International Journal of Farmacia

Journal Home page: www.ijfjournal.com

Phytochemical screening and antiulcer activity of *Jasminum mesnyi* and *Triticum aestivum* leaves in albino wistar rats

Mehnoor Farheen*, Sobia Farheen

Shadan Women's College of Pharmacy, Khairatabad, Telangana State, Hyderabad-500004

***E-mail: mehnoor_farheen@yahoo.com**

Abstract

The present study was aimed to investigate anti-ulcer activity of ethanolic extract of *Jasminum mesnyi* and *Triticum aestivum* leaves using NSAIDs (aspirin) induced ulceration and pylorus ligation ulceration in albino wistar rats. Omeprazole (30 mg/kg body weight) was used as standard antiulcer agent. The ulcer index and percentage protection was estimated individually for both plants in NSAIDs model. Volume of gastric secretion, free acidity, total acidity and pH was estimated in both models using combination 1:1 ratio of extracts of both plants. Ethanolic extract showed significant ($p<0.001$) ulcer protective action at the doses of 200 and 400 mg/kg body weight individually as well as in combined doses in both animal models. A significant reduction in volume of gastric juice, free acidity and total acidity, along with increase in pH was observed in both models of ulcerations. The antiulcer property of tests extracts was attributed due to the presence of flavonoids and tannins.

Keywords: *Jasminum mesnyi*, *Triticum aestivum*, Aspirin, Pylorus ligation, Gastroprotective.

INTRODUCTION

An ulcer is an open sore in the lining of the stomach or intestine, much like mouth or skin ulcers. Peptic ulcers are eventually caused by acid and pepsin, a digestive stomach enzyme. These ulcers can occur in the stomach, where they are called gastric ulcers or they can occur in the first portion of the intestine called as duodenal ulcers. "Peptic ulcer" is the term used to describe either or both types of ulcers. Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. [1]

Drugs of plant origin are gaining popularity as natural products are non-toxic, less side effects and available at affordable price and are being investigated for a number of disorders, including peptic ulcer. [2]

Jasminum mesnyi (Family: Oleaceae) commonly known as Primrose Jasmine is a native of China. It has been reported for anti-bacterial, anthelminthic, antioxidant, activity. *Jasminum mesnyi* leaves contains secoiridoids glucosides, syringin, rutin, coumarin, linalool with smaller quantities of α- terpineol, asarone, phytol and geraniol has been isolated from the leaves. [3-5]

Triticum aestivum (Family: Poaceae) common or bread wheat, is an annual grass native to the Mediterranean region and Southwest Asia. It has been reported to have hypolipidemic, anti-fungal, hypoglycemic activity and antioxidant activity. High content of bioflavonoids such as apigenin, quercitin, luteoline are present which also acts as potent antioxidants. Alkaloids, tannins, saponins, sterols,

indole compounds like choline and lactrileare also present. [6]

These two plants have been reported with potent antioxidant activity and have traditional claims for gastroprotective effect. Hence in present investigation comparative and combined effect of both plants is estimated in albino rats.

MATERIALS AND METHODS

All the chemicals and reagents used for the study were of analytical grade. Ethanol (Changshuyangyuan chemicals), Aspirin (USV Ltd Mumbai), Omeprazole (Alkem house, Mumbai) were incorporated in study.

Collection and authentication of plant material

The leaves of *Jasminum mesnyi* Hance and *Triticum aestivum* L. were collected from botanical gardens of Sri Venkateshwara University, Tirupati and authenticated by Dr. K. MadhavaChetty, Botanists and Assistant Professor at Sri Venkateshwara University, Tirupati.

Preparation of extract

The powdered plant material was extracted with analytical grade ethanol for 7 days. Fresh ethanol was added daily with occasional stirring in maceration process. After filtering the residue was left for solvent evaporation to get crude extract and was stored in air tight container. [7]

Phytochemical screening

The preliminary phytochemical screening was performed on plants extracts revealed the presence of Flavonoids, Terpenoids, Alkaloids, Glycosides, Tannins, Sterols, Carbohydrates, Saponins, Proteins and Gums and mucilage. [8]

Acute toxicity studies

Acute oral toxicity of Ethanolic extracts of *Jasminum mesnyi* and *Triticum aestivum* was carried out as per OECD guidelines 425. [9] Single dose of 2000mg/kg produced no toxicity or mortality in both extracts. Therefore, the LD50 was considered to be more than 2000mg/kg body weight. The LD50 of the extract as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000mg/kg. Dose selected for pharmacological studies are mentioned below:

The biological evaluation was carried out at doses of 200 and 400mg/kg body weight of individual plants and in combination as:

Test 1(200mg/kg): 100mg *Jasminum mesnyi* and 100mg *Triticum aestivum*.

Test 2(400mg/kg): 200mg *Jasminum mesnyi* and 200mg *Triticum aestivum*.

Assessment of antiulcer activity

Animals: Male Albino Wistar rats (150-200g) were used for the study. They were housed in plastic cages and were left for two days for acclimatization to animal room, was maintained under controlled conditions of (12 hours light and dark cycle at (22 ± 30^0C), and were kept on standard pellet diet and water and libitum. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on experiments on animals) with clearance no: 002/CEAD/SES/SWCP/14.

Aspirin induced gastric ulcers

Four groups of albino rats (n=6) were selected. Group 1, served as control, received distilled water. Group 2, served as standard received Omeprazole at dose of 30mg/kg. Group 3 received test extract 200mg/kg and group 4 received test extract 400 mg/kg of test extracts orally for 7 days. After 7 days of treatment, animals were fasted for 24 hours having access to water ad libitum. Ulcer was induced by administration of aqueous suspension of aspirin 250mg/kg on day of sacrifice. The animals were sacrificed after 6 hours using ether anaesthesia. The upper and lower ends of stomach were tied and stomach was isolated. Gastric juice was collected in test tube. Stomach was opened along the greater curvature and washed with distilled water and examined for determination of ulcer index. [10] Screening of ulcer was done as; (0: normal colored stomach, 0.5: red coloration, 1: spot ulcers, 1.5: haemorrhagic streaks, 2: deep ulcers, 3: perforation). [11] Ulcer index was calculated using formula

$$\mathbf{UI = U_N + U_S + U_P \; X \; 10^{-1}}$$

Where; (UI: Ulcer index, U_N: Average no. of ulcer per animal, U_S: Average of severity score and U_P: Percentage of animal with ulcer).

Percentage inhibition

Percentage inhibition of ulcer index was calculated as below;[12]

$$\text{Percentage inhibition} = \frac{\text{Control mean ulcer index- Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Antisecretory studies

The gastric contents were subjected to centrifugation at 1000 rpm for 10 min, and then analyzed for various estimations like pH, total volume of gastric secretion, free acidity, and total acidity.

Estimation of total gastric volume

After centrifugation total volume was directly read from marking of tubes.

Estimation of pH

The pH was estimated using pH strips.

Estimation of free acidity and total acidity

The gastric contents were centrifuged and subjected to titration for estimation of free acidity and total acidity. One milliliter of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the end point when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued by adding 1% solution of phenolphthalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. The sum of the two titrations was total acidity.

$$\textbf{Acidity} = \frac{\textbf{Volume of NaOH x Normality of NaOH}}{\textbf{100mEq/l}} \textbf{ x 0.1}$$

Pylorus ligation induced ulcers

Four groups of 24 hours fasted rats (n=6) with water ad libitum were selected. Group1, control received distilled water. Group 2, received Omeprazole 30mg/kg as reference drug for ulcer protective study. Group 3 and 4 received ethanolic test extracts 200 and 400 mg/kg. After 1 hour of drugs administration, pyloric ligation was done under slight ether anaesthesia, ligation was done without causing any damage to the blood supply of stomach. [13] Animals were allowed to recover and stabilize in individual cages and deprived of both food and water during post-operative period and care was taken to prevent coprophagy. At the end of 4h, stomach was dissected out and gastric contents were collected. The ulcer index was also calculated as described above.

Histopathology

Sections of stomach were examined histopathologically to study the antiulcerogenic potential. The stomach was washed thoroughly with saline, stored in 10%formalin. Dehydrated in alcohol, cleared in xylene in tissue processor and finally embedded in paraffin wax. Sections of 5µm thick were cut in a rotary microtome and mounted on glass slides using standard techniques. After staining with hematoxylin and eosin, the sections were examined under light microscope and photographed.

Statistical Analysis

Data are expressed as the Mean± Standard Error of Mean (S.E.M.) and statistical analysis was carried out employing One Way Analysis of Variance (ANOVA) followed by Dunnet's test.

RESULTS

Antiulcer activity:

Aspirin induced ulcer model

Ethanolic extracts of *Jasminum mesnyi* Hance and *Triticum aestivum* significantly decreased the occurrence of gastric lesions induced by aspirin and exhibited dose-dependent gastroprotection. (Table 1) And when administered in combination they produced additive effect due to same mechanism of action for gastroprotection(Table:2).Stomach ulcer representation is indicated (Figure 1,2)

Table 1: Aspirin induced Ulcer Index and Percentage Inhibition in Individual Plants Extract.

Treatment Groups	Dose(mg/kg b.wt, p.o)	Ulcer Index Mean ±SEM	Percentage Ulcer Inhibition
Control	250mg Aspirin	12.66±0.49	0
Standard	Omeprazole 30mg and Aspirin 250mg	1.66±0.33	86.88%
Jasminum mesnyi	200mg/kg test and Aspirin 250mg	5.11±0.57	59.63%
Triticum aestivum	200mg/kg test and Aspirin 250mg	4.89±0.21	61.37%
Jasminum mesnyi	400mg/kg test and Aspirin 250mg	2.89±0.44	77.17%
Triticum aestivum	400mg/kg test and Aspirin 250mg	2.45±0.28	80.64%

Values are expressed as Mean±S.E.M., (n=6). $p<0.001$ was found significant.

Table 2: Ulcer Index and Percentage Inhibition in Aspirin model in Combination Studies.

Treatment Groups	Dose (mg/kg b.wt, p.o)	Ulcer Index Mean ±SEM	Percentage Ulcer Inhibition
Control	250mg Aspirin	11.4±0.22	0
Standard	Omeprazole 30mg and Aspirin 250mg	1.76±0.47	84.56%
Test 1	200mg/kg test and Aspirin 250mg	4.2±0.53	63.15%
Test 2	400mg/kg test and Aspirin 250mg	2.31±0.49	79.73%

Values are expressed as Mean±S.E.M., (n=6). $p<0.001$ was found significant.

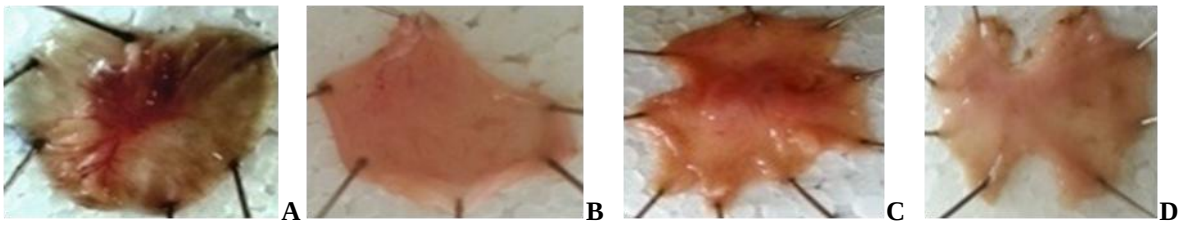

Fig 1: Aspirin Induced Stomach Ulcer Representation.

- **A. Control,** shows formation of hemorrhagic streaks, deep ulcers and significant damage of gastric mucosa.
- **B. Standard,** with less formation of ulcers.
- **C. Test extract (200mg/kg)** which shows spot ulcers and red colouration.
- **D. Test extract (400mg/kg)** which shows less ulcers formation when compared to animals treated with lower dose.

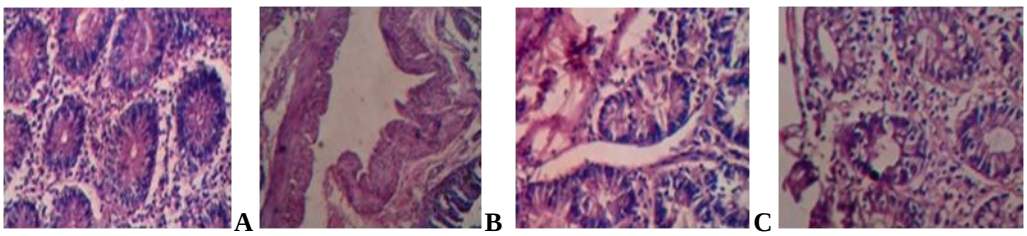

Fig 2: Histopathology images of Aspirin induced ulcers model.

- **A. Control,** showing deep erosions and congestion with RBC'S in eroded portion.
- **B. Standard,** showing stomach mucosa almost normal epithelium and submucosa.
- **C. Test (200mg/kg),** showing superficial and mucosal erosions with exudates in lumen.
- **D. Test (400mg/kg),** no deep ulcers are observed, thick muscularis was seen.

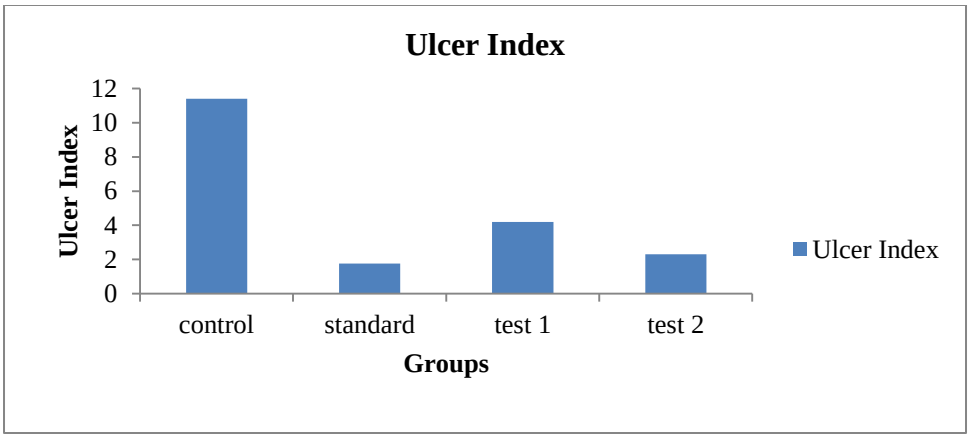

Fig 3: Representation of Ulcer Index of different groups of Aspirin method.

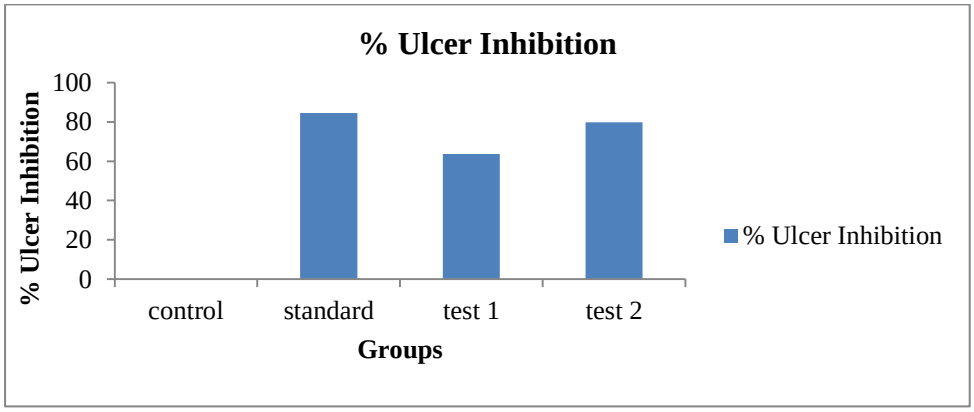

Fig.4: Representation of Percentage Ulcer Inhibition of different groups in Aspirin method.

Antisecretory studies

The reduction in various parameters like gastric volume, total acidity, free acidity and pH indicated the antisecretory effectof plant extracts. (Table 3)

Table 3: Effect of Test Drug (200, 400mg/kg) and Omeprazole 30mg on total gastric volume, free acidity, total acidity and pH of Gastric juice in Aspirin induced Ulcers model

Treatment Groups	Dose (mg/kg b.wt, p.o)	Total Gastric Volume (ml)	Free Acidity (mEq/Lt)	Total Acidity (mEq/Lt)	pH
Control	Aspirin 250mg	6.13±0.94	47.16±1.47	63.31±1.08	2.16±0.12
Standard	Omeprazole 30mg and Aspirin 250mg	3.10±0.56**	19.37±2.12**	30.21±1.16**	4.61±0.29**
Test 1	200mg/kg test and Aspirin 250mg	3.88±0.94**	32.01±1.57**	43.13±2.14**	3.97±0.16**
Test 2	400mg/kg test and Aspirin 250mg	3.34±0.88**	24.66±1.64**	39.22±2.79**	4.07±0.12**

**p<0.01, ***p<0.001 when compared to control, Dunnet's test.

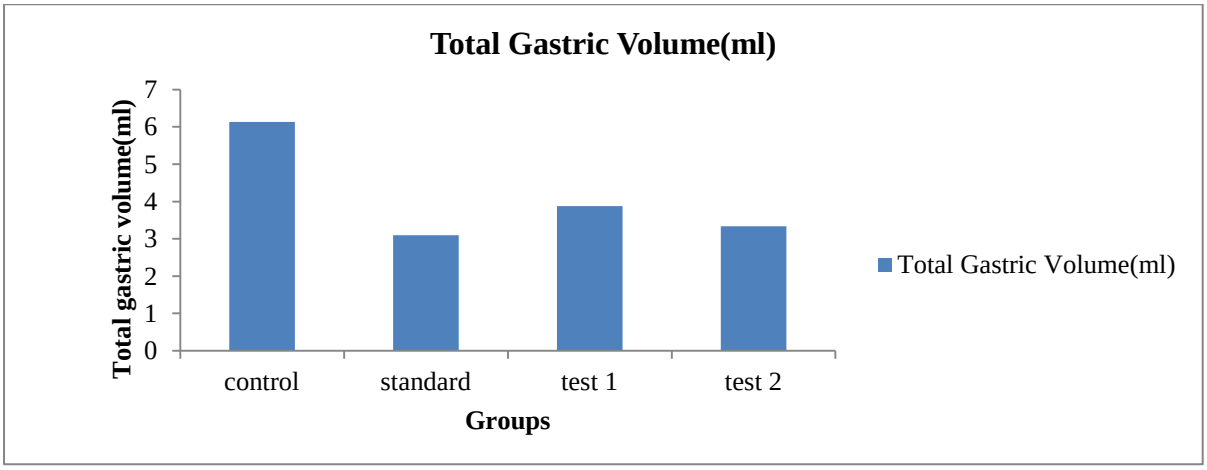

Fig 5: Representation of Total Gastric Volume of different groups in Aspirin method.

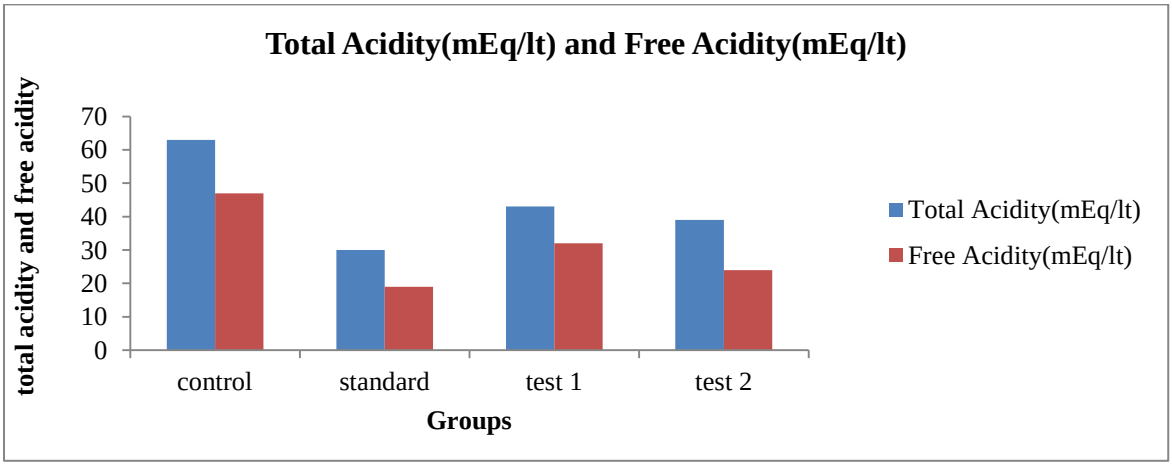

Fig 6: Representation of Free Acidity and Total Acidity of different groups in Aspirin method

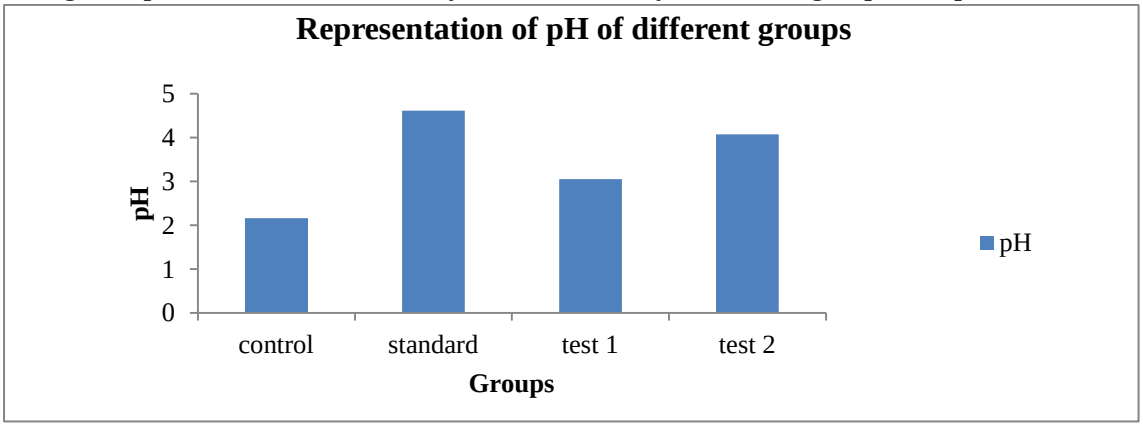

Fig 7: Representation of pH of different groups in Aspirin method

Pylorus ligation induced ulcer model

Gastroprotection was observed in dose dependent manner.

Table 4:Pylorus Ligation Induced Ulcer Index and Percentage Ulcer Inhibition in Combination Studies.

Treatment Groups	Dose(mg/kg b.wt, p.o)	Ulcer Index Mean ±SEM	Percentage Ulcer Inhibition
Control	Distilled Water	8.29±0.24	0
Standard	Omeprazole 30mg	0.98±0.71	88.17%
Test 1	200mg/kg test	2.07±0.88	75.03%
Test 2	400mg/kg test	1.45±0.45	82.50%

Values are expressed as Mean±S.E.M., (n=6). p<0.001 was found significant.

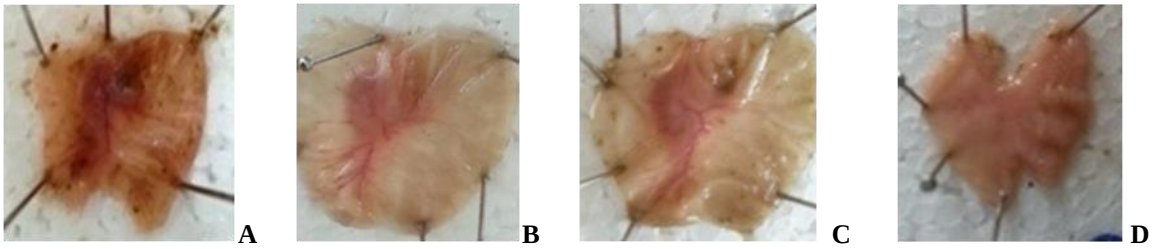

Fig 8: Pylorus Ligation Induced Stomach Ulcer Representation.

A. **Control**, shows deep ulcers and hemorrhagic streaks.
B. **Standard,**shows red colouration of stomach.
C. **Test extracts (200mg/kg)** with spot ulcers and few hemorrhagic streaks.
D. **Test extracts (400mg/kg)** shows less formation of ulcers when compared to animals treated with lower dose.

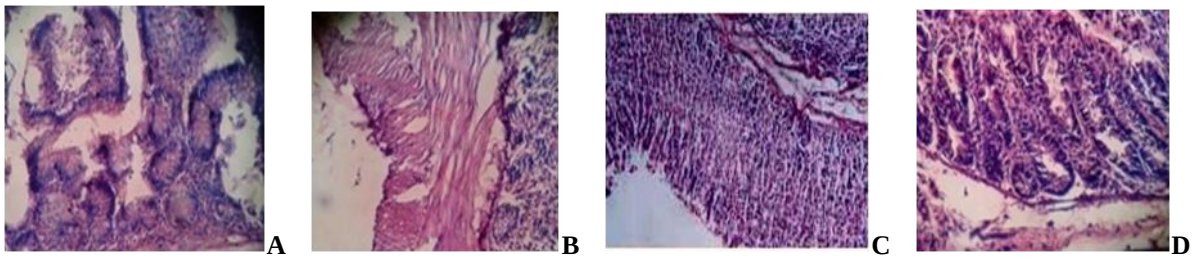

Fig 9: Histopathology images of Pylorus ligation induced ulcers method.

A. **Control**, showing hemorrhage and hyperplastic glands with inflammatory filtrate.
B. **Standard**, showing no ulcer formation, normal epithelium was observed.
C. **Test (200mg/kg),** showing small discontinuity and mild edematoussubmucosa.
D. **Test (400mg/kg),** showing normal mucosa with atropic glands.

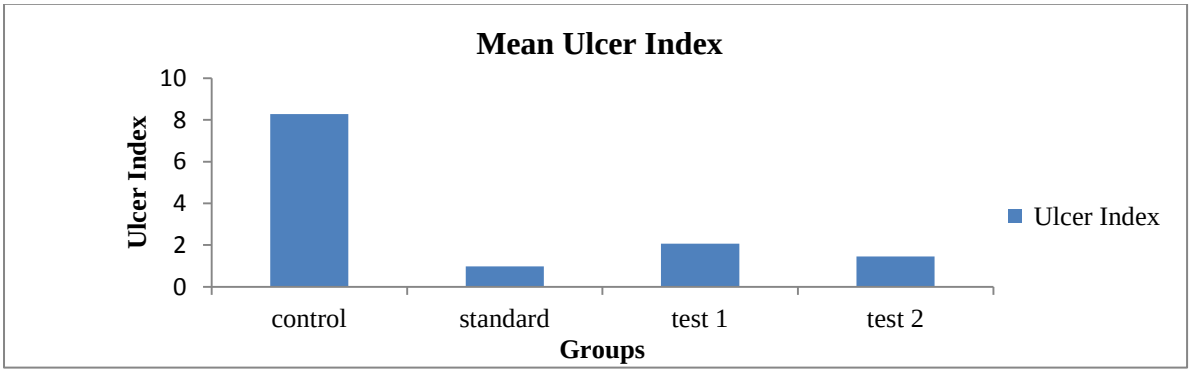

Fig 10: Representation of Ulcer Index of different groups of Pylorus ligation method

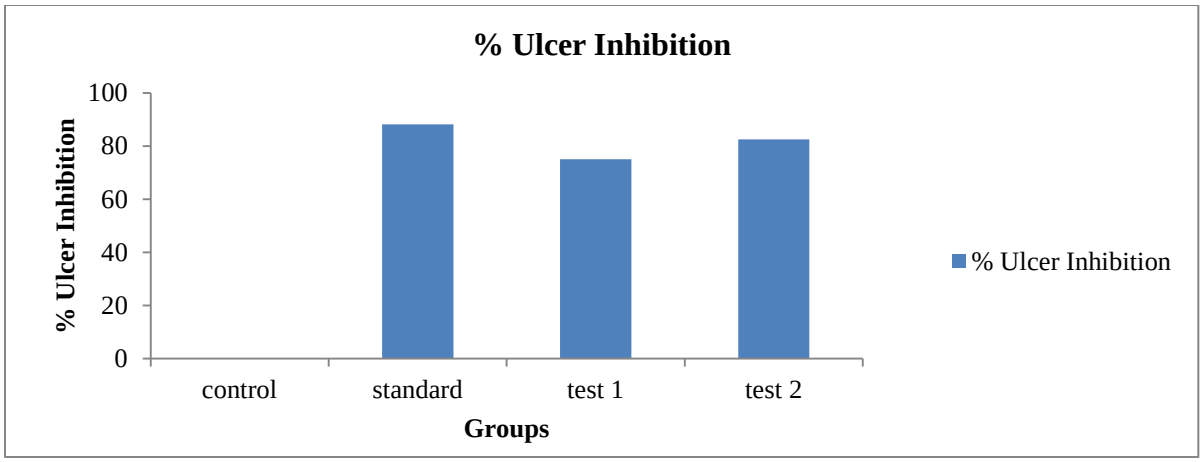

Fig 11: Representation of Percentage Ulcer Inhibition of different groups of Pylorus ligation method.

Antisecretory studies

The reduction in various parameters like gastric volume, total acidity, free acidity and pH indicated the antisecretory effectof plant extracts. (Table 5)

Table 5 : Effect of test Drug (200, 400mg/kg) and Omeprazole 30mg on total volume, total acidity, free acidity and pH of Gastric juice in Pyrolus Ligation Model.

Groups	Treatments	Total Gastric Volume (ml)	Free Acidity (mEq/Lt)	Total Acidity (mEq/Lt)	pH
Control	Distilled Water	4.50±0.24	43.21±2.63	94.03±1.12	2.11±0.13
Standard	30mg Omeprazole	2.34±0.16**	15.64±1.14**	33.25±3.64**	4.46±0.14**
Test 1	200mg/kg test	3.70±0.22**	34.83±1.03**	77.30±2.59**	3.21±0.18**
Test 2	400mg/kg test	2.90±0.18**	29.20±1.65**	69.53±2.66**	4.01±0.14**

**p<0.01, ***p<0.001 when compared to control, Dunnet's test.

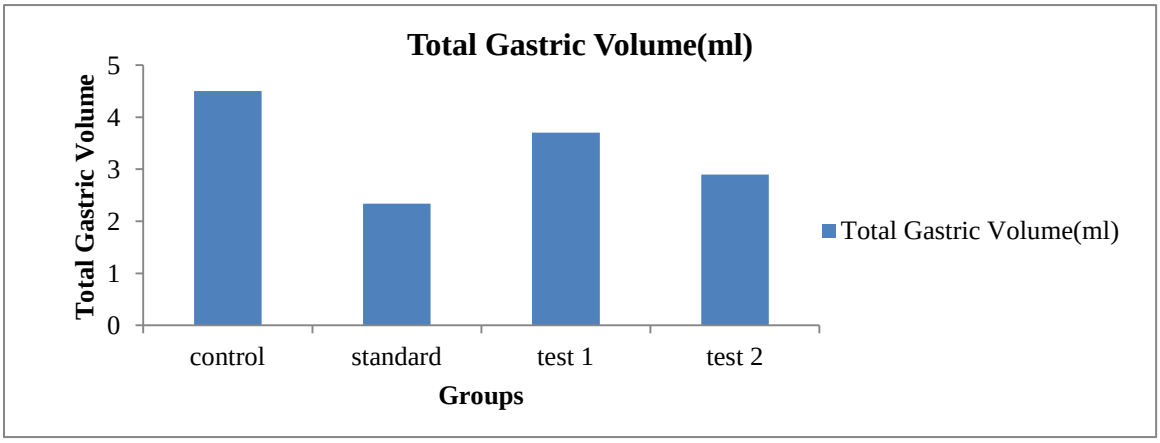

Fig. 12: Representation of Total Gastric Volume of different groups in Pylorus ligation method.
Representation of Total Gastric Volume of different groups in Pylorus ligation method.

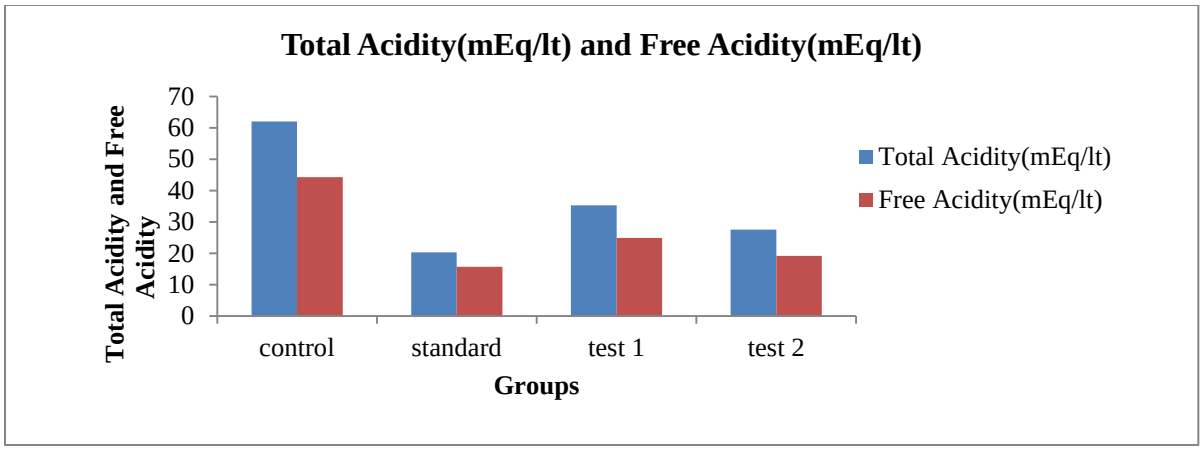

Fig. 13: Representation of Free Acidity and Total Acidity of different groupsinPylorus ligation method.

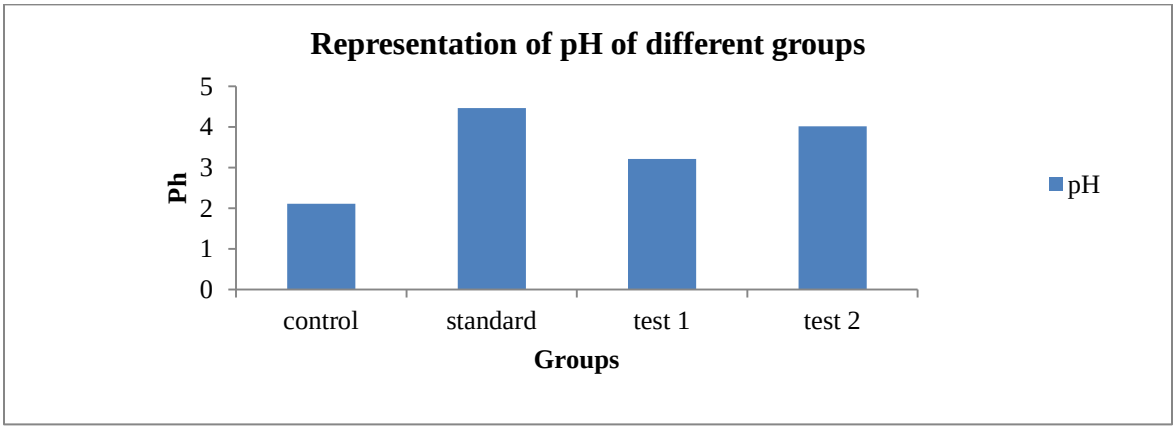

Fig. 14: Representation of pH of different groups inPylorus ligation method.

DISCUSSION

There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune gastrointestinal inflammation and gastric ulcer. Drugs with multiple mechanism of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent antioxidant action and are effective in healing experimentally induced gastric ulcers. [14-17]

The present study undertakes to evaluate the anti-ulcerogenic and gastroprotective activity of combined formulation of *Jasminummesnyi* and *Triticumaestivum* tested against NSAIDs (aspirin) induced and pylorus ligation induced ulcers in rats. These models, represents some of the most common causes of gastric ulcer in humans. The ulcerogenic effect of aspirin correlates well with its ability to suppress prostaglandin synthesis through their action on COX pathway. Deleterious effects of aspirin nonselective action on gastroprotection results from their inhibition of COX-1 isoform and thus brings about gastric ulceration [18] and thereby reduce the intrinsic ability of mucosa to resist injury by endogenous and exogenous aggressors. In Pylorus-ligation, ulcers are caused due to prolonged exposure and accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa. [19] In addition to gastric acid secretion, reflex or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcer in this model. [20]

Flavonoids are phenol compounds are found in many green plants. Since, flavonoids present in both plants antagonized aggressive factors which play a crucial

role in the pathogenesis of gastric lesions and also augment defensive factors to protect gastric mucosa from injury. Flavonoids decreases histamine secretion from mast cells by inhibition of histidine decarboxylase and stimulates prostaglandin biosynthesis, this mechanism of action is responsible for gastroprotective effect. The site of inhibition of acid formation by the parietal cells is the H^+-K^+ ATPase. Flavonoids and flavones were effective to stimulate PGE_2 production in gastric mucosal cells. Flavonoids reported to possess the property of preventing the formation of ulcers produced by various ulcerogens. [23, 24]Literature shows tannins may prevent ulcer development due to their vasoconstricting effects. [22]Flavonoids, tannins present in both plants have been reported with potent antioxidant activity and also exhibit antihistaminic and antisecretory activity which plays a major role in repairing the gastric damage in peptic ulcers and exhibits anti-ulcer and gastroprotective activity.

Findings of the present study clearly indicate that the oral administration of Ethanolic extract of *Jasminummesnyi* and *Triticumaestivum* displayed appreciable anti-ulcer and gastro protective effect as demonstrated by significant decrease in ulcer index and increased percent ulcer inhibition in both models. The present study therefore supports the claims of traditional medicinal practitioners as an antiulcer remedy. It could also be a prospective substitute for the existing synthetic antiulcer drugs which are to known to produce harmful adverse effects and hence for the development of a potent antiulcer agent having low-toxicity.

REFERENCES

[1]. Hoogerwerf, W.A., Pasricha, P.J., 2006. Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. In: Brunton, L.L., Lazo, J.S., Parker, K.L. (Eds.), Goodman &Gilman.s The Pharmacological basis of therapeutics, 11th ed. McGraw-Hill Medical Publishing Division, New York, pp. 967.981.

[2]. Dharmani P, Palit G. 2006. Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol 38:95-9.

[3]. Dullu V. Anthelmintic activity of ethanolic leaf extract of *Jasminummesnyi*. Asian Pac J Trop Dis 2014; 4(Suppl 1): S273-S275.

[4]. Poonia, P., Niazi, J., Chaudhary, G., Kalia, AN. 2011. In-vitro antioxidant potential of *Jasminummesnyi*Hance (leaves) extracts. Res J Pharm BiolChem Sci. 2(1): 348-357.

[5]. Satyal, P., Paudel, P., Lamichhane,B., Setzer WN. 2012. Volatile constituent and biological activities of the leaf essential oil of Jasminummesynigrowing in Nepal. Journal of chemical and Pharmaceutical Research. 4(1):437 – 439.

[6]. Singh, N., Verma, P., Pandey, BR. 2012. Therapeutic potential of organic *Triticumaestivum* Linn. (Wheat Grass) in prevention and treatment of chronic diseases: An overview. Int J Pharm Sci Drug Res. 4:10-4.

[7]. Khandelwal, K.R. Practical Pharmacognosy, 11th ed. NiraliPrakashan, Pune, 2004. p. 149-156.

[8]. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Niraliprakashan, Pune, 42 edition, 2008.

[9]. New OECD 425 Guidelines. OECD Guidelines for testing animals.2001, Dec 1/26,1-26.

[10]. Kulkarni S. K. 1997. Handbook of Experimental Pharmacology, 2nd ed., (VallabhPrakashan, Delhi), 69.

[11]. H.Gerhard Vogel. Drug Discovery and Evaluation, Springer-Verlag Berlin. Heidelberg, New York, 2002 p. 868.

[12]. Chitra, V., Madhuri, M., Rajalakshmi, V., 2013. Evaluation of Antiulcer Activity of Alcoholic Extract of GarugapinnataRoxb. Leaves in Experimental Animal Models. International Journal of Pharm Tech Research. 5(3):991-1000**.**

[13]. Shay H., Komarov S.A., Fels S.S., Meranze D., Gruenstein M. and Siplet H. 1945. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology. 48:43-61.

[14]. Repetto, M.G., Llesuy, S.F. 2002. Antioxidant properties of natural compounds used in popular medicines for gastric ulcers. Brazilian Journal of Medical and Biological Research. 35, 523–534.

[15]. Dhuley, J.N. 1999. Protective effect of Rhinax, an herbal formulation against physical and chemical factors induced gastric and duodenal ulcers in rats. Indian Journal of Pharmacology. 31,128–132.
[16]. George, S., Sathiamoorthy, A., Sathiamoorty, S., 1999. Effect of alpha tocopherol on gastric ulcers induced by pylorus ligation in rats. Indian Journal of Pharmacology 31, 431–433.
[17]. Goel, R.K., Sairam, K. 2002. Anti-ulcer drugs from indigenous sources with emphasis on Musa sapientum, Tamrabhasma. Asparagus racemosus and Zingiber officinale. Indian Journal of Pharmacology. 34:100–110.
[18]. Vane JR. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for asprin like drugs. Nat New Biol. 231:232-5.
[19]. Bafna, PA., Balaraman, R., 2005. Antiulcer and antioxidant activity of pepticare-A herbo mineral formulation. Phytomedicine. 15: 264-270.
[20]. Goel, RK., Bhattacharya, SK., 1991. Gastroduodenal mucosal defence and mucosal protective agents. Indian Journal of Experimental Biology. 29: 701-703.
[21]. Parmar, NS.,ShikhaParmar., 1998. Indian J PhysiolPharmacol. 48:343–351.
[22]. Suarez, J., Herrera, MD., Marhuenda, E., 1998. Hesperidin and neohesperidindihydrochalcone on different experimental models of induced gastric ulcer. Phytother Res. 50:616-8.
[23]. Aguwa, CN.,Nwako, SO., 1988. Preliminary studies of the root extracts of Nauclealatifolia Smith, for anti-ulcer properties. Niger J Pharm Sci. 48:16-23.