THE EFFECTS OF ACUTE SUPPLEMENTATION OF EVENING PRIMROSE OIL ON THE PREVENTION OF ETHANOL-INDUCED GASTRIC ULCER IN RATS

Masro Mohamad[a]*, Julian Ajeng[b]

Article History: **Received:** 22.05.2022 **Revised:** 18.08.2022 **Accepted:** 08.10.2022

Abstract: The present study aimed to evaluate the potential of Evening Primrose Oil (EPO) as an anti-ulcer agent following ethanol-induced gastric ulcer in rats. Thirty adult Sprague Dawley rats aged 8 weeks and weighed 250 ± 50 g were divided into 5 treatment groups with 6 rats in each group. All group rats were pre-treated with 0.9 % normal saline (Group 1), 200 mg / kg omeprazole (Group 2), 100 mg / kg EPO (Group 3), 500 mg / kg EPO (Group 4) and 1000 mg / kg EPO (Group 5). One hour after the pre-treatment, absolute ethanol (99.7%) was injected to induce gastric ulcer in all rats. All rats were culled and macroscopic examination was done on the stomach 1-hour post-ethanol injection. The stomach was weighed, examined using a magnifying glass and scoring of ulcer was given. There was no significant difference in the relative organ weight (ROW) of stomach observed in all group rats. Group 1 (0.9% normal saline) rats had the highest ulcer score with presence of ulcers and hemorrhagic streak which was not evidenced in all other group rats. This indicates the successful induction of gastric ulcer in the negative control group (Group 1). Group 2 (200 mg / kg omeprazole) and Group 5 (1000 mg / kg EPO) rats showed the lowest in the ulcer score indicating their anti-ulcer and protective effects against ulcer. This was also evidenced in all macroscopic examination. Group 2 (200 mg / kg omeprazole) and Group 5 (500 mg / kg EPO) rats also had the lowest ulcer index (risk of ulceration) and the highest percentage ulcer protection amongst all group rats. This indicates that EPO treatment at high dose (1000 mg / kg) is comparable to the standard treatment for gastric ulcer (omeprazole). In conclusion, this study shows that EPO treatment at high dose could reduce the risk of gastric ulcer if taken as a pre-treatment alternative medication and that EPO at high dose could be an alternative to the standard treatment used in the gastric ulceration. Further study has to be undertaken to evaluate the potential of EPO at high dose as anti-ulcer supplement.

Keywords: Evening primrose oil, gastric ulcer, peptic ulcer, ethanol-induced ulcer, anti-ulcer effects, protective effects against ulcer

[a]. Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Selangor, Malaysia
[b]. Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Selangor, Malaysia

***Corresponding Author**

E-mail: masro@cyberjaya.edu.my

DOI: 10.31838/ecb/2022.11.10.003

INTRODUCTION

Gastric Ulcer

Peptic ulcer is the sore on the lining on the gastrointestinal tract. The most common ulcer are the gastric ulcer and duodenal ulcer. Peptic ulcer is caused by the imbalance between the aggressive factors such as hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species and defensive factors (Leslie et al., 1972). The defensive factors are the function of the mucus bicarbonate barrier, prostaglandins (PG), mucosal blood flow, cell renewal, migration and some growth factors. There are other factors implicated in the pathogenesis of peptic ulcer. This includes sedentary life style, alcohol intake, spicy food, drugs and bacterial infections, moreover, there are other endogenous substance implicated in the ulcer formation (Robert et al 1990). The more important ones include bacteria infection, drugs, neuropeptides, chemicals, inflammatory substance and reactive oxygen species. Oxidative stress is one of the most important factors in ulcer formation. Oxidative stress might cause organelles damage include the mitochondria, lysosome and other organelles.

Ethanol is a 2-carbon compound with the chemical formula C_2H_5OH. It is an alcohol. It is a by-product of yeast fermentation. It can also be found in overripe fruit. Ethanol is produced by the symbiotic yeast can be found in Bertam Palm blossom. In the human body, ethanol can be removed via oxidation by alcohol dehydrogenase. The mechanism of ethanol-induced lesion is varied, inclusive of depletion of gastric acid content, damaged mucosal blood flow and mucosal cell injury. It has been documented that ethanol can causes severe damage to the gastrointestinal mucosal (Scab et al., 1998). It starts with microvascular injury results in increased vascular injury, enema formation and epithelial lifting. According to Scab et al. (1998), after the intragastric administration of the ethanol, a rapid and time-dependent release of endothelin-1 into the systemic circulation preceded the development of haemorrhagic mucosal erosions by vasoconstrictions. Besides, by having to decrease the secretion

of bicarbonate and mucus production which are protective agents, ethanol can produce necrotic lesion in the gastric mucosal. Furthermore, ethanol has been found to be able to activate TNF-α and mitogen activated protein kinase (MAPK). Apart from that, ethanol is able to initiate apoptosis which lead to cell death. Ethanol metabolism is able to release superoxide anion and hydroperoxyl free radical which can lead to the lipid peroxidation. Increased in the lipid peroxide content and oxygen derived free radicals results in marked changes in the cellular levels and causes membrane damage, cell death, exfoliation and epithelial erosion.

Evening Primrose Oil

Evening Primrose Oil (EPO) is an oil extracted from the seeds of the Oenothera biennis, a wild flowering plant that bloom in the evening native to North America and found in parts of Asia and Europe which is rich in omega-6 essential fatty acids; the linolenic acid (LA) and gamma-linolenic acid (GLA) (Hudson, 1984). A study by Riley (1949) found that the Evening Primrose Oil contained palmitic 8.8%, stearic 1.3%, C_{22+24} 1.0%, oleic 7.0%, linolenic 71.7% and GLA 10.2% (Gunstone, 1992). To be exact, the seeds of the EPO contained about 65% linolenic acid and 8% to 10% GLA (Hardy, 2000). Despite the low level of GLA, EPO remain the most commonly used GLA oil and was the first commercially available product (Gunstone, 1992). In human body, GLA is converted into di-homo gamma linolenic acid (DGLA) and arachidonic acid (AA). DGLA is the main precursors of eicosanoids and prostaglandins (PGH1) (Chilton et al., 1996). PGF1 then form PGE1 and thromboxane A1 (TXA1) via the action of cyclooxygenase enzyme (COX). Both PGF1 and TXA1 are anti-inflammatory mediators. TXA1 promote vasodilation and inhibit platelet aggregation. Another metabolites of AA is 15-hydroxyeicosatrienoic acid (15-HETrE). GLA is converted to DGLA, which in turn is converted to AA. One of the possible fates of AA is to be transformed into a group of metabolites called eicosanoids; a class of paracrine hormones. The three types of eicosanoids are prostaglandins, thromboxane A2 and leukotrienes. Through the action of 5-Lipoxygenase (5-LOX), leukotrienes B4 (LTB4), 15-HETE; lipoids (LXs) are produced.

A study by the Blumenthal et al. (2012) stated that sales of herbs botanical dietary supplements in the United States increased to an estimated 4.5 %. The sales is greater than the previous year which was also conducted by Blumenthal et al. (2011). In the food, drug and mass market (FDM) for 2011, EPO was ranked in the top 25 among herbs and botanical supplements sold; making over 5 million USD.

Despite the widespread usage of EPO, the potential effects of EPO on gastric ulceration were not well studied. Thus, this study provided insights on the effects of EPO as anti-ulcer agent following ethanol-induced gastric ulcer in rats.

MATERIALS AND METHOD

Experimental Animals

30 healthy adult Sprague-Dawley rats of mixed gender weighing 230 ± 50 g were used in the present study. The rats were classified into 5 groups as shown in Table 2.1.

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

Group	n	Treatment	Dose given
1 (Negative control)	6	0.9% Normal saline	10 mg/kg b.w
2 (Positive control)	6	Omeprazole	20 ml/kg b.w
3 (Low dose-EPO)	6	Low dose-EPO	100 mg/kg b.w
4 (Medium dose-EPO)	6	Medium dose-EPO	500 mg/kg b.w
5 (High dose-EPO)	6	High dose-EPO	1000 mg/kg b.w

Ethanol-Induced Gastric Ulceration

The ethanol administration to induce gastric ulceration was performed 1 hour after the pre-treatment with either normal saline / omeprazole / EPO. The gastric ulcer was induced by administering absolute ethanol (99.7%) by force-feeding. All rats were fasted 12 hour before the administration of the absolute ethanol at a dose of 1ml / 200g b.w. After the induction with absolute ethanol, the rats were kept in cages to prevent coprophagia during and after the experiment.

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 as shown in the Figure 2.1. 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.

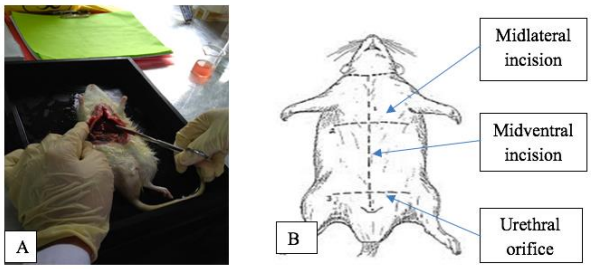


Figure 2.1: (A) Dissection process and (b) Ventral position of the rat. Dashed lines indicate point of incision.

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. Figure 2.3 shows the macroscopic appearance of a normal stomach.

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

Figure 2.2

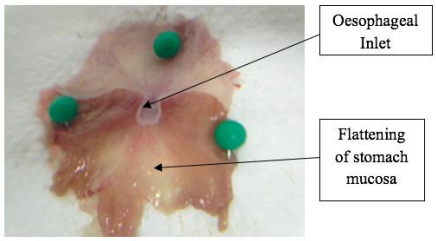
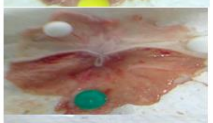
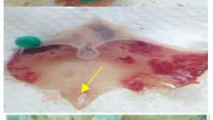
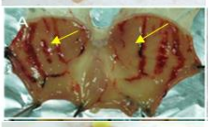
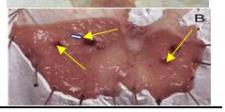


Figure 2.3: Macroscopic appearance of a stomach.

Ulcer Scoring

Ulcer scoring used in the present study was according to Jyoti et al. (2011) and Hojage et al. (2010). Table 2.2 below shows the criteria of the ulcer scoring.

Table 2.2. The description and the ulcer scoring according to Jyoti et al. (2011) and Hojage et al. (2010).

Descriptions	Ulcer score	Examples
Normal stomach with no sign of ulceration, discolouration, haemorrhage streak or perforation.	0	
Presence of red discoloration.	0.5	
Presence of spot ulcer as indicated by arrow.	1.0	
Presence of haemorrhagic streak as indicated by arrows.	1.5	
Presence of Ulcer as indicated by arrow.	2.0	
Perforation as indicated by arrows.	2.5	

Calculation of Ulcer Index and Percentage Protection

The calculation for the percentage of ulcer protection was based on Gerhard (2002) and Hojage (2010). The calculation was done according to the formula as shown in Figure 2.4.

$$\%\ \text{Protection} = \frac{\text{Control Mean Ulcer index - Test Mean Ulcer index}}{\text{Control Mean Ulcer index}} \times 100$$

Figure 2.4

Meanwhile, the ulcer index was calculated based on the formula shown in Figure 2.5.

$$\text{Ulcer Index} = (UN + US + UP) \times 10^{-1}$$

Where;
UN= Average number of ulcer per animals
US = Average severity of ulcer
UP = Percentage of animals with ulcer

Figure 2.5

Statistical Analysis

Difference between the mean of the relative organ weight and the mean of the scoring of ulcer in the treatment groups and the negative control group was determined using the one-way Analysis of Variance (ANOVA) from SPSS. A post Hoc test was carried out to determine the difference within the group. Significant difference between means was determine at 95% significant correlation ($p<0.05$).

RESULTS & DISCUSSION

Relative Organ Weight

It was found that the mean of ROW of stomach in the Negative control, Positive control, Low dose-EPO, Medium dose- EPO and the high dose-EPO were 0.77 (±0.06), 0.76 (±0.08), 0.81 (±0.08), 0.73 (±0.08) and 0.78(±0.06) respectively. There is no significant difference when the ROW of each group was compared between each other. The bar chart of the ROW in all groups is as shown in Figure 3.1.

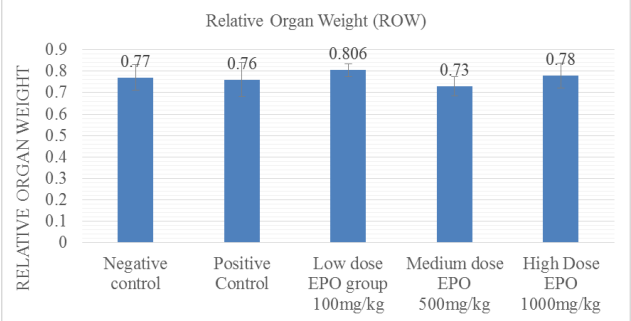


Figure 3.1: Bar chart of relative organ weight of all group rats. Data is expressed as mean (±SEM). When the ROW from all groups were compared between each other, the difference is not significant.

Macroscopic Appearance of Stomach

In the Negative control group rats, 3 out of 6 (50%) had presence of ulcer and were graded as 2 using the ulcer score. 3 other (50%) rats had presence of haemorrhagic streak and were graded as 1.5 by the ulcer score. Figure 3.2 shows the macroscopical appearance of all stomachs of the Negative control rats. In the Positive control group rats, 3 out of 6 rats (50%) had only presence of discolouration of the stomach while the 3 others showed a normal stomach with no discolouration, presence of ulcer, presence of haemorrhagic streak and presence of perforation. Figure 3.3 shows the macroscopic

appearance of all stomach of the Positive control rats. In the Low dose-EPO group rats, 3 out of 6 (50%) had presence of ulcer as were graded as 1 using the ulcer score and the 3 others had presence of discolouration in the stomach. Figure 3.4 shows the macroscopic appearance of all stomachs of the Low dose-EPO group rats, 3 out of 6 rats (50%) had presence of ulcer and were graded as 1 by the ulcer score and the 3 others had presence of discolouration in the stomach. In the Medium dose–EPO group rats, 4 out of 6 rats (66.7%) had presence of discolouration and 2 others were normal. Figure 3.5 shows the macroscopic appearance of all stomachs of the Medium dose-EPO group rats, only 2 rats has sign of presence of discolouration with 2 other normal in appearance. Figure 3.6 shows the macroscopic appearance of all stomachs of the High dose-EPO rats.

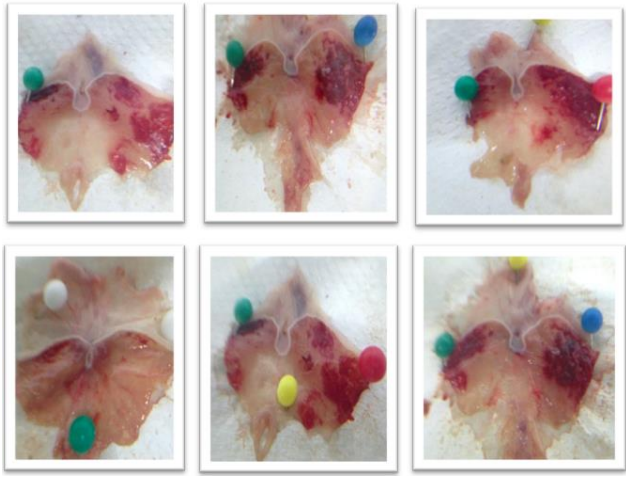


Figure 3.2: Macroscopic appearance of stomachs in the Negative control group rats.

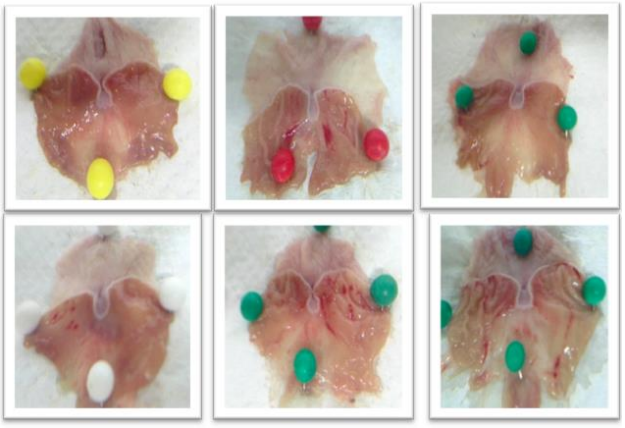


Figure 3.3: Macroscopic appearance of stomach in the Positive control group rats.

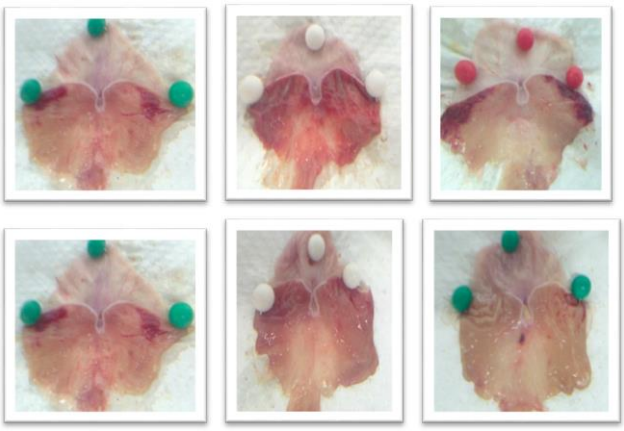


Figure 3.4: Macroscopic appearance of stomach in the Low dose-EPO rats.

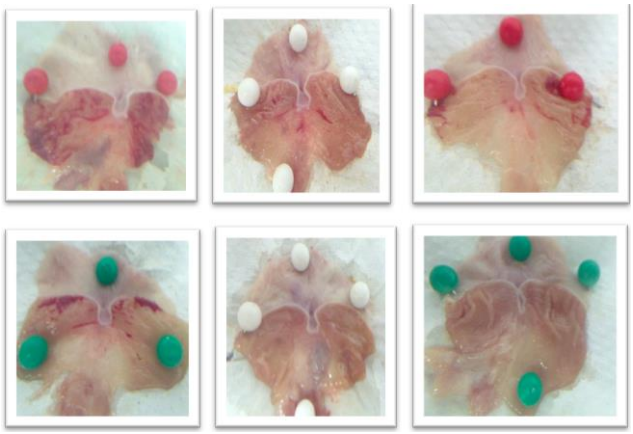


Figure 3.5: Macroscopic appearance of stomach in the Medium dose-EPO rats.

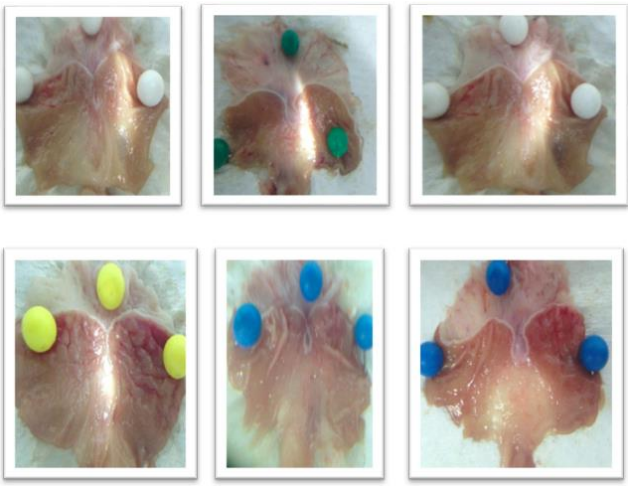


Figure 3.6: Macroscopic appearance of stomach in High dose-EPO control group rats.

Scoring of Ulcer

The scoring of ulcer was given based on the scoring outlined in 2.11. Figure 3.7 shows a bar chart of mean ulcer score in all group rats. The mean ulcer score for the Negative control,

Positive control, Low dose-EPO, Medium dose-EPO and High dose-EPO were 4.66 (±0.11), 0.25 (±0.11), 1.5 (±0.11), 0.33 (±0.105) and 0.17 (±0.10) respectively. When the mean ulcer score was compared between each group, It was found out that Positive control, Low does-EPO, Medium dose-EPO and the high dose-EPO group are significantly different with that of the Negative control group ($p<0.05$, Mann-Whitney test). This indicates that even with the supplementation of Low dose–EPO, ulcer could be reduced significantly. It was noted that the High dose-EPO group has the lowest ulcer score of all group rats was as compared to other group.

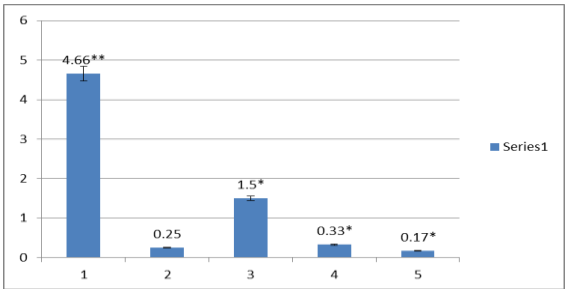


Figure 3.7: Mean ulcer score of all group rats. Data is expressed as mean (±SEM). When the mean ulcer score of all groups were compared between each other, it was found that the Positive control, Low dose-EPO, Medium dose-EPO and the High dose-EPO groups are significantly different with that of Negative control group ($p<0.05$, Mann-Whitney test).

Ulcer Index

Ulcer index was calculated based on the formula outlines in 2.12. Basically, the ulcer index also evaluated the downside risk of contracting ulcer. In the present study, the ulcer index for the Negative control, Positive control, Low dose-EPO, Medium dose-EPO and the High Dose-EPO was 10.360, 0.0025, 10.200, 6.790 and 0.120 respectively. This indicates that the risks of getting ulcer is very low if pretreatment of omeprazole and high dose-EPO is given. Although the Low dose-EPO group had a significant difference in the ulcer score between the Negative controls, the risk is still similar to the negative control group. Figure 3.8 shows the ulcer index for all group rats.

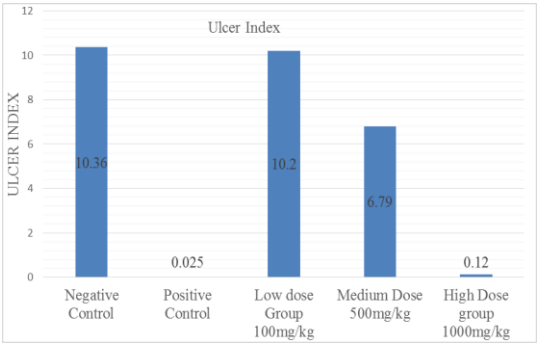


Figure 3.8: Bar chart of ulcer index of all groups. Negative control group recorded the highest ulcer Index (10.360) followed by the Low dose-EPO group (10.200), while the lowest ulcer index was recorded in the Positive control group (0.025) followed by the High dose-EPO group (0.120).

Percentage Protection

The percentage protection indicates the percentage of protection of the treatment against ulcer. It was found that the percentage ulcer protection obtained from the Negative control, Positive control, Low dose-EPO, Medium dose-EPO and the High dose-EPO groups was 0.00, 99.75, 1.54, 34.45 and 98.39 respectively, As suspected, the Positive control and the High dose-EPO group had the highest in the protection against ulcer in the present study. Figure 3.9 shows the percentage ulcer protection for all group rats.

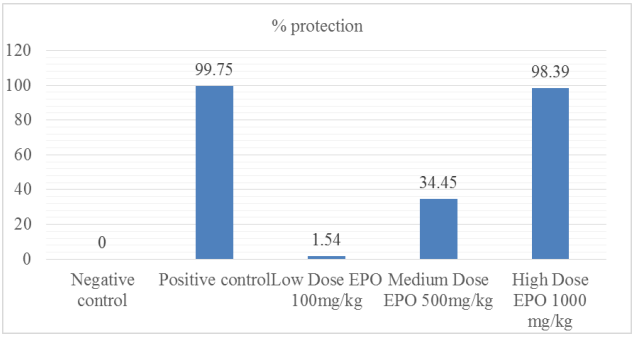


Figure 3.9: Bar chart of percentage ulcer protection of all groups. The Positive Control group showed the highest percentage protection (99.75%) followed by the the High dose-EPO group (98.39%). Meanwhile, the Low dose-EPO depicted percentage protection of 1.54%.

Limitation of Study

In the present study, the duration of supplementation of EPO in the rat is one hour before the induction with absolute ethanol. The time limit is the limitation of the study in order to obtain a more accurate result as time is required for the EPO to be metabolized into its constituents. A high tech gadget with better resolution is required to obtain more accurate macroscopic photo of the rat stomach.

CONCLUSION

In the present study, there is no significant difference in the relative organ weight (ROW) of stomach observed between all the groups. The ROW was also relatively comparable with previous reports. The Positive control and the High dose-EPO group rats showed the lowest in the ulcer score indicating their protective effects against ulcer. This was also evidenced in all the macroscopic examination. The Negative control had the highest ulcer score with some of the rats had presence of ulcer and also haemorrhagic streak which was not evidenced in all other group rats. The Positive control and the High dose-EPO group rats also had the lowest ulcer index (risk of ulceration) and the highest percentage ulcer protection amongst all other group rats. This indicates that EPO treatment at high dose is comparable to the standard treatment for gastric ulcer. As expected, the Negative control and the Low dose-EPO had the highest ulcer index and minimal percentage ulcer protection. This present study shows that EPO treatment at high dose could reduce the risk of getting gastric ulcer if taken as a pre-treatment alternative medication and that EPO at high dose could be an alternative to the standard treatment used in the gastric

ulceration. Further study has to be undertaken to evaluate the potential of EPO at high dose as anti-ulcer supplement.

REFERENCES

i. Chilton, L., Surette, M.E., and Swan, D.D.1996. Metabolisme of gamma linoleic acid in human neutrophils. J Immunol 156:2941-7.

ii. Blumenthal, M, Lindsrom, A, Lynch, M.E, Rea, P.2001. Herb sales continue growth – Up3.3% in 2010. HerbalGram.90:64-7

iii. Blementhal, M, Lindstorm, a, Ooyen, and Lynch, M.E 2012. Herb supplement sales increase 4.5% in 4.5% 2011.HerbalGram. 95:60-4

iv. Gunstone, F. D. (1992). Gamma linolenic acid--occurrence and physical and chemical properties. Progress in Lipid Research, 31(2), 145–61.

v. Hardy, M. L. (2000). Herbs of Special Interest to Women. Journal of the American Pharmaceutical Association (1996), 40(2), 234–242.

vi. Hudson, B. J. F. (1984). Evening primrose (Oenothera spp.) oil and seed. Journal of the American Oil Chemists' Society, 61(3), 540–543.

vii. Hojage MG, Hriprassanna RC, Patil KS, Matha PAti S, Wadkar G, and Rao KP. Antiulcer effects of alcoholic exrracts of Murus Alba Lim. Leaves in rodents. Indian Drugs .2010 : 47(6):64-68

viii. Jyoti Gupta, Dinesh Kumar., Ankit Gupta,(2012) . Evaluation of gastric anti-ulcer activity of methanolic extract of cayratiatrifolian experimental animals. Asian pacific journals of tropical Disease: 99-102

ix. Leslie W. Peptic Ulcer: Reappraisal for its peptic ulcer etiology. Ann Roy Coll Surg Engl 1972; 50:146-163

x. Robert A.Olafsson AS. Lancaster C. Zhang. Interleukin-1 is cytoprotective, antisecretory, stimulate PGE2 synthesis by the stomach, and retard gastric emptying. Life sci 1990; 48:123-134