https://doi.org/10.33472/AFJBS.6.Si3.2024.820-841

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Insilico, Invivo Antiulcer Activity of *Panicum Sumatrense*

Akula Sowjanya[*1], Shaik mallika[2], Uma Mahesh[3], Dr. Amreen Sultana[4], Dr. K.lakshmi Surekha[5], Dr.P.Ramya[6]

[1*, 2, 3] Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana.

[4,5,6] Department of pharmacy practice, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana.

***Correspondence:** Mrs Sowjanya Akula**,**Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana. akula.sowjuu@gmail.com,Phone: +91-8106445852, Orcid: (https://orcid.org/0000-0002-6560-4505).

Article Info
Volume 6, Issue Si3, June 2024
Received: 13 Apr 2024
Accepted: 11 May 2024
doi: 10.33472/AFJBS.6.Si3.2024.820-841

Abstract: Ulcers are occurs due to imbalances between aggressive factors and defensive factors. According to WHO guidelines 7.8 million people are affected every year with peptic ulcers. An available marketed drug shows side effects like arrhythmia, hypersensitivity reactions. Hence there is a need to develop safe, therapeutic, cost effective anti-ulcer drugs. Various efficient natural herbal remedies to treat ulcers but there are no scientific evaluations and explanations for those kinds of remedies. In this scenario *Panicum sumatrense* is one of the natural sources to boost up gastro-intestinal health status and protective agent for ulcers. The present study was designed to evaluate the antiulcer activity of *Panicum sumatrense* by using *invivo* and *insilico* methods. In-silico studies has done using Autodock 4.2 version with the chemical constituents of panicum sumatrense and with various proteins and we have found that gallic acid, ferulic acid have high docking score with urease protein and gastric proton pump respectively. The milk extract of Panicum sumatrense significantly decreases ulcer index when compared with methanolic extract in aspirin and pyloric ligation induced ulcer models. Significantly increases the pH and decreases the total and free acidity of gastric juice in pyloric ligation induced ulcer model. presence of ferulic acid present in panicum sumatrense which get attached with gastric proton pump. As described earlier in *insilico* docking scores (table no-11) ferulic acid(docking score-6.4) has binding affinity to alpha chain of gastric proton pump and has an ability to show action on it. By the above mentioned two protective measures like boosting mucus production by nourishment to gut flora and proton pump inhibiting activity, *panicum sumatrense* showing anti-ulcer activity by increased mucus production and decreased gastric secretions by administering methanolic and milk extracts of *panicum sumatrense* in dose dependent manner.

Keywords: *panicum sumatrense,Ulcer,In Silico.*

I. INTRODUCTION:

An ulcer is an open sore caused by damage to the lining of the mucus membrane. Combination of gastric ulcers and duodenal ulcers are termed as peptic ulcer. peptic ulcers are characterized by burning epigastric pain, belching, indigestion, nausea and vomiting, passing excessive amount of gas, loss of appetite, hiccupping following meals, etc. Mostly ulcers are formed due to imbalances between aggressive factors (like NSAIDS, Helicobacter pylori, Gastric secretions, Pepsin, etc) and defensive factors (like Prostaglandins, Mucosal blood flow, Bicarbonates, Regeneration of epithelial layers, etc). According to WHO guidelines 7.8 million people are affected every year with peptic ulcers, mostly people with age between 35-60 years are more prone because of the usage of NSAIDS more in this age. Compared to Women, Men are prone to ulcer disorders. Nowadays mostly adults and children are affected by ulcerative gastritis because of their changed food habits like intake of junk food, spicy food, preservative foods etc. Smoking, skipping meals and alcohol consumption also triggers the formation of ulcers.

NSAIDS-induced ulcers are asymptomatic because analgesic action of NSAIDS may mask the pain of ulcers. People generally experience ulcerative gastritis within 3-6 months of continuous usage of these drugs. As we know our body plays a good defensive mechanism, such that when a person affected by the NSAID induced ulcer our body defensive mechanism activates and produces Endogenous prostaglandins which are responsible for production of mucus which in-turn produces bicarbonate ions from gastric and duodenal epithelium. Hence treatment with prostaglandins analogues and anti-oxidants can decrease the NSAIDS induced gastric mucosal damage. Not only prostaglandin analogues but also other drugs are used in the treatment of ulcers which includes proton pump inhibitors (effectively reduce the gastric acid secretion by inhibiting the H+/K+ ATPase pump from parietal cell membrane),antacids(effectively neutralizes the stomach acids), antibiotics(mainly act by killing harmful bacteria in the stomach which causes ulcer ex: helicobacter pylori). Among all antiulcer drugs Proton pump inhibitors and H2-receptor antagonists are most widely used in the treatment of peptic ulcers. These drugs shows hypersensitivity, arrhythmia, impotence and haemopoietc changes gynaecomastia, alopecia along with the anti-ulcer activity with an increased rate of possible reoccurrence. Hence there is a need to develop safe, therapeutic, cost effective anti-ulcer drugs. In recent times, people have become more conscious about their health and they are slowly identifying that health is wealth. Nowadays people are looking for natural food remedies and drugs from herbal sources to cure and prevent diseases and disorders in advance. As there are many efficient natural herbal remedies to treat ulcers but there are no scientific evaluations and explanations for those kinds of remedies. At the same time people became educated, they are mostly considering the scientifically proven facts. New anti-ulcerative agents with proven scientific results are required in the present scenario. In this scenario *Panicum sumatrense* is one of the natural sources to boost up gastro-intestinal health status and protective agent for ulcers caused by external factors with less side effects.

Panicum sumatrense belongs to the family poaceae, It is a herbaceous plant which is widely grown throughout the world. It has many biologically active compounds, nutrients, proteins, high fibre, Vitamins (like B), Micro elements (like iron ,zinc, magnesium) and also

wide range of phytochemical constituents like hydroxybenzoic acid, cinnamic acid, gallic acid, vanillic acid, gentisic acid, proto-catechuic acid, myricetin which can be utilized as functional group food ingredients. All these high content phytoconstituents have been considered to be responsible for biological activities that have been reported earlier like radical scavenging activity, antioxidant activity etc. The present experiment was designed to evaluate the anti -ulcer activity of *Panicum sumatrense* containing acid, tannins, flavonoids, phenolic compounds and also DPPH free radical scavenging activity and antioxidant properties which are mainly responsible for the anti-ulcer activity. Till now there is no scientifically reported evidence or activity on anti-ulcer activity by *Pancum sumatrense,* hence the present experiment was designed to evaluate the antiulcer activity of *Panicum sumatrense* by using *invivo* and *insilico* methods. The main objective of this study is to compare the anti-ulcer activity of milk and methanolic extract of *panicum sumatrense* with standard and control.

II.*IN SILICO* METHOD:

Docking studies: Docking studies was performed on few of the chemical constituents of Panicum sumatrense (like gallic acid, ferulic acid, protocatechuic acid, gentisic acid, vanillic acid) against the targets (like urease protein, gastric proton pump) to estimate the binding affinity of chemicals i.e. ligands with the proteins (targets). Docking studies, a lamarckian genetic algorithm method executed in autodock 4.2 version was in the works to find out the orientation of ligands with proteins respectively. All the ligands were downloaded from pubchem Cid along with their ID'S respectively, that are Gallic acid(pubchem CID 370), Ferulic acid(pubchem CID 445858), protocatechuic acid(72), gentisic acid(3469), vanillic acid(8468). Now we need to get proteins which act as targets, proteins are downloaded from PDB and uniport with their respective IDs, that are urease protein(PDB ID: 4H10), cyclo-oxygenase-2(PDB ID: 4RRW), and gastric proton pump[alpha chain(UNIPORT ID:P20648), beta chain(UNIPORT ID:P51164)]. Now by using Auto dock Vina 4.2 version we are docking the ligands with protein and obtaining result as docking score.

III.MATERIALS AND METHODS:

The present Anti-Ulcer activity was conducted on healthy albino rats of weight 150-250 gm (which was imported by VAB BIOSCIENCE, HYDERABAD labs) and kept isolated for few days under standard conditions (room temperature 24º-27ºC) and subjected to 12 hours of day cycle and 12 hours of light cycle. Food is provided in the form of dry pellets and clean tap water for their survival. The rats of either sex were divided into five groups of six animals each.

1) Chemicals and drugs: Aspirin, HCL, Toffers reagent, NaOH, ether, omeprazole 50mg, Panicum sumatrense (local market).

2) Equipment and Apparatus: Mixer, Soxhlet, Scissors, Needle

3) Preparation:

3.1. Preparation of milk extract of Panicum sumatrense: The millet is collected from the local market and identified by the Department of Pharmacognosy of our institute collected millets are seeds of Panicum sumatrense (little millet). To enhance properties of millets they are subjected to soaking(24hr) and germination(36hr) and then grind to obtain aqueous extract.

3.2. Preparation of methanolic extract of Panicum sumatrense: The collected millet are subjected to roasting to enhance chemical properties of flavonoids and subjected to mixer/grinder to make powder and then sieved with a 40# sieve. After that powder is subjected to Soxhlet apparatus for crude drug extraction. The extract was evaporated by using a rotary evaporator.

4) Procedure: In this experiment ulcers are induced by two methods that are NSAID induced and Pylorus ligation method. A detail description of process to compare the anti-ulcer activity of milk extract and methanolic extract of panicum sumatrens (i.e. test), with standard (i.e. Omeprazole) and with vehicle treated as follows:

4.1. ASPIRIN INDUCED ULCERS: Chronic usage of NSAIDS causes the destruction of the mucosal lining of the stomach, which leads to the formation of ulcers that causes the discomfort and burning sensation in the stomach. In this method, ASPIRIN is used to induce ulcers to rats of groups MEPS and Milk EPS, their ulcer index is compared with control group and standard group of rats by following procedure. Animals were subjected to fasting 36 hours before performing the experiment. And different group of animal studies along with the doses which have to be given up to 7days are mentioned in the table no.-1.

Table no -1: Experimental groups and treatment given in aspirin induced ulcer

GROUP NUMBER	GROUP NAME	DOSAGE GIVEN
1	Control group	
2	Standard group(omeprazole)	50mg/kg
3	MEPS low dose	200mg/kg
4	MEPS high dose	400mg/kg
5	Milk EPS low dose	200mg/kg
6	MILK EPS high dose	400mg/kg

On the day of experiment doses (saline, MEPS, Milk EPS, RANTIDINE) were administered to the respective groups of rats and after completion of 1hour high dose of aspirin was administered with a dosage of 250 mg/kg to induce ulcers in rats stomach by over dosing of aspirin (NSAIDS). Rats were sacrificed by cervical dislocation after 4 hours and the stomach was opened by making a small incision on the stomach. Isolated stomach was rinsed with normal saline and ulcer index was calculated. The ulcer index of the test group is compared with control and standard groups.

4.2. PYLORIC LIGATION INDUCED ULCERS: Pyloric ligation method is mostly used to induce more ulcers by the ligation of pylorus part, due to this ligation the stomach acids were not able to move into intestine part and causes ulcers due to over accumulation of stomach acids. During the pyloric ligation, the rat's stomach was opened through a small incision on the abdomen region and the pylorus part was ligated out and tied with a surgical thread and stomach was closed by sutures with the help of a curved needle and surgical thread. The rats were allowed to recover and were sacrificed after 5 hours to collect gastric fluids and to observe stomach ulcers. Gastric acids from the stomach were collected and measured then subjected to centrifugation to get supernatant fluid. In conical flask, 1ml of supernatant fluid is taken and diluted with 10 ml of distilled water for titration against NaOH

using phenolphthalein and topfers reagent as indicator. The titration values (volume consumed by NaOH) were used to estimate the free and total acidity. The empty stomach was cleaned by normal saline and ulcers were noticed and ulcer index was calculated. And different group of animal studies along with the doses which have to be given up to 7days are mentioned in the table no.-2.

Table no -2: Experimental groups and treatment given in pyloric ligation induced ulcer

GROUP NUMBER	GROUP NAME	DOSAGE GIVEN
1	Control group	
2	Standard group(omeprazole)	50 mg/kg
3	MEPS low dose	200mg/kg
4	MEPS high dose	400mg/kg
5	Milk EPS low dose	200 mg/kg
6	Milk EPS HIGH DOSE	400 mg/kg

Estimated parameters: (of every animal in groups)

A) Volume of gastric content: All the gastric contents are collected into a measuring cylinder and the volume of gastric contents were measured.

B) Volume of gastric juice: Gastric contents are transferred into centrifuge tubes and subjected to centrifuge at 1000 rpm for 10 mins to get supernatant fluid which is used in the titration.

C) pH: Concentration of collected supernatant fluid was measured by using digital pH meter.

D) Determination of free acidity: Value of Initial colour change is taken as free acidity.

E) Determination of total acidity: Value of saturated colour change is taken as total acidity.

F) ULCER INDEX: Based on the severity of gastric mucosal lesions ulcer index of rats were calculated and graded the differences between them with the following formula.

$$UI=UN+US+UP*10^{-1}$$

WHERE: UI=ulcer index

UN=average number of ulcers per animal

US=average of severity score

UP=percentage of animals with ulcer

PERCENTAGE OF GASTRIC ULCER PROTECTION: Ulcer protection was calculated by using the below formula.

Gastro protection=(UIC-UIT)/UIC*100

WHERE: UIC=ulcer index of control

UIT=ulcer index of test

G) Histopathological studies: After the collection of gastric content, a cut to the stomach tissue was done from greater curvature to lower curvature and stretched with pins to observe ulcer. After cleaning of stomach tissue with saline ulcers were observed and noted for clear count. Then the following stomach tissues were stored in sampling bottles with 10 percent formalin solution and sent to patho labs for histopathological studies with a given code to the samples for identification. Histopathological studies from the labs state that the stomach

tissues after staining with hematoxylin and eosin stain, and observed under 100x the following studies were observed.

5. Statistical Analysis: The statistical data was expressed as mean ± S.E.M. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. Differences between the data were considered significant at P<0.05.

IV.RESULTS:

IV.1. Effect of methanolic extract and milk extract of panicum sumantrense in ASPIRIN induced gastric ulcer:

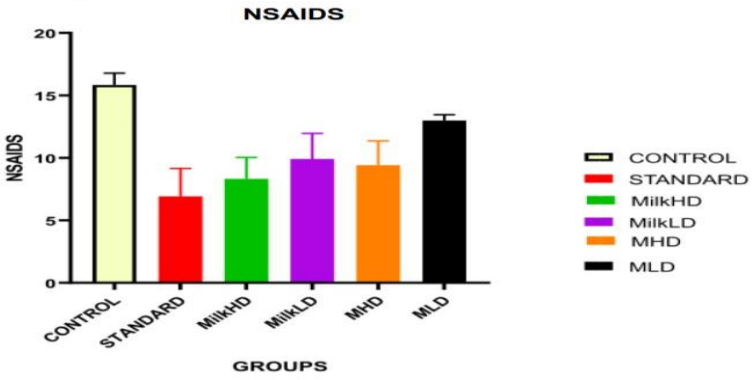
In the aspirin induced ulcer model, the administration of high doses of aspirin induces hemorrhagic gastric lesions and deep ulcers on the stomach walls of rats of all groups. The ulcer index of the control group is 15.833±0.93575. But the animals which are treated with methanolic extract at 200mg/kg and 400 mg/kg show ulcer index 13±0.44721 and 9.421±1.93617 respectively. The methanolic extract at 400 mg/kg showed significant (P< 0.001) reduction in the ulcer index by 47.441 percentage protection of gastric mucosa. Omeprazole at 50 mg/kg scored an ulcer index of 6.92±2.236 and showed a significant reduction in the ulcer index by 56.28 percentage protection of gastric mucosa. Administration of panicum sumantrense 1 week before the induction of gastric lesions by aspirin showed reduced ulcer index. The milk extract of Panicum sumatrense significantly decreases ulcer index when compared to methanolic and control groups.

Table no-3: Effect of MEPS and Milk EPS on ulcer index and ulcer protection in aspirin induced gastric ulcer :

GROUP NUMBER	TREATMENT	ULCER INDEX	ULCER PROTECTION
I	Control	15.833±0.93575	0
II	Standard group (50mg/kg) omeprazole	6.922±2.236	56.28
III	MEPS low dose (200 mg/kg)	13±0.44721	37.39
IV	MEPS high dose (400 mg/kg)	9.421±1.9367**	40.49
V	Milk EPS low dose (200 mg/kg)	9.9166±2.0429	17.87
VI	Milk EPS high dose (400 mg/kg)	8.3233±1.7057****	47.44

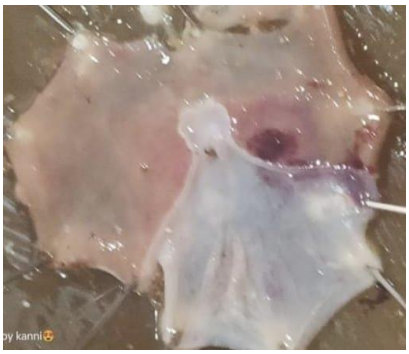
The above values are expressed as MEAN±SEM, n=6(no. of animals in each group). Statistical values are calculated by one-way anova and they are significant at p>0.05.

Figure IV.1-i : Effect of MEPS and Milk EPS on ulcer index in aspirin induced gastric ulcer :

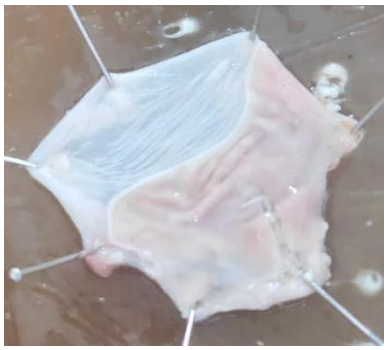


The above values are expressed as MEAN±SEM, n=6(no. of animals in each group). statistical values are calculated by one-way annova and they are significant at p>0.05.

Figure V.1-ii. Macroscopic view of stomach walls in aspirin induced ulcer:



A. CONTROL

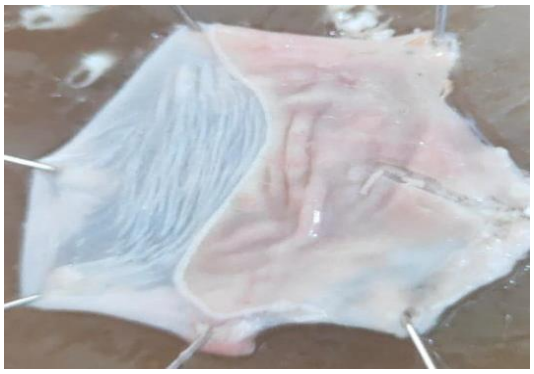


B. STANDARD

C.MEPS LOW DOSE

D. MEPS H0IGH DOSE

E. MILK EPS LOW DOSE



F. MILK EPS HIGH DOSE

IV.2 Effect of methanolic extract and milk extract of panicum sumantrense in pyloricligation method induced gastric ulcer:

In the pyloric-ligation method induced ulcer model, the ligation to the pylorus part of the stomach induces hemorrhagic gastric lesions and deep ulcers on the stomach walls of rats of all groups due to the accumulation of all gastric acid secretions. The ulcer index of the control group is 25.666±0.8191. The animals treated with methanolic extract at 200mg/kg and 400 mg/kg showed ulcer index 18.5±69139 and 15.166±0.7923 respectively. The milk extract at 400 mg/kg showed non-significant($P<0.0068$) reduction in the ulcer index by 63.32 percentage protection of gastric mucosa. Omeprazole at 50 mg/kg scored an ulcer index of

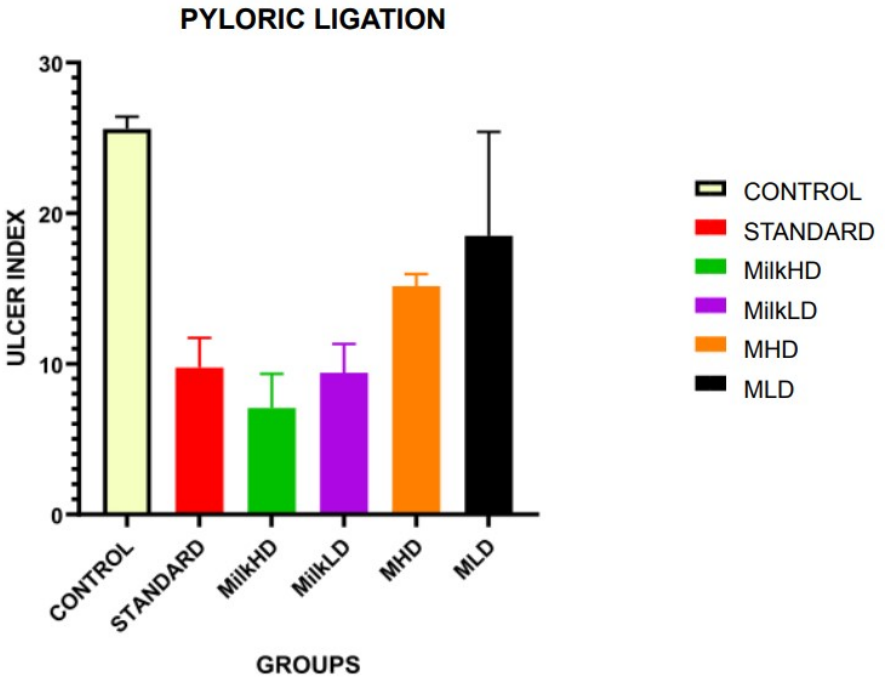
9.75±1.9771 and showed a significant reduction in the ulcer index by 62 percent protection of gastric mucosa. Administration of panicum sumantrense 1 week before the induction of gastric lesions by pyloric ligation method showed reduced ulcer index. The milk extract of Panicum sumatrense significantly decreases ulcer index when compared to methanolic and control group.

Table-4: Effect of MEPS and Milk EPS on ulcer index and ulcer protection in pyloric ligation induced gastric ulcer model :

GROUPS	TREATMENT	ULCER INDEX	ULCER PROTECTION
I	Control group	25.666±0.8191	0
II	Standard group (omeprazole)	9.75±1.9771	62
III	MEPS low dose (200 mg/kg)	18.5±6.9139	27.9
IV	MEPS high dose (400 mg/k	15.166±0.7923 ns	40.896
V	Milk EPS low dose (200 mg/kg)	9.4166±1.9184	63.2
VI	Milk EPS high dose (400 mg/kg)	7.0666±2.2789**	72.48

The above values are expressed as MEAN±SEM , n=6(no.of animals in each group). statistical values are calculated by one-way anova and they are significant at p>0.05.

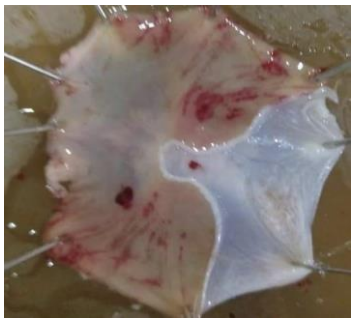
Figure IV.2-i: Effect of MEPS and Milk EPS on ulcer index and ulcer protection in pyloric ligation induced gastric ulcer model :



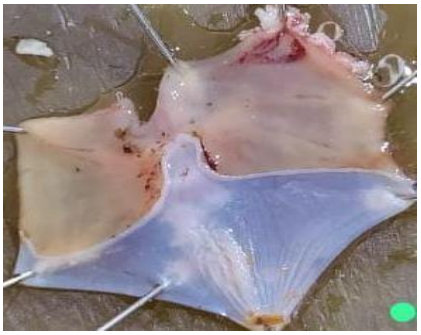
The above values are expressed as MEAN±SEM , n=6(no. of animals in each group). statistical values are calculated by one-way anova and they are significant at p0.05.

Figure IV.2-ii : Macroscopic view of stomach walls in pyloric induced ulcer:

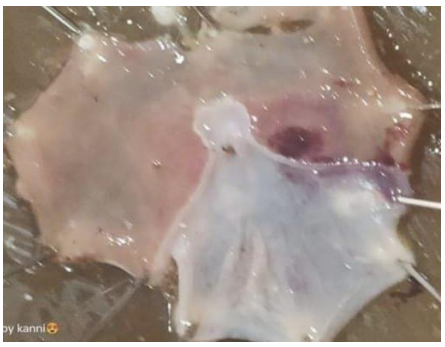
A.CONTROL



B. STANDARD



C.MEPS LOW DOSE



D. MEPS HIGH DOSE

E. Milk EPS LOW DOSE

F. Milk EPS HIGH DOSE

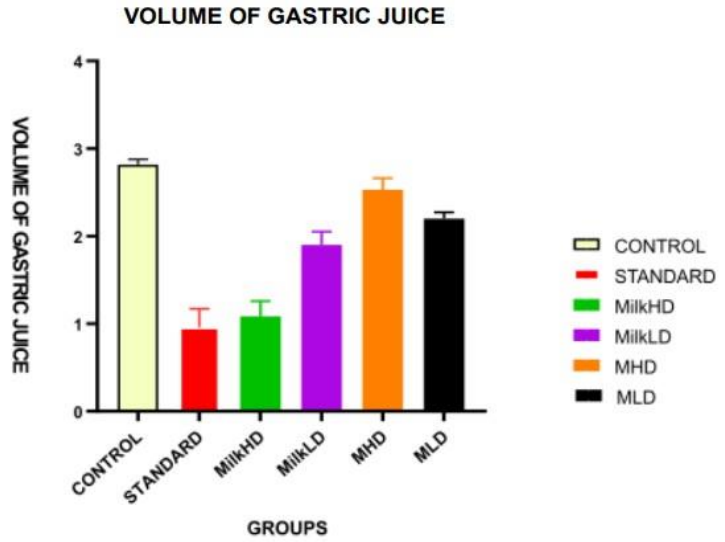
IV.3 Pylorus ligation parameters

A. volume of gastric juice: In pyloric ligation induced gastric ulcer model, the volume of gastric fluids are high in control group (2.175 ± 0.0760). Gastric juice was significantly decreased in milk extract group at 200mg/kg (1.4066±0.0645) and 400 mg/kg (1.3583±0.06754) where as no significant in methanolic extract group at 200mg/kg (1.888±0.0346) and 400 mg/kg (1.67±0.473) . Significantly decreased volume of gastric fluids were recorded in the standard group treated with Omeprazole

Table no-5: Effect of MEPS and AEPS of *panicum sumantrense* on gastric juice.

GROUP NUMBER	TREATMENT	VOLUME OF GASTRIC FLUIDS
I	Control	2.175 ± 0.0760
II	Standard(omeprazole)	1.236±0.0343
III	MEPS low dose (200 mg/kg)	1.888±0.0346
IV	MEPS high dose (400 mg/kg) ns	1.67±0.473
V	Milk EPS low dose(200 mg/kg)	1.4066±0.0645
VI	Milk EPS high dose(400mg/kg) **	1.3583±0.06754

FigureIV.3-ii: Effect of MEPS and Milk EPS of *panicum sumantrense* on gastric juice.



50 mg/kg (1.236±0.0343) when compared to the control group.

The above values are expressed as MEAN±SEM , n=6(no .of animals in each group). statistical values are calculated by one-way annova and they are significant at $p<0.0001$**** to that of control group $p>0.05$.

B. Volume of gastric contents: In pyloric ligation induced gastric ulcer model, the volume of gastric contents are high in control group(2.816 ± 0.060).Gastric content was significantly decreased in milk extract at 200mg/kg (2.2 ±

Figure V.3-iii: **Effect of MEPS and Milk EPS of panicum sumantrense on gastric pH.**

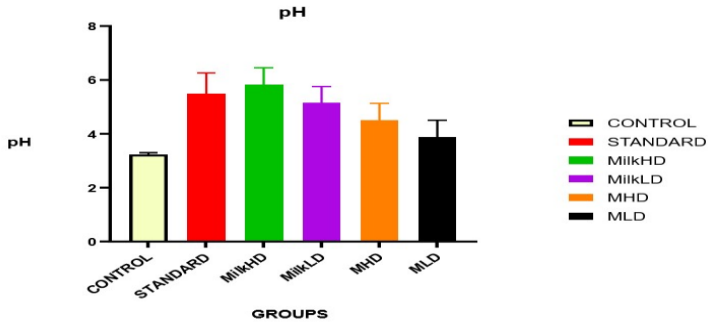


Table no-6: **Effect of MEPS and MilkEPS of *panicum sumantrense* on gastric content.**

GROUP NUMBER	TREATMENT	VOLUME OF GASTRIC CONTENT
I	Control	2.816 ± 0.060
II	Standard(omeprazole)	0.96 ± 0.21
III	MEPS low dose (200 mg/kg)	2.2 ± 0.0723
IV	MEPS high dose (400 mg/kg) **	2.53 ± 0.13
V	Milk EPS low dose(200 mg/kg)	1.9 ± 0.152
VI	Milk EPS high dose(400mg/kg) ****	1.0833 ± 0.174

0.0723) and 400mg/kg(2.53 ± 0.13) also significantly decreased volume of gastric contents were noticed in methanolic group at 200mg/kg (1.9 ± 0.152) and 400 mg/kg (1.0833 ± 0.174) . Significantly decreased volume of gastric contents were recorded in standard group treated with omeprazole at 50 mg/kg (0.96±0.21) when compared to the control group.

C. pH: In pyloric ligation induced gastric ulcer models, the pH of the gastric juice is low in the control group (3.25 ± 0.05). pH of gastric juice was significantly increased in milk extract group at 200mg/kg (4.50 ± 0.64) and 400mg/kg(3.90 ± 0.60) whereas non-significantly increases in methanolic extract group at 200mg/kg (5.16 ± 0.60) and 400 mg/kg (5.830 ± 0.63. significantly increased values of pH were noticed in the standard group treated with omeprazole at 50 mg/kg (5.5 ± 0.76) when compared to the control group.

Table no-7: **Effect of MEPS and AEPS of *panicum sumantrense* on gastric pH.**

GROUP NUMBER	TREATMENT	PH
I	Control	3.25 ± 0.05
II	Standard(omeprazole)	5.5 ± 0.76
III	MEPS low dose (200 mg/kg)	4.50 ± 0.64
IV	MEPS high dose (400 mg/kg) ns	3.90 ± 0.60
V	Milk EPS low dose(200 mg/kg)	5.16 ± 0.60
VI	Milk EPS high dose(400mg/kg) **	5.830 ± 0.63

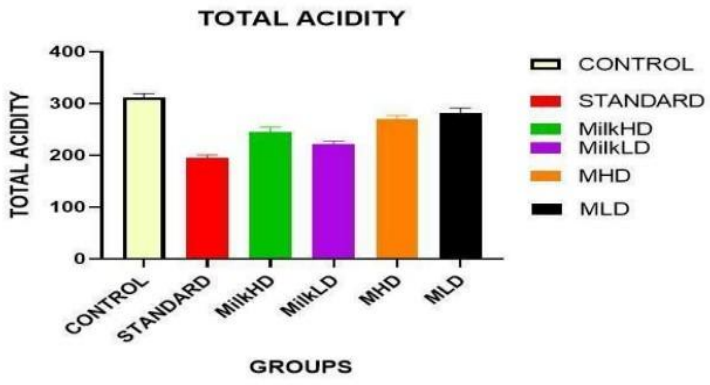
The above values are expressed as MEAN±SEM , n=6(no. of animals in each group). statistical values are calculated by one-way annova and they are significant at p>0.05.

D. Total acidity: In pyloric ligation induced gastric ulcer model the total acidity is high in control group(312.166 ± 7.3199).Total acidity was significantly decreased in milk extract group at 200mg/kg (282.666 ± 8.905) and 400mg/kg(269.8333 ± 7.336) whereas non-significantly increased in methanolic extract group at 200mg/kg (222 ± 5.977) and 400 mg/kg (245 ± 9.750) . Significantly decreased values of total acidity were recorded in the standard group treated with omeprazole at 50 mg/kg (195.333 ± 5.161) when compared to the control group.

Table no-8: **Effect of MEPS and Milk EPS of panicum sumantrense on total acidity.**

GROUP NUMBER	TREATMENT	TOTAL ACIDITY
I	Control	312.166 ± 7.3199
II	Standard(omeprazole)	195.333 ± 5.161
III	MEPS low dose (200 mg/kg)	282.666 ± 8.905
IV	MEPS high dose (400 mg/kg) ns	269.8333 ± 7.336
V	Milk EPS low dose(200 mg/kg)	222 ± 5.977
VI	Milk EPS high dose(400mg/kg) **	245 ± 9.750

Figure V.3-iv: Effect of MEPS and Milk EPS of panicum sumantrense on total acidity.



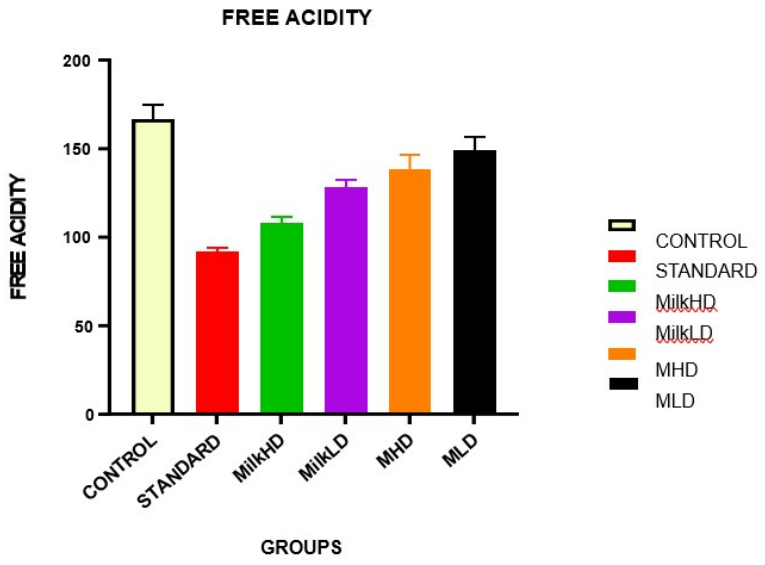
The above values are expressed as MEAN±SEM , n=6(no .of animals in each group). statistical values are calculated by one-way annova and they are significant at p>0.05.

F. Free acidity: In pyloric ligation induced gastric ulcer model the free acidity is high in control group(167 ± 7.827).Free acidity was significantly decreased in milk extract groups at 200mg/kg (149.5 ± 7.401) and 400mg/kg(138.666 ± 8.046) whereas non-significantly increased in methanolic group at 200mg/kg (128.5 ± 4.248) and 400 mg/kg (108.166 ±4.248). Significantly decreased values of free acidity were recorded in the standard group treated with omeprazole at 50 mg/kg (91.833± 2.05) when compared to the control group.

Table no-9: **Effect of MEPS and AEPS of *panicum sumantrense* on free acidity.**

GROUP NUMBER	TREATMENT	FREE ACIDITY
I	Control	167 ± 7.827
II	Standard (omeprazole)	91.833± 2.056
III	MEPS low dose (200 mg/kg)	149.5 ± 7.401
IV	MEPS high dose (400 mg/kg) ns	138.666 ± 8.046
V	Milk EPS low dose(200 mg/kg)	128.5 ± 4.248
VI	Milk EPS high dose(400mg/kg) **	108.166 ± 4.248

Figure V.3-v: Effect of MEPS and Milk EPS of *panicum sumantrense* on free acidity.



The above values are expressed as MEAN±SEM, n=6(no.of animals in each group). statistical values are calculated by one-way anova and they are significant at p>0.05.

Table no-10; **Results of Parameters of pyloric ligation**

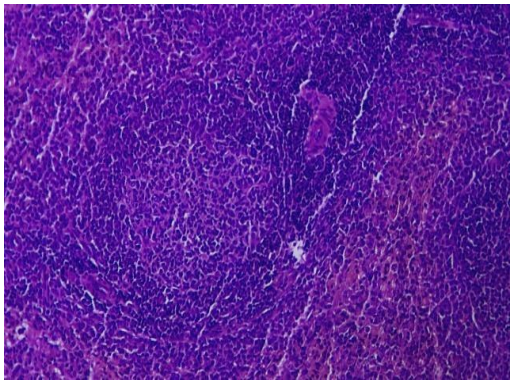
Groups	Treatment	Volume of gastric content	Volume of gastric juice	pH	Total acidity (mEq/lit)	Free acidity (mEq/lit)
1	control	2.816 ± 0.060	2.175±0.0760	3.25 ± 0.05	312.166± 7.3199	167 ± 7.827
2	standard	0.96 ± 0.21	1.236±0.0343	5.5 ± 0.76	195.333 ± 5.161	91.833± 2.056
3	MEPS 200mg/kg	2.2 ± 0.0723	1.888±0.0346	4.50 ± 0.64	282.666 ± 8.905	149.5 ± 7.401
4	MEPS 400 mg/kg	2.53 ± 0.13**	1.67±0.473ns	3.90 ± 0.60 ns	269.8333± 7.336ns	138.666 ± 8.046ns
5	Milk EPS 200 mg/kg	1.9 ± 0.152	1.4066±0.0645	5.16 ± 0.60	222 ± 5.977	128.5 ± 4.248
6	Milk EPS 400 mg/kg	1.0833±0.174****	1.3583±0.06754**	5.830±0.63**	245 ± 9.750**	108.166± 4.248**

IV.4 Histopathological studies

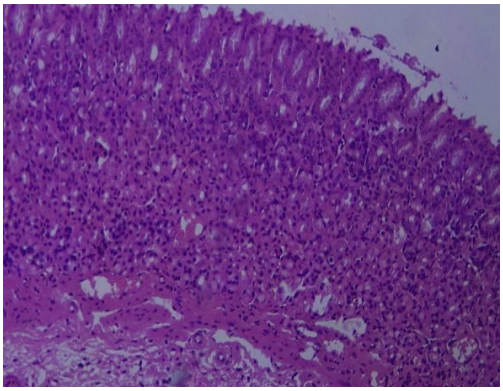
NSAIDS TREATED GROUP:

- CONTROL GROUP: : Histopathological studies of rats which are pre-treated with saline before induced ulcers have noticed, multi focal damage to mucus, Deep ulceration of glandular epithelium and damage to sub mucosa.
- STANDARD GROUP: Histopathological studies of rats which are pre-treated with omeprazole before induced ulcers have noticed normal morphology of glandular stomach.
- MEPS 200mg/kg: Histopathological studies of rats which are pre-treated with 200mg/kg of MEPS before induced ulcers have noticed numerous erosions, multi-focal damage to mucus superficial ulcers.
- MEPS 400mg/kg: Histopathological studies of rats which are pre-treated with 400mg/kg of MEPS before induced ulcers have noticed slightly 2-3 spots.
- Milk EPS 200 mg/kg: Histopathological studies of rats which are pre-treated with 200mg/kg of Milk EPS before induced ulcers have noticed normal morphology of glandular stomach.
- Milk EPS 400mg/kg: Histopathological studies of rats which are pre-treated with 200mg/kg of Milk EPS before induced ulcers have noticed normal morphology of glandular stomach.

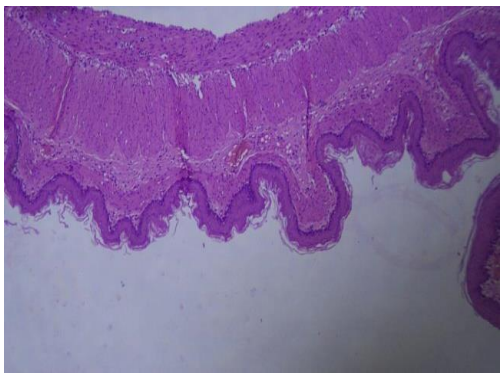
IV.4 Figure-i : Histology of NSAID group stomachs under 100x magnification



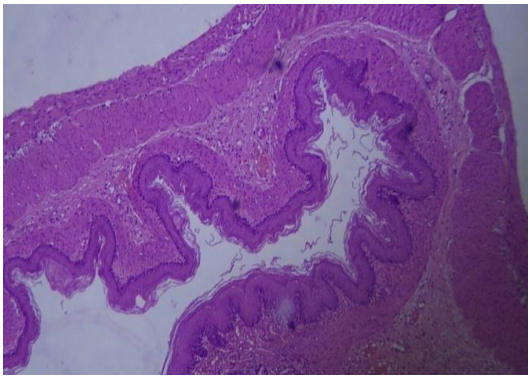
A. CONTROL GROUP



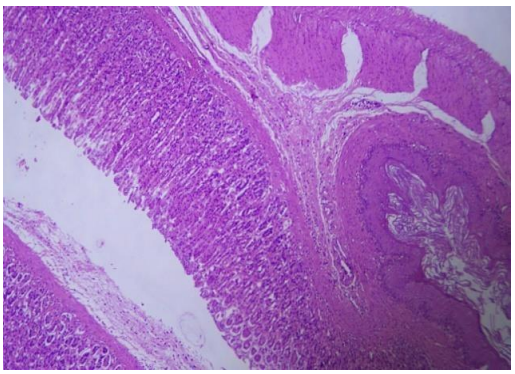
B.STANDARD GROUP



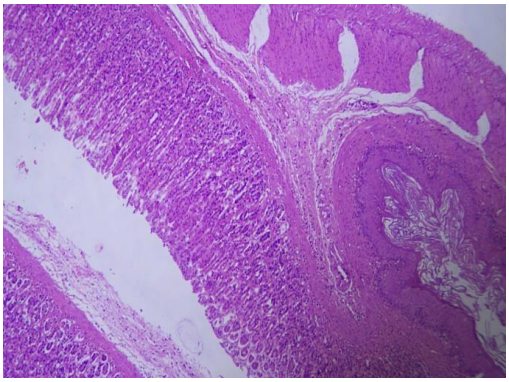
C.MEPS LOW DOSE



D. MEPS HIGH DOSE



E. MILK EPS LOW Dose



F. MILK EPS HIGH DOSE

Pyloric ligation group:

CONTROL GROUP: Histopathological studies of rats which are pre-treated with saline before induced ulcers have noticed, perforation occurred with great damage to mucus lining.

STANDARD GROUP: Histopathological studies of rats which are pre-treated with omeprazole before induced ulcers have noticed superficial ulcers with protective mucus lining.

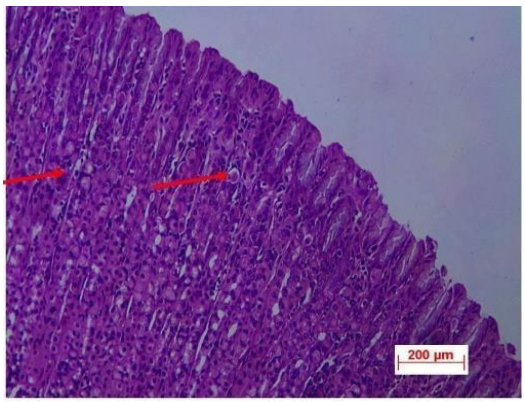
MEPS 200mg/kg: Histopathological studies of rats which are pre-treated with 200mg/kg of MEPS before induced ulcers have noticed damage to mucus lining with deep ulcers.

MEPS 400MG/KG: Histopathological studies of rats which are pre-treated with 400mg/kg of MEPS before induced ulcers have noticed superficial ulcers, slight damage to glandular epithelium.

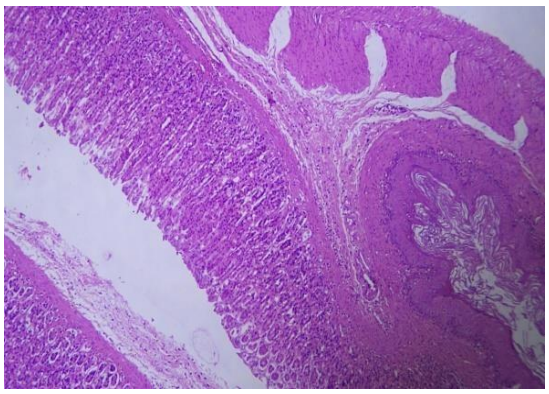
Milk EPS 200mg/kg: Histopathological studies of rats which are pre-treated with 200mg/kg of Milk EPS before induced ulcers have noticed normal morphology of glandular stomach.

Milk EPS 400MG/KG: Histopathological studies of rats which are pre-treated with 200mg/kg of Milk EPS before induced ulcers have noticed normal morphology of glandular stomach.

V.4 Figure-ii: Histology of NSAID group stomachs under 100x magnification

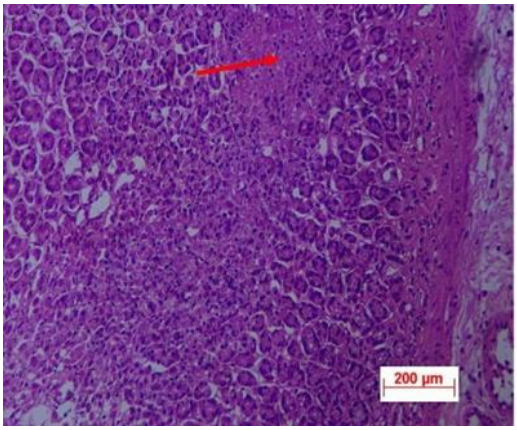


A. Control

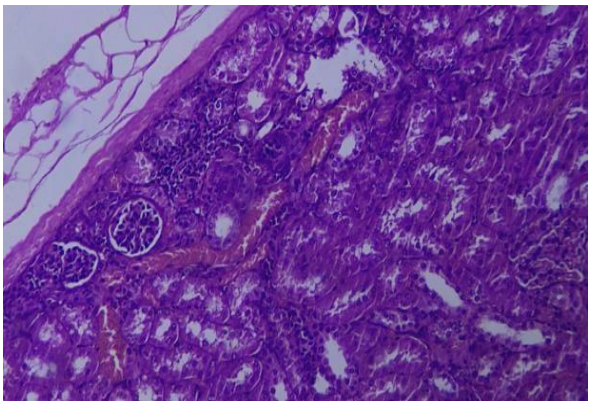


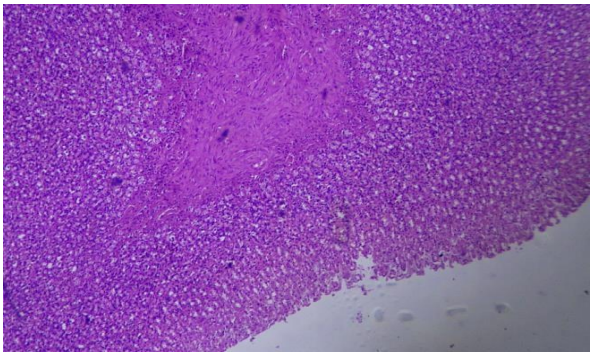
B. STANDARD

C.MEPS LOW DOSE

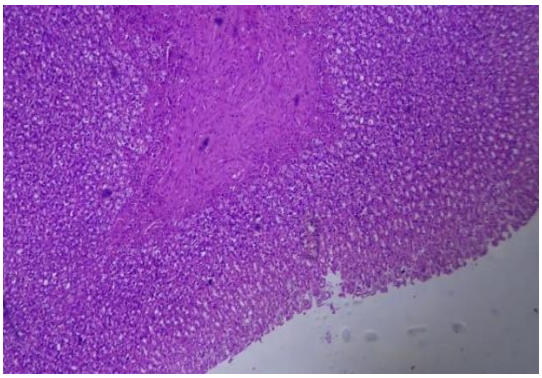


D. MEPS HIGH DOSE





E. MILK EPS LOW DOSE



F. MILK EPS HIGH DOSE

V.5 *INSILICO* DOCKING SCORES

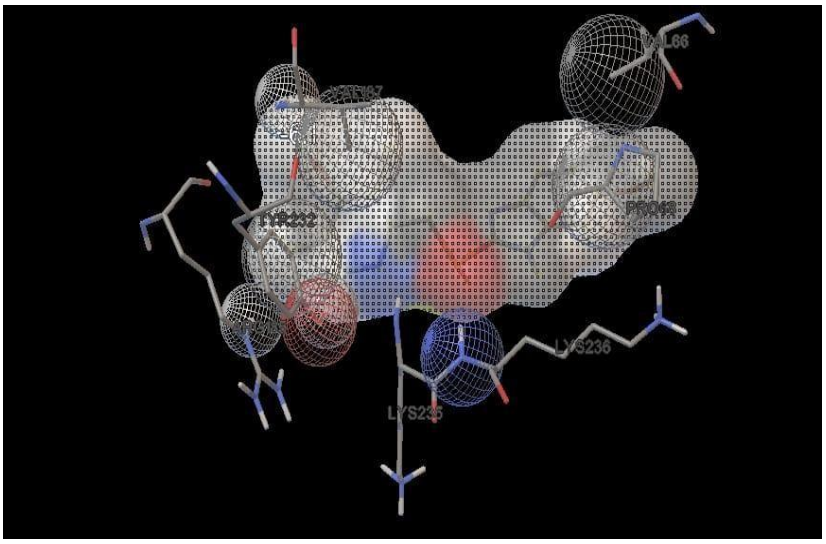
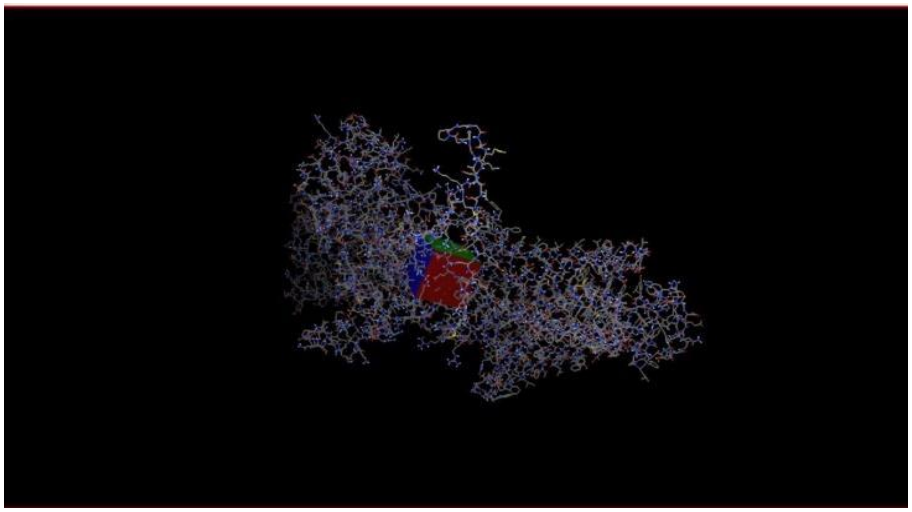


Table no-11: Binding affinity of ligands with the proteins and their binding scores

PROTEIN NAME	LIGAND NAME	DOCKING SCORE
UREASE PROTIEN	Gallic Acid	-6.7
	Gentisic Acid	-6.4
	Proto-Catechuic Acid	-6.3
	Vanillic Acid	-6.3
	Ferulic Acid	-5.9
GASTRIC PROTON PUMP (ALPHA CHAIN)	Ferulic Acid	-6.4
	Omeprazole	-7.3
	Gallic Acid	-6.3
	Proto-Catechuic Acid	-6.1
	Gentisic Acid	-6.0
GASTRIC PROTON PUMP (BETA CHAIN)	Gallic Acid	-5.3
	Ferulic Acid	-5.2
	Proto-Catechuic Acid	-5.1
	Gentisic Acid	-4.9
	Vanillic Acid	-4.8
	Omeprazole	-6.1

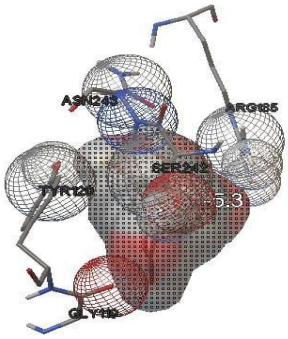
V.5 Figure-i: Binding affinity of ligands with proteins:

Omeprazole(-6.1) Binded with gastic proton pump (β-chain) at tyrosine-

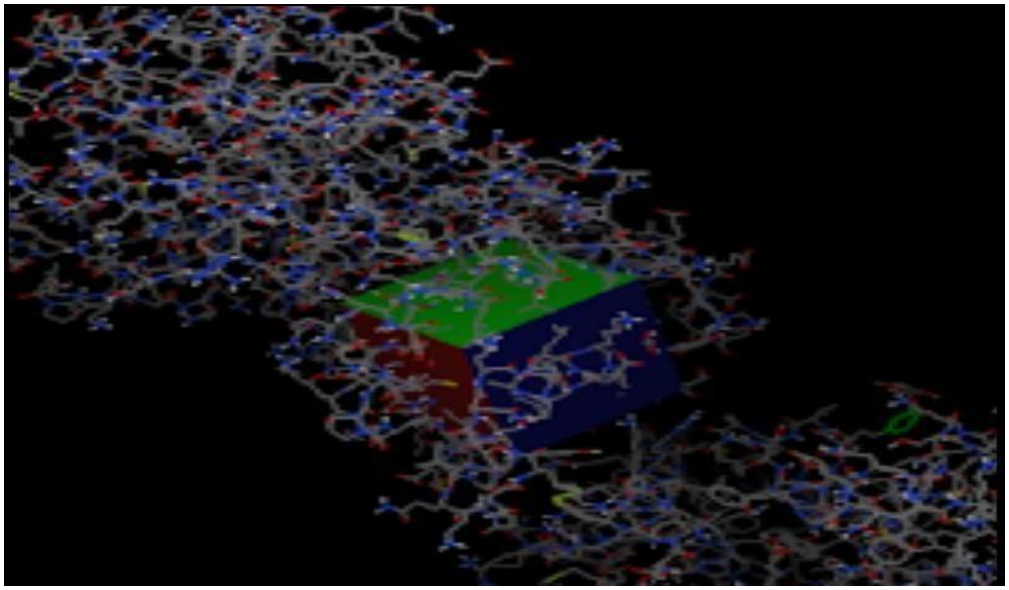


232,lysine235 and 236).

Gallic Acid (-6.3)) Binded with gastric proton pump(α-chain) at tyrosine 120,



arginine 185, glycine 118.



Gallic Acid (-6.7) Binded with urease protein.

V. DISCUSSION:

Mucus lining on the walls of the stomach provides a protective environment to the stomach from strong acids, chemicals, bacteria. Damage to the mucus lining which in turn causes wounds in the stomach called ulcers. The main reason for the formation of ulcers is unknown, but the major causes for the formation of ulcers are imbalances between the levels of gastric juices like HCL, sodium bicarbonate, and pepsin. Mostly in people with high usage of aspirin causes ulcers. Aspirin is the most commonly used NSAID among all the patients for minor pain reliefs across the world. Aspirin is also used in most of the combination drugs for effective results, along with the positive therapeutic result intake of aspirin possesses so many adverse effects in those forming ulcers are one of the considered side effects of aspirin caused by erosion of the mucus lining. Intake of aspirin alters the production and secretion of prostaglandins, increased secretion of gastric acids and back diffusion of H^+ ions. Exogenous prostaglandins are responsible for inhibition of acid secretion and stimulates mucus production which in turn secretes the bicarbonate ions responsible for pH balance (i.e., Acid/Alkaline balance) in the stomach. Alterations in the production and secretion of prostaglandins caused by chronic aspirin intake leads to reduced secretions of mucus and bicarbonate ions causes acute gastritis which results in formation of ulcers. In our study methanolic and milk extract of Panicum sumatrense showed the significant anti -ulcer activity compared to control group that may be due to presence of ferulic acid present in panicum sumatrense involved in the enhancement of prostaglandin production results in mucus and bicarbonate ion secretion from faveolar cells of stomach epithelium.

In pylorus ligation method, all the gastric juices are accumulated in the stomach region as we ligated the pylorus part of the stomach which results in the formation of ulcers because high acid content has destroyed the gastric mucosa. Gastrin is a group of digestive hormones released from the pyloric end of the stomach into the bloodstream and it is carried out to stomach walls, where it triggers the release of gastric juices. Gastric juices primarily consist and release hydrochloric acid which helps to break the fibrous matter in the food and kills bacteria ingested along with the food and converts pepsinogen into pepsin, which helps in digestion of proteins into peptides. As discussed earlier hydrochloric acid aims to breaks the fibrous food in the stomach, fibrous food consists of cellulose, non-cellulosic polysaccharides such as hemicellulose, gums, mucilages and non-carbohydrate component called lignin. All these components present in the fibrous food causes slow digestion in the stomach which provides many beneficial things such as it provides nourishment to the gut flora and act as prebiotics, so that gut flora don't turn on to the mucus lining for food .On the other hand fibrous food in the stomach absorbs all liquid contents to provide soft bowel movement, here the excessive amount of stomach acids are also absorbed by the fibrous food. .As stomach acids are in work with the digestion of fibrous food ,there is no side effect of gastric acids on the walls of stomach. So when the effect of gastric acids are less on the stomach the total acidity and free acidity will be decreased in the stomach which results in the protective environment for mucus lining on the stomach walls, hence mucus lining reduces the formation of ulcers. On the other hand Urea is the common byproduct of cellular metabolism which is discovered in gastric secretions as a result of paracellular and transcellular diffusion across the epithelium. Sometimes helicobacter pylori bacteria became the causative factor for the formation of ulcers. Helicobacter pylori is present in the mucus lining of stomach walls

and duodenum walls. It secretes an enzyme called urease that converts urea into ammonia present in gastric juices. Then converted ammonia protects the H.PYLORI bacteria from stomach acids, the bacteria will be well colonized in the stomach and duodenum and engulfs the mucus lining and stomach tissue which leads to the formation of ulcers. As we discussed earlier in *insilico* studies(table no-11), few of the chemicals present in panicum sumantrense has binding affinity with urease proteins, and act as urease inhibitors, like gallic acid with highest docking score(6.7) which inhibits the activity of urease enzyme which causes the ceasation in the conversion of urea into ammonia. When urea is not converted into ammonia, the protective environment for H.pylori bacteria will be absent and they can be demolished by gastric juices then the gastric walls were not affected by H.pylori bacteria.SO no formation ulcers.

Also proton pump inhibitors play a major role in acid secretions. Proton pump inhibitors block the gastric H+/K+-ATPase enzyme which intern inhibits the heavy gastric secretions in the stomach. As a result acids are released in required amounts and the balanced environment between gastric acids and bicarbonate ions takes place which results in the maintenance of good mucus levels on the stomach walls, which act as protective measurement from ulcers. In our experimental study, milk extract of Panicum sumatrense showed the significant anti - ulcer activity compared to methanolic and control group that may be due to presence of ferulic acid present in panicum sumatrense which get attached with gastric proton pump. As described earlier in *insilico* docking scores (table no-11) ferulic acid(docking score-6.4) has binding affinity to alpha chain of gastric proton pump and has an ability to show action on it. Further phytochemical screening is required to evaluate the composition ,strength of chemical constituents present in milk extract of *panicum sumatrense.*

By the above mentioned two protective measures like boosting mucus production by nourishment to gut flora and proton pump inhibiting activity, *panicum sumatrense* showing anti-ulcer activity by increased mucus production and decreased gastric secretions by administering methanolic and milk extracts of *panicum sumatrense* in dose dependent manner.

VI.CONCLUSION

From the above experiment we have concluded that methanolic and milk extract of *panicum sumatrense* has effective anti-ulcer activity by the different mechanisms of actions and by providing protective measurements like production of mucus lining, inhibiting heavy secretions of gastric acids. Milk extract of *panicum sumatrense* has significant anti-ulcer effect when compared to ethanolic extract of *panicum sumatrense*. Nutraceuticals and chemical constituents present in the extracts show their therapeutic effects in the body and produce anti- ulcer activity which was supported by the docking scores of chemical constituents of *Panicum sumatrense* in *Insilico* studies.

REFERENCE

1. Deshpande SS, Shah GB, Parmar NS. Antiulcer activity of Tephrosia purpurea in rats. Indian journal of Pharmacology. 2003 May 1;35(3):168-72.
2. Omansen TF, Erbowor-Becksen A, Yotsu R, van der Werf TS, Tiendrebeogo A, Grout L, Asiedu K. Global epidemiology of Buruli ulcer, 2010–2017, and analysis of 2014 WHO programmatic targets. Emerging infectious diseases. 2019 Dec;25(12):2183.

3. Phillips TJ. Chronic cutaneous ulcers: etiology and epidemiology. Journal of Investigative Dermatology. 1994 Jun 1;102(6).
4. De Wet JM, Prasada Rao KE, Brink DE. Systematics and domestication of Panicum sumatrense (Graminae). Journal d'agriculture traditionnelle et de botanique appliquée. 1983;30(2):159-68.
5. Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. A potential of some medicinal plants as an anti-ulcer agents PharmacognCY Rev. 2010 Jul;4(8):136-46. doi: 10.4103/09737847.70906. PMID: 22228953; PMCID: PMC3249913.
6. Melcame L,Garcia -Iglesis P,Calvet X.Management of NSAID -associated peptic ulcer Disease.Expert Rev Gastroenterol Hepatol. 2016 Jun;10(6):723-33. doi:
7. Kim BW. [Diagnosis and Treatment of Peptic Ulcer Disease: Present and Future Perspective]. Korean J Gastroenterol. 2016 Jun 25;67(6):318-20. Korean. doi: 10.4166/kjg.2016.67.6.318. PMID: 27312832.
8. Wang A, Yerxa J, Agarwal S, Turner MC, Schroder V, Youngwirth LM, Lagoo-Deenadayalan S, Pappas TN. Surgical management of peptic ulcer disease. Curr Probl Surg. 2020 Feb;57(2):100728. doi: 10.1016/j.cpsurg.2019.100728. Epub 2020 Jan 7. PMID: 32138833.
9. MacAllister CG. A review of medical treatment for peptic ulcer disease. Equine Vet J Suppl. 1999 Apr;(29):45-9. doi: 10.1111/j.2042-3306. 1999. tb05168. x. PMID: 10696293.
10. Deshpande, S. S., G. B. Shah, and N. S. Parmar. "Antiulcer activity of Tephrosia purpurea in rats." Indian journal of Pharmacology 35.3 (2003): 168-172.
11. Raju, D., et al. "Evaluation of Anti-ulcer activity of methanolic extract of Terminalia chebula fruits in experimental rats." Journal of Pharmaceutical Sciences and research 1.3 (2009): 101.
12. Shrivastava, N., A. Srivastava, A. Banerjee, and M. Nivsarkar. "Anti-ulcer activity of Adhatoda vasica Nees." Journal of herbal pharmacotherapy 6, no. 2 (2006): 43-49.
13. Liu, X. M., M. N. M. Zakaria, M. W. Islam, R. Radhakrishnan, A. Ismail, H. B. Chen, K. Chan, and A. Al-Attas. "Anti-inflammatory and anti-ulcer activity of Calligonum comosum in rats." Fitoterapia 72, no. 5 (2001): 487-491.
14. Pal, Saroj K., Pulok K. Mukherjee, and B. P. Saha. "Studies on the antiulcer activity of Moringa oleifera leaf extract on gastric ulcer models in rats." Phytotherapy research 9, no. 6 (1995): 463-465.
15. Asuzu, I. U., and O. U. Onu. "Anti-ulcer activity of the ethanolic extract of Combretum dolichopetalum root." International Journal of Crude Drug Research 28, no. 1 (1990): 27-32.
16. Pal, S. and Chaudhuri, A.N., 1991. Studies on the anti-ulcer activity of a Bryophyllum pinnatum leaf extract in experimental animals. Journal of ethnopharmacology, 33(1-2), pp.97102.
17. Gregory, M., Divya, B., Mary, R.A., Viji, M.H., Kalaichelvan, V.K. and Palanivel, V., 2013. Anti–ulcer activity of Ficus religiosa leaf ethanolic extract. Asian Pacific journal of tropical biomedicine, 3(7), pp.554-556.

18. Malairajan, P., Gopalakrishnan, G., Narasimhan, S. and Veni, K.J.K., 2008. Evalution of anti-ulcer activity of Polyalthia longifolia (Sonn.) Thwaites in experimental animals. Indian journal of pharmacology, 40(3), p.126.
19. Prabha, P., Karpagam, T., Varalakshmi, B. and Packiavathy, A.S.C., 2011. Indigenous antiulcer activity of Musa sapientum on peptic ulcer. Pharmacognosy research, 3(4), p.
20. . B.Ramu et al, Formulation Of Lamotrigine Orodispersible Tablets By Using New Generation Superdisintegrants World Journal Of Pharmacy And Pharmaceutical Sciences Volume 4,2015, Issue 06, 631- 643.
21. Ramu B, Sathish Kumar M, Ramakrishna M (2015) Current Regulatory Scenario for Conducting Clinical Trials in India. Pharmaceut Reg Affairs. 4:137. doi: 10.4172/2167-7689.1000140.
22. Mounika, I y Ramu, B. 2018. "Lifestyle drugs: concept and impact on society." Journal of Human Virology & Retrovirology, 6(2): 46-49. https://doi.org/10.15406/ jhvrv.2018.06.00194
23. Ramu B, Saibaba SV. Role of community pharmacist in management of anaemia. Pharm Pharmacol Int J., 2018; 6(3): 216–220. DOI: 10.15406/ppij.2018.06.00178.
24. Alothman, M., Bhat, Rajeev., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry, 115, 785–788.
25. Pradeep SR, Guha M. Effect of processing methods on the nutraceutical and antioxidant properties of little millet (Panicum sumatrense) extracts. Food chemistry. 2011 Jun15;126(4):1643-7.
26. Deshpande SS, Shah GB, Parmar NS. Antiulcer activity of Tephrosia purpurea in rats. Indian journal of Pharmacology. 2003 May 1;35(3):168-72.
27. Niharika M, Reddy VN, Manisha R, Raju MG. Anti-ulcer Activity of Ethanolic Extract of Alstonia scholaris Bark: An in vivo and in silico Approach. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2022 Jan 15:14-32.
28. Alam S, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of Momordica charantia L. in rats. Journal of ethnopharmacology. 2009 Jun 25;123(3):464-9.
29. Umre R, Ganeshpurkar A, Ganeshpurkar A, Pandey S, Pandey V, Shrivastava A, Dubey N. In vitro, in vivo and in silico antiulcer activity of ferulic acid. Future Journal of Pharmaceutical Sciences. 2018 Dec 1;4(2):248-53.