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Research Article

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ANTI-ULCER INVESTIGATION OF THE DIFFERENT EXTRACTS OF BARK OF *BAUHINIA VARIEGATA* LINN (*CAESALPINIACEAE*) BY PYLORIC LIGATION & ASPIRIN PLUS PYLORIC LIGATION MODEL

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ABSTRACT

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, present study was carried out to investigate antiulcer activity of chloroform & methanol extract of bark of Bauhinia variegata Linn (Family: Caesalpiniaceae) in pylorus ligation and Aspirin + pylorus ligation induced ulceration in albino rats. Two dose levels 300 and 600 mg/kg were selected for the study. In both ulcer models, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined. Ranitidine 50 mg/kg was used as standard drug.

KEYWORDS

Bauhinia variegata, anti-ulcerogenic, pyloric ligation, Aspirin plus pyloric ligation.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity (Falk, G.W, 2001). Several factors are implicated in the pathogenesis of gastric ulcer including increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer (Glavin, G.B., *et al,* 1992; Kent-Lioyd, K.C., *et al,* 1994). There is a balance between the aggressive (i.e. acid, pepsin, active oxidants, H. pylori) and the mucosal protective (i.e. mucus, bicarbonate, prostaglandin's) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive one (Tepperman, B.L., *et al,* 1994). Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects (Mahendran, P., *et al,* 2002; Anoop, A., *et al,* 2003). However, screening plants for active drugs is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies (Borrelli, F., *et al* 2000).

Traditional Uses

Folk medicine

The bark of the plant is medicinally more important and used by tribals for cure of variety of ailments. The bark is used in fever, as tonic and astringent (Kapoor, S.K. *et al* 1980), as antileprotic, in skin diseases and wound healing (Sahu,T.R, 1981), antigoitrogenic (Thakur, M.J., *et al* 1992), and as antitumour (Singh, P.B. and Aswal, B.S. 1992). The leaves are used in treatment of skin diseases and stomatitis (Balajirao, N.S., *et al,* 1995). The roots of the plant are used as an antidote for snake

poisoning, in dyspepsia, flatulence and as carminative . They are also reported to be useful as antitumour and in obesity (Shah, N.C., *et al,* 1971).

Ayurveda (Kapoor,L.D. 2005)

In Ayurvedic literatures the plant is known by various names Kanchnar, Gandari, Yugmapatra and Karbudara. It is reported to have Kasaya rasa, Ruksha guna, Shita virya and Katu vipaka. The stem bark of Bauhinia variegata is used in the treatment of krimiroga (worm infestation), gandamala (scrofula), apaci (cervical lymphadenitis) and vrana (wounds).

Unani (Nadkarni,A.K, 1954; Chopra, R.N, *et al,* 1956)

In Unani system of medicine bark of the plant is described as astringent to the bowels, tonic to the liver. It is reported to be useful in treatment of leucoderma, leprosy, menorrhagia, asthma, wounds and ulcers. The flower buds are claimed useful in piles, cough, eye diseases, liver complaints and as styptic in haematuria and menorrhagia.

MATERIALS AND METHODS

Plant material:

The plant bark of *Bauhinia variegata* was collected from in and around of Warangal, Andrapradesh, India. The collected bark was washed with tap water to removing adhering dust followed by distilled water, shade dried, then subjected to size reduction to make powder by using mechanical grinder. The crushed mass of bark was then carried out for the process of extraction. The coarse powder was extracted successively with chloroform and Methanol using a soxhlet apparatus. After the effective extraction the solvent was distilled off (Simple distillation). The extracts were dried using a rotary vacuum evaporator (Model no-CR-2001; Cyber Club Corporation; Water Bath: HS-3001) and subjected to routine chemical test for the presence of phyto constituents (Kokate,, 1991).

Animals

Wistar albino rats weighing 180-200 gms of either sex were used in the study. Animals were procured from Laboratory Animal House of Talla Padmavathi College of Pharmacy. All animal experiments strictly complied with the approval of Institutional Animal Ethical Committee and approved by CPCSEA 1505/P0/9/11/ CPCSEA. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 hr light–12 hr dark cycle. The food was withdrawn 24 hours before the experiment but allowed free access of water. Care should be taken to avoid copulation and fighting.

Chemicals: CMC, Ranitidine

Reagents: Topfer's reagent & Phenolphthalein.

Acute oral toxicity studies (Ecobichon, D.J., 1997)**:**

A safe dose of the extract was determined by acute oral toxic class method of Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines.

EXPERIMENTAL DESIGN

The animals were numbered, weighed and then divided into six groups with 6 animals in each as follows:

Group- I : Control Carboxy Methyl Cellulose (CMC)) 10ml/kg. p.o.)

Group- II : Standard (Ranitidine 50 mg/kg p.o.)

Group- III : CEBV (200mg/kg) is suspended with CMC (0.5%)

Group-IV : CEBV (400mg/kg) is suspended with CMC (0.5%)

Group-V : MEBV (200m g/kg) is suspended with CMC (0.5%)

Group-VI : MEBV (400mg/kg) is suspended with CMC (0.5%)

Gastric ulcer induced by pylorus ligation (PL) in rats: [21, 22]

Wistar Albino rats of either sex were housed in individual cages and fasted (water allowed) for 24 hours prior to pyloric ligation, care being taken to avoid copulation. Under light ether anaesthesia the abdomen is opened by a small midline incision below the xiphoid process, pyloric portion of the stomach is slightly lifted out, and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is kept carefully, and the abdominal wall closed by interrupted sutures. The drugs are administered orally one hour prior to pyloric ligation.

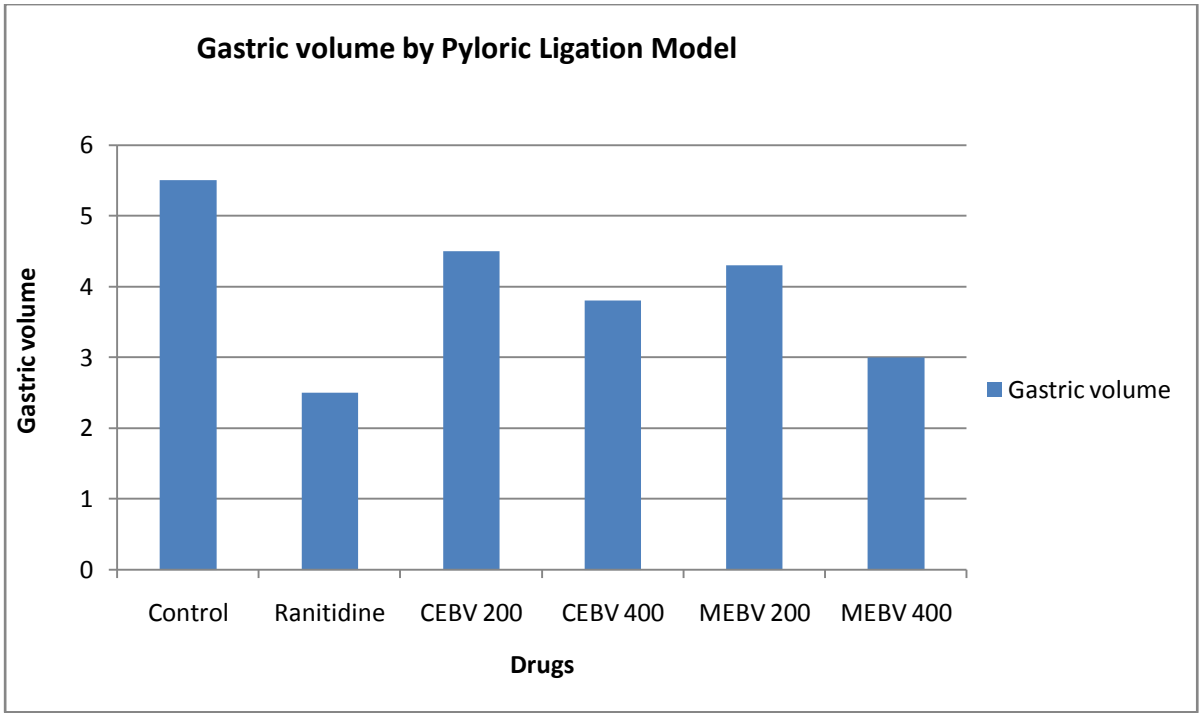
They are deprived of both food and water during the postoperative period, rats were sacrificed by an over dose of anaesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a measuring cylinder. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity. The ulcer index and the percentage (%) of protection was calculated and the results were summarized and depicted in the **Table No.-1** and **Figure No.-1: (a, b, c, d, e, f, g, h).**

Table No.-1: Effect of extracts of *Bauhinia variegata* Linn bark on pyloric ligation ulcer model in rats.

Groups	Treatment (mg/kg)	Volume of gastric juice (VGJ) (ml)	P^H	Free Acidity mEq/L (FA)	Total Acidity mEq/L (TA)	Ulcer score	Ulcer Index	% Protection
Group I	Control	5.5 ± 0.25	3± 0.034	95 ± 0.037	125 ± 0.23	2.76	18.14	-
Group II	Raniditine (50mg/kg)	2.5 ± 0.09**	6.5±0.07**	40 ± 0.0477*	70 ± 0.04**	0.33**	3.41	81.2%
Group III	CEBV 200 (200mg/kg)	4.5 ± 0.09	3.8± 0.061	78 ± 0.066	113 ± 0.09	2.11	12	33.84%
Group IV	CEBV 400 (400mg/ kg)	3.8 ± 0.12	4.6±0.060	55 ± 0.0428	92 ± 0.0763	1.33	10	44.87%
Group V	MEBV 200 (200mg/kg)	4.3 ± 0.076*	4.3±0.060	65 ± 0.0428	102 ± 0.049	1.06	7.42	59.05%
Group VI	MEBV 400 (400mg/kg)	3 ± 0.0980*	6±0.042*	42 ± 0.066**	82 ± 0.06**	0.36**	4.22	76.7%

CEBR: Chloroform extract of *Bauhinia variegata*;
MEBR: Methanolic extract of *Bauhinia variegata*;
Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnets't' test, Note: n=6 in each group (*P value < 0.0001).(**P value <0.01). (*P value <0.05)**

Figure No. 1 (a):



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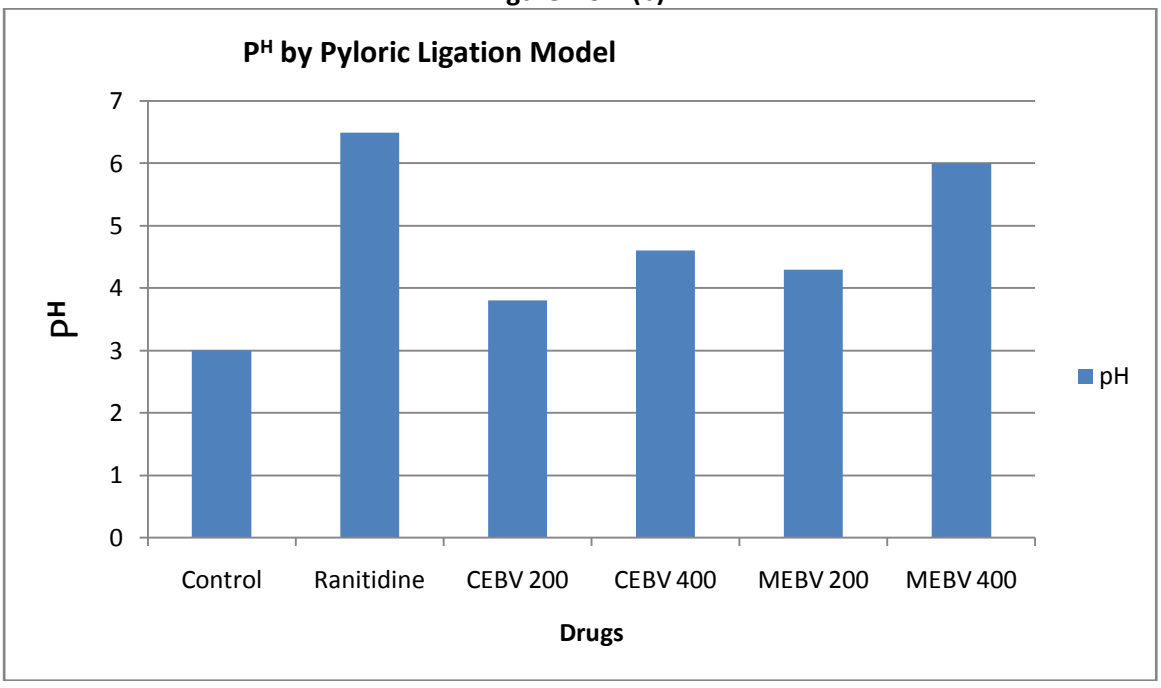

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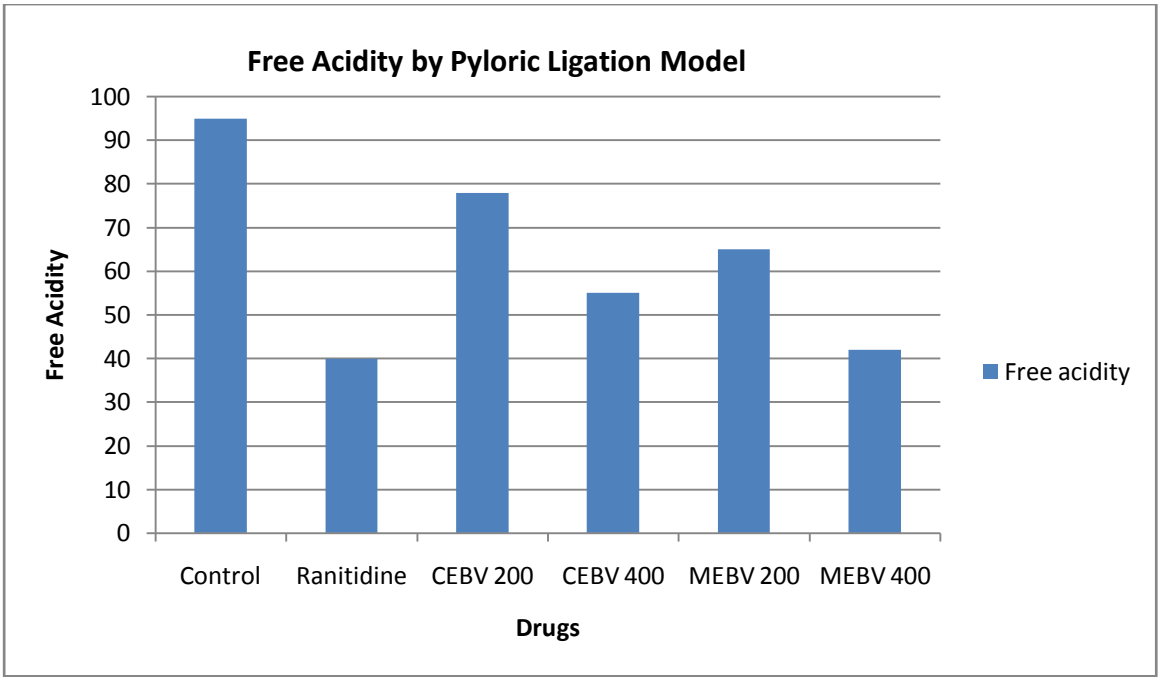

Free Acidity by Pyloric Ligation Model
Free Acidity
100
90
80
70
60
50
40
30
20
10
0
Control
Ranitidine
CEBV 200
CEBV 400
MEBV 200
MEBV 400
Drugs
Free acidity

Figure No.1 (d):

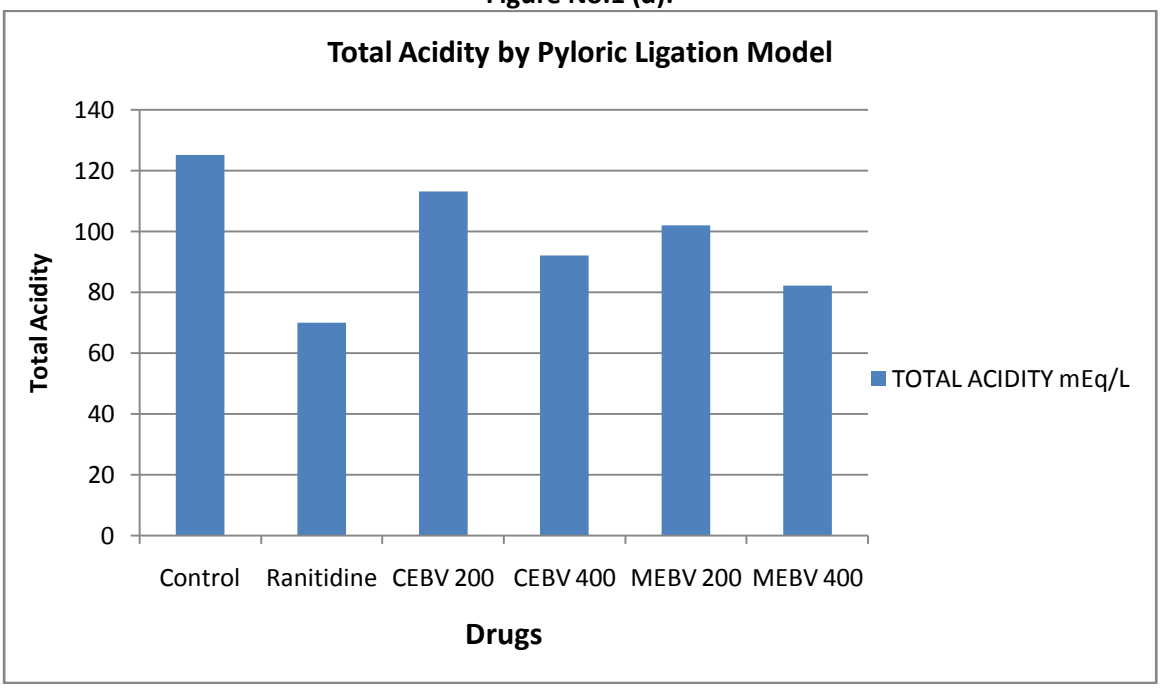
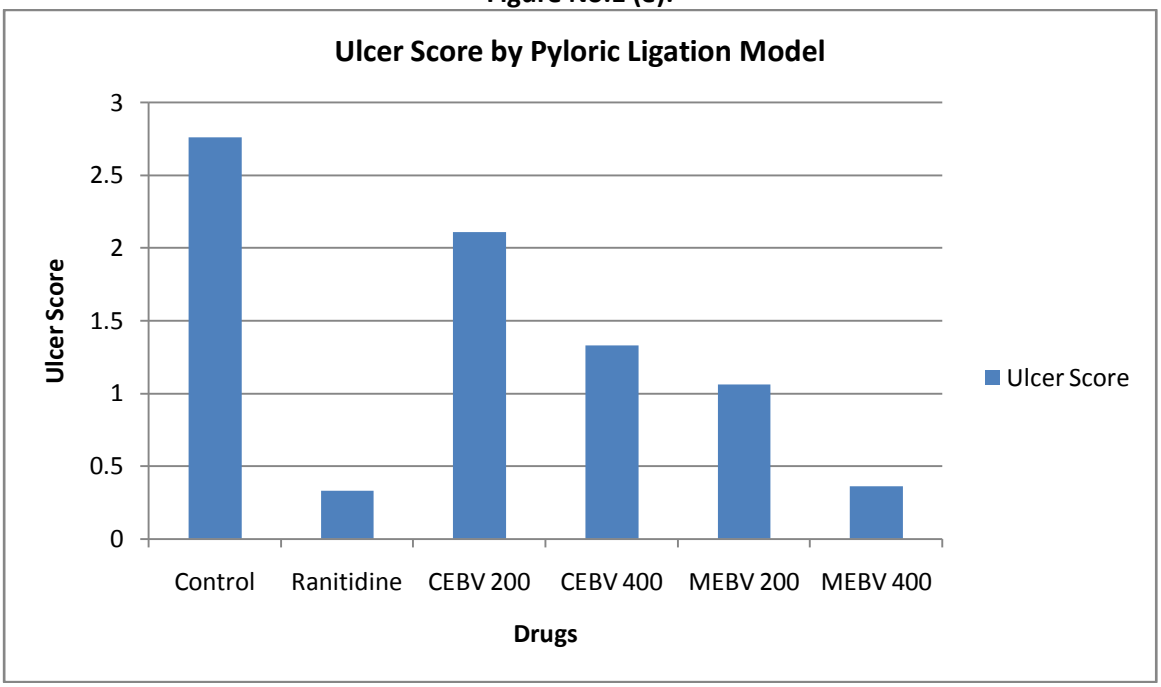

Total Acidity by Pyloric Ligation Model
Total Acidity
140
120
100
80
60
40
20
0
Control
Ranitidine
CEBV 200
CEBV 400
MEBV 200
MEBV 400
Drugs
TOTAL ACIDITY mEq/L

Figure No.1 (e):



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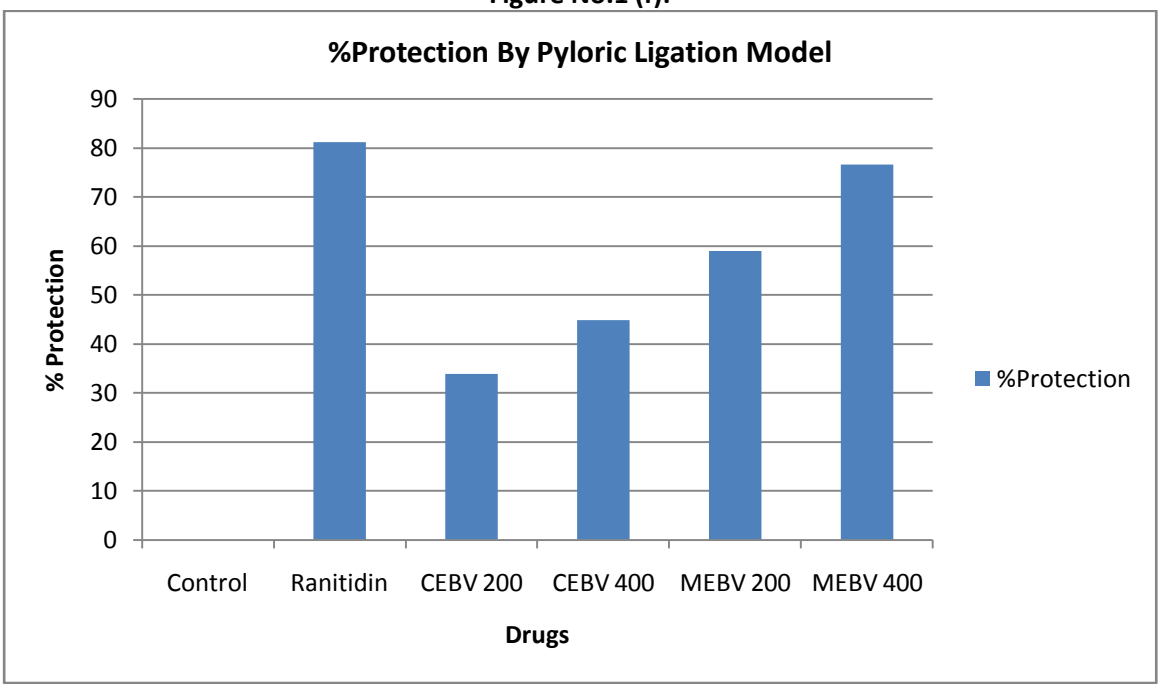

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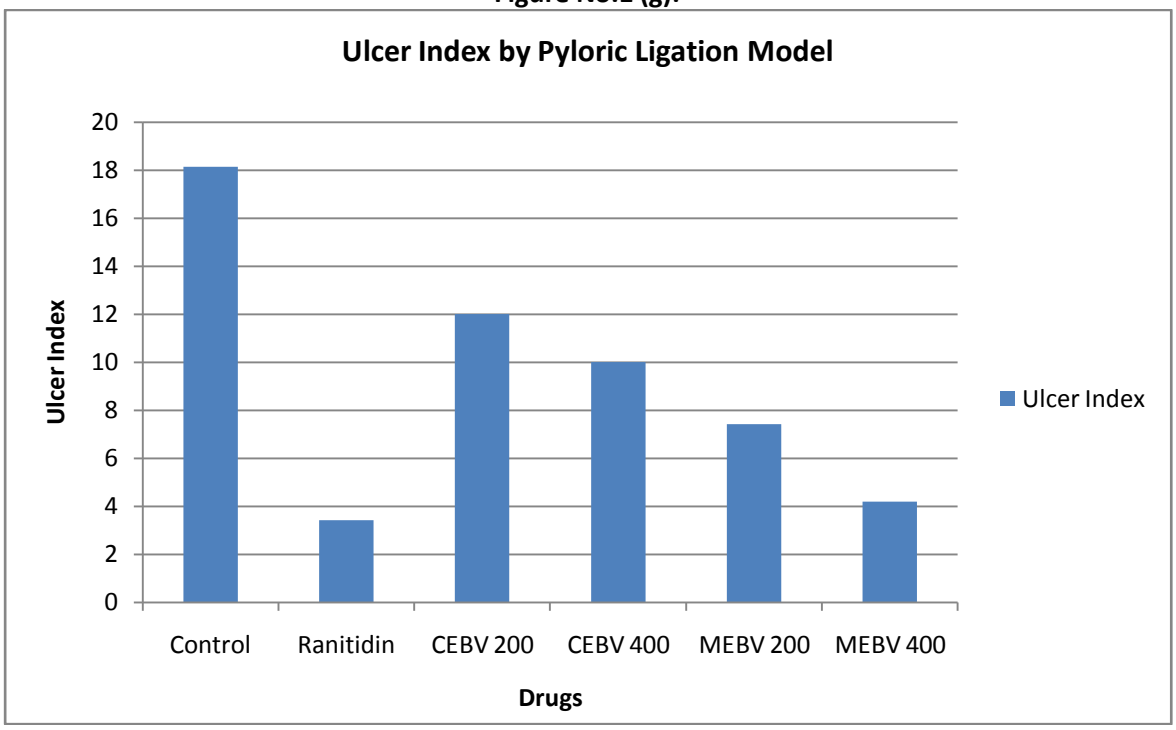
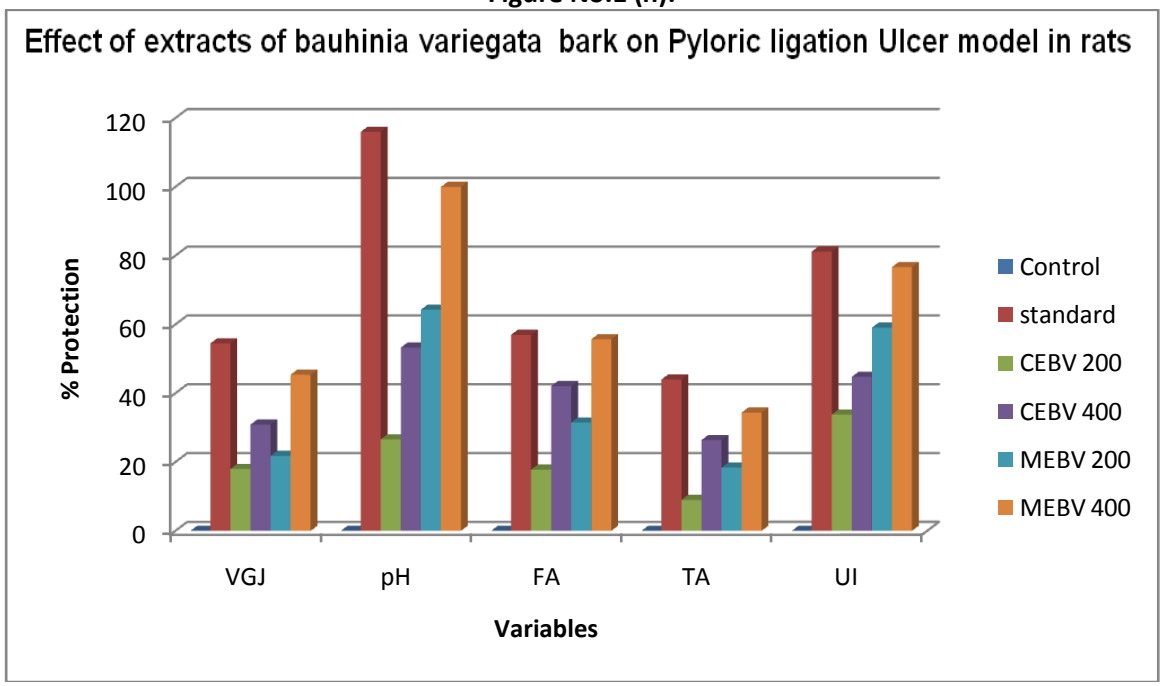


Figure No.1 (h):



6.3.1.2. Gastric ulcer induced by Aspirin + Pyloric ligation model:

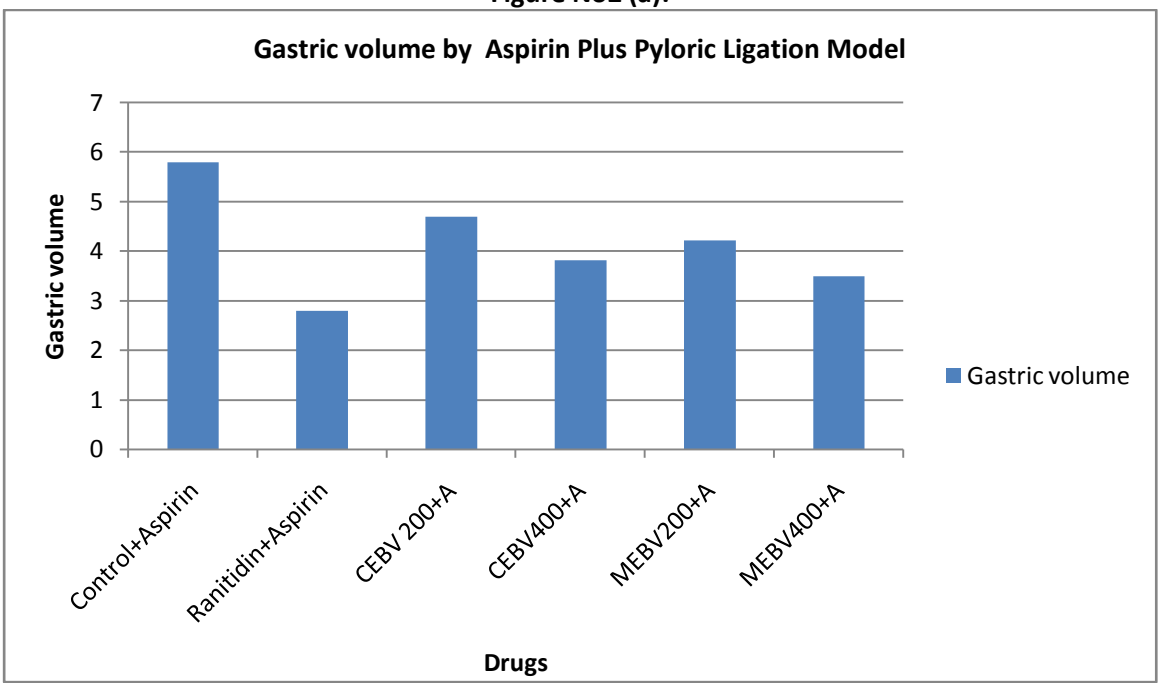
Thirty six rats of either sex were divided in to six groups. All the animals received Standard drug ranitidine (50 mg/kg) and extracts of *Bauhinia variegata* bark at dose levels of 200 & 400 mg/kg taken as test solutions. Treatment along with 500mg/kg of Aspirin high dose at a time. After 1hr, the 24 hr fasted rats were subjected to pyloric ligation. After 4 hours the animals were sacrificed after pyloric ligation by using anesthetic ether. The stomach was removed carefully and the contents were emptied in to a graduated centrifuge. The collected gastric juice was centrifuged at 2000 rpm for 10 min and the volume of the gastric juice was measured. Total acidity in the supernatants was determined with 0.01N NaOH and expressed as m.Eq/L gastric juice. The stomach was excised, opened along the greater curvature and examined for ulcer lesions by a 10X-magnifier. The ulcer index and the % of protection was calculated and the results were summarized and depicted in the **Table No.-2 and Figure No.-2 (a, b, c, d, e, f, g, h).**

Table No. 2: Effect of extracts of *Bauhinia variegata* Linn bark on Aspirin + pyloric ligation ulcer model in rats

Groups	Treatment (mg/kg)	Volume of gastric juice (VGJ) (ml)	P^H	Free Acidity mEq/L (FA)	Total Acidity mEq/L (TA)	Ulcer score	Ulcer Index	% Protection
Group I	Aspirin +Control	5.8 ± 0.256	2 ± 0.034	98 ± 0.037	130 ± 0.237	2.88	20.12	-
Group II	Aspirin + Raniditine	2.8 ± 0.09**	5.8 ± 0.0703	42 ± 0.0477	70 ± 0.0428	0.36**	3.98**	80.21%
Group III	Aspirin + CEBV 200	4.7 ± 0.09	2.6 ± 0.061	80 ± 0.066	118 ± 0.091	2.32	13.52	32.803%
Group IV	Aspirin + CEBV 400	3.82 ± 0.129	4 ± 0.060	66 ± 0.0428	104 ± 0.0763	1.44	11.88	40.95%
Group V	Aspirin + MEBV200	4.22 ± 0.076	3.5 ± 0.060	72 ± 0.0428	108 ± 0.0494	1.13*	8.5*	57.65%
Group VI	Aspirin + MEBV400	3.5 ± 0.098**	5 ± 0.042	48 ± 0.0666	84 ± 0.0601	0.44**	4.8**	76.09%

CEBR: Chloroform extract of bark of *Bauhinia variegata*;
MEBR: Methanolic extract of bark of *Bauhinia variegata;*
Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnets 't' test , Note: n=6 in each group (*P value < 0.0001).(**P value <0.01). (*P value <0.05).**

Figure No2 (a):



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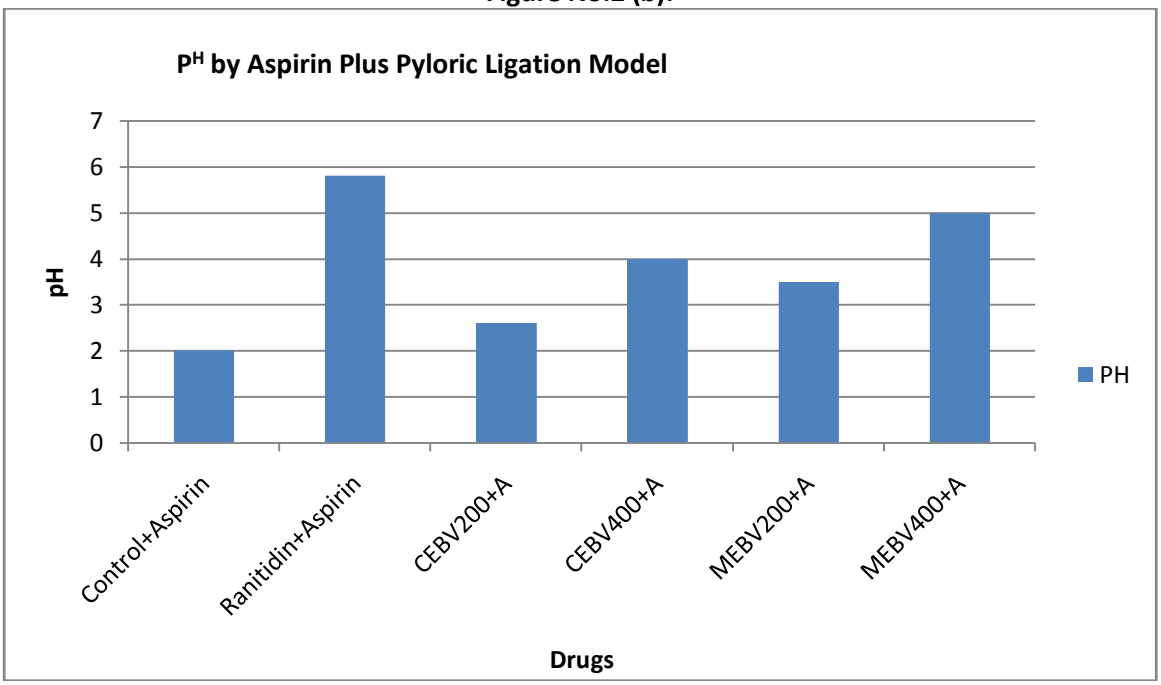


Figure No.2(c):

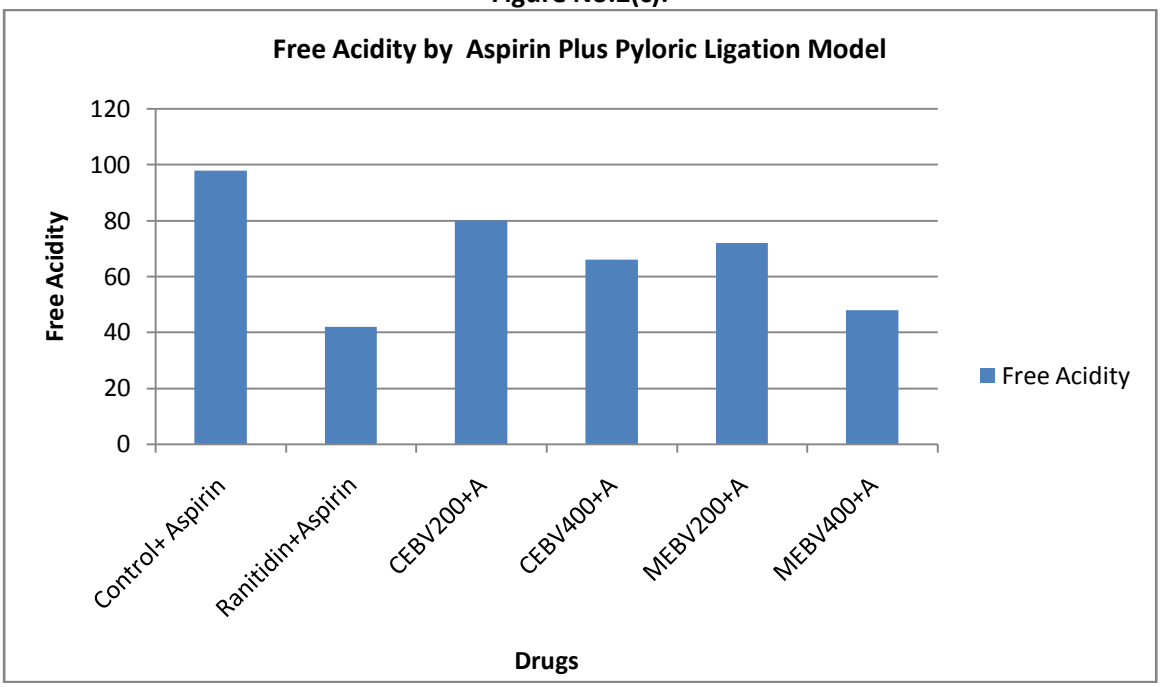
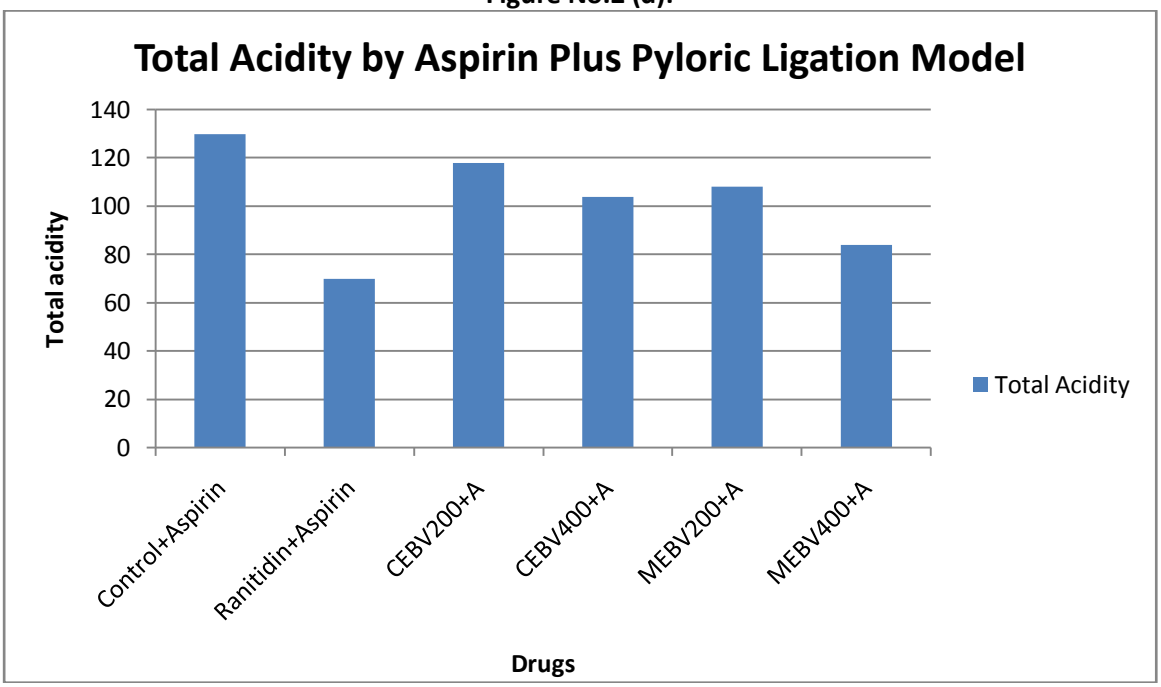


Figure No.2 (d):



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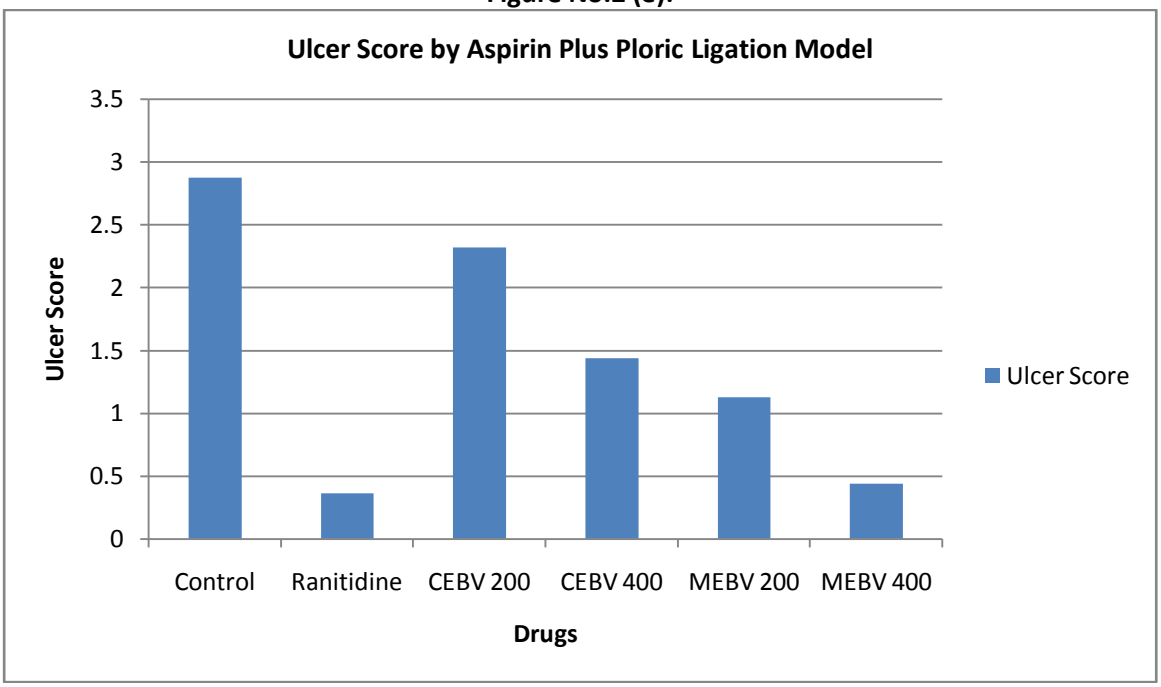

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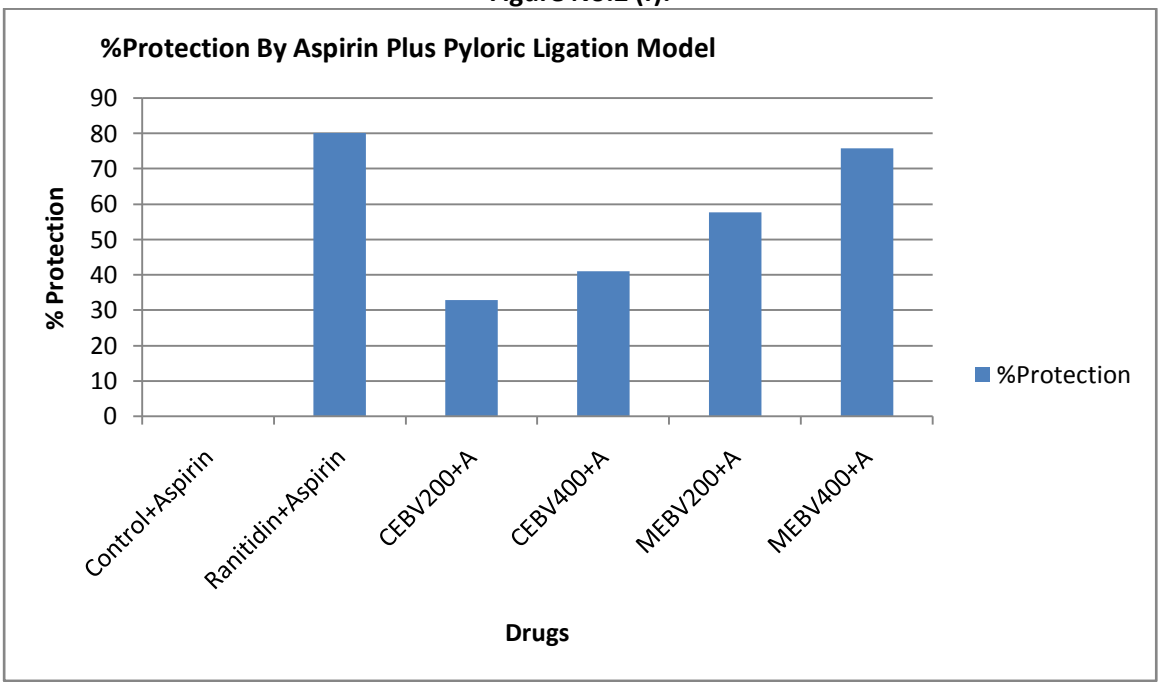

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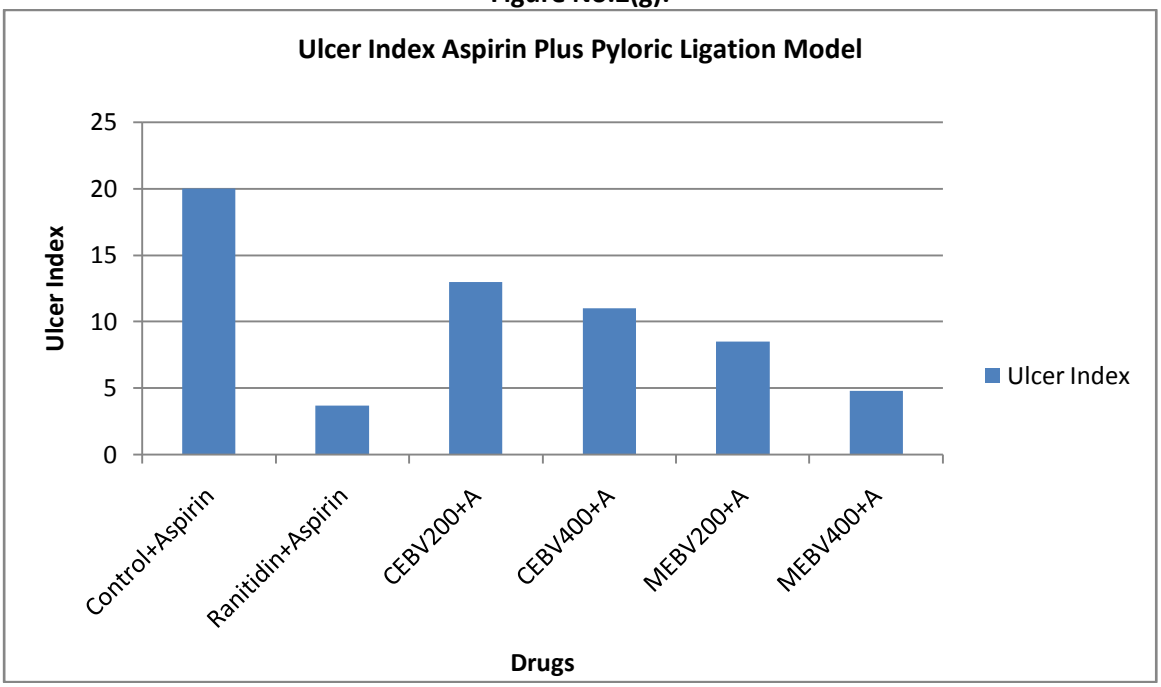
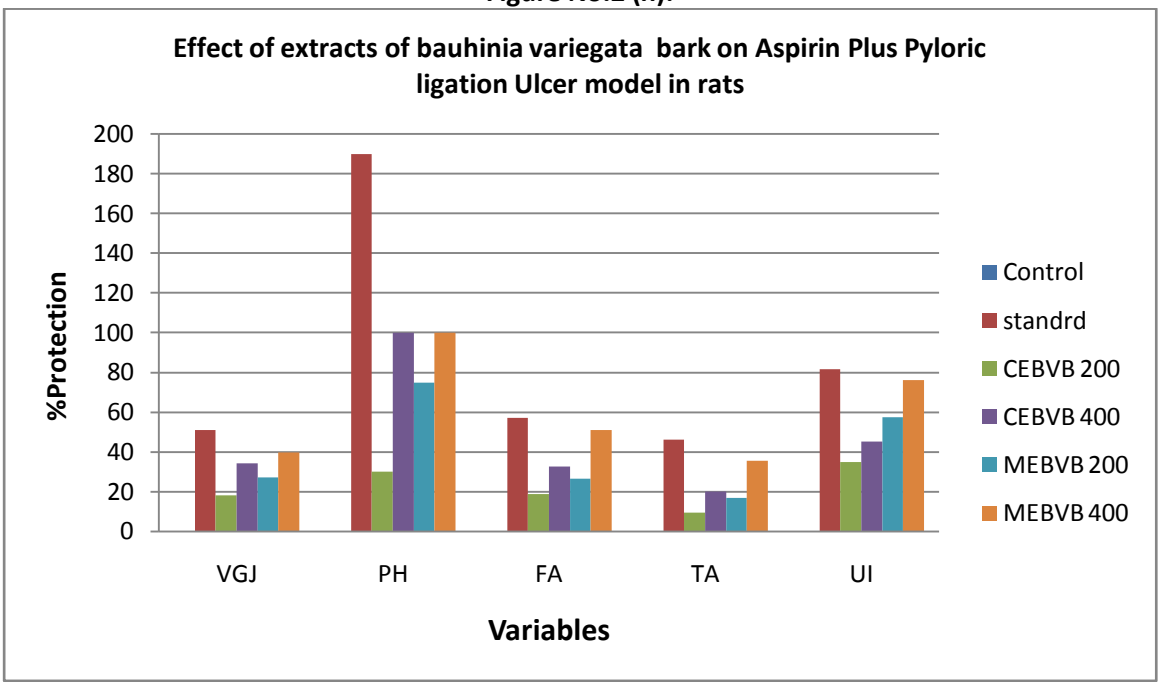


Figure No.2 (h):



Macroscopic evaluation of stomach: [24, 25]

The stomachs were opened along the greater curvature, rinsed with saline solution to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted. Scoring of ulcer will be made as follows:

Normal colored stomach..... (0)
Red coloration..... (1)
Spot ulcer..... (2)
Hemorrhagic streak..... (3)
Deep Ulcers..... (4)
Perforation..... (5)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

Lesion severity was determined by measuring ulcer index. It was calculated as follows.

$$Ulcer\ index = \frac{10}{x}$$

Where **x** is total mucosal area/total ulcerated area.

The mean ulcer index values obtained for each group were compared with the results of standard group.

Percentage protection of ulceration was calculated as below:

$$\%Protection\ of\ ulceration = \frac{Ulcer\ index\ Control - Ulcer\ index\ Test}{Ulcer\ index\ Control} \times 100$$

Determination of pH:

An aliquot of 1ml of gastric juice was diluted with 1ml of distilled water and P^H of the solution was measured using pH meter.

Determination of Total Acidity:

An aliquot of 1ml gastric juice diluted with 9ml of distilled water was taken into a 50 ml conical flask and two drops of Phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as m.Eq/L by the following formula.

$$Acidity = \frac{Vol.of\ NaOH \times N \times 10\ m.Eq/L}{0.1}$$

Determination of Free Acidity:

Instead of Phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

RESULTS AND DISCUSSIONS

The extracts of *Bauhinia variegata* (CEBV and MEBV) at dosse levels of 200 & 400 mg/kg showed statistically significant ($p<0.01$) anti-ulcer activity in pylorus-ligated rats and in aspirin plus pylorus-ligated rat model are shown in **Table No.1 & 2** Similarly, the volume of gastric content and total acidity value of the extracts treated groups also statistically significantly reduced the values ($p<0.01$) when compared with control. The extracts MEBV shown better results than other extracts of this plant both in pylorus-ligation model and aspirin plus pylorus-ligation rat models. All the extracts treated animals parametric values were comparable to that of standard Ranitidine. In order to assess the statistical significance between treated and a control group was evaluated by One-way analysis of variance (ANOVA) followed by Dunnett's t-test. The extracts (CEBV and MEBV) at the dose level of 200 & 400 mg/kg showed dose response antiulcer effect.

In the present study, the extracts of *Bauhinia variegata* (CEBV and MEBV) shown significant reduction in gastric acid secretion and gastric ulcer formation. The reduction in ulcer formation could be directly correlated to reduction in gastric acid secretion. Prostaglandins are known to play a very important role in gastric ulcer formation. NSAIDs like aspirin are known to induce gastric ulcer by inhibiting prostaglandin synthesis. Prostaglandins are cytoprotective agents. In the present study, extracts of *Bauhinia variegata* produced reduction in gastric ulcer formation in pylorus ligated rats treated with aspirin suggesting that it has gastric cytoprotective action. It appears that the extracts reduce gastric acidity and ulceration is due to H_2 receptor blockade. It may be concluded that the anti-ulcer activity of the

extracts of *Bauhinia variegata* could be attributed to fixed oil present in the plant which block the cyclooxygenase and lipoxygenase.

CONCLUSION

The extracts of *Bauhinia variegata* bark (CEBV and MEBV) at dose levels of 200 & 400 mg/kg showed statistically significant ($p<0.001$) Anti-ulcer activity in pylorus-ligated rats and in aspirin plus pylorus-ligation rat model. Significantly reduced the values Gastric volume, free acidity & total acidity when compared with control. The methanol extracts shown better results than chloroform extracts of this plant both in pylorus-ligation Shay rat model and aspirin pylorus-ligation rat model.

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