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Eurycoma longifolia in Radix™ for the Treatment of Ethanol-induced Gastric Lesion in Rats

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Abstract: The effect of treatment with Radix™ on ethanol-induced gastric lesions was investigated. The main ingredient of Radix™ is *Eurycoma longifolia*. Twenty-four rats of the *Sprague-Dawley* species were randomly divided into four groups. Three groups were given 0.5 mL 100% ethanol orally. Another group was used as a control and was given only distilled water orally (control). After 6 h all the rats were fed with normal diet. One group that was administered with ethanol was only given distilled water orally (no treatment). Another two groups that were administered with ethanol were treated with oral Radix™ 0.128 mg g^{-1} b.wt. (Radix) and oral ranitidine 21.4 mg kg^{-1} b.wt. (Ranitidine), respectively. After one week, all the rats were fasted overnight and sacrificed. The stomach was isolated and examined for the presence and severity of gastric lesions. Measurements for malondialdehyde content and gastric acid concentration were also done. It is found that the ulcer index was lower in the Radix and ranitidine group compared to the no treatment group whereas in the control group there was no lesion. There was no difference in ulcer index between the Radix and ranitidine group. The gastric MDA content was significantly higher in all the groups that were induced with ethanol compared to the control group but no difference between all the ethanol-induced groups. There was no difference in the gastric acid concentration in all groups. Hence it is concluded that *Eurycoma longifolia* in Radix™ is as effective as ranitidine in the treatment of ethanol-induced gastric lesions in rats.

Key words: *Eurycoma longifolia*, ethanol, ulcer index, malondialdehyde, gastric acid

INTRODUCTION

About 80% of the population is still using traditional medicine to treat health problem (Diallo *et al*., 1996). Traditional medicine includes consuming herbs, plants, animals or minerals and also practices according to religious belief and norms. It is often termed alternative, complementary or non-conventional medicine. Radix™ is a product by HPA Industries from Malaysia. The main ingredient of Radix™ is *Eurycoma longifolia*, which is also known as Tongkat Ali, Long jack, pasak bumi, Piak or tung saw. Its usage is mainly focused on its aphrodisiac characteristic. Among the active components in *Eurycoma longifolia* are alkaloid, saponins, erycomanone and eurycomalactone which are able to stimulate testosterone production in male. Tada *et al*. (1991) revealed the presence of quassinoids in *Eurycoma longifolia* which has anti-ulcer effect. Ulcer is a break on an organ surface or tissue due to superficial tissue inflammation. Peptic ulcer is ulcer that occur at the gastrointestinal tract either stomach (gastric ulcer) and duodenum (duodenal ulcer).

The mechanism of ethanol-induced gastric lesion is complicated and multifactorial. Ethanol causes oedema, hyperemia especially to the superficial epithelial cells, venoconstriction, arteriol dilatation, necrosis and hemorrhage on gastric mucosal and submucosal surface (Dinoso *et al*., 1976; Guth *et al*., 1984). A few studies relate the lesion formation due to its free radicals and lipid peroxidation (Hernandez-Munoz *et al*., 2000; Pan *et al*., 2008; Salim, 1990). Malondialdehyde (MDA) is the end product of lipid peroxidation. Oxyradical produced during ischaemia-reperfusion causes severe changes at the cellular level. It causes cell death because it attacked the cell component which is essential such as nucleic acid, protein and lipid. The oxy-radical also stimulates the membrane lipid peroxidation process forming toxic products such as epoxide, aldehyde and new free radicals (Glavin and Szabo, 1992).

This study was carried out to determine the effects of *Eurycoma longifolia* in Radix™ for the treatment of ethanol-induced gastric ulcer in rats considering the

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importance and widespread usage of traditional and complementary medicine in the community.

MATERIALS AND METHODS

In this study, twenty-four rats of the *Sprague-Dawley* species (200-250 g) were randomly divided into four groups. Three groups were given 0.5 mL 100% ethanol by oral gavage. Another group was used as a control and was given only distilled water by oral gavages (control). After six hours all the rats were fed with normal rat diet. One group that was administered with ethanol was only given distilled water by oral gavage (no treatment). Another two groups that were administered with ethanol were treated with Radix™ 0.128 mg g^{-1} b.wt. diluted in distilled water (Radix) given by oral gavage and ranitidine 21.4 mg kg^{-1} b.wt. (Ranitidine) given by oral gavage, respectively. Food and water were given *ad libitum* throughout the experiment. After one week, all the rats were fasted overnight and sacrificed. The stomachs were isolated and examined for the presence and severity of gastric lesions. Measurements for MDA and gastric acid concentration were also done. This study was conducted in the Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia and has been approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee.

Measurement of gastric lesion: The lower end oesophagus and pylorus were clamped and the stomach was removed. It was then opened up and the gastric lesions was assessed using semi quantitative scale by Berry *et al.* (1988) which have been modified by Nafeeza *et al.* (1999) (Table 1).

Measurement of malondialdehyde: Gastric tissue MDA content was measured using a method modified by Ledwozyw *et al.* (1986). The gastric tissue was homogenised in distilled water, centrifuged and the diluted supernatant was added with trichloroacetic acid. After 15 min at room temperature, thiobarbituric acid was added and the samples were incubated in 100°C water bath for 30 min. After cooling, n-butanol was added and the absorbency of the upper phase was read.

Table 1: Gastric Lesion according to semi quantitative scale

Score	Lesion
5	Multiple hemorrhagic lesion throughout gaster
4	Lesion covers 80% of gastric fold
3	3 lesions 1-4 mm length over 80% of gastric fold
2	≥2 lesions of 2 mm length
1	1 lesion with generalised erythema
0.5	Dotted hemorrhage
0	No lesion

Source: Berry *et al.* (1988)

Measurement of gastric acid concentration: Samples of gastric juice were collected and centrifuged at 3000 rpm for 10 min. Aliquots of each sample were titrated with 0.01N NaOH to a pH of 7.0. The concentration of hydrogen ion was calculated as described by Shay *et al.* (1954).

Statistical analysis: All results were expressed as Mean±SEM. Statistical analysis was performed using SPSS version 12.0 and Excel by student t-test and two-way ANOVA. A p-value of less than 0.05 was considered statistically significant for all parameters.

RESULTS

Measurement of gastric lesion: The ulcer index was significantly lower in the Radix (0.17±0.11) ($p = 0.041$) and ranitidine group (0.08±0.08) ($p = 0.013$) compared to no treatment (0.67±0.11) group. There was no lesion in the control group. There was no difference in ulcer index between the Radix and ranitidine group (Fig. 1).

Measurement of malondialdehyde (MDA): The gastric MDA content was significantly higher in all the groups that were induced with ethanol i.e., no treatment (0.3273 nmol mg^{-1} protein±0.04), Radix (0.4107 nmol mg^{-1} protein±0.07) and ranitidine (0.3341 nmol mg^{-1} protein±0.02) compared to the control group (0.1776 nmol mg^{-1} protein±0.02). There was no difference in the gastric tissue MDA concentration between all the ethanol-induced groups (Fig. 2).

Measurement of gastric acid concentration: There was no significant difference in the gastric acid concentration

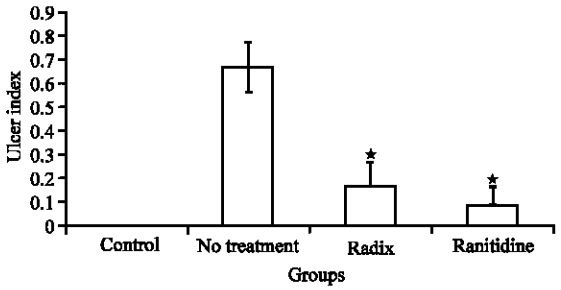

Fig. 1: Effects of Radix™ on gastric ulcer index after one week of treatment, The data is expressed as Mean±S.E.M (n = 6), *$p<0.05$ compared to control

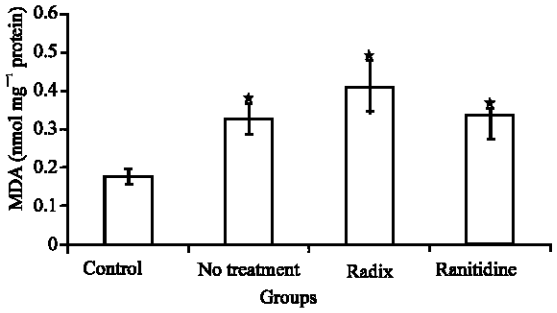

Fig. 2: Effects of Radix™ on gastric tissue MDA, content after one week of treatment. The data is expressed as Mean±SEM (n = 6). *p<0.05 compared to control

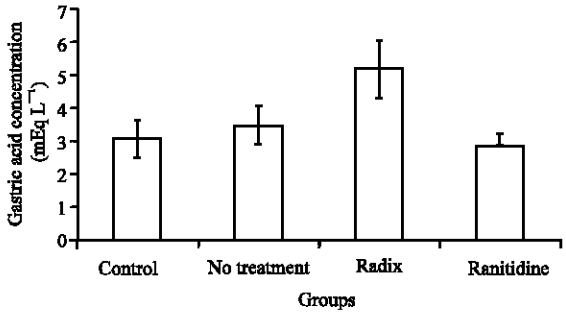

Fig. 3: Effects of Radix™ on gastric acid concentration after one week of treatment, The data is expressed as Mean±SEM (n = 6)

among all the groups i.e., control group (3.073 mEq L^{-1} ±0.56), no treatment (3.47 mEq L^{-1}±0.59), Radix (5.1717 mEq L^{-1}±0.91) and ranitidine (2.832 mEq L^{-1}±0.39) (Fig. 3).

DISCUSSION

This study showed that Radix™ was able to reduce 75% of the gastric ulcer index compared with no treatment group. Radix™ is as good as ranitidine in the treatment of ethanol-induce gastric lesion as there is no significant difference between the ulcer index in the Radix and ranitidine group.

In this current study, we found that MDA was increased after challenged with ethanol. Elevated gastric MDA reflects an intensification of lipid peroxidation process and further prove the involvement of free radical in ethanol-induced gastric lesions. Antioxidant such as *Eurycoma longifolia* used in this study is expected to retard lipid peroxidation process but this was not the case in this study. Amongst the factors causing the lack of antioxidant effects of *Eurycoma longifolia* could be the dose of ethanol used. If, in fact the dose of ethanol used is high and ethanol increases the production of free radical, it is highly possible that the amount of *Eurycoma longifolia* in Radix™ used is insufficient to scavenge the excessive free radical. Even with no reduction in the lipid peroxidation process, Radix™ is able to reduce the gastric lesion; most probably the mechanism is not via its antioxidant properties.

This study showed that 100% ethanol did not increase gastric acid concentration. Previous study also showed that ethanol at higher concentration has either no effect or mildly inhibits gastric acid concentration whereas lower concentration up to 5% stimulates gastric acid secretion (Chari *et al*., 1993). We found that Radix™ has no effect on gastric acid concentration. No study has shown the effect of *Eurycoma longifolia* on gastric acid. It could be that *Eurycoma longifolia* in Radix™ has no effect on gastric acid. Ranitidine is a competitive H_2 histamine receptor antagonist to parietal cell. As expected it has no effect on the gastric tissue MDA content. Dose used in this study is according to the oral dose given to human (4.3 mg kg^{-1} day^{-1}). Ranitidine inhibits basal, nocturnal and food stimulated acid secretion depending on the dose given (Segawa *et al*., 1991; Yeomans, 2000). Study done by Segawa *et al*. (1991) using ranitidine 100 mg kg^{-1} day^{-1} subcutaneously was able to inhibit basal gastric acid secretions. This study showed that ranitidine was unable to inhibit gastric acid secretion. It could be that one week duration is not enough to reduce gastric acid significantly. Previous study done by Jaarin *et al*. (1999) reported that after one week of treatment there is no reduction of gastric acid but the reduction occurred after three weeks of treatment with ranitidine. If the dose is increased or use a longer treatment period with ranitidine, there could be a reduction in the gastric acid.

In conclusion, *Eurycoma longifolia* in Radix™ given as oral gavages at 0.128 mg g^{-1} b.wt. was able to treat ethanol-induced gastric lesions in rats. The mechanism for the ulcer healing is not through reduction of the MDA or gastric acid. There could be other mechanism for the ulcer healing such as increased in gastric blood flow, gastric mucous production or prevention in prostaglandin inhibition. Further study needs to be carried out to determine its mechanism for ulcer healing and further assess its potential as an anti-ulcer agent.

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