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Research Article

Evaluation of anti-ulcer activity of the leaf extract of *Solanum pubescens* willd. (Solanaceae) in Wistar albino Rats

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Abstract

The present study showed that the methonolic extract of *Solanum pubescens willd* leaves possess Anti-ulcer in animal model. The anti-ulcer activity was evaluated against Pylorus ligated ulcers. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate,mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Oral administration of methanol extract of *solanum pubescens willd* at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 36.28% and 52.3% ($p<0.001$) respectively compared to the ulcer control, proving the anti-ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 70% when compared with ulcer control. Extract treated and ulcer control group was compared with normal control group. Result showed a significant decrease in the ulcer development in the animal models (pylorus ligated model) used in the study. In pylorusligation, both the doses showed significantant ulcer activity by reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. The intensity of heamorrhage and lesions was significantly reduced upon pretreatment with the extract, revealing the protective effect of MESP. Flavonoids and tannins are the major constituents that are present in the leaves of *Solanum pubescens willd* which may be responsible for its Ulcer- protective and Anti-ulcer activity.

Keywords: Peptic ulcer, *Solanum pubescens*, Wistar rats, Acute Oral toxicity, ANOVA

INTRODUCTION

Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide[1], representing a major burden for health care resources. Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives[2]. As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care, reinforcing the theory that plant extracts can be good sources of new drugs. Ethiopia is a country characterized by a wide range of climatic and ecological conditions possessing enormous diversity of flora and fauna, including a wide range of potentially useful medicinal plants[3].

In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share[4,5]. In this modern era also 75–80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects[6]. Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity[7].

The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors)[8]. Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors and the real causes have been found by research to include bacterial infection (Helicobacter pylori) or reaction to various medications, particularly NSAIDS (non steroidal anti-inflammatory drugs)[9]. Helicobacter pylori, NSAIDS drugs, emotional stress, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer[10]. The Gram-negative bacterium Helicobacter pylori remains present between the mucous layer and the gastric epithelium and is strategically designed to live within the aggressive environment of the stomach. Initially, Helicobacter pylori resides in the antrum but over time migrates toward the more proximal segments of the stomach[11].

Many synthetic anti-ulcer drugs are available in the market but most of them are associated with many adverse and unwanted effects like Gastro-intestinal irritation, ulceration and fluid retention. In addition to aggravating the ulcers, NSAIDS also produce hepato-toxicity and nephro-toxicity[12]. Since ancient times people have been relying on plants either as prophylactic of therapeutic agents to restore and maintain the health. Medicinal plants have been used in the development of new drugs which may have an invaluable role

in the progress of drug discovery[13]. Therefore a need for the development of anti-ulcer agents from natural source with more powerful activity and lesser side effects.

MATERIAL AND METHODS

Plant collection[14]:

The leaves of *Solanum pubescens willd* were collected from Satupra MP. It was identified and authenticated by Department of Botany, Safiya College, and Bhopal M.P.

Preparation of extract:

The leaves of *Solanum pubescens* willd were shade dried and then ground till they became coarse powder in a motar-pestle. The powdered material thus obtained was subjected to extraction using Methanol[15]. The extracts obtained were distilled to remove excess of the solvent and then evaporated at 40°C to get a semi-solid mass. These extracts were subjected to phytochemical tests which have been described below.

Animals:

Wistar rats of either sex (150-200gms) were housed in separate cages at controlled room temperature (24 ±2°C; relative humidity 60-70%) in a 12hr light- dark cycle. They were fed with standard pellet diet and water dlibitum[16].

Qualitative Phytochemical analysis:

Determination of Acute Oral toxicity (LD50) of *Solanum pubescens willd*

Table 1: Experiment protocol:

Name of the study	**Acute toxicity**
Guideline followed	OECD425 method-acute toxic classMethod
Animals	Healthy young adult non-pregnantSwiss albino mice.
Bodyweight	25-30 g
Sex	Male
Administration of dose and volume	2000 mg/kg body weight, single doseIn 0.2 ml
Number of groups and animals	2 groupsand6animals
Route of administration	Oral by using mice oral feeding needle
Vehicle	Carboxy Methyl Cellulose (CMC)

Housing and feeding conditions:

Room temperature	22°C±3°C
Humidity	40-60%
Light	12 h: 12h (light:darkcycle)
Feed	Standard laboratory animal food pellets with water ad libitum

Study period and observation parameters:

Initial observation	First 30 minutes
Special attention	First 1-4hrsafterdrugadministration
Long term observation	Upto 14days
Direct observation parameters	Diarrhea, sitting in the corners, sniffing excessively, standing onhind limbs,
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory,autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

Study procedure:

Acute oral toxicity was performed as per Organization for Economic Cooperationfor Development (OECD) guideline 425 methods[17]. The extract was administered in a single dose by gavages using specially designed mice oral needle. Animals are fasted 24h prior to dosing (food was withheld, but not water). (OECD Guideline for testingof chemicals 425).

Pharmacological evaluation:

Animal selection:

Healthy adult male Wistar albino rats weighing between 150 and 200gms wereselected for the anti-Ulcer studies[18].

Housing and feeding condition:

The temperature in the experimental animal room was kept

22±30⁰C. Artificial lighting was provided. The animals were acclimatized to standard laboratory conditions of temperature (22±30°C) and maintained on 12:12 h light: dark cycle[19]. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They were provided with regular rat chow diet and distilled water adlibitum.

Preparation of animals:

The animals were randomly selected and kept in their cages for at least 5 days prior todosing to allow for acclimatization to the laboratory conditions.

Extracts & Standards drug used:

Extract used: Methanolic extract o f *Solanum pubescens* willd leaves. Standard drug used: Omeprazole: 20mg/kgb.w. Drugs, Omeprazole and the test extract of Solanum pubescens willd were suspended in 0.5% CMC and used for anti-ulcer studies. Each drug suspension was freshly prepared just before administration[20].

Preparation and administration of doses

The extract was solubilised in 0.5% Carboxy Methyl Cellulose prior to experimental use to obtain the desired concentrations (200 and 400mg/kg body weight) in 1ml. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 hrs.

Anti-ulcer activity:

Pyloru sligation induced Gastric ulcers:

Rats were divided into four groups of six animals each[21]. All the animals selected forthe study were of weight between 200-250gms.

Group I (control), received, 0.5% CMC.

Group II (reference standard) was treated with 20mg/kg omeprazole.Group III treatedwith400mg/kg methnolic extract of S*olanum pubescens* willd.

Group IV was treated with 200mg/kg methanolic extract of Solanum pubescens willd.

Animals in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and *pyloru sportion* of stomach was lifted out and ligated[22]. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Four hours after the pyloricligation, the animals were sacrificed by an excess dose of ether. The stomach was carefully removed and the gastric contents were collected. The gastric juice was centrifuged at 1000rpm and gastric volume was measured[23]. Free and total acidites of the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/L/100 gms. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the gastric ulcers and t calculate ulcer index. Ulcer scoring is done according to the scale mentioned below.

Ulcer Index:

After the incision of the stomach at the greater curvature the ulcers were observed. And the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using venire calipers[24]. The following arbitrary scoring system was used to grade the incidence and severity of lesions.

Normal coloration–0

Red coloration – 0.5

Spot ulcer – 1

Hemorrhagic streaks–1.5

Ulcer - 2

Perforation-3

Determination of Free Acidity and Total Acidity:

The gastric contents were centrifuged at 1000 rpm for 10mins. 1ml of supernatant was diluted with 9ml distilled water. A volume of 2ml diluted gastric juice was treated with 0.1N sodium hydroxide run from a micro burette using 3-4 drops of Topfer's reagent as indicator until a canary yellow colour was observed. The volume of NaOH run down was noted[25]. This corresponds to free acidity. Further, 2-3 drops of phenolphthalein was added and titrated with NaOH until pinkcolour was restored. This gives total acidity. Free acidity and Total acidity are expressed in terms of ml of 0.1N HCl per 100 gms of gastric contents. This is the same as mEq/lit. Acidity may be calculated by using. The following formula:

Acidity= volume of NaOH X Normality of NaOH X100 mEq/lit Histopathological

Evaluation:

The gastric tissue samples were fixed in neutral buffered formalin solution for duration of 24 hrs. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of methanolic extract of *Solanum pubescens*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor[26,27]. These sections were stainedwith hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes (P. Thirunavukkarasu et al.,2009).

Statistical Analysis:

Statistical analysis was carried out using Graph Pad Prism 5 software version 5.04 (Graph Pad prism software Inc.) The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's t-test. P values< 0.05 were considered significant[28].

RESULTS AND DISCUSSION

The methonolic extract of *Solanum pubescens* willd was subjected to Phytochemical and pharmacological screening. Pharmacological screening involved the evaluation of anti-ulcer activity[29]. The results of the methanolic extract of *solanum pubescens* has been documented below.

Preliminary Phytochemical

The colour of the Alcoholic test extract (methanolic extract of *Solanum pubescens* willd) was found to be dark green in colour and the consistency was found to be sticky. The extracts were subjected to phytochemical screening for the presence of type of phyto-constituents[30]. The phytochemical screening revealed that methanolic extract contains resins, alkaloids, Phytosterols, Phenols and Flavonoids of the phytosterols, the ones identified were tri-terpenes. The results have been tabulated in the table 2 showing various phyto constituents in the extract.

Table 2: Preliminary phytochemical analysis of Extract

Phyto constituents	Extracts	
	Petroleum ether	Ethanol
Carbohydrates	-	+
Glycosides	-	-
Alkaloids	-	+
Phytosteroids	-	_
Flavonoids	-	+
Protein and amino acids	-	-
Saponin	-	_
Phenols & tannins	-	+
Anthaquinone Glycosides	-	-
Triterpenoids	-	+

(-) represents Absence; (+) represents Pre sence

Acute toxicity Study:

Methanolic Extract of *Solanum pubescens* willd did not produce any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight[31]. There was neither change in behavioral pattern or any sign of toxicity during the observations up to 24hrs for mortality. Thus the extract was considered to be safe for pharmacological evaluation. Biological evaluation was carried out at doses of 200 and 400mg/kg.

Result: From acute toxicity study it was observed that the administration of Methanolic extract of *Solanum pubescens willd* to mice did not induce any toxicity of extract and mortality in the animal's upto 2000mg/kg orally.

Pharmacological evaluation:

Evaluation of Anti-ulceractivity was carried out for the methonolic extract of the plant. Anti-ulcer was performed by Pylorus ligation induced ulcer.

Anti-Ulcer Evaluation:

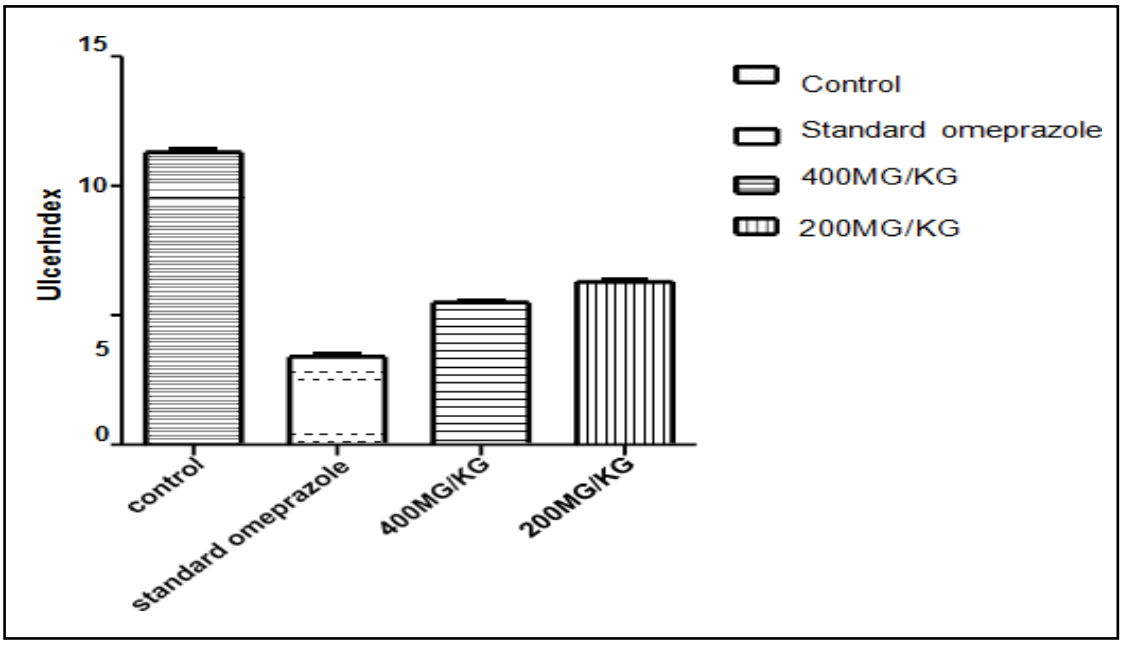
Pylorus Ligation method:

In pyloric ligation induced ulcer model, Oral administration of MESP in two different doses (200 mg/kg and 400 mg/kg) showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group[32]. MESP was showing protection index of 52.3% and 36.28%at the dose of 400mg/kg and 200mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug a protection percentage of 70% has been observed. Since the ulcer protective percentage of MESP at 200mg/kg is 36.28% it can be considered to be less significant in the context of the study[33].

Table 3: Ulcer index and % protection

Groups	Ulcer Index	% Protection
Group I-Control	11.3±0.11	--
Group II Standard (omeprazole)	3.39±0.07***	70%
Group III - *S.pubescens willd* Extract 400mg	5.48±0.07***	52.3%
Group IV - *S.pubescens* willd Extract 200mg	7.2±0.06*	36.28%

All values represent Mean ± SEM, n=6 in each group. P <0.001. Control group is



Graph 1: compared with standard and extract doses

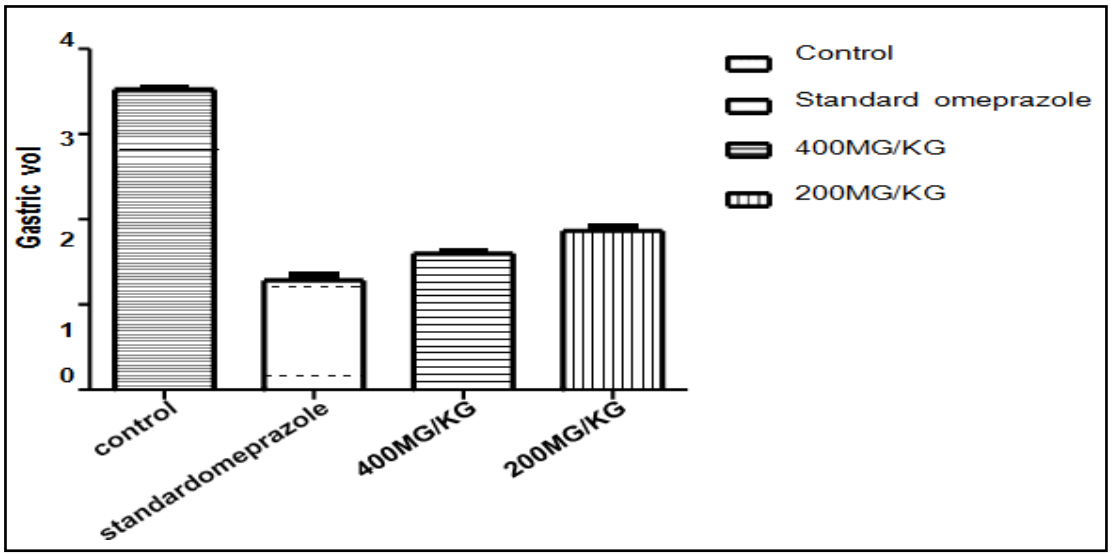
Gastric volume, Free Acidity and Total Acidity:

The results of various acid secretory parameters such as Gastric volume, pH, free acidity and Total acidity of methanolic extract of *Solanum pubescens* willd on pylorusligation induced gastric ulcer in rats are summarized in Table 4. Estimation of acid secretory parameters was increased significantly in the control group. Administration of MESP exhibited a significant ($p < 0.001$) reduction in all the parameters and the results were comparable with the standard drug Omeprazole 20mg/kg. In control group the mean gastric juice was 3.52ml. Omeprazole, the standard drug decreased the mean gastric volume (1.28ml), which is statistically significant[34]. Apart from the standard, ethnologic extract also showed decrease in the mean gastric juice at both the doses of 400 and200 mg/kg. The extracts reduced them gastric juice volume to 1.59ml and 1.86 ml respectively. The test extracts showed the decreased in gastric juice volume on comparison to control group and thus indicate their anti-secretary mechanism[35]. This demonstrates the dose dependent effect of MESP.

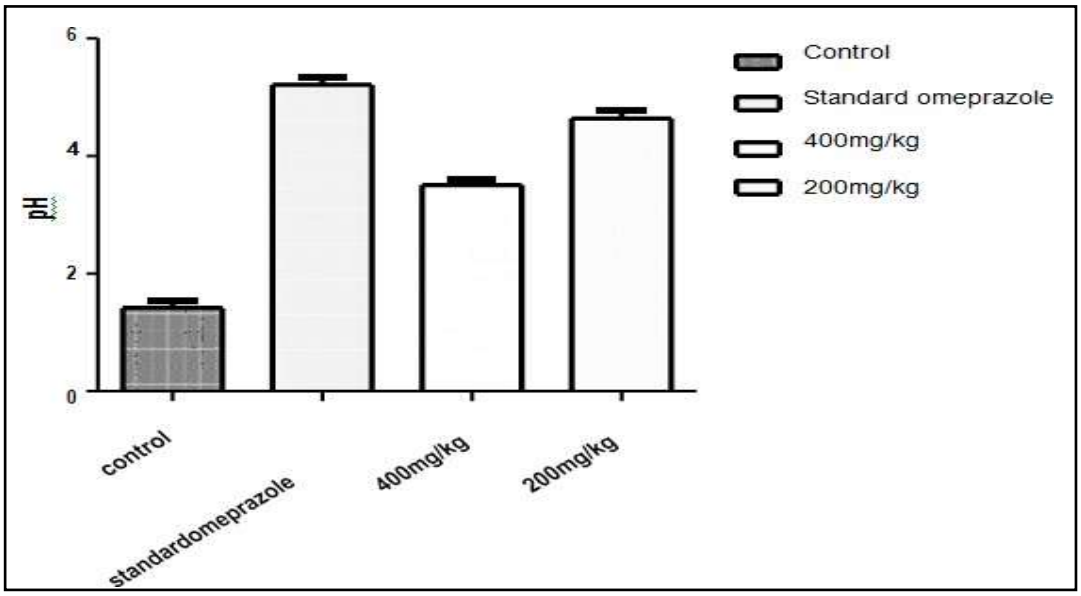
Table 4: Gastric volume, pH, free acidity and Total acidity

Group	Gastric Volume	pH	Free Acidity	Total Acidity
Control	3.52±0.02	1.41±0.12	55.01±2.28	67.79±1.31
Std(Omeprazole)	1.28±0.05***	5.21±0.13***	20.90±0.76***	32.06±3.316***
Extract 400mg	1.598±0.03**	3.5±0.08***	29.86±0.77**	41.96±0.715***
Extract 200mg	6±0.04**	4.63±0.14***	39.66±1.14*	55.56±0.99***

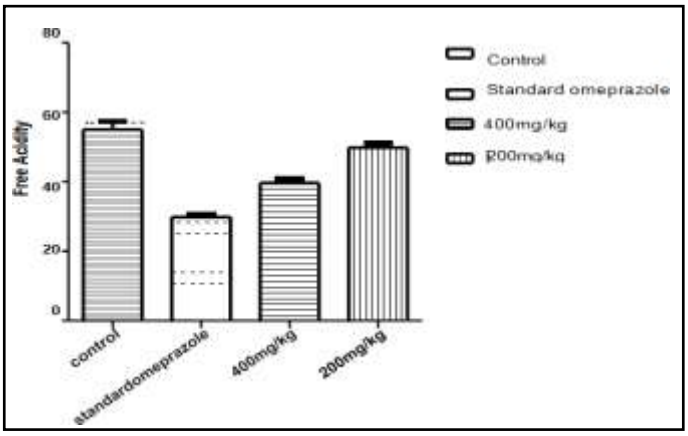
All values represent Mean ± SEM, n=6 in each group. P <0.001. Control group iscompared with standard and extract doses.



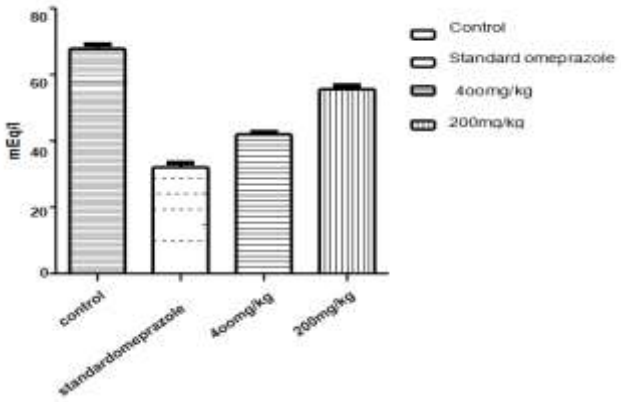
Graph 2: Comparing the amount of Gastric volume collected from each of theControl, Standard and the Extract (400 and 200mg/kg) groups.



Graph 3: Graph Comparing the pH of Gastric contents collected from each ofthe Control, Standard and the Extract (400 and 200mg/kg) groups



Graph 4 Comparison of Free Acidity of the Control, Standard and theExtract (400 and 200mg/kg) groups.



Graph 5: Comparison of Total Acidity of the Control, Standard and theExtract (400 and 200mg/kg) groups.

ULCER IMAGES (*Pylorus Ligation*):

(A)

(B)

(C)

(D)

Figure 1: Ulcer Images (Pylorus Ligation)

Histopathology images–Pylorus ligated ulcer

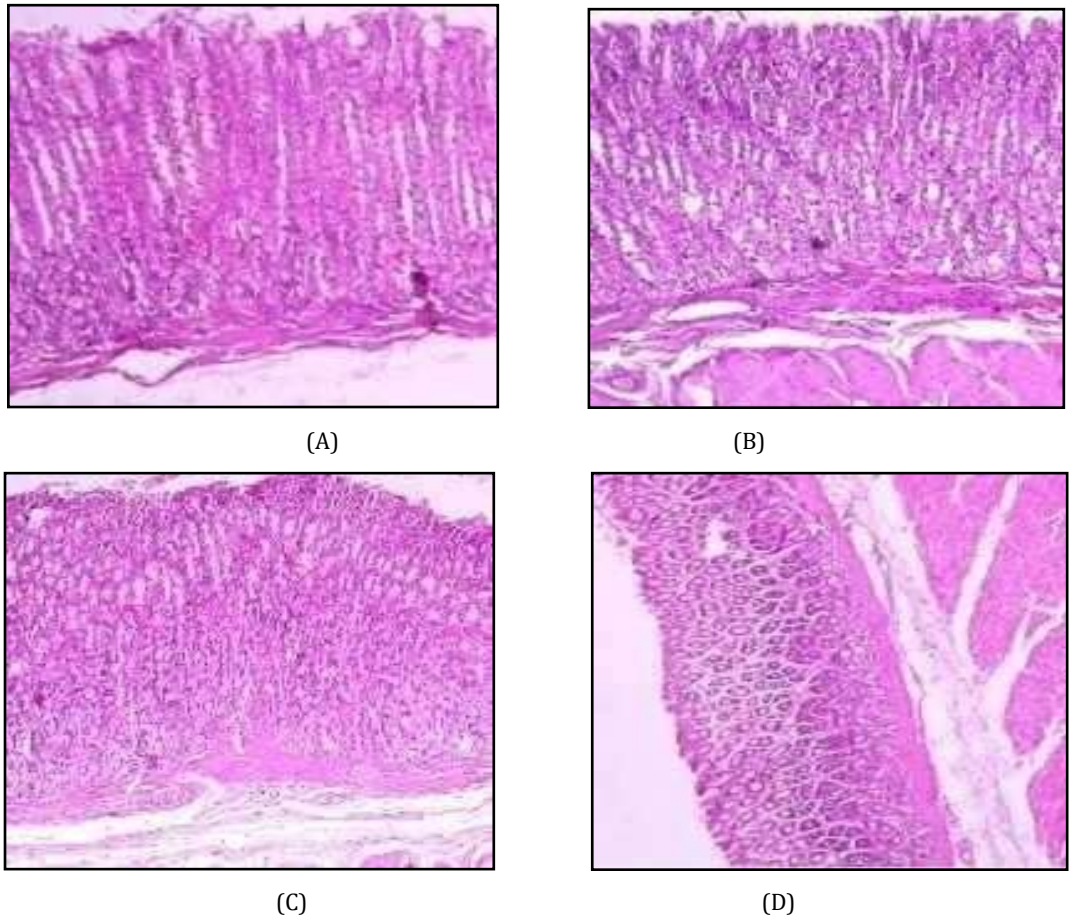


Figure 2: (A)–Control; (B)–Standard; (C)–Extract400mg; (D)–Extract200mg

Histopathology examination:

The histopathological examination of stomachs of the rats showed a better picture of the gastric lesions and the damage occurred to the stomach mucosa[36,37]. Acute ulceration of stomach was observed in group-I, Group (A) (control group rats) show mucosal ulceration consisting of necrosis, cellular debris, neutrophils and degenerated epithelial cells Group-II (B) shows gastric mucosa with intact epithelium, lamina propria and muscularis mucosa[38]. Group (C) shows intact mucosa with plenty of regenerative epithelial cells Group (D) shows focal mucosal ulceration consisting of degenerated cells and also contains few regenerated epithelial cells.

DISCUSSION

In this study, the anti-ulcer activity of methanolic extract of Solanum pubescens willd has been studied. The anti-ulcer activity was evaluated against Pylorus ligated ulcers. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Ulcer caused by pylorus ligation is due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier. The activation of the vagus- vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion.

Antiulcer effect is supported by the decrease in the aggressive factors like gastric volume, decrease in free and total acidity and an increase in the resistance factors like pH showing the anti-secretary mechanism. It is significant to note when the pH was nearing 5.2 (Std) and 3.5 (Ext 400mg/kg), the ulcer score appeared less. The antiulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. The mucosal defense mechanism maybe due to the epithelial cells of the gastric mucosa, which are impermeable to H^+ ions thereby forming a physical barrier

The methonolic extract of *solanum pubescens* willd was evaluated by using pylorus ligation method, Oral administration of methanol extract of *solanum pubescens willd* at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 36.28% and 52.3% ($p<0.001$) respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 70% when compared with ulcer control. Extract treated and ulcer control group was compared with normal control group.

CONCLUSION

The present study showed that the methonolic extract of *Solanum pubescens willd* leaves possess Anti- ulcer in animal model. *Solanum pubescens willd* showed a significant decrease in the ulcer development in the animal models (pylorus ligated model) used in the study. In pylorusligation, both the doses showed significantant ulcer activity by reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. The intensity of heamorrhage and lesions was significantly reduced upon pretreatment with the extract,

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REFERENCES

1. Young CN, Koepke JI, Terlecky LJ, Borkin MS, Boyd SL, Terlecky SR, Reactive oxygen species in tumor necrosis factor-α-activated primary human keratinocytes: implications for psoriasis and inflammatory skin disease, J Invest Dermatol, 2008; 128(11):2606-2614. https://doi.org/10.1038/jid.2008.122 PMid:18463678 PMCid:PMC4102307

2. Jeong YH, Oh YC, Cho WK, Lee B, Ma JY, Anti-inflammatory effects of Melandrii herba ethanol extract via inhibition of NF-κB and MAPK signaling pathways and induction of HO-1 in RAW 264.7 cells and mouse primary macrophages, Molecules, 2016; 21(6):818. https://doi.org/10.3390/molecules21060818 PMid:27338335 PMCid:PMC6272949

3. Rahman et al., Antioxidant and anti-inflammatory potentials of Solanum pubescens, Willd Journal of Research in Pharmacy Research, 2019; 23(2):187-197 196. https://doi.org/10.12991/jrp.2019.124

4. Shu Z, Xing N, Wang Q, Li X, Xu B, Li Z, Kuang H, Antibacterial and anti- inflammatory activities of Physalis alkekengi var. franchetii and its main constituents. Evidence-Based Complement Altern Med. 2016; 2(1):12-26. https://doi.org/10.1155/2016/4359394 PMid:27057196 PMCid:PMC4748097

5. Souza EP, Faria RX, Rocha LM, Clinical trials studies of plant extracts with anti-inflammatory activity, J App Pharm Sci. 2016; 6(12):224-232. https://doi.org/10.7324/JAPS.2016.601233

6. Gonzalez R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martinez- Augustin O, Medina FS. Effects of flavonoids and other polyphenols on inflammation, Crit Rev Food Sci Nutr. 2011; 51(4):331-362. https://doi.org/10.1080/10408390903584094 PMid:21432698

7. Kaur A, Kumar S. Plants and plant products with potential antipsoriatic activity,A review Pharm Biol., 2012; 50(12):1573-1591. https://doi.org/10.3109/13880209.2012.690430 PMid:22971237

8. Kushwaha S, Shah SK, Patel N, Tyagi CK, Effects of hydroalcoholic extract of Allium sativum on STZ induce hyperglycemia, The Pharma Innovation Journal 2016; 5(8):106-110

9. Boakye YD, Agyare C, Abotsi WK, Ayande PG, Ossei PP, Anti-inflammatory activity of aqueous leaf extract of Phyllanthus muellerianus (Kuntze) Exell. and its major constituent, geraniin, J Ethnopharmacol, 2016; 187:17-27. https://doi.org/10.1016/j.jep.2016.04.020 PMid:27103113

10. Bairagi SM, Pathan IB, Nitin NE., Analgesic and anti-inflammatory activity of crude leaf and bark extract of Lantana camara, Marmara Pharm J. 2017; 21(4): 810-817. https://doi.org/10.12991/mpj.2017.7

11. Rahman HU, Mahmood R, Haris M, Rahman N. Phytochemical profiling of successive extracts of fruit and stem bark of Solanum pubescens. Int J Pharm Pharm Sci., 2014; 6(9):147-153.

12. Nagaraju N, Rao KN. A survey of plant crude drugs of Rayalaseema, Andhra Pradesh, India. Journal of Ethnopharmacol., 1990; 29(2):137-158. https://doi.org/10.1016/0378-8741(90)90051-T PMid:2374428

13. Kumari GK, Rao LJ, Rao NP., Myricetin methyl ethers from Solanum pubescens, Phytochemistry, 1984; 23(11):2701- 2702. https://doi.org/10.1016/S0031-9422(00)84140-0

14. Wang Y, Qian J, Cao J, Wang D, Liu C, Yang R, Li X, Sun C. Antioxidant capacity, anticancer ability and flavonoids composition of 35 Citrus (Citrus reticulata Blanco) varieties. Molecules. 2017; 22(7):1114. https://doi.org/10.3390/molecules22071114 PMid:28678176 PMCid:PMC6152254

15. Adhikari B, Dhungana SK, Ali MW, Adhikari A, Kim ID, Shin DH. Antioxidant activities, polyphenol, flavonoid, and amino acid contents in peanut shell. J Saudi Soc Agric Sci. 2018, 1(1):20-26.

16. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000; 52(4):673-751.

17. Shaikh RU, Pund MM, Gacche RN. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system in vitro as well as in vivo. J Tradit Complement Med. 2016; 6(4):355-361. https://doi.org/10.1016/j.jtcme.2015.07.001 PMid:27774419 PMCid:PMC5067865

18. Rahman H, Mahmood R, Rahman N, Haris M. Antibacterial activity of Solanum pubescens-an ethnomedicinal plant from South Western region of Andhra Pradesh. Int J Appl Sci Biotechnol. 2014; 2(4):501-509. https://doi.org/10.3126/ijasbt.v2i4.11280

19. Pal M, Shah SK, Patel N, Tyagi C.K, Evaluation of Anti-Arthritis Activity of Butea monosperma Bark Extract on Albino Wistar Rats, Ijppr. Human, 2018; 12 (4):416-428

20. Hossain MA, Rahman SM. Isolation and characterisation of flavonoids from the leaves of medicinal plant Orthosiphon stamineus. Arabian J Chem. 2015; 8(2):218-21. https://doi.org/10.1016/j.arabjc.2011.06.016

21. Dhawan D, Gupta J. Comparison of Different solvents for phytochemical extraction potential from Datura metel plant leaves. Int J Biol Chem. 2017; 11:17-22. https://doi.org/10.3923/ijbc.2017.17.22

22. Matani SK, Al-Wahaibi RN, Hossain MA. Total flavonoids content and antimicrobial activity of crude extract from leaves of Ficus sycomorus native to Sultanate of Oman. Karbala Int J Med Sci. 2015; 1(3):166-171. https://doi.org/10.1016/j.kijoms.2015.11.007

23. Rahman N, Rahman H, Haris M, Mahmood R. Wound healing potentials of Thevetia peruviana: Antioxidants and inflammatory markers criteria. J Tradit Complement Med. 2017; 7(4):519-525. https://doi.org/10.1016/j.jtcme.2017.01.005 PMid:29034202 PMCid:PMC5634754

24. Joseph JM, Sowndhararajan K, Manian S. Evaluation of analgesic and anti- inflammatory potential of Hedyotis puberula (G. Don) R. Br. ex Arn. in experimental animal models. Food Chem Toxicol. 2010; 48(7):1876-1880. https://doi.org/10.1016/j.fct.2010.04.027 PMid:20417244

25. Wang P, Xie K, Wang C, Bi J. Oxidative stress induced by lipid peroxidation is related with inflammation of demyelination and neurodegeneration in multiple sclerosis. Eur Neurol. 2014; 72(3-4):249-254. https://doi.org/10.1159/000363515 PMid:25277682

26. Rahman et al. Antioxidant and anti-inflammatory potentials of Solanum pubescens Willd Journal of Research in Pharmacy, 2019; 23(2):187-197. https://doi.org/10.12991/jrp.2019.124

27. Jou JM, Lewis SM, Briggs C, LEE SH, De La Salle B, McFadden S. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol. 2011; 33(2):125-132. https://doi.org/10.1111/j.1751-553X.2011.01302.x PMid:21352508

28. Anosike CA, Obidoa O, Ezeanyika LU. The anti-inflammatory activity of garden egg (Solanum aethiopicum) on egg albumin-induced oedema and granuloma tissue formation in rats. Asian Pac J Trop Med. 2012; 5(1):62-66. https://doi.org/10.1016/S1995-7645(11)60247-2 PMid:22182646

29. More SK, Lande AA, Jagdale PG, Adkar PP, Ambavade SD. Evaluation of anti-inflammatory activity of Solanum xanthocarpum Schrad and Wendl (Kaṇṭakāri) extract in laboratory animals. Anc Sci Life. 2013; 32(4): 222-226. https://doi.org/10.4103/0257-7941.131976 PMid:24991071 PMCid:PMC4078473

30. Ogunnaike BF, Okutachi IR, Anucha ES, Gbodi OO, Shokunbi OS, Onajobi FD. Comparative anti-inflammatory activities of Jatropha curcas, Ocimum gratissimum and Solanum scabrum leaves, J Nat Prod Plant Resour. 2013; 3(1):59- 66.

31. Deng Li H, Z, Liu R, Loewen S, Tsao R. Bioaccessibility, in vitro antioxidant activities and in vivo anti-inflammatory activities of a purple tomato (Solanum lycopersicum L.), Food Chem. 2014; 159:353-360. https://doi.org/10.1016/j.foodchem.2014.03.023 PMid:24767066

32. Sharma OP, Bhat TK. DPPH antioxidant assay revisited, Food Chem.,2009; 113(4):1202-1205. https://doi.org/10.1016/j.foodchem.2008.08.008

33. Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from Clerodendrum Infortunatum, L. Environ Toxicol Pharmacol, 2010; 30(1):11- 18. https://doi.org/10.1016/j.etap.2010.03.005 PMid:21787623

34. Dasgupta N, De B. Antioxidant activity of Piper betle L. leaf extract in vitro, Food Chem, 2004; 88(2):219-224. https://doi.org/10.1016/j.foodchem.2004.01.036

35. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity, Saudi Pharm J., 2013; 21(2):143-152. https://doi.org/10.1016/j.jsps.2012.05.002 PMid:24936134 PMCid:PMC4052538

36. Debjit B., Chiranjib C., Tripathi K.K., Pankaj, K. P. Recent trends of treatment and medication peptic ulcerative disorder, International Journal of PharmTech Research, 2010; 2(1):970-980.

37. Vyawahare N. S., Deshmukh V.V., Godkari M. R., Kagathara V.G.,Plants with anti-ulcer activity, Pharmacognosy Review, 2009; 3:108-115.

38. Hoogerwerf W.A. Pasricha P.J., Agents Used for Control of Gastric Acidity and Treatment of Peptic Ulcers and Gastro Esophageal Reflux Diseaseedition, pp. 1005-19, McGraw-Hill, New York, NY, USA, 10th edition, 2001.