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Assessment of Anti-Ulcer Activity of Psidium Guajava Ethanolic Flower Extract in Albino Rats

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ABSTRACT
Gastric ulcers are a prevalent gastrointestinal disorder characterized by the erosion of the stomach lining, often resulting from factors such as stress, non-steroidal anti-inflammatory drug use, and infection.. This study aimed to assess the anti-ulcer potential of Psidium guajava ethanolic flower extract in albino rats.
The research involved inducing gastric ulcers in albino rats using a well-established experimental model. The rats were divided into different groups, including a control group, a reference group treated with a standard anti-ulcer drug, and several experimental groups treated with varying doses of Psidium guajava ethanolic flower extract. The rats were administered the extracts orally for a specified duration, and their gastric ulcer parameters were evaluated.
Various parameters were assessed to determine the anti-ulcer activity of the Psidium guajava ethanolic flower extract, including ulcer index, gastric volume, gastric pH, mucin secretion, and histopathological changes. The results were statistically analyzed using appropriate methods.
The findings of the study indicated that the Psidium guajava ethanolic flower extract exhibited significant anti-ulcer activity in albino rats. The extract effectively reduced the ulcer index, gastric volume, and acidity levels, while promoting mucin secretion and demonstrating favorable histopathological changes in the gastric mucosa. These results support the traditional use of Psidium guajava in the treatment of gastric ulcers and highlight its potential as a natural alternative or adjunct therapy to conventional anti-ulcer drugs.
In conclusion, this study provides scientific evidence for the anti-ulcer activity of Psidium guajava ethanolic flower extract in albino rats. The findings support further research to identify and isolate the bioactive compounds responsible for the observed therapeutic effects.
Keywords; Ethanol, anti-ulcer, Psidium guajava, non-steroidal anti-inflammatory drug

I. INTRODUCTION

Peptic ulcer (H. pylori) is chronic of development disease. It is characterize by imbalance linking the different factor that damage the internal mucosal an those for the its protection & resulting to a lesion of digestive tract[1]. It have two of the mainly prevalent disease in the all world, & some of complication major causes of the morbidity & mortality. The incidence of duodenal and gastric ulcers varies across the global population, and the average age of those who have the condition is between 30 and 60 years old, while it can strike at any age. among Africa, duodenal ulcers are uncommon among black individuals, but in the United States, both blacks and whites experience the same prevalence of duodenal ulcers. Additionally, males are more likely than females to get duodenal ulcers. The causes of peptic ulcers might be complicated. Alcohol and nicotine, among other environmental factors, can prevent or lessen the release of mucus and bicarbonate, which increases the secretion of acid.

Children, adult & old who have duodenal ulcers are more than two times more likely to get the condition general population.The discovery of Helicobacter pylori and ulcers linked to long-term use of anti-inflammatory medications have improved our understanding of the circumstances surrounding the development of peptic ulcers in recent years. A good diet is defined by nutrition and its guidelines, and it has long been understood that setting nutritional benchmarks is essential for promoting health and preventing and treating diseases.

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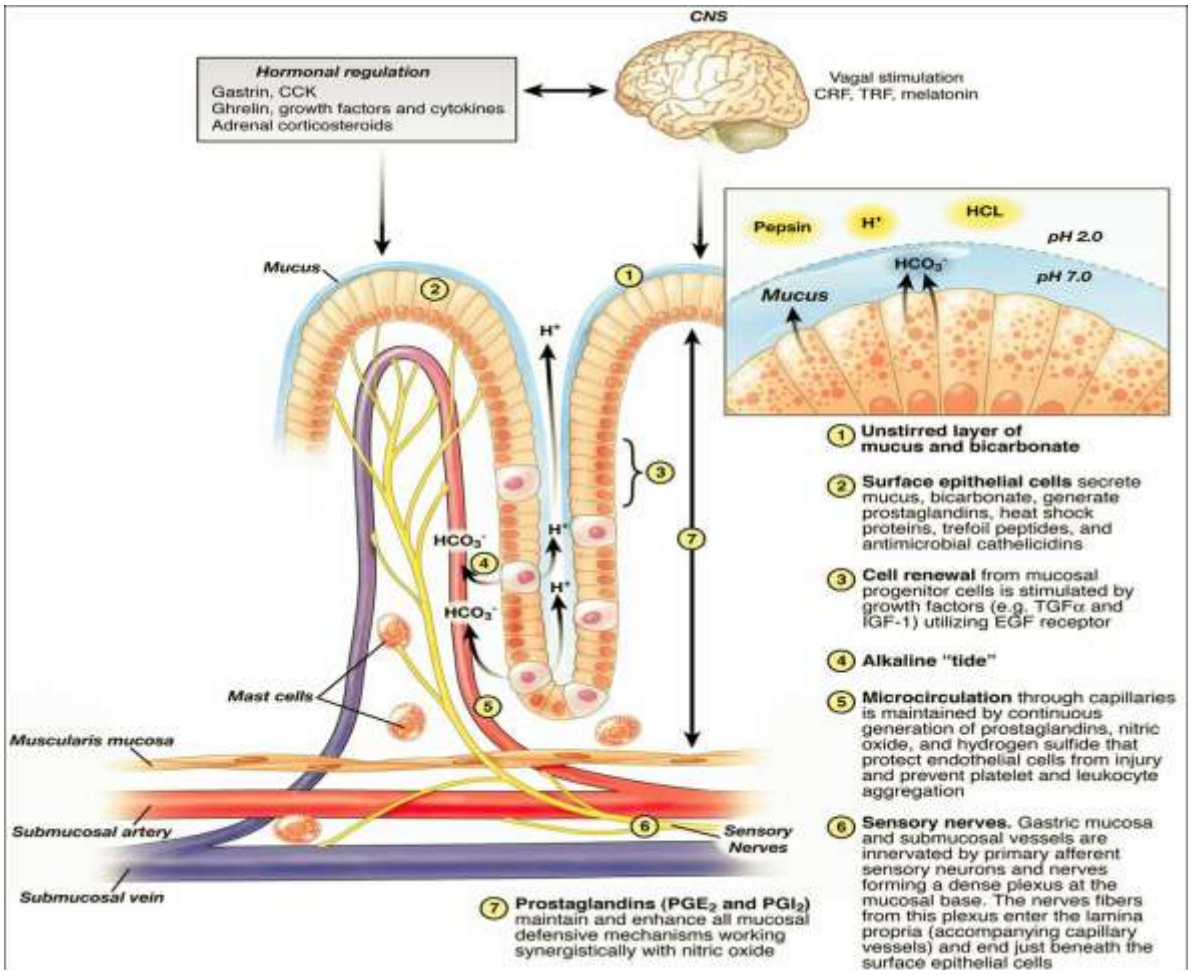

PLAN OF WORK

1. Preliminary work
2. Literature of survey
3. Selection of plant
4. Collection andauthentication of plant material
5. Drying and Extraction of plant
6. Percentage yield
7. Acute oral toxicity
8. Phytochemical Investigation
- Qualitative Phytochemical Estimation
9. Antioxidant Activity
- DPPH Assay
10. In-vitro study antibacterial activity
- Well diffusion assay
11. In-vivo study anti-ulcer activity
- Indomethacin induced Ulcer model

Plant collection

The medicinal plant Psidium guajava flower (300 gm) was collect from Bhopal, M.P. After collection & cleaning, the part of plants were dried under dimness at the room temperature for 4 days or oven dried at the temperature 45°C till complete drynes.

Extraction of plant material

Flower powered are plant parts of Psidium guajava (300 gm) was extracted by the used of method extraction & dissimilar organic with solvents used with ether (50-60^{0}C) and consecutively extracted in methanol for 30 hours using the soxhlet apparatus. To make certain the complete extraction of each Psidium guajavaextract evaporate to the compact pressure with used rotary air evaporator. It results are dried excess container for additional use.

Formula;

$$\% \text{ yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x} 100$$

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Figure 1: Soxhlet apparatus

Phytochemical investigation in extraction

To identify the experimental extraction absence or presence of another phytoconstituents by complete the qualitative & quantitative photochemical analysis. The identify colour strength precipitate configuration it use for the medical purposes to tests.

Test for Carbohydrates

- **Molisch'sTest:** The aqueous solution of the Psidium guajavaextract to 1 ml were mixed with little drops of Molish reagent with conc. H_2SO_4 was further drop wise added along with the wall of test tube. When two liquid mixes up, occurs. It observed the colour present of carbohydrates.
- **Benedict's test:** All equal level amount of Benedict's sample reagent & Psidium guajava extract to be mixed & heated for 8-15 minutes on water bath. appears green, yellow or red which shows the present of reducing sugar.
- **Barfoed's Test: In** this aqueous level is solution of Psidium guajavaextract, 2 ml oil used Benedict solution with added and excited for boiling. In the presence of monosaccharides red colour indication was seen due to formation of cupric oxide.

Tests for Alkaloids

- **Dragendorff's Test:** Extract 2 ml of Psidium guajava was taken. With acohol was mixed & w shaken with little drops of acetic acid Dragendroff's reagent. This alkaloids presence indicates of an orange red precipitate.
- **Wagner's Test:** In acetic acid 1ml of Psidium guajavaextract was dissolved. Few drop of Wagner's sample reagent are mix. This indicate presence of alkaloids reddish-brown ppt.
- **Mayer's Test:** 2 ml of Psidium guajavaextract was dissolved with acetic acid & few drop with reagent added to it. The alkaloids presence and indicated by all formation of a dull white color.

Test for Saponins

- **Froth Test:** 2ml of Psidium guajava extract was added in water & shaken well. The occurrence of saponin was indicate by stable froth arrangement.

Test for Triterpenoids & Steroids

- **Libermann-Burchard Test:** The Psidium guajava extraction was dissolved with ccl4. To it 2 ml of acetic acid & 1 ml of acetic anhydride added, after that boiled on a water bath & next cooled. This Indication Presence of steroids by bluish green colour.
- **Salkowski Test:** The Psidium guajavaextract was dissolve in a chloroform & the same volume with normal concentrated of sulphuric acid with added ccl4. (green colour)

Test for Tannin and Phenolic Compounds

- **Ferric Chloride Test:** The quantity of Psidium guajavaextract are dissolve in water. Add to the little drop of diluted solution with fe+ chloride. dark blue presence of tannins.

Test for Flavonoids

- **Shinoda's Test:** A few little drops of concentrated HCL to 1 ml of Psidium guajavaextract in alcohol were added. It was heated on the water bath. when the (red to pink colour) occurred presence of flavonoids.

Test for Glycosides

- **Borntragers Test:** Dilute sulphuric acid was added to 5 ml with test solution dilute to the sulfuric acid be added. It was warm for approx 10 min. and then filtrate was obtained. It cold filtrate, the same amount of benzene or chloroform be added to the cold filtrate with shaken well. Separation of organic chemical layer was obtain & after that NH3 was added. occurrence of anthraquinone glycosides are indicating, the (pink and red colour) in ammonical layer.

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- **Keller Killiani Test:** 2 ml of test solution are added in a test tube, 5 ml of mix glacial acetic acid & 5 drop with 5% fe use chloride. Add carefully 0.1 ml of concentrated sulphuric acid. The presences of Cardiac glycosides were indicated by the creation of blue colour .

Test for fats and oils

- **Solubility test**

➢ This test 4-6 ml of mix alcoholic solution of Psidium guajava extract, and little ml. of chcl3 & solubility is seen.

Acute Toxicity Study

This toxicity study method position to the guideline method with the use 3aprox animals of a only per step.This study depending on the death & disease rate status of animal, with a average all hours may be used for necessary to the allow for the judgment of on toxicity study. The material (Psidium guajava) is administered by orally group of experimental animal dose. The material is experienced using method, all doses using 3 animals are using single sex. [78]

DPPH

This method determine antioxidant activity of Psidium guajava extract by using DPPH free radical scavenging assay. 1 mg/ml Eethanolic solutions are extracts/standard was prepared.

Different concentration of Psidium guajavaextracts/standard (20 – 100µg/ml) were prepared from 1mg/mL stock solution and 2mL of 0.1mM solution with DPPH was added. This mixture were and incubated for in 30 min in the room [79]

Percentage antioxidant activity of sample/standard was calculated by their using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Experimental work

Animals Protocol

IAEC Approval All animals used experiment approved by Institutional Animal Ethics Committee (IAEC).

Animal used

Weight 200±50 gm

Strain Wistar rat

Housing Condition- Animals are housed in group of the six inseparate cage under proscribed condition temperature (23 ± 3°C). All are the animals are given the standard and maintain diet & water change regularly.

Induction of ulcer in rats:

Male Wistar rats weighing 230±20 were fasted for 24 hr with liberated admission to water and rats randomly divide into five group. The control groups received a normal (water, 6 ml/kg, p.o.) and Second group is inducer group which was treated only Indomethacin 30 mg/kg bw. And treatment groups III and IV were given Indomethacin 40 mg/kg bw & test sample (Extract-100, 500 mg/kg body weight) respectively 70 min prior to the Indomethacin (20 mg//kg with route of p. o.) for seven days. Standard group (V) was treated antiulcer drug
(omeprazole 20 mg/kg, p.o,). The rats were sacrificed after one hours of Indomethacine**.**

Experimental design

Rats (n=30) was randomized into following groups:
Group 1- Normal control
Group 2- Inducer group Indomethacin 20 mg/kg
Group 3- Psidium guajava extract Treated 200 mg/kg
Group 4- Psidium guajava extract Treated 400 mg/kg
Group 5- Standard drug Treated (Omeprazole) 20 mg/kg

Parameters assessed for anti-ulcer activity

- Determination Ulcer Index
- Determination Volume and urine ph & gastric juice
- Free acidity determination

Ulcer index

The next random score system are used for grade to the prevalence & severity of the lesion. The stomach be then incise along to the superior curving and rinsed with the normal water and remove the gastric ulcer contents, with examine by the using a 45x and 10x magnifier used lens to assess the formationin ulcers. No. of ulcers are counted by Kulkarni method (0 = no ulcer, 0.5 = red coloration, 2 = spot ulcers, 3 = Haemorrhagic streaks, 4 = Ulcers > 3 but < 5 and 5 = Ulcers > 5)**.**
The ulcer Index & percentage of ulcer inhibition were resolute as follows:
Ulcer index = UN + US + UP × 10−1
Where,
UN = Average number of Normal ulcers per animal,
US = Average of the severity score,

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UP = Percentage of the animals with count ulcers

Volume of gastric juice

This quantity of juice in each animal be calculated past centrifugation with aprox 1000 rpm for 12 min. & analyzed. The quantity of used centrifuged sample is expressed as ml/ 100g body weigh.

pH of gastric juice

This pH meter used of gastric juice for important pH 2 ml of gastric juice with 2 ml of distilled water.

Determination of free acidity

2 ml used of distilled water & dilute 1 ml of juice aliquot & transferred to the conical flask (60 ml) with adding of 3 drops phenolphthalein indicator. 0.02 N NaOH used for the titration pending a undying pink color resulted its extreme volume was determined**.** The acidity are calculated by this formula:

$$\text{Acidity} = \frac{\text{VolumeofNaOH} \times \text{NormalityofNaOH} \times 100}{0.1}$$

II. RESULTS

Percentage Yield

This extraction used for percentage yield is a extremely critical in a order to the determine for standard effectiveness for extraction , & specific plant various sections plant solvents are used. The yield of extracts traditional from Psidium guajavais given away in Table: 5

Table 1: Percentage Yield of crude extracts of Psidium guajava extract

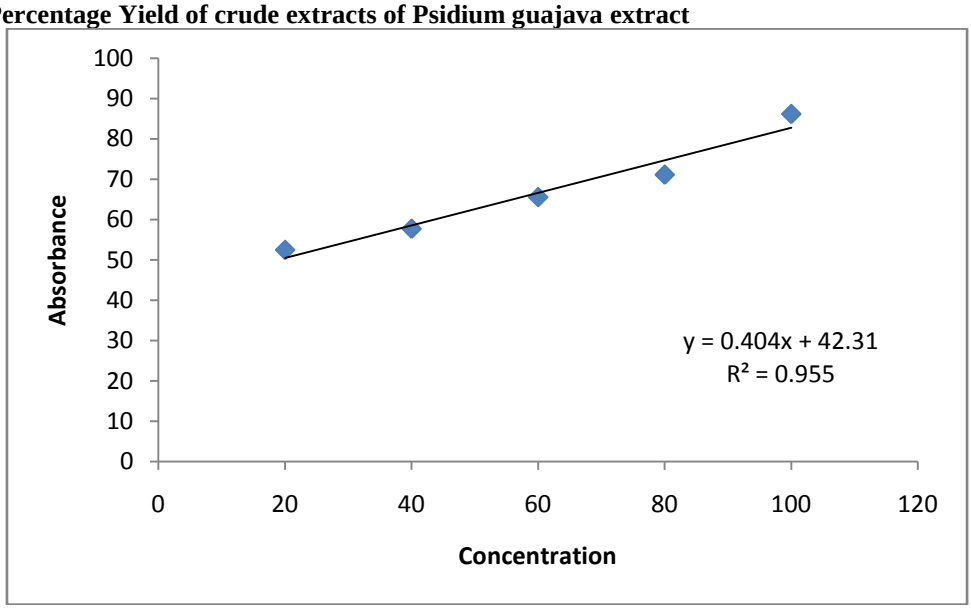

Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid

Table DPPH radical scavenging activity of methanol extract of Psidium guajava

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.521	42.4371
40	0.486	46.2923
60	0.458	49.3928
80	0.425	53.0321
100	0.365	59.6634
Control	0.905	
IC50		**59.22**

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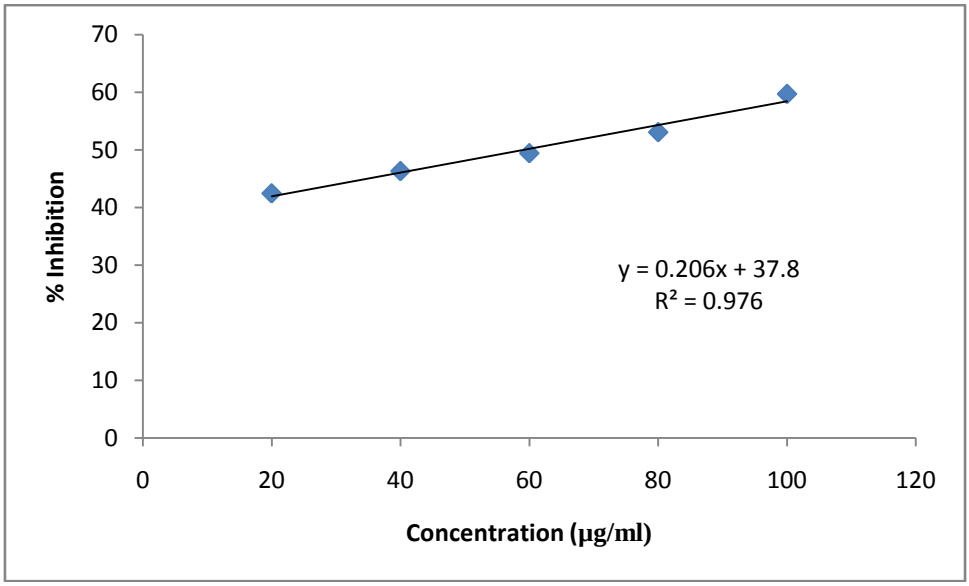
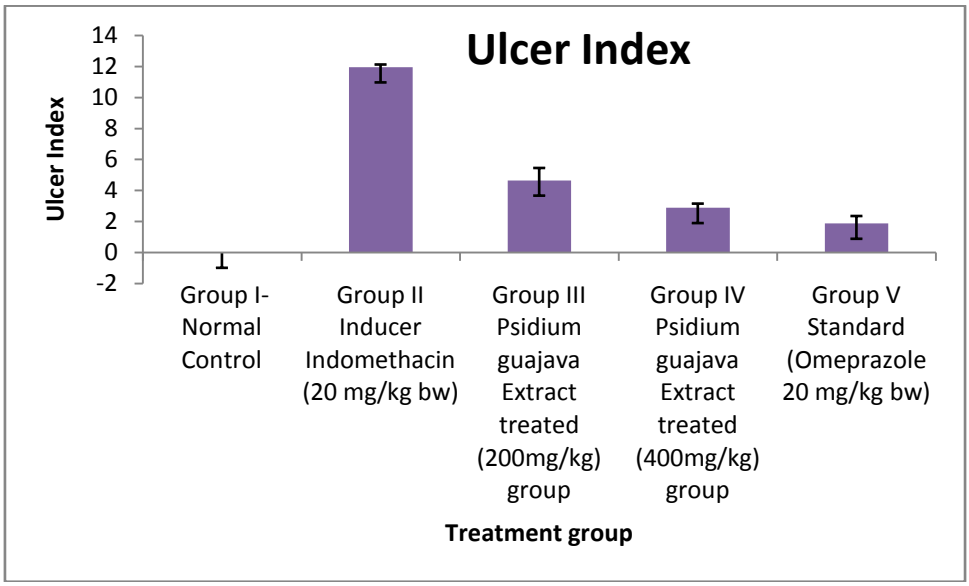


Figure: represents the Percentage Inhibition Vs Concentration of extract of Psidium guajava

Table: **Observation of Ulcer Index**

Groups	**Ulcer Index**
	Mean
Group I- Normal Control	0
Group II Inducer Indomethacin (20 mg/kg bw)	11.957±0.154
Group III Psidium guajavaExtract treated (200mg/kg) group	4.655±0.785
Group IV Psidium guajavaExtract treated (400mg/kg) group	2.886±0.256
Group V Standard (Omeprazole 20 mg/kg bw)	1.873±0.468



Graph: **Bar chart represents ulcer index in indomethacine induced ulcer in rats**

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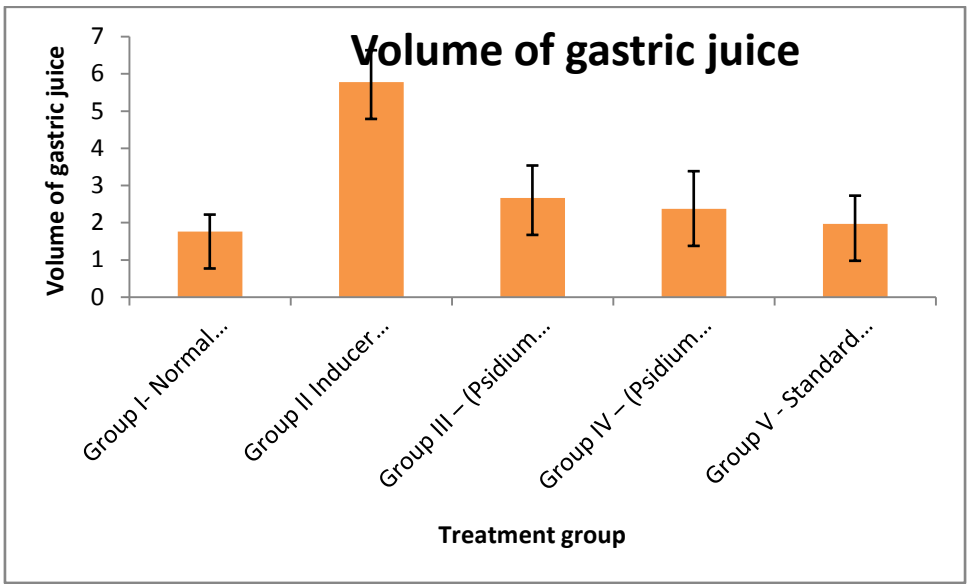
Determination of Volume of gastric juice

Table: Observation of volume of gastric juice

Treatment Group	Volume of gastric juice
Group I- Normal Control(Saline)	1.766± 0.448
Group II Inducer Indomethacine 20 mg/kg bw)	5.783± 0.846
Group III – (Psidium guajavaExtract treated 200 mg/kg bw)	2.668± 0.864
Group IV – (Psidium guajavaExtract treated 400 mg/kg)	2.373± 1.007
Group V - Standard (Omeprazole 20 mg/kg bw)	1.973± 0.75



Graph: Bar chart represents gastric volume in indomethacine induced ulcer in rats

Determination of pH of gastric juice:

Table: Observation of pH of gastric juice

Treatment Group	pH of gastric juice
Group I- Normal Control(Saline)	4.257±0.676
Group II inducer Indomethacin 20 mg/kg bw)	2.375±0.915
Group III – (Psidium guajavaExtract treated200 mg/kg bw)	3.437±0.698
Group IV – (Psidium guajavaExtract treated400 mg/kg)	3.846±1.019
Group V- Standard (Omeprazole 20 mg/kg bw)	4.027±0.783

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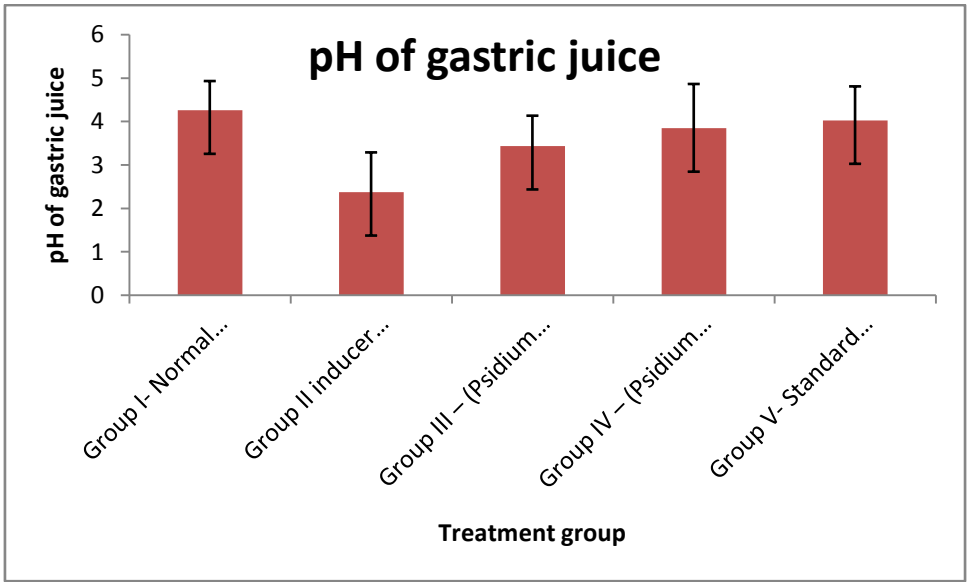
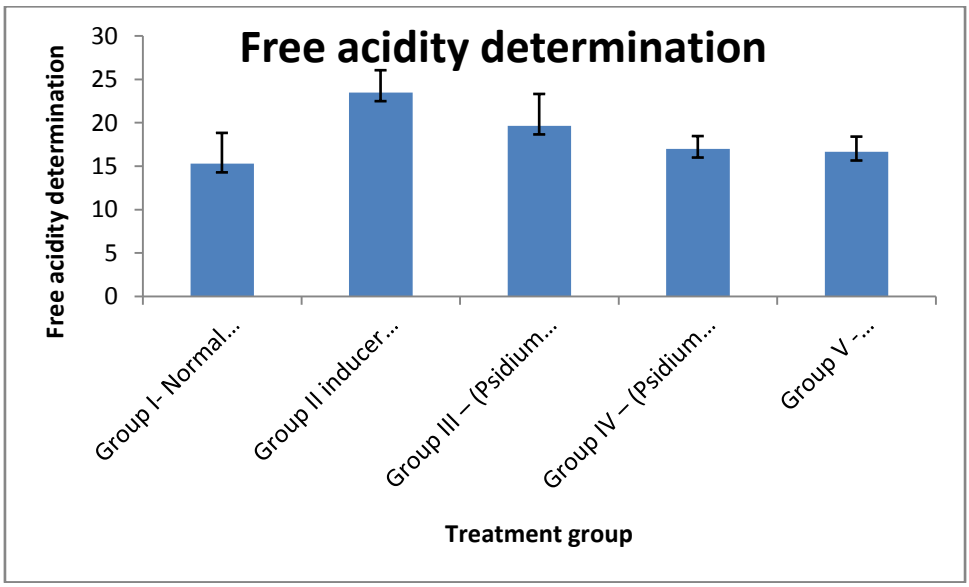


Graph: **Bar chart represents pH in indomethacine induced ulcer in rats**

Free acidity determination:

Table: **Observation of free acidity in indomethacine induced peptic ulcer in rats:**

Treatment Group	**Free acidity determination (mE/L)**
Group I- Normal Control(Saline)	15.287±3.544
Group II inducer Indomethacin 20 mg/kg bw)	23.485±2.562
Group III – (Psidium guajavaExtract treated 200 mg/kg bw)	19.658±3.656
Group IV – (Psidium guajavaExtract treated 400 mg/kg)	16.996±1.471
Group V - Standard(Omeprazole 20 mg/kg bw)	16.655±1.751



Graph: **Bar chart represents free acidity determination in indomethacine induced ulcer in rat**

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Images

Figure: Normal stomach of rat

Figure: Ulcer is induced in rat stomach

III. DISCUSSION

Peptic ulcer, is considered for current age epidemics, It has been distressing around 13% of world population**.** Previous study optional that ulcer to difference between acid , lipid & pepsin along with failing of the mucosa membrane blockade**.** Due to the lesion it normally for connected with injure of stomach, which is the

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simply generate via overkill creation of endogenous & exogenous lively oxygen with free radicals used . a few % main causes of ulcers exclude chronic alcoholic damage beverages & $NASID_S$, as well as soon stress & ulcer infection**.**

The Indomethacin drug induced the ulcer model was engaged for screening & anti-ulcerogenic activity. This model shows gastric acid secretion & cytoprotection activities**.** It is induced gastroduodenal ulceration through its facility to inhibit prostaglandin synthesis**.** The inhibition of prostaglandin, which acting a vital role in exhilarating the discharge of bicarbonate & mucus, maintain flow of blood & bendabl cell proceeds & repair, results increase receptiveness to mucosal injury & gastroduodenal ulceration.

oral acute toxicity studies, Psidium guajavawas create to be protected as did not cause any mortality rate up to 2000 mg/kg. Hence, 300 and 600 mg/kg doses were selected for the present study.

DPPH radical scavenging activity of Psidium guajavaextract exhibit % of inhibition 59.66% and its IC 50value was found to 59.22µg/ml. Ascorbic acid used for a reference composition which exhibit % reticence 86.17% and show IC 50 value of 19.03µg/ml.

Five group of wistar rats was taken . Rats be divided into different five group each containing to 6 animals. Group first received of saline for 7 days. Group second is indomethacin inducer group (20 mg/kg bw). Group third is Psidium guajavaextract (200 mg/kg bw). Fourth group is Psidium guajavaextract (400mg/kg bw) and Group fifth is standard (Omeprazole 20 mg/kg bw). The extract of Psidium guajavawas evaluated by using indomethacin induced peptic ulcer model. Ulcer produced model was seen as red sores. The stomach of rats in the indomethacin induced peptic ulcer showed higher inductions of gastric ulcers.There are a important reduce in the calculated gastric ulcer index. It is treated animals are compared with the Psidium guajava(200 mg/kg bw) treated. The volume of gastric juice was observed as 2.373 ml of Psidium guajava(400 mg/kg bw) in decreased level as compared to Psidium guajava(200 mg/kg bw) treated group showed gastric volume of 2.668. The pH of gastric juice was observed as 3.846 of Psidium guajava(400 mg/kg bw) treated group and it showed the reduction in acidic pH as compared to Psidium guajava(200 mg/kg bw) showed 3.437 pH. The free acidity was observed as mE/L of Psidium guajava(400 mg/kg bw) treated group 16.996and it showed the reduction in acidity as compared to Psidium guajava(200 mg/kg bw) showed 19.658 mE/L.

IV. CONCLUSION

This result plant sample extraction kingdom is represents to rich store house of some organic compound, several used for the medicinal purposes & can serve as front for the growth of new agents having excellent efficacy & pathological disorders.

The fresh extract of Psidium guajavawas extracted. These bio-active components like carbohydrates, glycosides, flavonoids, phenolic components alkaloids & triterpenoids are present in ethanolic extract Psidium guajava. Experimental results have revealed that the extract of Psidium guajavahas different degrees of anti-ulcer activity depending leading to the dose level and the bioactive components present in it.

This study present treatment of Psidium guajava extract flower maintains the normal range of acidity & also preserve pH level of stomach. It study chains use of Psidium guajava extract by confined healers as a fixed medicine in treatment of ulcer. This effect can be use qualified to occurrence of a variety of bioactive components present on extract due to protecting potential of extract confirm the mechanism of anti-ulcer activity against indomethacin induced peptic ulcer.

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