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Phytochemical & Pharmacological evaluation of pet-ether and chloroform extract of leaves of *Cassia mimosoide* Linn (family-C*aesalpiniaceae*)

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Research Article

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ABSTRACT

Cassia mimosoides Linn, Family- Ceasalpiniaceae found in the different parts of India and widely distributed in South Odisha and extensively used for treatment for the aphrodisiac, cough, jaundice etc. The present study is an attempt for the preliminary investigation of phytochemical constituents and to explore the anti ulcer activity of petroleum either and chloroform extracts of leaves of Cassia mimoseides by developing gastric ulcer by pyloric ligation and aspirin with pyloric ligation model. The extracts were administered to experimental animals orally at the doses of 100mg & 200mg per kg with ulcer developed animals. Ranitidine (5mg/kg) was administered as standard drug. All the test doses were administered orally by suspending in 0.5% carboxy methyl cellulose. The results shown, the extracts have protective activity over the ulceration. The parameters like volume of gastric juice, pH of the gastric juice, free acidity, total acidity and ulcer index are compared with standard drug & extracts.

KEYWORDS: *Cassia mimosoides, pyloric ligation, aspirin, pet-ether & chloroform extracts, phytochemical tests, anti-ulcer activity.*

INTRODUCTION

The southern India is enriched with natural flora and as per folklore report the rural people restrict themselves with herbal therapy without being suffered from chronic side effects. The leaves are used for the treatment of asthma, typhoid fever, stomach problems etc. The roots and seeds are useful in whooping cough and antispasmodic, seeds can be used as stimulating drink[1].

Chrysephanol isolated from leaves, *n-hetriacontanol*, and an unidentified anthraquinone isolated from aerial parts of this plant[2]. Leaves contain emodin, its glycosides and lueolin-7-glucosides and free emodic acids were also isolated[3,4].

Material & methods[5]:

Cassia mimosolides leaves were collected from South Odisha, in the month of February. Prof. S.K.Dash., PG department of Biosciences, CPS, mohuda identified it. The leaves were dried under shade and pulverized into powder by a mechanical grinder. Dried powdered leaves taken for the present study.

Preparation of extracts:

The dried, powered leaves of *Cassia mimosoides* (1.2kg) was extracted with pet-ether (60-80) a dark greenish black coloured extract was obtained (yield 4.8% with respect to dry powder leaves). The marc after drying was extracted with chloroform to produce a dark greenish semisolid (yield 5.2%) collected. These extracts were collected and concentrated for various chemical group identification and pharmacological evaluation.

Phytochemical screening [6,7,8,9]**:**The chemical tests were carried out on the extracts for the qualitative identification of phytochemical constituents and showed in **Table No.1**

Table No.1 Phytochemical tests for the presence of active constituents in *Cassia mimosoides* leaves extract.

Test for Active constituents	Petroleum ether (60-80°c) extract	Chloroform extract
Acidic compounds	+	+
Steroidal compounds	+	+
Glycosides	+	+
Carbohydrates	+	+
Alkaloids	+	+
Flavonoids	-	-
Tannins	-	-
Saponins	-	-
Protein & amino acids	-	-
Antraquinones	+	+

+ = present, -= absent

Pharmacological Evaluation:

Experimental Animals:

Adult wister albino rats (180-220gm) of both sexes were used for the experiments, were housed in standard poly propylene cages at room temperature of 30 ± 2^0c and 60-65% relative humidity and had tree access to food and water adlibitum. The rats were used for the experiment after an acclimatization period of two week. The animals were divided into 6 groups and 6 animals each group.

Acute Toxicity study:

Acute toxicity study was carried out and LD_{50} at the extracts were determined [10,11,14]. From the result 100mg & 200mg of the extracts were selected as the therapeutic dose for the study [12]. All procedures described were reviewed and approved by the University Animal Ethical committee (reference code: 990/C/CPCSEA/2006) University Department of Pharmaceutical Sciences (UDPS), Bhubaneshwar, Odisha.

Anti-ulcer activity of extracts of cassia mimitosides leaves by pyloric ligation model in rats[10, 13]

The antiulcer activity was done by using wister rats. Rats were divided into various groups, each containing six animals. Food was withdrawn for 24hrs and free access to water. Under eather anaesthesia pyloric ligation was made[14,15]. The extracts at *cassia mimosoides* at a dose levels of 100,200mg/kg taken as tests and ranitidine (5mg/kg) as a standard drug, 0.5% carboxy methyl cellulose used as a vehicle for the preparation of suspension and administered orally, prior to ligation.

After 4hr abdomen was opened and the stomachs was isolated after suturing the lower oesphageal

end, and the animals were sacrified. The stomach was opened with the greater curvature. The gastric juice were collected and measured both volume & pH.. The stomach was washed with 1ml of distilled water and the washings were added to the gastric juice. The gastric contents were centrifuged at 2000rpm for 10min. 1ml of the supernatant liquid taken and diluted and titrated against 0.01 N NaoH. The column of 0.01N NaoH required corresponds to the total acidity (meq/L/100gm) was calculated[16].

Acidity -volume of NaoH X normality X 100 meq/1/100/0.1

The results were shown in **table No 2** and **Figure Nos 1 to 6.**

Table – 2: Effect of extracts of *Cassia mimosoides* Linn leaves on pyloric ligation ulcer model in rats **[% of protection** $\left(1-\frac{t}{c}\right)$ **x100]**

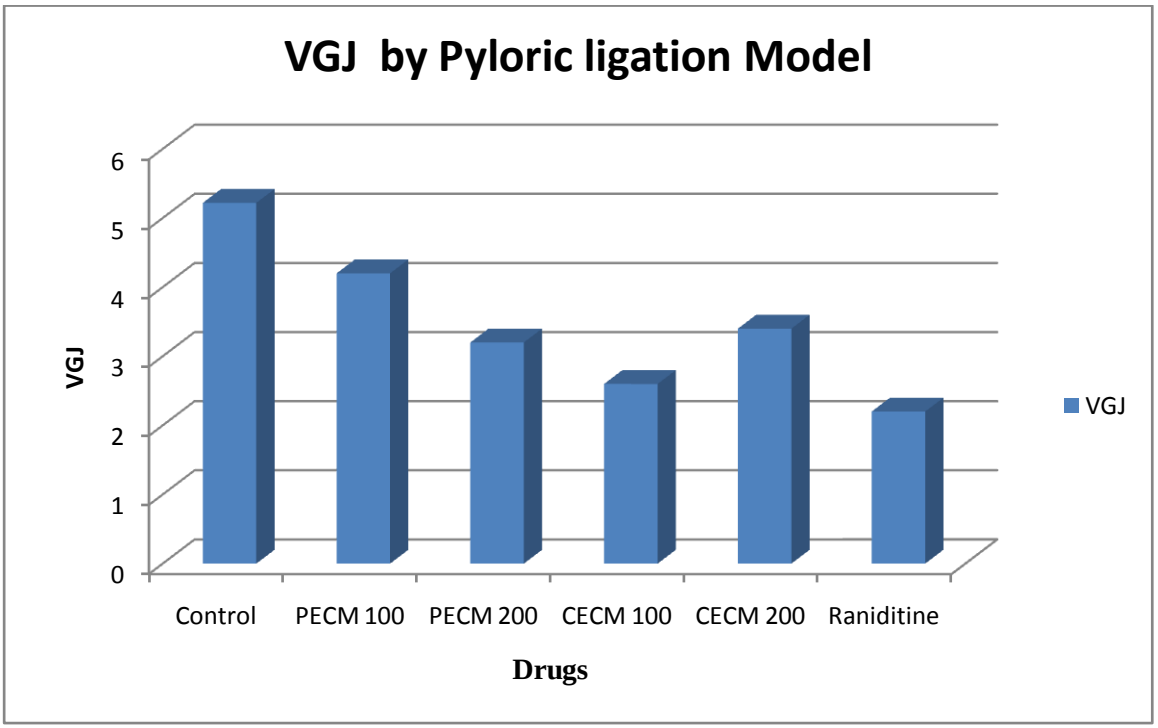
Group	Drug Treated	Volume of gastric juice (VGJ) (ml/100gm)	PH	Free Acidity mEq/L (FA)	Total Acidity mEq/L (TA)	Ulcer Index (UI)
I	Vehicle control (0.5% CMC) p.o	5.22 ± 0.0101	2.2 ± 0.428	2.7 ± 0.0601	12.4±0.0557	5
II	PECM (p.o) 100mg/kg	3.2±0.0843[a](39%)	3.3 ± 0.477[a](50%)	2.6 ± 0.0516(4%)	9.7±0.577[a]	
III	PECM (p.o) 200mg/kg	2.6±0.0634[a](50%)	3.9±0.0577[a](77%)	1.8±0.0749[a](33%)	8.6±0.0577[a]	
IV	CECM 100 (p.o) 100mg/kg	3.4±0.0601[a](35%)	2.9±0.0577[a](32%)	2.4 ± 0.098(11%)	9.7 ± 0.070[a]	3.33(33%)
V	CECM 200 (p.o) 200mg/kg	2.9 ±0.042[a](44%)	3.9±0.049[a](77%)	1.8 ± 0.47[a](33%)	8.4 ± 0.066[a]	1.66(67%)
VI	Ranitidine 5 mg/kg	2.2 ±0.0919[a](58%)	5.4±0.0703[a](100%)	0.4±0.0477[a](85%)	5.2 ± 0.0428[a]	00(100%)

Results are expressed mean ± SEM of six readings; Significance evaluated by One-way analysis of variance (ANOVA) followed by Dunnett's t-test. versus control group, [a]P < 0.001, [b]P < 0.005 (n = 6) Figures in parentheses indicates the % of protection.

PECM– Petroleum Ether Extract of ***Cassia mimosoides*** **Linn**

CECM– Chloroform Extract of ***Cassia mimosoides*** **Linn**

Figure No.1:



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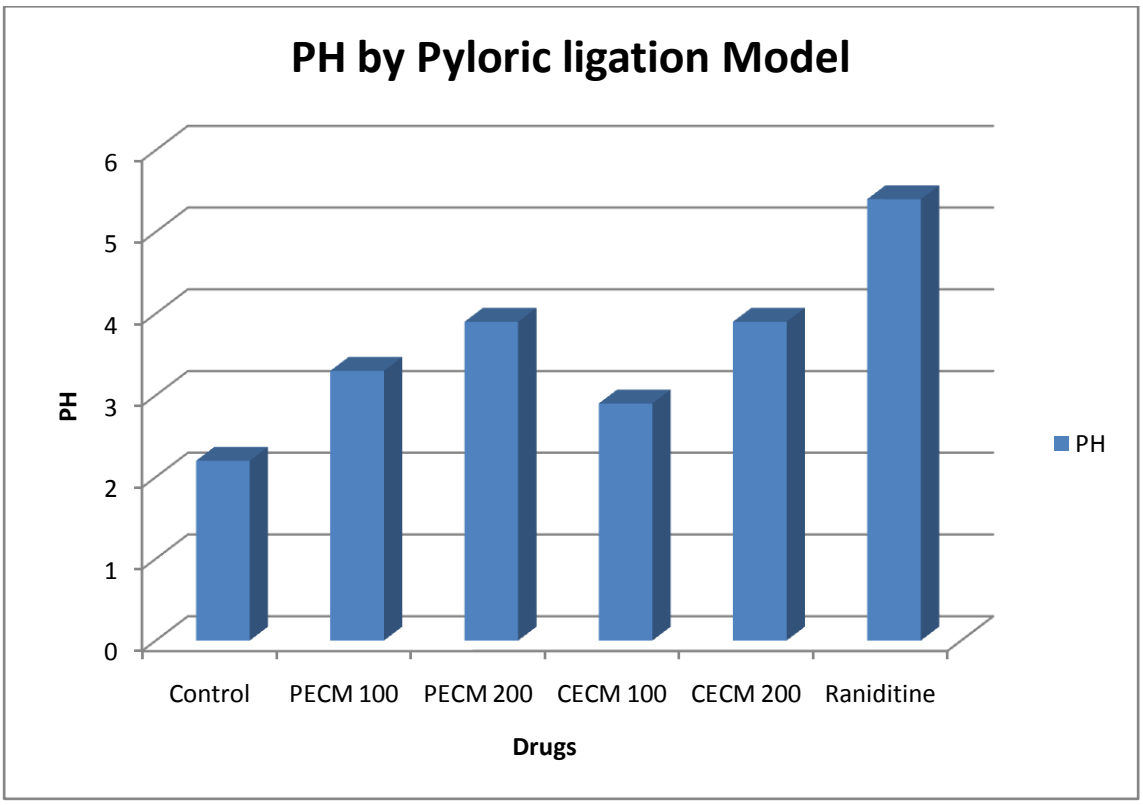

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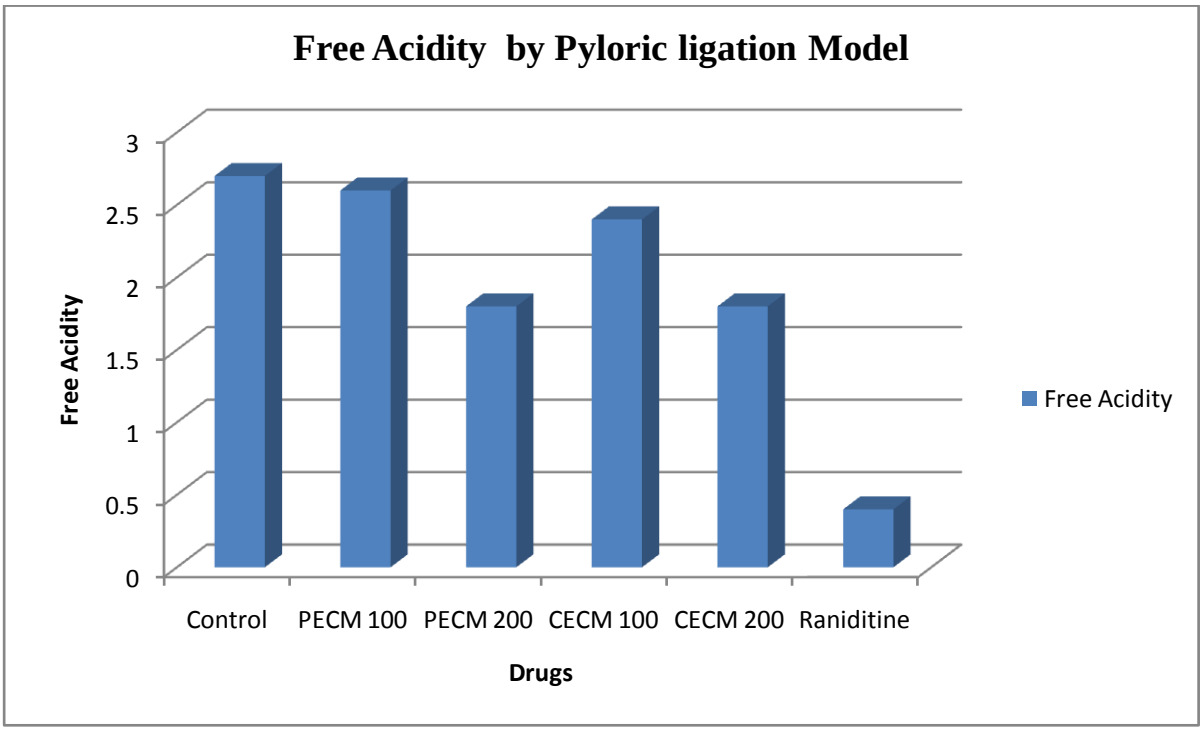

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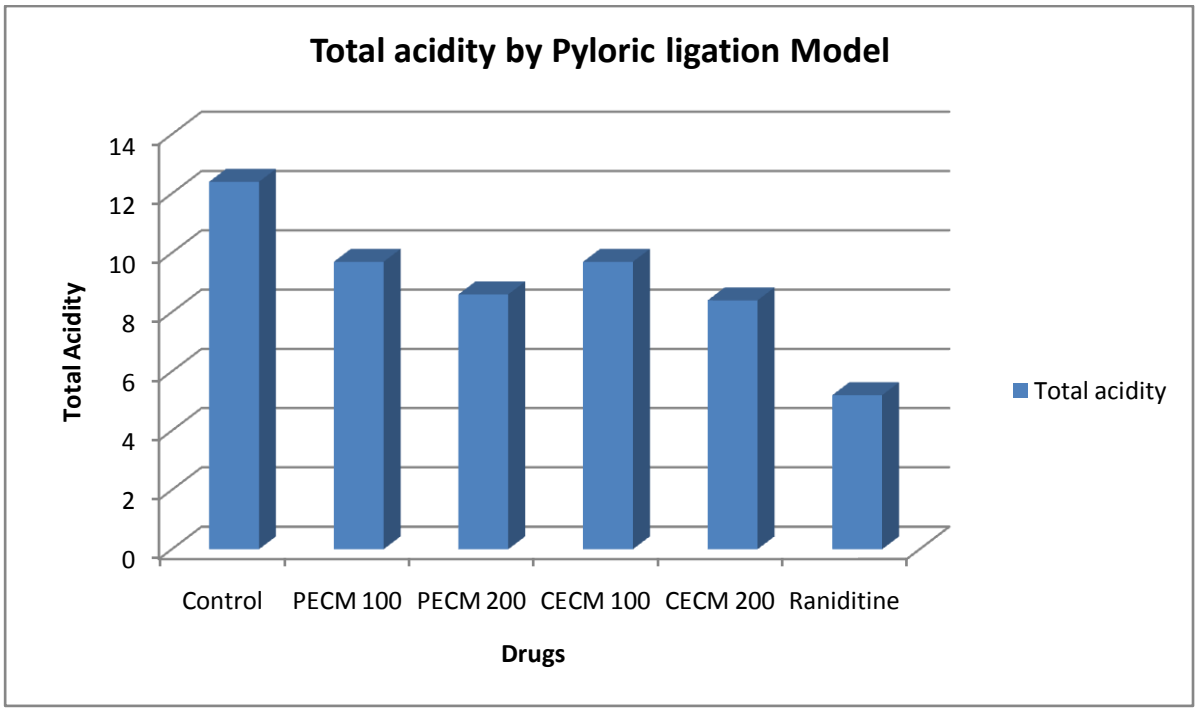

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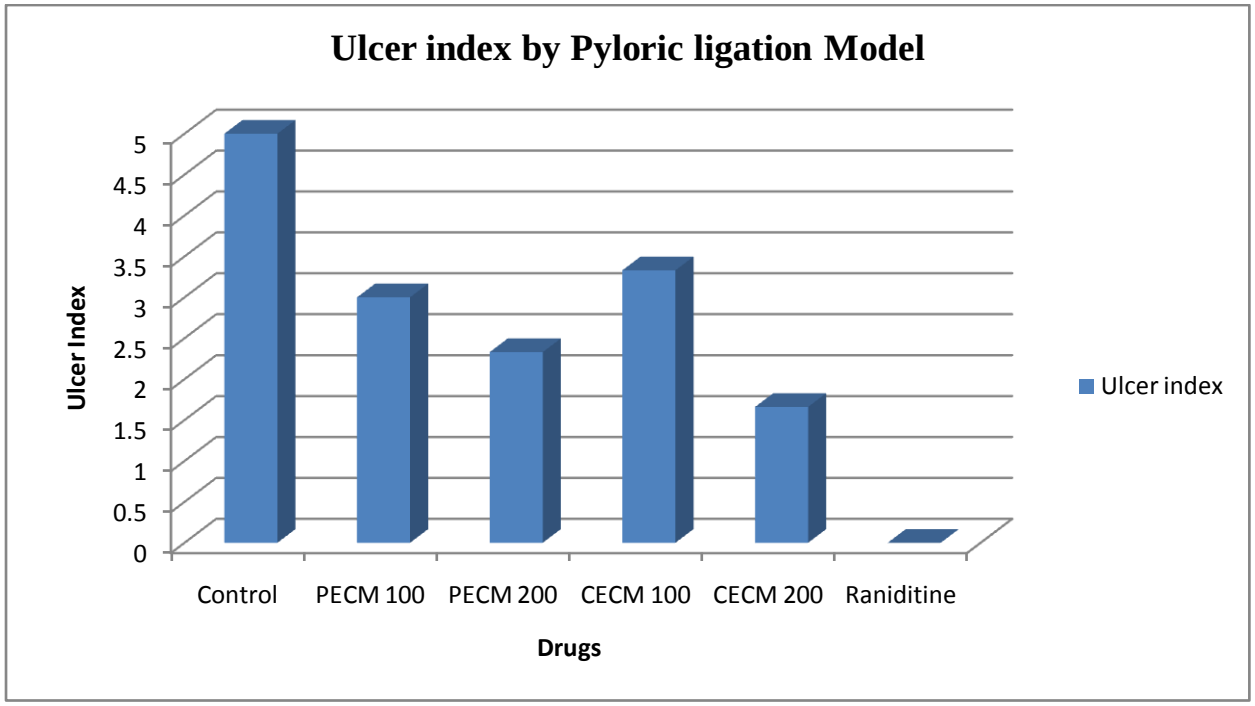

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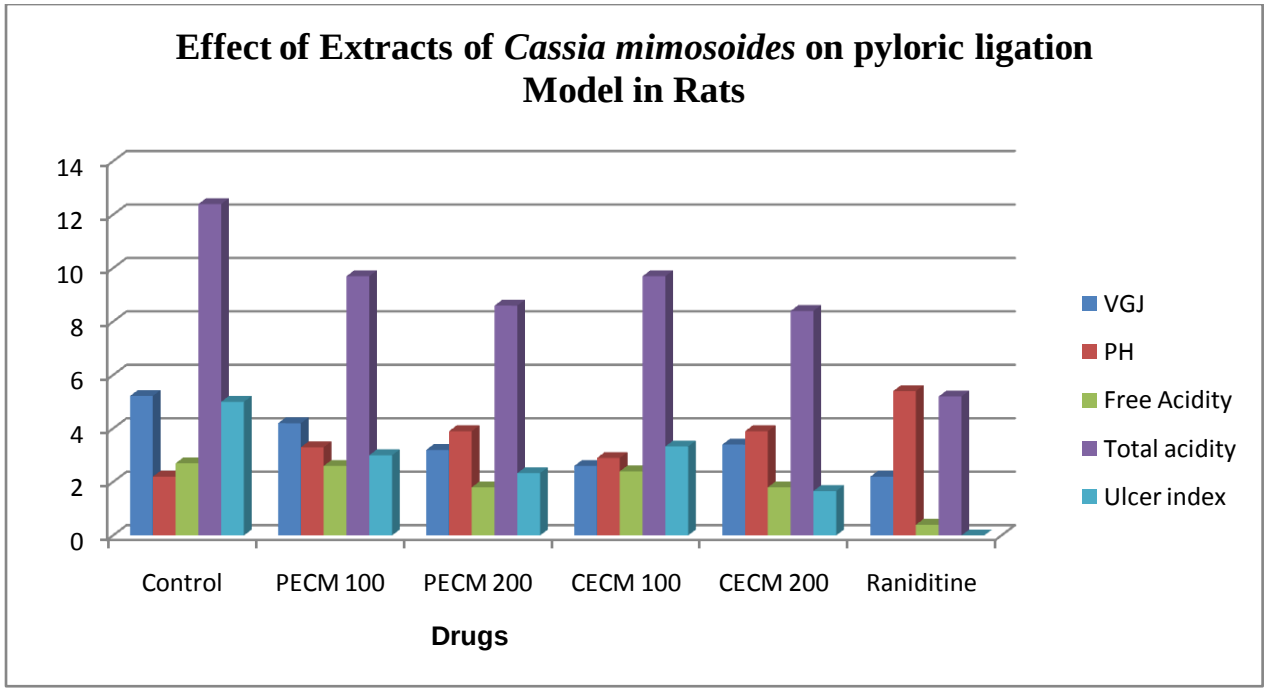


Evaluation of anti-ulcer activity of extracts of *cassia mimosoides* leaves by Aspirin Plus Pyloraus ligation model in rats.[14, 15, 16]

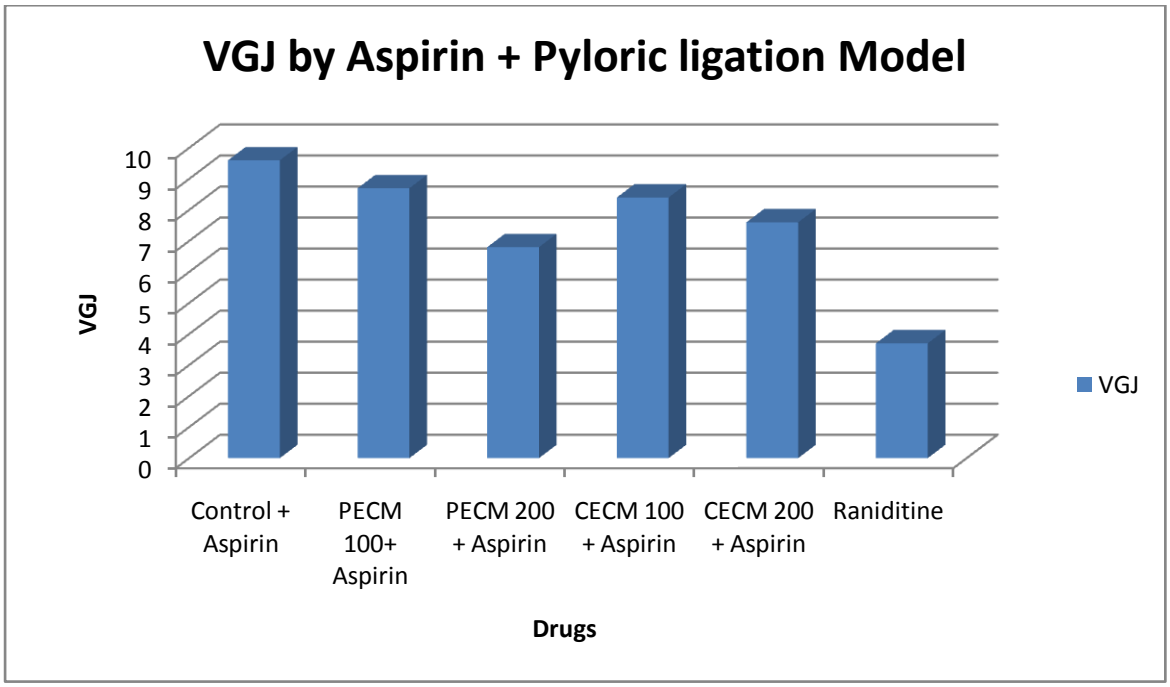
36 wister albino rats of both the sexes of 180-220gm were taken and divided into 6 groups and each group contains 6 animals. All the animals received standard drug ranitidine (5mg/kg) and extracts of *cassia mimosoides* at dose levels of 100 and 200mg/kg taken as test drugs. Treatment with aspirin 200mg/kg daily once for five days to all the animals. On the 6th day the rats were kept fasting for 24 hrs before and under ether anaethesia rats were anaesthetized pyloric ligation. After 4 hrs the animals were sacrified after ligation of oeso-phageal end of stomach. The stomachs were taken out and opened at greater curvature. The volume of gastric juice measured and centrifuged at 2000rpm for 10min. Total acidity of the supernatants liquid determined by litrating with 0.01 NaoH and expressed as meq/L of gastric juice. The excised stomachs were examined for ulcer lesions by a 10 X magnifier. The ulcer index and the % of protection was calculated.
The results were summarized and shown in the **Table-3 and figure-7-13.**

Table–3 : Effect of *Cassia mimosoides* Linn on Aspirin + pyloric ligation ulcer model in rats [% of protection $\left(1-\frac{t}{c}\right)$ x100]

Group	Drug Treatment	Volume of gastric juice VGJ (ml/100gm)	PH	Free Acidity mEq/L (FA)	Total Acidity mEq/L (TA)	Ulcer Index (UI)
I	Vehicle control (0.5% CMC) + Aspirin (p.o)	9.6 ± 0.1542	2.6 ± 0.0494	4.6 ± 0.0472	16.5 ± 0.137	5
II	PECM 100 mg/kg +Aspirin (p.o)	8.7±0.0428[b](9%)	2.8±0.0042(8%)	3.3±0.0277[a](28%)	11.8 ± 0.0307[a]	2.66(47%)
III	PECM 200 mg/kg +Aspirin (p.o)	6.8±0.0477[a](29%)	3.7±0.0601[a](42%)	2.7±0.0210[a](41%)	10.6 ± 0.0501[a]	2.33(53%)
IV	CECM 100 mg/kg +Aspirin (p.o)	8.4±0.0542[a](13%)	3±0.0516[c](15%)	3.5±0.0477[a](24%)	11.3 ± 0.0807[a]	2.33(53%)
V	CECM 200 mg/kg +Aspirin (p.o)	7.6±0.0477[a](21%)	3.9±0.0428[a](50%)	2.8±0.0258[a](39%)	10.8 ± 0.0763[a]	2(60%)
VI	Ranitidine 5 mg/kg +Aspirinn (p.o)	3.7±0.0516[a](61%)	4.8±0.166[a](85%)	0.8±0.0421[a](83%)	5.8 ± 0.0954[a]	0(100%)

Results are expressed mean ± SEM of six readings; Significance evaluated by One-way analysis of variance (ANOVA) followed by Dunnett's t-test. versus control group, [a]P < 0.001, [b]P < 0.005 (n = 6) Figures in parentheses indicates the % of protection.
PECM– Petroleum Ether Extract of ***Cassia mimosoides*** **Linn**
CECM– Chloroform Extract of ***Cassia mimosoides*** **Linn**

Figure No.7:



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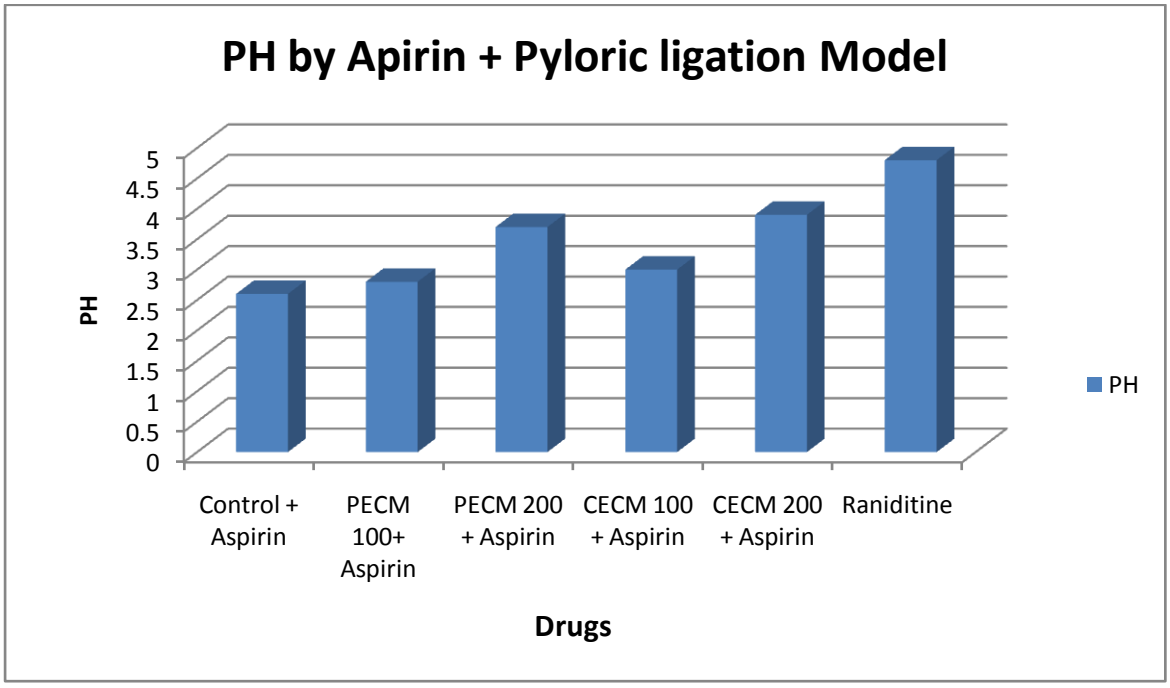

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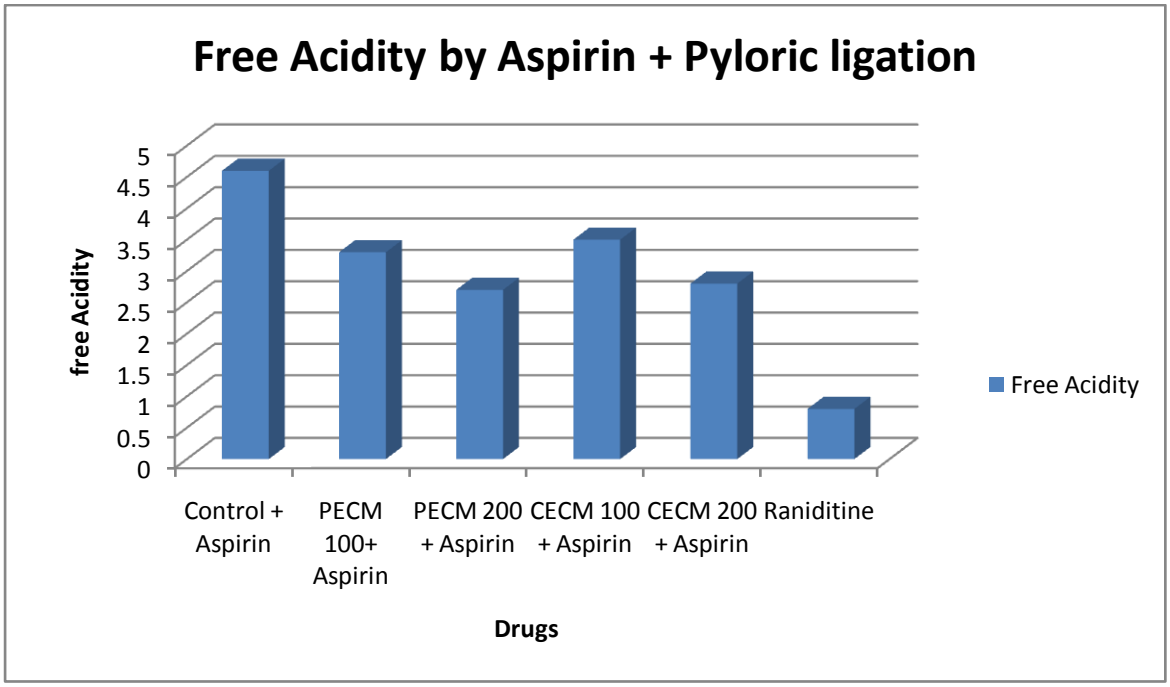

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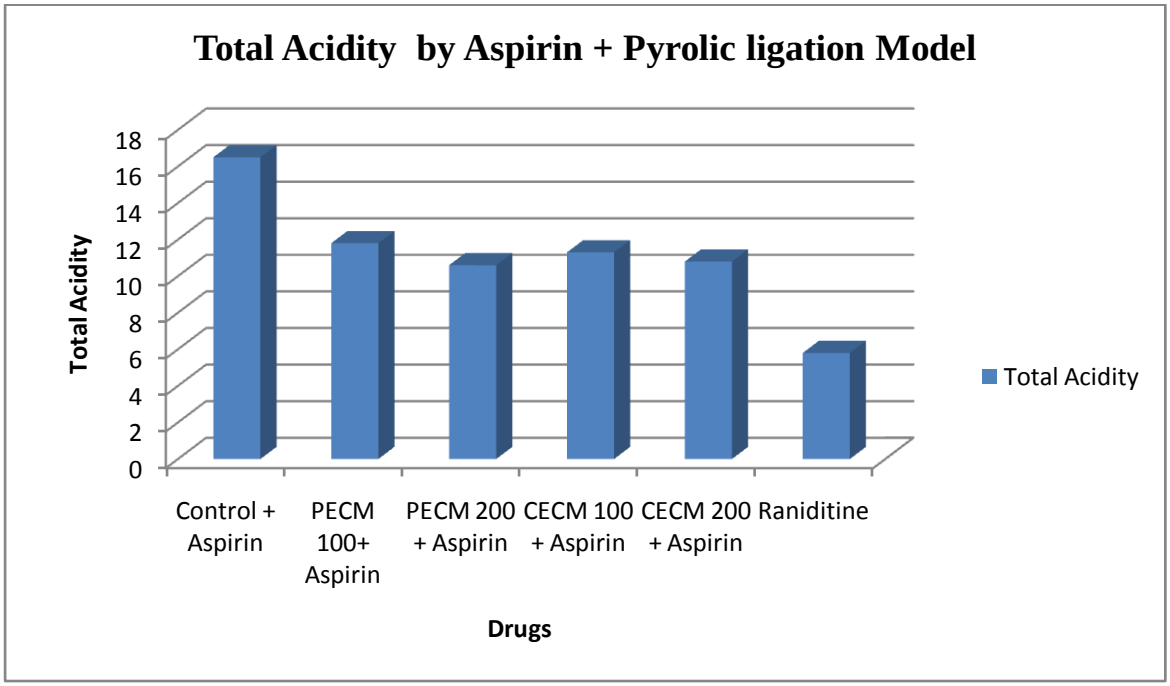

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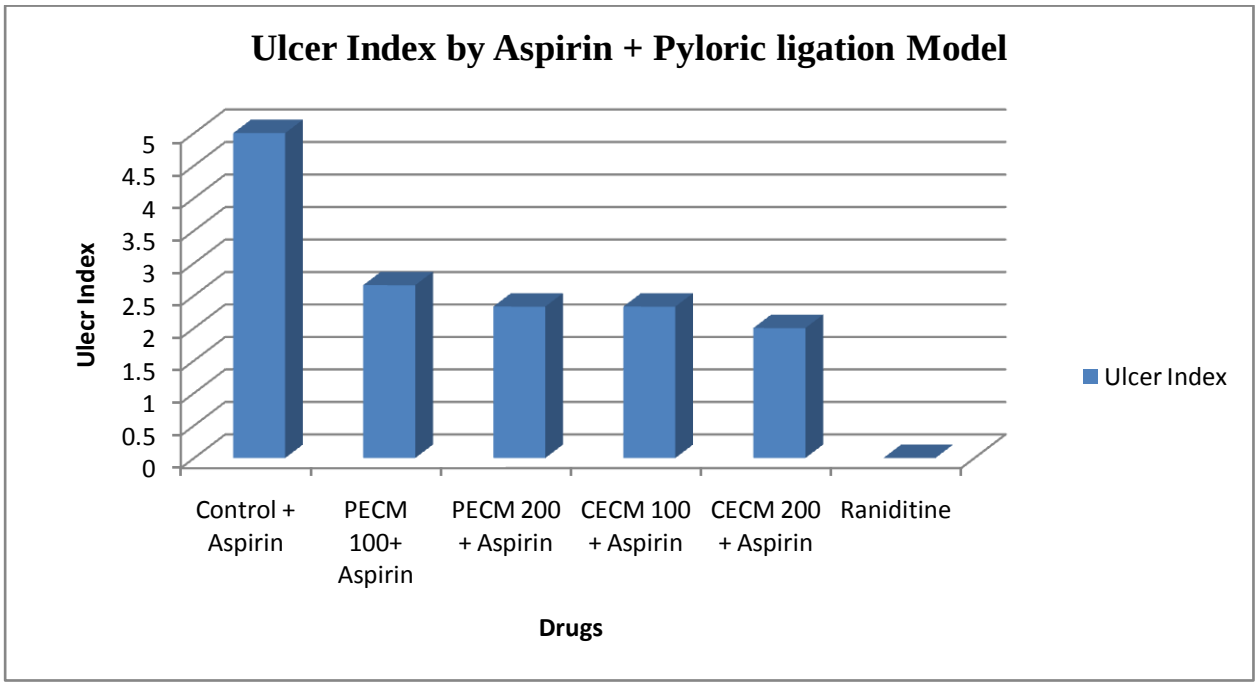

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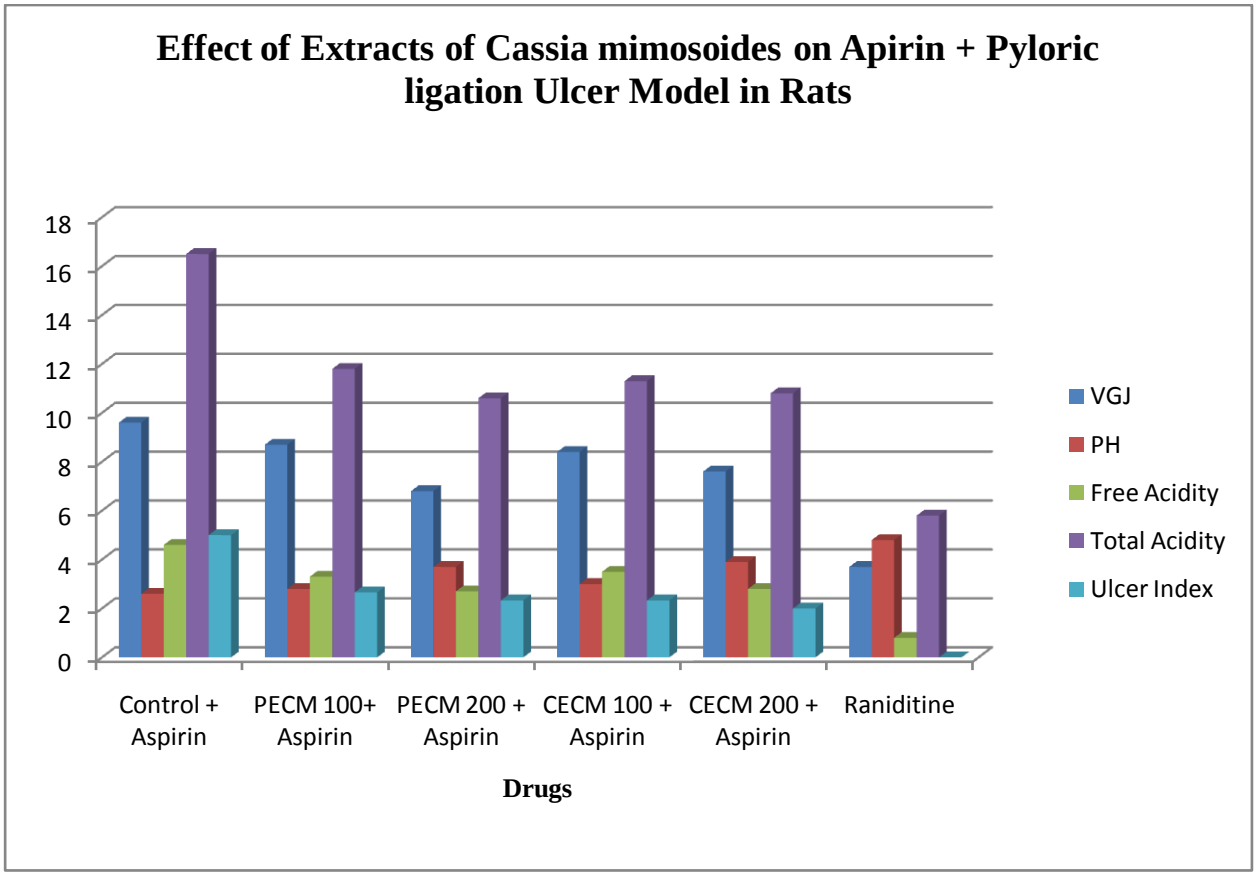

Statistical Analysis:

The results were presented as mean ± SEM and statistical significance between and control group evaluated by one-way ANOVA followed by Dunnett's t-test[16].

RESULTS & DISCUSSION:

The findings of the present study shown the extracts of *cassia mimosoides* at dose levels of 100mg and 200mg/kg having remarkable anti-ulcer activity, in pyloric ligation ulceration (shay rat model) other than aqueous extract ($P<0.01$) found to reduce the volume of gastric juice to a significant when compared to the control and standard group. Similarly the total acidity, free acidity and ulcer indexes are the major parameter for evaluation of anti-ulcer activity was also reduced significantly when compared to control group.The H_2-receptor antagonists inhibit acid secretion elicited by gastrin and to a lesser extent by muscarinic againsts[18-]. The present investigation therefore indicates that the extracts of cassia mimosoides posses anti-ulcer activity which may be due to inhibition of gastrin and hence act as anti-secretory and anti-ulcer agent.

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