THE EFFECTS OF ACUTE SUPPLEMENTATION OF EURYCOMA LONGIFOLIA (TONGKAT ALI) ON THE PREVENTION OF ETHANOL-INDUCED GASTRIC ULCER IN RATS

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Article History: **Received:** 24.05.2022 **Revised:** 19.08.2022 **Accepted:** 09.10.2022

Abstract: This study was done to investigate the prevention of ethanol-induced ulcer of Eurycoma longifolia (E. Longifolia). E. longifolia aqueous extract was obtained from a commercialised product (Nu-Prep Lelaki). A total of 36 rats (n=6 for each group) which were separated into 6 groups were used in the present study. All rats were fasted from food for 24 hours prior to the pre-treatment. For pre-treatment, Group 1 (normal group) and Group 2 (negative control group) received 0.9% normal saline, Group 3 (positive control group) received 150 mg of ranitidine, Group 4 (low dose group), Group 5 (intermediate dose group) and Group 6 (high dose group) each received 250 mg/kg, 500mg/kg and 1000mg/kg of E. longifolia respectively. Rats from Group 2, Group 3, Group 4, Group 5 and Group 6 were induced with ulcers by administration of 5 ml/kg of absolute ethanol an hour after the pre-treatment. The rats were euthanized an hour after ulcer induction. The stomachs were weighed and the degree of ulcerations was scored. The mean ulcer score for Group 2 was found to be the highest (3.83) followed by Group 4 (1.83), Group 3 (1.5), Group 5 (0.83), Group 6 (0.33) and Group 1 (0). The mean ulcer score of Group 2 is significantly higher ($p<0.05$) when compared to the mean ulcer score of other groups. Mean ulcer score of stomachs of rats from Group 5 and 6 scored lower when compared to Group 3 and this suggests that Group 6 with the highest dose is the most effective dose. The relative organ weight (ROW) of Group 2 was the highest (0.75%) and is significantly higher ($p<0.05$) when compared to other groups. This is followed by Group 1 (0.64%), Group 4 (0.59%), Group 3 and Group 6 scores the same (0.58%) and the lowest is Group 5 (0.56%). In conclusion, E. longifolia extract can be used to prevent gastric ulcers and is comparable to ranitidine. However, further studies should be done to elucidate the effects especially on histological evidence.

Keywords: Eurycoma longifolia, E. Longifolia, gastric ulcer, peptic ulcer, ethanol-induced ulcer, anti-ulcer effects, protective effects against ulcer

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DOI: 10.31838/ecb/2022.11.10.004

INTRODUCTION

Peptic Ulcer

Peptic ulcer disease refers to the ulceration of the stomach lining (gastric ulcer) or the first part of the small intestine (duodenal ulcer) as defined by Ratini (2014). Advanced peptic ulcer may cause upper gastrointestinal (GI) bleeding. A study by Müller et al., (2009) reported that mortality from upper GI bleeding was higher among inpatients when compared to outpatients. Symptoms of ulcer include nausea, vomiting, heartburn, bloating and burning pain in the middle or upper part of the stomach between meals or at night (Ratini, 2014). However, in severe cases, patients may lose weight, vomit blood or have dark black stools due to bleeding of the ulcer.

The healing of gastric ulcers depend on the duration of therapy whereas for duodenal ulcer, the healing depends on the degree and length of inhibition of gastric secretion (Deakin & Williams, 1992). According to Laursen et al. (2014) in the National Consensus on Management of Peptic Ulcer Bleeding, blood transfusion is recommended for haemodynamically stable patients without any serious ischaemic disease. Patient should undergo endoscopy within 24 hours of bleeding which will reduce surgery rate, bleeding rate and duration of inpatient stay. If the patient is suspected to have serious ulcer bleeding with blood in their gastric aspirations, endoscopy should be done within 12 hours. This may reduce the need of blood transfusions and decrease length of hospitalization. Surgery is only needed if complications such as perforation of the GI lining or severe bleeding develops (Kenny, 2012).

The use of H2-receptor antagonist blocks the action of histamine at all H2-receptors and its aim is mainly to stop the gastric acid secretion. It is also particularly effective against nocturnal acid secretion (Clark et al., 2012). This group of drugs reduces the intracellular concentrations of cyclic adenosine monophosphate by blocking the binding of histamine to the H2-receptors competitively, thus, reducing the secretion of gastric acid. However, the action is fully reversible. According to

Harrison (2014), a study done in a medical surgical intensive care unit (ICU) showed that of 8562 patients, 57% received an H2 receptor antagonist whilst the remaining received a proton pump inhibitor for stress ulcer prophylaxis. A study done by Konturek et al. (1983) showed that oral administration of prostaglandin E2 and ranitidine both have a protective action against aspirin-induced gastric micro bleeding in eight healthy subjects.

Eurycoma longifolia

In Malaysia, there are extensive use of herbal medicines among adults. Aziz & Tey (2009) reported that about 33.9% out of 1601 adult respondents claimed to have used herbal medicine in the 12 months. The famous herbal medicines includes Eurycoma longifolia (E. longifolia) Jack which is also known as Pasak Bumi in Indonesia and Tongkat Ali in Malaysia. This herbal plant originates from South-East Asia. E. longifolia is a shrub plant from the family of Simaroubacceae. It can grow up to 15 m in height on sandy soil (Kumar et al., 2014).

E. longifolia is most known for its aphrodisiac properties (Aziz & Tey, 2009). A significant increase in sperm concentration and the percentage of sperm with normal morphology was reported after consuming water soluble extract of E. longifolia Jack (Tambi & Imran, 2010). E. longifolia was also reported to increase the sexual performance of sluggish rats after acute and sub-acute oral administration of the root powder. The effect was ascribed to the increased testosterone levels (Zanoli et al., 2009). Furthermore, according to a study done by George et al., (2013), a 12 week supplementation of freeze-dried water extract Physta® (E. longifolia) improved the muscle strength of men between the age of 30-55 years old, but it is not classified as a doping violation in sports as the ratio of testosterone to epitestosterone remained unchanged from the baseline. This is the reason why E. longifolia is favoured by athletes, as it is safe to use in sports according to the World Anti-Doping Agency (WADA).

E. longifolia was also found to have antiulcer properties. A study done by Qodriyah & Asmadi (2013) reported that there was no difference in ulcer index reported between the groups treated with Radix coffee containing E. longifolia as compared to the group treated with ranitidine which served as the positive control. This led to the conclusion that Radix coffee which contains E. longifolia is as effective as ranitidine in treating ethanol-induced gastric lesions in rats. However, there are lack of studies on the antiulcer properties of E. longifolia of different solvents.

E. longifolia is widely used especially by men for its aphrodisiac properties. As reported by Jamia (2006), E. longifolia is amongst the most popular Malaysian plants that is subjected to extensive research. Currently, there is only limited number of studies done on the antiulcer properties of E. longifolia. Thus, this study was done to evaluate the potential benefits of the plant on gastric ulcer in rats.

MATERIALS AND METHOD

Experimental Animals

36 healthy adult Sprague-Dawley rats of mixed gender weighing 230 ± 50 g were used in the present study. The rats were classified into 6 groups as shown in Table 2.1. The rats were housed in plastic cages in the Animal House, University of Cyberjaya (Selangor, Malaysia). The temperature of the cabin was controlled temperature at 23 ± 0.5°C. The animals were randomly divided into 6 groups. The rats were supplied with food chow and water ad libitum during acclimatization period of 7 days.

Table 2.1. Grouping of the rats and the treatments based on the dose of the treatment of the rats received.

Group	Indication	Number (n)	Treatment	Ulcer induction
1	Normal	6	10 mg/kg b.w of 0.9% Normal saline	No
2	Negative control	6	10 mg/kg b.w of 0.9% Normal saline	Yes
3	Positive control	6	150 mg/kg b.w of ranitidine	Yes
4	Low dose of *E. longifolia*	6	250 mg/kg b.w of *E. longifolia*	Yes
5	Intermediate dose of *E. longifolia*	6	500 mg/kg b.w of *E. longifolia*	Yes
6	High dose of *E. longifolia*	6	1000 mg/kg b.w of *E. longifolia*	Yes

Preparations of aqueous extract of Eurycoma longifolia

The extract of E. longifolia used in this study was obtained from a commercial product; Nu-Prep Lelaki (Batch number: NE 140313) as shown in Figure 2.1. The product was manufactured by Phytes Biotech Sdn Bhd. This product was purchased from Guardian, Shaftsbury, Cyberjaya. Each 350 mg capsule contained 100 mg of E. longifolia extract. To prepare the aqueous extract of E. longifolia, the vegetative capsules were first cut to obtain the root extracts of E. longifolia powders. The powders were then weighed according to each rat's body weight prior to the force feeding procedure. Due to the hygroscopic effect of the powder, the aqueous extracts of E. longifolia were freshly prepared prior to the force feeding procedure. The powders were diluted in 10 ml of normal saline for every 1 kg of the rat's weight. Doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg were prepared accordingly for Group 4, Group 5 and Group 6 respectively.

Figure 2.1: Commercial product containing E. longifolia extract, Nu-prep Lelaki.

Ethanol-Induced Gastric Ulceration

The ulcer induction was performed 1 hour after the pre-treatment procedure and it was done through administration of absolute ethanol (99.75%). In the present study, the normal group rats (Group 1) received 10 ml/kg of normal saline as a replacement for the absolute ethanol while the other groups received 5 ml/kg of absolute ethanol each. The procedure and the use of absolute ethanol to induce gastric ulcer was according to De Pasquale et al. (1995). After the ulcer induction, the

animals were kept in their respective cages.

Animal Culling and Macroscopic Inspection

After 1 hour of absolute ethanol administration, the rats were first given a mixture of the ketamine (100mg/kg) and xylazine (10mg/kg) via intraperitoneal injection until the rats were fully sedated. When the rats were fully sedated, the rat was placed on its back on a dissection tray. The legs of the rats were laterally spread and were secured to the dissection board with pins poked down on the paws. The tip of the scissor was then inserted between the skin and the underlying tissue at the opening of skin at the throat. A midventral incision was made through the skin extending to the genital region. As the skin was cut through, it was pulled away from the underlying muscles. This could prevent cutting into the underlying muscles. Then, another incision was made through the skin around the genital and the base of the tail down the lateral side of the limb. The resulting flaps of tissue was removed or pinned to the lateral site. Fascia was first washed with normal saline and the adipose was then removed. Stomach was washed with 0.9 % normal saline, collected and weighed. Using a magnifying glass (with magnification 10X), the physical appearance of the stomach was carefully examined and ulcer was properly observed and scored. Picture were also taken for each rat.

Gross inspection of the stomach

To inspect the internal part of the stomach, an incision was made along the greater curvature to empty the stomach contents. Stomach weights were taken and recorded according to formula in Figure 2.2.

It was followed by placing the stomach firmly and examined using a magnifying glass (with 10X magnification). Ulcer pit were observed along the glandular region and the number of ulcers was recorded.

$$\text{ROW} = \frac{organ\ weight\ (g)}{body\ weight\ before\ culling(g)} \times 100$$

Figure 2.2.

Ulcer Scoring

During the ulcer scoring process, the stomachs were macroscopically inspected for the ulcer severity. Each stomach was scored based on the number and the length of the ulcers using the ulcer scoring as outlined by Huligol et al. (2012). The ulcer scoring is as shown in Table 2.2. Pictures of the stomachs were taken for each rat and the stomachs were kept in 10% formalin in an air-tight container for further studies.

Table Error! No text of specified style in document..**1.** Ulcer scoring based on Huligol et al. (2012).

Scoring	Description
0	No lesions
1	Haemorrhagic suffusions
2	1-5 small ulcers of 3 mm size
3	1 ulcer of more than 3 mm size
4	Many ulcers of more than 3 mm size
5	Perforated ulcer

Statistical Analysis

Statistical analysis was done by using Statistic Package for Social Sciences Programmes (SPSS) version 20.0. The results for the mean ROW and mean ulcer score in this study was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Significant difference between means was determine at 95% significant correlation ($p<0.05$).

RESULTS & DISCUSSION

Relative Organ Weight

The relative organ weight (ROW) was calculated based on the Equation in Figure 2.2. In the present study, it was found that the mean ROW of stomach in the normal, negative control, positive control, low dose of E. longifolia, intermediate dose of E. longifolia and high dose of E. longifolia group rats were 0.64±0.05%, 0.75±0.09%, 0.58±0.05%, 0.59±0.05%, 0.56±0.05% and 0.58±0.06% respectively as shown in Figure 3.1 below.

The mean ROW of stomach of the negative control group rats was found to be the highest (0.75%) as compared to the other groups whilst the intermediate dose of E. longifolia group rats had the lowest mean ROW of stomach (0.56%) as shown in Figure 3.1. The mean ROW of the negative control group rats showed a significant different ($p<0.05$) when compared to that of the mean ROW of other groups. When comparing the mean ROW of stomach between the low dose of E. longifolia, intermediate dose of E. longifolia and the high dose of E. longifolia groups, it was found that the intermediate dose of E. Longifolia group had the highest mean ROW and the low dose of E. Longifolia group had the lowest mean ROW (0.59%).

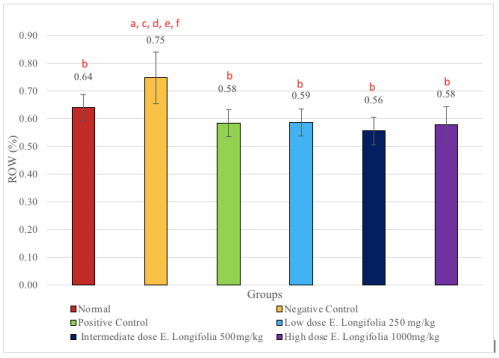

Figure 3.1: Mean relative organ weight of all group rats. a: $p<0.05$ with normal; b: $p<0.05$ with negative control; c: $p<0.05$ with positive control; d: $p<0.05$ with low dose of E. longifolia (250mg/kg); e: $p<0.05$ with intermediate dose of E. longifolia (500mg/kg); f: $p<0.05$ with high dose of E. longifolia (1000mg/kg).

ROW of all group rats was calculated in order to remove bias due to different body weight of each rats (Stevens, 1976). Administration of ethanol has been shown to induce ulcer in many studies (Khare et al., 2008; Motawi et al., 2012; Qodriyah & Asmadi, 2013). A study by Chandra et al. (2012) found out that administration of absolute alcohol to rats without any pre-treatment resulted in ulceration of the mucosal cells with some inflammatory changes and necrosis. Study done by Ramakrishnan & Salinas (2007) also stated that inflammation causes water retention in the tissue and this causes swelling. The swollen tissue weighs more than that of the normal tissues. This proves why the negative control group which was pre-treated with only normal saline prior to ulcer induction, has the highest mean ROW as shown in Figure 3.1. Varghese et al.

(2013) stated that E. longifolia possess anti-inflammatory properties and this could be the reason why the mean ROW of all group rats treated with the E. longifolia had a lower mean ROW when compared to that of the normal group. This result is consistent with the findings by Hummadi et al.. (1999) where it was found that the stomach weight increased in the ulcerated (negative control) group.

Macroscopic Appearance of Stomach

In the normal group, all rats had normal appearance of stomach with no perforation or haemorrhagic ulcer as shown in Figure 3.2. All stomachs were graded as 0. In the negative control group rats, all rats showed presence of ulcers with more than 3 mm in size. Five out of 6 (83.33%) rats were graded as 4 while 1 out of 6 (16.66%) rats were graded 3. Figure 3.3 shows the macroscopic appearance of all stomachs in the negative control rats. In the positive control group rats, 2 out of 6 (33.33%) rats showed no perforation or haemorrhagic ulcer, 3 out of 6 (50%) rats showed 1-5 ulcers of 3 mm in size and 1 out of 6 (16.66%) rats showed ulcer more that is more than 3 mm in size. Figure 3.4 shows the macroscopic appearance of all stomachs in positive control rats. In the low dose of E. longifolia group, 2 out of 6 (33.33%) rats showed no perforation or haemorrhagic ulcer. Two out of 6 (33.33%) rats showed ulcers with 3 mm size and were graded as 2. The remaining 2 (33.33%) rats were graded as 3 and 4 respectively. Figure 3.5 shows the macroscopic appearance of all stomachs in the low dose of E. longifolia group rats. In the intermediate dose of E. longifolia group rats, 4 out of 6 (66.66%) rats had normal appearance of stomach with no perforation or haemorrhagic ulcer and were graded as 0. One out of 6 (16.66%) were scored 2 with small ulcers of 3 mm in size and the remaining rats were scored 3 with an ulcer more than 3 mm in size when observed. Figure 3.6 shows the macroscopic appearance of all stomachs in the intermediate dose of E. longifolia group rats. In the high dose of E. longifolia, 5 out of 6 (83.33%) rats were scored 0 with no perforation or haemorrhagic ulcer observed. Only 1 out of 6 (16.66%) was scored 2 with small ulcers of 3 mm in size. Figure 3.7 shows the macroscopic appearance of all stomachs in the high dose of E. longifolia group rats.

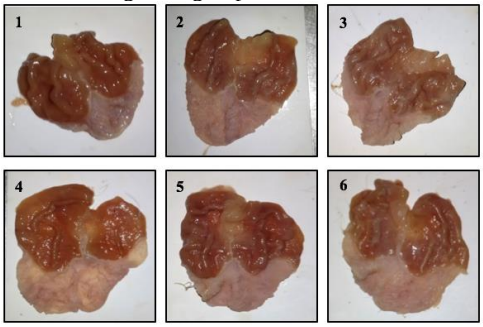

Figure 3.2: Macroscopic appearance of stomachs of Group 1, pre-treated with normal saline without ulcer induction.

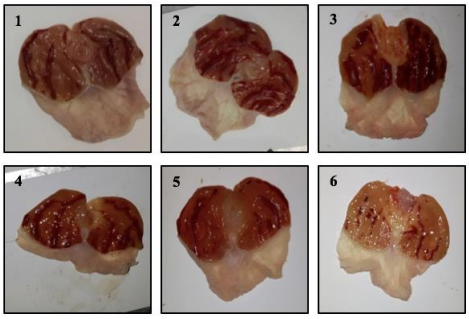

Figure 3.3: Macroscopic appearance of stomachs of Group 2, pre-treated with normal saline with ulcer induction.

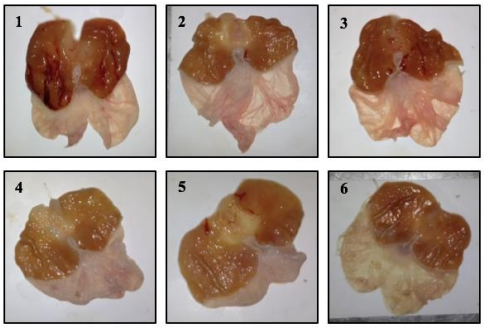

Figure 3.4: Macroscopic appearance of stomachs of Group 3, pre-treated with ranitidine with ulcer induction.

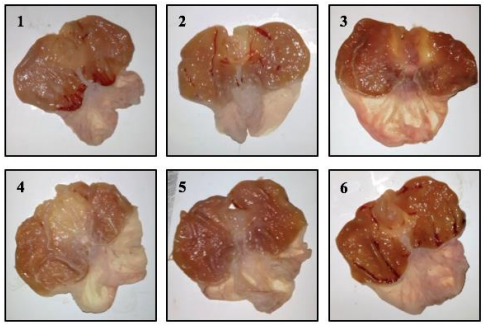

Figure 3.5: Macroscopic appearance of stomachs of Group 4, pre-treated with low dose of E. Longifolia (250mg/kg) with ulcer induction.

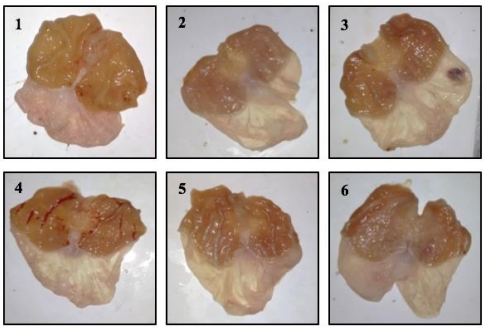

Figure 3.6: Macroscopic appearance of stomachs of Group 5, pre-treated with intermediate dose of E.

Longifolia (500 mg/kg) with ulcer induction.

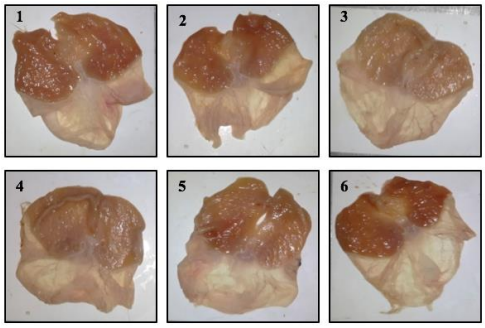

Figure 3.7: Macroscopic appearance of stomachs of Group 6, pre-treated with high dose of E. Longifolia (1000 mg/kg) with ulcer induction.

Scoring of Ulcer

The scoring of ulcer was given based on the scoring outlined in 2.11. Figure 3.8 shows a bar chart of mean ulcer score in all group rats. The mean ulcer score for the normal, negative control, positive control, low dose of E. longifolia (250 mg/kg), intermediate dose of E. longifolia (500 mg/kg) and high dose of E. longifolia (1000 mg/kg) group rats were 0.00±0, 3.83±0.4, 1.50±1.2, 1.83±1.6, 0.83±1.3 and 0.33±0.8 respectively.

Figure 3.7 shows that the mean ulcer score in the negative control group was the highest among all groups and the mean ulcer score in the negative control group is significantly higher compared to the other groups. The mean ulcer score of the normal group was the lowest among all groups. When comparing the mean ulcer score of the low dose, intermediate dose and high dose of E. longifolia, it was found that the high dose of E. longifolia had the lowest mean ulcer score.

Ethanol was used for ulcer induction in the present study as ethanol has been used to induce ulcer in many previous studies (Khare et al., 2008; Motawi et al., 2012; Qodriyah & Asmadi, 2013). It was stated by Bode & Bode (1997) that alcohol abuse is one of the major cause of haemorrhagic gastric lesions which destroys the mucosal lining of the stomach. Depending on the alcohol concentration, beverages with more than 15% of alcohol concentration was found to inhibit gastric motility and delays the stomach emptying process. This may cause longer exposure of the ethanol administered to the rat's stomach lining and thus causing gastric ulcer in the rats. Jamal et al. (2006) stated that the ethanol causes protein precipitation of the cytoplasmatic components in the superficial cells of the stomach mucosa. This reaction releases components of vasoactive mediators such as leukotrienes C4 (LTC4) and histamine.

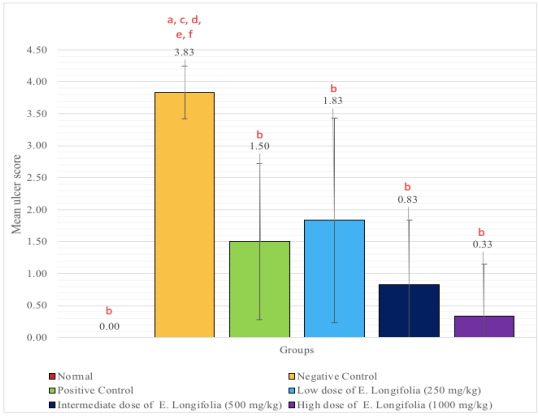

Figure 3.8: Mean ulcer score of all rats group. a: p<0.05 with normal; b: p<0.05 with negative control; c: p<0.05 with positive control; d: p<0.05 with low dose of E. longifolia (250mg/kg); e: p<0.05 with intermediate dose of E. longifolia (500mg/kg); f: p<0.05 with high dose of E. longifolia (1000mg/kg).

The LTC4 formation begins as early as 30 seconds, maximal within 5 minutes and returned to normal level after 3 hours (Peskar, 1991). As shown in Figure 3.8, the normal group had mean ulcer score of 0 and it was proven that the 24 hours duration of fasting does not cause any perforation or haemorrhagic ulcer. A study done by Pillai et al. (2010) also proves that water itself does not cause lesion on the stomach with 0 ulcer index and no ulcerated surface.

Ranitidine, a H_2 receptor antagonist works by inhibiting histamine. Jamal et al. (2006) stated that administration of ethanol releases histamine, thus, the use of Ranitidine in this current study prevented gastric acid secretion and lowered the incidence of lesions. The mean ulcer score of the positive control was found to be lower than the mean ulcer score of low dose of E. longifolia. However, the mean ulcer score of the positive control group was found to be higher than the mean ulcer score of the intermediate dose of E. longifolia and high dose of E. longifolia as shown in Figure 3.8. Ethanol-induced ulcers are not inhibited by antisecretory agents but is inhibited by agents that enhance the mucosal defensive factors such as prostaglandins (Morimoto et al., 1991). Thus this explains why stomachs of rats in the positive control group were ulcerated even when pre-treated with Ranitidine.

The trend of decreasing in mean ulcer score as concentration increases seen in the present study, are consistent with previous studies done in preventing ulcers (Akuodor et al., 2010; Dhanaraj & Jegadeesan, 2013). However, the results found in this present study are not consistent with a study done by Adeniyi & Olowookorun (1989) whereby it was observed that in rats where testosterone synthesis was inhibited, the gastric acid secretion reduces. The study showed that decrease in testosterone would decrease the risk of lesion in the stomach. Although E. longifolia which is widely known to increase testosterone (Tambi et al., 2012), it did not worsen the lesions but instead the intermediate dose and high dose of E. longifolia had a lower mean ulcer score when compared to the positive control group. It is therefore postulated that E. longifolia has chemical constituents that prevents the severity of ulcer progression.

A bioassay study done by Tada et al., (1991) found out that E. longifolia contains quassionoids such as Pasakbumin A (eurycomanone) and Pasakbumin B and that both exhibit potent antiulcer activity. The mechanism of action on the antiulcer properties of E. longifolia are still unknown. Varghese et al. (2013) has proven that the extract of E. longifolia root possess the anti-inflammatory properties and that it is proven that E. longifolia prevented and decreased the formation of ulcer indulced by ethanol.

LIMITATION OF STUDY

A further consideration that can be made is to compare the effectiveness of the commercialised product containing E. longifolia against the fresh extract of E. longifolia that is done in a lab. This is because there are many factors in the extraction process that can affect the performance of the extracts. Such factors are temperature, type of extraction solvents and the extraction time. All these factors will affect the amount and type of chemicals that are obtained, and thus will affect the performance of the extract.

CONCLUSION

This study was able to demonstrate the antiulcer properties of E. longifolia tested in prevention of ethanol-induced ulcer in rats. A 500mg/kg and 1000mg/kg dose of E. Longifolia was proven to be as effective as ranitidine in prevention of ethanol-induced-ulcer. All the groups treated with E. longifolia had a significantly lower mean ulcer score and mean ROW as compared to that of the negative control group and that the mean ulcer score and mean ROW in the E. longifolia group were incomparable to that of the positive control group which was given ranitidine as the pre-treatment.

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