effects are important in preventing the formation of gastric mucosa necrosis. *Rafflesia hasseltii*, used in traditional aboriginal Malay treatment of postpartum women, was evaluated for its cytoprotective effects. Both aqueous and ethanol extracts of the plant were evaluated to determine which extract offers better protection of the gastric mucosa in terms of inhibiting ulcer damage. Gastric ulcers were induced by oral administration of absolute ethanol (5mL kg^{-1}) to 4 groups of fasting rats: the ulcer control rats which received no pre-treatments (Group 1); rats pre-treated with oral aqueous extracts of *R. hasseltii* (Group 2); rats pre-treated with oral ethanolic extracts of *R. hasseltii* (Group 3) and rats pre-treated with oral cimetidine (Group 4). Oral pre-treatments were given 30 minutes prior to oral induction of ulcers by absolute alcohol. Animals in Group 1 experienced severe gastric damage following absolute ethanol administration, with absolutely no inhibition. Group 2 and Group 3 rats exhibited the highest degree of inhibition to ethanol-induced ulcer damage, by a percentage 99.2 and 98.9% inhibition, respectively. Animals in Group 4, the cimetidine group, had only 59.4% inhibition to ethanol-induced gastric damage. The results suggested that *R. hasseltii* possess novel cytoprotective activity on gastric mucosal cells and undeniably beneficial for treating gastric ulcer.

Key words: Cytoprotection, rate, *Rafflesia hasseltii*, cimetidine

INTRODUCTION

Rafflesia, a genus of 20 species, is notable for producing the largest flowers in the world, measuring up to 7 kg. These massive blooms smell of rotting flesh and attract carrion flies for pollination. As endophytes growing completely embedded within their hosts, Rafflesia lack leaves, stems and roots and emerge only to flower for sexual reproduction. These Southeast Asian endemic holoparasites rely exclusively on their host plants, namely the tetrastigma species of the grapevine family, Vitaceae, for all nutrients, including carbohydrates and water[1].

Rafflesia hasseltii flower buds, known locally as forest cabbage, are harvested by aborigines (the Orang Asli), who use the extracts to treat haemorrhoids and dysentery. A tea, prepared by boiling Rafflesia buds in water, is prepared for recuperating post-partum women so as to restore their health and strength[1,3].

Wiart *et al.*,[4], reported that *R. hasseltii* displays a broad spectrum of antibacterial activity against 4 micro organisms: Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus. However, there is currently little known research on the active compounds or properties of *R. hasseltii*. Its applications in traditional medicine suggest that there might be other uses, one of which could be as a cytoprotectant. It is postulated that *R. hasseltii* might show anti-ulcer properties besides its principal traditional use in postpartum treatment.

Gastric ulcers are a major cause of morbidity and mortality. Current evidence implicates Helicobacter pylori in the development of duodenal and gastric ulcers[5], but the underlying mechanisms of ulcerogenesis remain unclear. The gastric wall layers consist of the mucosa, muscularis mucosa and submucosa and mucosal defence against the acidity of the stomach include the secretion of mucus bicarbonate and the surface epithelium barrier itself. It is likely that a breakdown of normal mucosal defence mechanisms provides the opportunity for gastric acid and pepsin to damage susceptible gastric mucosa. Increasing mucosal protection would be one way of preventing ulcers.

Rats are commonly used as the animal-model for screening of compounds for effective cytoprotectants. Gastric ulcers are induced by oral administration of absolute ethanol[6,7], a necrotizing agent that rapidly promotes the formation of hyperemic lesions in the gastric mucosa, an acute inflammatory reaction wherein there is increased vascular permeability contributing to

Corresponding Author: Noor, S.M., Department of Molecular Medicine, Faculty of Medicine, University Malaya 50603 Kuala Lumpur, Malaysia

Table 1: Effects of *R. hasseltii* extracts on ethanol-induced gastric lesions in rats

Group	Pre-treatment	Oral dosage	Conc. mg/mL	Ulcer area (mm2) mean ± S.E.M)	Inhibition (%)
1	Distilled water (Control)	5 mL kg^{-1}	-	389.0±55.6	-
2	Alcoholic extract	5 mL kg^{-1}	400.00	4.17±1.05*	98.9
3	Aqueous extract	5 mL kg^{-1}	400.00	3.00±0.45**	99.2
4	Cimetidine	5 mL kg^{-1}	400.00	158.0±24.86***	59.4

*p<0.001 significant from control **p<0.001 significant from control and Group 2 ***p<0.001 significant from control, Group 2 and Group 3

haemorrhage. Plant compounds such as flavonoids, terpenoids and xanthines that are known for their anti-inflammatory activity have been shown to prevent gastric lesions induced by ethanol. The anti-inflammatory effects of these compounds have been attributed to their ability to scavenge oxygen free radicals

MATERIALS AND METHODS

R. hasseltii: A bud of *R. hasseltii* was collected from an Orang Asli village in Perak. Ethanol and aqueous extracts of *R. hasseltii* were prepared, based on previously established protocols.

Cimetidine: Cimetidine is a histamine H_2-receptor antagonist that effectively inhibits gastric acid production. Cimetidine used in this study was obtained from the University of Malaya Medical Centre (UMMC) pharmacy. The dose for each rat was 50 mg kg^{-1}.

Experimental animals and induction of ulcer: Twenty-four adult *Sprague-Dawley* female rats, which weighed 150-200 g. each, were obtained from the Animal House, Faculty of Medicine, University of Malaya. Rats were caged individually and fed standard food pellets supplemented with bottled water. Forty-eight hours to the ulcer experiments, the rats were assigned six to a group. The four study groups were: the positive control group (Group 1); group pre-treated with aqueous extracts of *R. hasseltii* (Group 2); group pre-treated with ethanol extracts of *R. hasseltii* (Group 3) and group pre-treated with cimetidine (Group 4). At the forty-eight hour mark, the rats were induced to fast by the removal of food from the cages, but the rats still had access to bottled water till up to two hours before the experiments. By the time the experiments commenced, the rats' stomachs would have been cleared of food and water.

Group 1 rats were given oral administration of 1.0 mL of distilled water; Group 2 rats were given oral administration of 1.0 mL of aqueous *R. hasseltii* extracts; Group 3 rats were orally administered with 1.0 mL of ethanol *R.hasseltii* extracts; Group 4 received 1.0 mL, orally, liquid cimetidine. After a 30-minute wait, rats in all four groups were given oral administration of absolute ethanol to induce gastric ulceration. Fifteen minutes later, the rats were sacrificed by overdoses diethyl ether gas and then dissected. The rats' stomachs were removed for gross and histology examinations.

Statstical analysis: Data are expressed as means±SEM. Statistical analysis were performed using a tow-tailed t-test and values of $p<0.05$ were regarded as indicating significant differences.

RESULTS

Macroscopical (gross) examination: Gross mucosal lesions were identified as haemorrhage or linear breaks (erosions), indicating damage to the mucosal surface, i.e. ulceration. Upon dissection, the stomach was sliced open to reveal the mucosal lesions, which could be observed macroscopically under a dissecting microscope (×20) with the aid of a square grid eyepiece (10×10 mm^2). The Ulcer Area (UA) in mm^2 was determined as the total sum of gastric lesions for each stomach in the group. The inhibition percentage was expressed as a percentage of the ulcer area in control group rats (Group 1) and was calculated by the following formula[8] Inhibition (%) = [(UA control-UA pre-treated group)/(UA control) × 100%

Histological examination: Histological observations were done on H and E stained stomach sections. The degree of damage varied according to the experimental groups, but generally, histological damage was seen in the form of necrosis of the mucosal layer, with exfoliation of mucosal cells and presence of red blood cells in the gastric mucosa, which was indicative of haemorrhagic lesions. The submucosa was oedematous and there was visible infiltration of polymorphonuclear leucocytes.

Table 1 summarises the outcome. Rats in the control group (Group 1) suffered very severe lesions, as seen in the photo of a representative dissected stomach (Fig. 1). The severity of the ulcer lesions were assuaged in rats that were pre-treated with *R. hasseltii* extracts and cimetidine, with *R. hasseltii*-treated rats showing a significantly higher degree of inhibition against ulcerogenesis compared to the degree of inhibition offered by cimetidine, as seen in Fig. 2.

Fig. 1: Macroscopic view of severe alcohol-induced necrosis of a rat's gastric mucosa. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in the control group

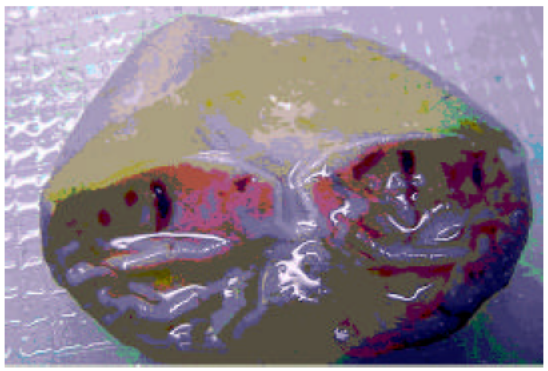

Fig. 2: Reduced necrosis of the gastric mucosa. Cytoprotection by *R. hasseltii* extracts, whereby there is visibly reduced formation of the gastric lesions induced by absolute ethanol

DISCUSSION

The results of the current study showed that both aqueous and alcoholic extract of *R. hasseltii* protect the rat gastric mucosa against hemorrhagic lesions produced by absolute ethanol compared with control. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Ethanol-induced gastric ulcer has been widely used for the experimental evaluation of anti-ulcer activity. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus[9]. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol[10]. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury[11].

Cimetidine offered 59.4% inhibition against the necrotic damage induced by absolute ethanol. However, *R. hasseltii* extracts were found to be significantly more effective in inhibiting ulcer formation. Ethanolic *R. hasseltii* extracts offered 98.9% inhibition, while aqueous *R. hasseltii* extracts provided 99.2% inhibition protection. This outcome is encouraging enough to suggest that *R. hasseltii* might indeed possess active compounds that make it a suitable cytoprotectant. It might be postulated at this early juncture that the *R. hasseltii* active compounds dissolve well in both alcohol and aqueous solutions. Traditional medical practice involves the making of tea by boiling *R. hasseltii* in water, but whether this has to do with any anecdotal increase in efficiency is not known.

The next step now is to decipher the cytoprotective mechanisms of *R. hasseltii* and in the meantime, continuing animal work could be done on determining the optimum doses, as well as a toxic dose or adverse effects.

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