

Antiulcer Activity of Aqueous Extract of Avipattikar churna

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Summary

The aqueous extract of Avipattikar churna was evaluated for gastroprotection in rats using the ibuprofen, ethanol and pylorus ligation induced ulcer models. Efficacy was assessed by determination of mean ulcer size, ulcer number and gastric irritancy index (GII). Oral administration of aqueous extract (500mg/kg) of Avipattikar churna significantly protected against gastric lesions as compared to ranitidine in ibuprofen, ethanol and pylorus ligation induced ulcer models. Histopathological examination of stomach mucosa showed the protective action of aqueous extract of Avipattikar churna against mucosal epithelial damage caused by ibuprofen, ethanol and pylorus ligation. The present study provides a strong evidence of antiulcer activity of aqueous extract of Avipattikar churna against gastric lesions. The antiulcer activity is recognised by a reduction in acid secretory parameters (i.e. total and free acid), gastric volume and gastric irritancy index (GII).

Keywords: Anti-ulcer, avipattikar churna, ethanol, ibuprofen, pylorus ligation

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Introduction

“Avipattikar churna” is a polyherbal Ayurvedic medicine used as remedy for hyperacidity, indigestion, anorexia, urinary retention, constipation and piles¹. According to ayurvedic physicians ulcer formation is due to improper digestion of food. Though Avipattikar churna is claimed to decrease hyperacidity thereby being useful in curing peptic ulcers, there is no scientific proof to support this claim^{1,2}. The present study reports the antiulcer activity of aqueous extract of Avipattikar churna on ibuprofen, ethanol and pylorus ligation induced peptic ulcer models in rats by comparison with reference to the standard drug ranitidine.

Materials and Methods

Plant material

Avipattikar churna consists of fourteen ingredients viz., *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Terminalia chebula*, *Terminalia bellerica*, *Embelica officinalis*, *Cyperus rotundus*, salt (*vida lavana*), *Embelia ribes*, *Elettaria cardamomum*, *Cinnamomum tamala*, *Syzygium aromaticum*, *Operculina terpehthum*, and *Saccharum officinarum*². All these ingredients were procured from the local market of Udupi, India and were authenticated by botanist V. Aravinda Hebbar, Professor and Head of the department of botany, M.G.M College, Udupi. A voucher specimen of the same has been deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal for future reference.

Preparation of Avipattikar churna

The churna was prepared as per the procedure given in Ayurvedic Formulary of India¹. All the ingredients viz., *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Terminalia chebula*, *Terminalia bellerica*, *Embelica officinalis*, *Cyperus rotundus*, salt (*vida lavana*), *Embelia ribes*, *Elettaria cardamomum*, *Cinnamomum tamala*, *Syzygium aromaticum*, *Operculina terpehthum*, and *Saccharum officinarum* were powdered separately, passed through 80 # sieve and then mixed together in proportions as specified in Ayurvedic Formulary of India.

Preparation of the extract

Avipattikar churna powder (100 g) was extracted with 2 l of chloroform water (1: 1000) by maceration. The filtered extract evaporated under vacuum gave a dry yield of 18.4% (w/w) and was stored in desiccator until further use. The aqueous extract of churna obtained hereafter is referred as AQEAC.

Animals

Healthy Wistar adult albino rats of either sex, 2-3 months old and weighing between 150-200 g were used in the experiment³. Animals were housed individually in polypropylene cages, maintained under standard conditions (12:12 L:D cycle; 25° ± 3° C and 35-60 % humidity); the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance (IAEC/KMC/01/2009-2010).

Acute toxicity

Inbred male Swiss albino mice weighing 40-50 g were starved overnight and divided into 6 groups of 6 each. They were fed with AQEAC suspended in 2% gum acacia, in increasing dose levels of 100, 500, 1000, 3000, 6000 and 10,000 mg/kg body weight⁴. The mice were observed continuously for 2 hr for behavioural, neurological and autonomic profiles and after a period of 24 and 72 hr for any lethality⁵.

Gastroprotective studies

Ibuprofen, ethanol and pylorus ligation induced ulcer models were chosen for the experiment. Each model consisted of three groups of six rats each. The aqueous extract suspended in 2% gum acacia was administered orally at a dose level of 500mg/kg. Ranitidine (25mg/kg) suspension in acacia solution was used as the reference standard drug in all the three models.

Ibuprofen induced gastric ulcers

Ibuprofen in the dose of 300 mg/kg was administered orally 15 hr intervals to fasted rats to produce gastric ulcers⁶. The animals were sacrificed 6 hr after the second dose of ibuprofen. Stomachs were incised along the greater curvature and ulceration was scored.

The stomachs were then stretched over a frog-board and with the help of magnifying glass and millimetre scale, the number of ulcers and length of each ulcer were measured⁷. Test drug and gum acacia were administered 1 hr before the dose of ibuprofen.

Ethanol induced gastric ulcers

Rats fasted for 14-16 hr were orally administered with absolute alcohol (1ml/animal) 1 hr after the administration of test drug and gum acacia alone to the vehicle control group animals⁸. Animals were sacrificed 3 hr after alcohol administration. Stomachs were incised along the greater curvature and ulceration was scored.

Pylorus ligation induced ulcers

This test was performed as suggested by *Shay et al*⁹. The selected animals were divided randomly into six groups of six animals each. Each group of animals received one of the following test samples through oral route: 2% v/v gum acacia in distilled water (2ml/kg), ranitidine (25mg/kg), test extracts (each 500mg/kg) respectively, twice daily for two days. One hour after the last treatment, pylorus ligation was done under ether anaesthesia. The animals were then returned to the observation chamber. The animals were deprived of both food and water during the post operative period. After 4 hr the animals were sacrificed following the ether overdose. The abdomen of each animal was opened and the stomach was isolated. The gastric juice was collected by giving a small cut to the pyloric region just above the knot in a measuring cylinder and stomach was opened along the greater curvature. The mucosal layer was washed with one ml distilled water and washings were added to the gastric secretions. The gastric contents were centrifuged at 2000 rpm for 10 minutes. The supernatant fluid (1ml) was diluted with 9 ml of distilled water and then titrated against 0.1 N sodium hydroxide solution using Topper's reagent till the solution turns orange in colour. The volume of sodium hydroxide required corresponds to free acidity. The solution was further titrated till the solution regained pink colour. The volume of sodium hydroxide required corresponded to the total acidity. Each stomach was then examined carefully for ulcers. The ulcer number and ulcer size were noted down.

Collection of Gastric Juice

The stomach was excised carefully by keeping the oesophagus closed and opened along the greater curvature and luminal contents were removed. The gastric contents were collected and centrifuged at 1000 rpm for 10 min; the volume of supernatant was expressed as ml/100 gm body weight and the centrifuged samples were decanted and analysed for gastric volume, free acidity and total acidity.

Estimation of Total and Free Acidity

It was measured by the method of Hawk *et al*¹⁰. One ml of supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The pH of this solution was noted with the help of pH meter. The solution was titrated against 0.01N sodium hydroxide using topfer's reagent as indicator. The end point was titrated when the solution turned to orange colour. The volume of NaOH was noted, which corresponds to free acidity. Further, it was titrated till the solution regains pink colour. The total volume of NaOH was noted, which corresponds to the total acidity.

Gastric irritancy index (GII)

The method used in the present study was that of Goburdhun *et al*¹¹. The rat stomachs were stretched over a frog-board and with the help of magnifying glass and mm scale, the length of each ulcer was measured. By totalling length of all ulcers in a stomach gastric irritancy size (GIS) was estimated¹¹, and by totalling number of ulcers per stomach, the ulcer number (UN) was estimated¹². The product of GIS and UN was called gastric irritancy index (GII). Group mean and SE of GII, GIS and UN were later calculated.

Histopathological evaluation

Animals of all the groups were sacrificed and the stomachs were immediately isolated, washed in saline and preserved in 10% formaldehyde solution for histopathological studies. The study was carried out within two days after the storage in formalin. The central part of damaged or ulcerated tissue was cut into half along the long diameter and 2-5 tissue samples were taken. After standard processing¹³, the cut tissue (Eliot Inc.) embedded in paraffin and 4 µm thick were cut using a rotary microtome (Reichert Inc.), stained with haematoxylin-eosin and then examined under microscope (Olympus FHY).

Statistical analysis

The results were analysed by using one way ANOVA followed by post hoc Sheff's test using 11.5 version of SPSS computer software.

Results

Acute toxicity was carried out and the extract was found to be safe up to a dose of 10,000 mg/kg. Treatment with 1 ml of absolute ethanol, 300 mg/kg dose of ibuprofen and pylorus ligation showed formation of ulcers (Figure 1, 4 & 7). Treatment with 500 mg/kg AQEAC showed significant reduction in mean ulcer size, ulcer number and ulcer index (Table 1, 2 & 4) as compared to ranitidine in all the three models tested. Histopathological examination of stomach mucosa further confirmed that pre-treatment with AQEAC (500 mg/kg) protected the mucosal epithelial from the damage caused by ethanol (Figure 6), ibuprofen (Figure 9) and pylorus ligation (Figure 3) in different models. Ibuprofen, ethanol treated groups and pylorus ligated groups shows the ulcerated mucosa with haemorrhage and discontinuity of lining of epithelium while AQEAC (500 mg/kg body weight) shows normal mucosa with mild hyperplasia and mild oedematous submucosa, compared to ranitidine treated group (Figure 2,5 & 8) which shows the normal mucosa with no ulcer.

Table 1: Ibuprofen induced ulceration model.

Group	Drugs	Dose (mg/kg)	MUN Mean \pm SE	MGIS(mm) Mean \pm SE	MGII Mean \pm SE
1.	Control (n=6)	--	6.33 \pm 0.715	21.17 \pm 1.99	134.83 \pm 30.67
2.	Ranitidine treated group	25	2.5 \pm 0.837 ^b	4.17 \pm 0.654 ^b	11.00 \pm 2.646 ^c
3.	Churna treated group	500	3.833 \pm 0.477 ^a	6.5 \pm 0.563 ^b	26.5 \pm 4.507 ^c

MUN: Mean ulcer number, MGIS: mean gastric irritancy size, MGII: mean gastric irritancy index and a-p < 0.05, b-p<0.001 and c-p<0.01

Table 2: Ethanol induced ulceration model.

Group	Drugs	Dose (mg/kg)	MUN Mean ± SE	MGIS (mm) Mean ± SE	MGII Mean ± SE
1.	Control (n=6)	--	5.17 ± 0.307	3.50 ± 0.428	298.33 ±39.57
2.	Ranitidine treated group	25	3.17 ± 0.401 ^a	3.74 ± 0.295 ^b	39.00 ±7.113 ^b
3.	Churna treated group	500	3.50 ± 0.428 ^a	4.33 ± 0.242 ^b	59.5 ±13.043 ^b

MUN: Mean ulcer number, MGIS: mean gastric irritancy size, MGII: mean gastric irritancy index and a-p<0.05 and b-p<0.001

Table 3: Pylorus ligation induced ulceration model.

Group	Drugs	Dose (mg/k)	Volume of gastric juice (ml)/100g body weight	Free acidity (mEq/l)	Total acidity (mEq/l)
1.	Control	--	4.25 ± 0.466	27.336 ± 3.083	62.343 ± 3.316
2.	Ranitidine treated group	25	1.916±0.176 ^a	8.26 ± 1.01 ^a	21.004 ± 2.138 ^a
3.	Churna treated group	500	2.466±0.166 ^b	14.946 ±2.543 ^a	31.86 ±2.259 ^a

a-p<0.001 and b-p<0.05

Table 4: Pylorus ligation induced ulceration model.

Group	Drugs	Dose (mg/k)	MUN Mean ±SE	MGIS(mm) Mean ±SE	MGII Mean ± SE
1.	Control (n=6)	--	4.83 ±0.477	20.50±1.118	100.83±13.422
2.	Ranitidine treated group	25	1.83 ±0.307 ^a	9.67± 1.116 ^a	18.83±4.636 ^a
3.	Churna treated group	500	3.17±0.477 ^b	12.17± 1.078 ^a	14.50±9.458 ^b

MUN: Mean ulcer number, MGIS: mean gastric irritancy size, MGII: mean gastric irritancy index, a-p<0.001 and b-p< 0.05

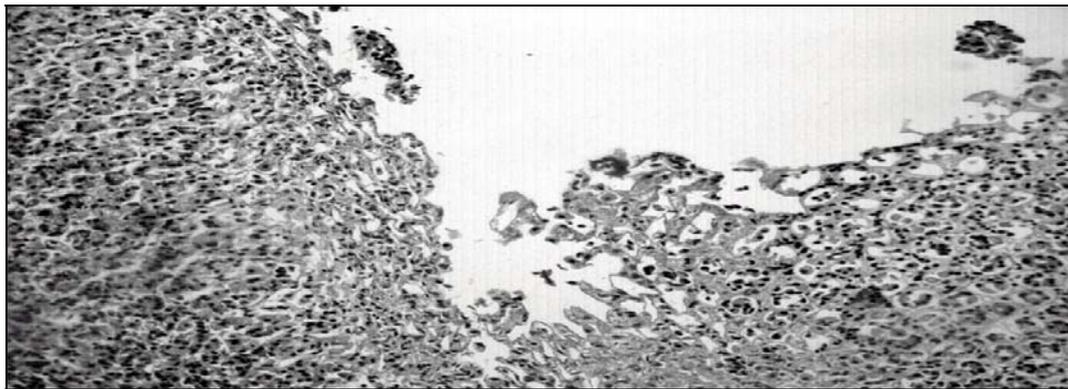


Figure 1- Photomicrograph of Control Gastric tissue, showing damaged mucosal area in Pylorus ligation model (Hematoxyline & Eosin stain, 20 x 10)

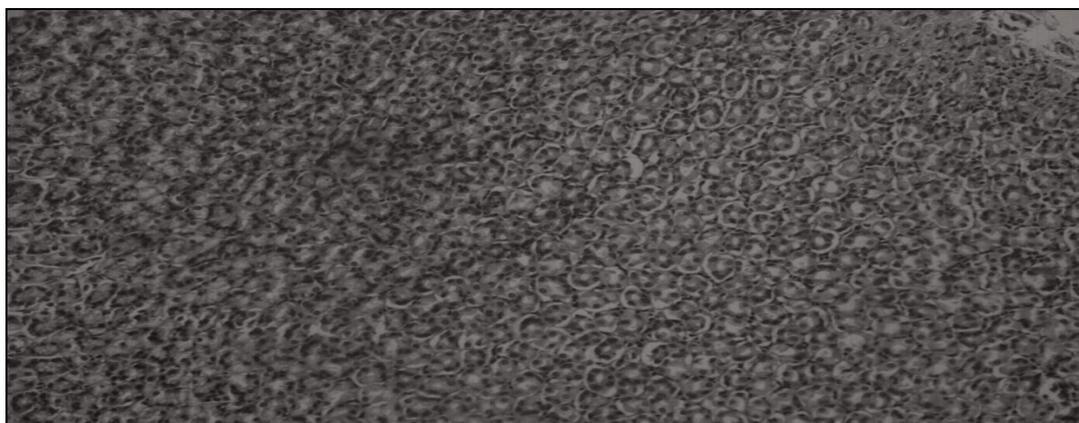


Figure 2- Photomicrograph of Ranitidine treated gastric tissue, showing mucosal Protection in pylorus ligation model (Hematoxyline & Eosin stain, 20 X 10)

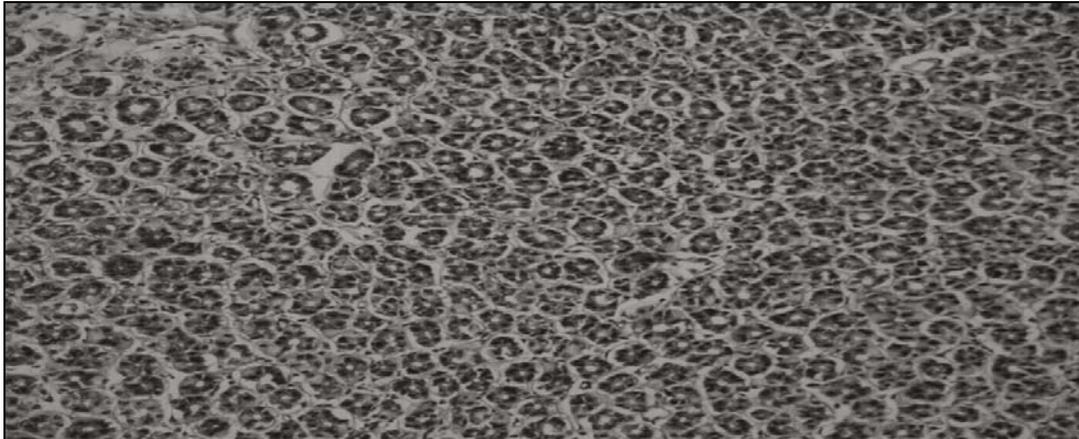


Figure 3- Photomicrograph of Churna treated gastric tissue, showing mucosal Protection in pylorus ligation model (Hematoxyline & Eosin stain, 20 X 10)

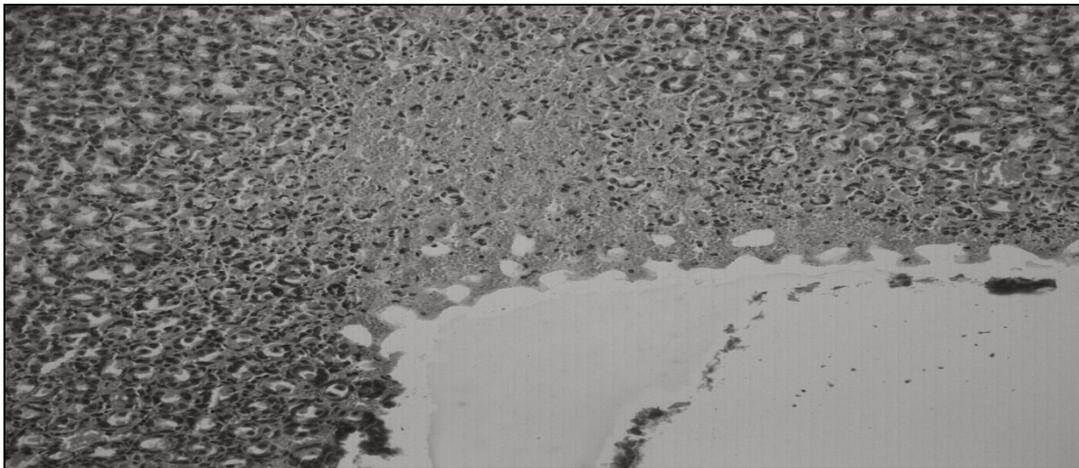


Figure 4 - Photomicrograph of Control Gastric tissue, showing damaged mucosal area in Ethanol induced ulcer model (Hematoxyline & Eosin stain, 20 x 10)

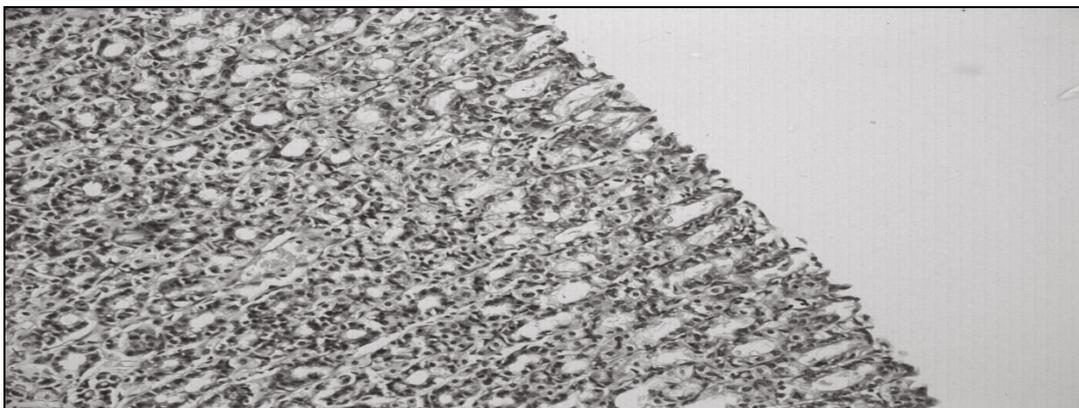


Figure 5-Photomicrograph of Ranitidine treated gastric tissue, showing mucosal Protection in Ethanol induced ulcer model (Hematoxyline & Eosin stain, 20 X 10)

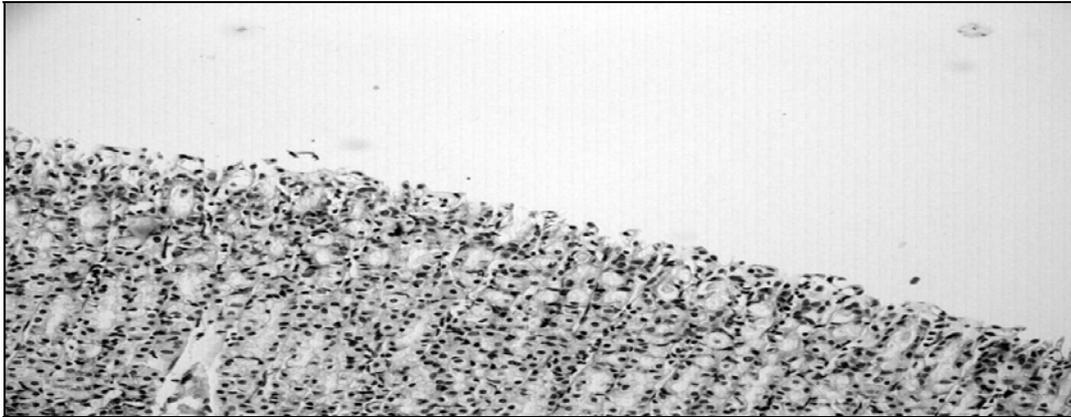


Figure 6 - Photomicrograph of Churna treated gastric tissue, showing mucosal Protection in Ethanol induced ulcer model (Hematoxyline & Eosin stain, 20 X 10)

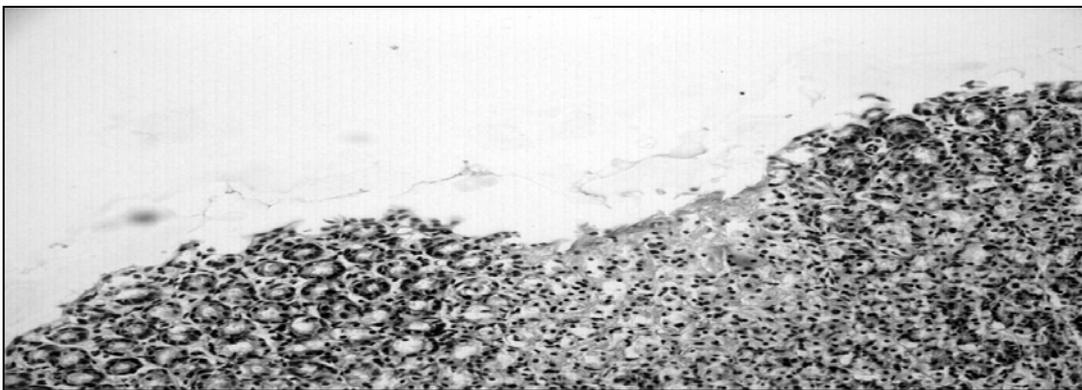


Figure 7- Photomicrograph of Control Gastric tissue, showing damaged mucosal area in Ibuprofen ulcer model (Hematoxyline & Eosin, 20 x 10)

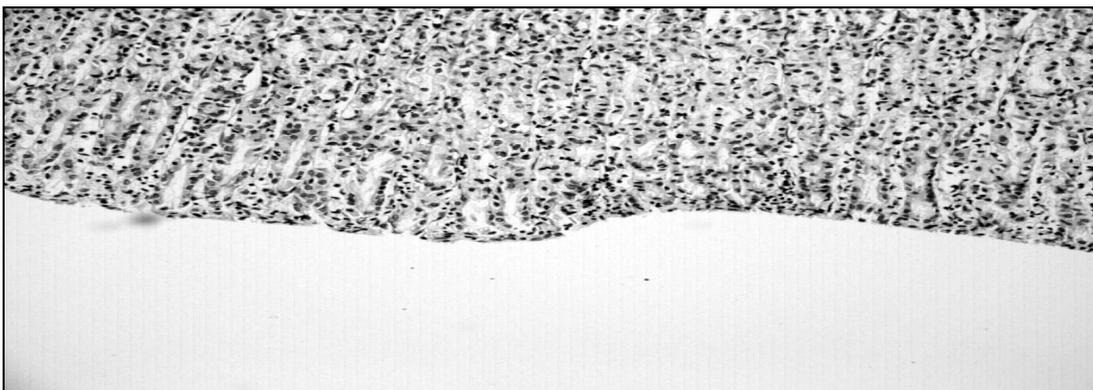


Figure 8 -Photomicrograph of Ranitidine treated gastric tissue, showing mucosal protection in Ibuprofen ulcer model (Hematoxyline & Eosin, 20x10)

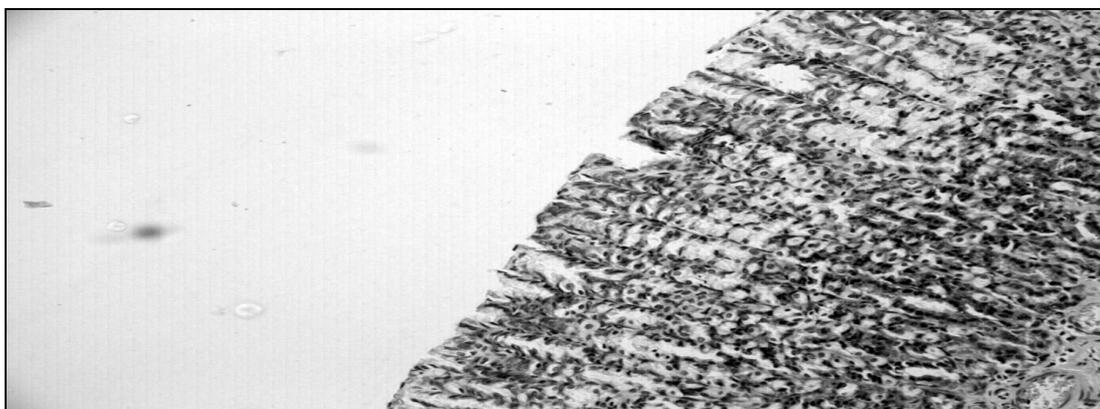


Figure 9- Photomicrograph of Churna treated gastric tissue, showing mucosal protection in Ibuprofen Ulcer model (Hematoxyline & Eosin, 20 x 10)

Discussion

NSAID's are known to induce peptic ulcer not only by denaturing mucous glycol-proteins but also by free radical formation^{14, 15}. Similarly, alcohol is also known to produce free radicals and induce peptic ulcers¹⁶. The free radicals produced cause lipid peroxidation, leading to membrane fluidity which in turn increases the influx of Ca^{2+} ions and results in the reduced membrane integrity of surface epithelial cells, thereby generating gastric ulcers^{17,18}. Free radicals have been demonstrated as contributing factor in tissue injury and in the modulation of pain¹⁹. The incidence of ethanol induced ulcers, predominant in the glandular part of the stomach has been reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products²⁰ and reactive oxygen species²¹ resulting in the damage of rat gastric mucosa²². Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier²³. The increase in gastric volume of the pylorus ligated group (Table 3) is undoubtedly due to increased production of HCl as it is evident from the total acidity of the gastric juice. In the present study administration of AQEAC (500 mg/kg) exhibited marked gastro protection in ibuprofen, ethanol and pylorus ligation induced ulcer models. The tissue protection by AQEAC was further confirmed by histopathological studies. The effects of AQEAC were found to be comparable with that of ranitidine in reducing the ulcer number and gastric irritancy index.

To conclude, AQEAC holds promise as an adjunct to existing drugs used in peptic ulcer therapy. Further work is necessary to elucidate the actual mechanism involved in the anti-ulcer activity of this polyherbal formulation.

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