IJPSR (2014), Volume 5, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 March 2014; received in revised form, 22 May 2014; accepted, 13 July 2014; published 01 October 2014

FRUITS OF *SCINDAPSUS OFFICINALIS* ATTENUATES PYLORUS LIGATION INDUCED ULCER IN RATS

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Keywords:

Scindapsus officinalis, Peptic ulcer, Antioxidant, Ulcer index

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ABSTRACT: Objective: *Scindapsus officinalis* has been reported to have antioxidant, analgesic, anti-inflammatory, antibacterial, and antihistaminic activity. Peptic ulcer disease involves inflammation and oxidative stress. So, the present study was carried out to evaluate the potential of hydroalcoholic extract of *Scindapsus officinalis* fruits as an antiulcer agent. **Materials and Methods:** In this pharmacological study evaluation of the antiulcer effect of hydroalcoholic extract of *Scindapsus officinalis* fruits was performed by using pyloric ligation induced gastric ulcers model. Ranitidine (50 mg/kg p.o.) was used as standard. The preliminary phytochemical investigation, estimation of phenolic and flavonoid content and *in-vitro* antioxidant activity were also estimated. **Results:** The hydroalcoholic extract of *Scindapsus officinalis* fruits has a significant effect on scavenging free radicals as found in DPPH free radical scavenging assay and NO scavenging assay. In pylorus ligation induced ulcer model, the plant extract showed gastric ulcer healing effect and gastric antisecretory effect. The high dose of (500 mg/kg) of hydroalcoholic extract of *Scindapsus officinalis* fruits was more efficacious in reducing ulcer index. The result was supported by morphological and histopathological study findings. **Conclusion:** The hydroalcoholic extract of *Scindapsus officinalis* fruits have antiulcer activity, which can be attributed to its antioxidant mechanism of action.

INTRODUCTION: The World Health Organization (WHO) has estimated that approximately 80% of the world population relies primarily on traditional medicines as a source for their primary health care [1]. A peptic ulcer is the most predominant of gastrointestinal diseases. The etiological factors behind the disease are inadequate dietary habits, prolonged use of NSAIDs, stress, *H. pylori* infection, and some genetic factors [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(10).4351-58

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4351-58

The basic pathophysiology of gastric ulcer results due to an imbalance between some endogenous aggressive factors (such as hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species *etc*.) and cytoprotective factors (surface-active phospholipids, nitric oxide, bicarbonate barrier, prostaglandins, antioxidants like catalase, some growth factors *etc*) [3].

Peptic ulcer diseases involve the increased expression of IL-8 [4] and the pathogenesis of gastric inflammation, ulcerogenic, and carcinogenesis with *H. pylori* infection lies in increased production of reactive oxygen species (ROS) [5]. Based on these findings, substances with anti-oxidant and anti-inflammatory property can be utilized for the treatment of peptic ulcer. *Scindapsus officinalis* (Roxb.) Schott fruits have been widely used in

many parts of India for the treatment of various diseases and ailments. *Scindapsus officinalis* has previously reported possessing antioxidant [6], analgesic, anti-inflammatory [7], antibacterial [8], antidiabetic [9] and antihistaminic activity [10]. Therefore, the present study was undertaken to evaluate the antiulcer activity of the hydroalcoholic extract of *Scindapsus officinalis* fruits.

MATERIALS AND METHODS:

Plant Material and Extraction: Fruits of *Scindapsus officinalis* were collected from the local market and were authenticated at Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar, India. Dried fruits of the plant were weighed accurately and subjected to cold maceration extraction using alcohol and distilled water in 1:1 ratio.

Drugs and Chemicals: Ammonium molybdate, sodium thiosulphate, potassium iodide, aluminum chloride, potassium ferricyanide, and Folin ciocalteu phenol reagent were obtained from Loba Chemie Pvt. Ltd. DPPH was obtained from Himedia Pvt. Ltd. Vitamin C was obtained from S.d Fine Chem Ltd.

Preliminary Phytochemical Screening: The hydroalcoholic extract of the plant fruits was qualitatively screened for the presence of different phytoconstituents [11, 12]. Total flavonoid content was determined by aluminum chloride method [13, 14,] and total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent method [15] using quercetin standard solution.

In vitro Study:

Total Antioxidant Capacity: The total antioxidant capacity of the hydroalcoholic extract was measured by the spectrophotometric method as described by Preito *et al.* [16] Briefly, 0.1ml of the extract, dissolved in water, was added to 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 ºC for 90 min. After cooling to room temperature, the absorbance of the aqueous solution was measured at 695 nm against a blank. Using ascorbic acid as the standard, the total antioxidant capacity of the extract was expressed as milligram equivalent of ascorbic acid/g of extract.

DPPH Free Radical Scavenging Activity: The free radical scavenging activity of the extract was measured in terms of hydrogen donating ability using DPPH as described by Blois *et al.*, and Chowdhary *et al.* [17, 18] Briefly, 2 ml of DPPH solution in methanol (0.1mM) was added to different concentrations of the extract solution. The mixture was incubated at room temperature for 45 min, and the absorbance was measured at 518nm against blank (methanol) using ascorbic acid as standard. Percentage inhibition of DPPH free radical was calculated using the equation:

$$\text{DPPH Radical Scavenging Activity (\%)} = [(Ac - As)/Ac] \times 100$$

Where Ac is the absorbance of the control (*i.e.*, DPPH radical alone), As is the absorbance of DPPH radical with sample extract/standard ascorbic acid.

Nitric Oxide [NO] Scavenging Assay: The NO scavenging potential of the hydroalcoholic extract was determined using the method of Mandal *et al.*, and Green *et al.* [19, 20] Briefly, different concentrations of the plant extract were mixed with sodium nitroprusside solution (10 mM), and the mixture was incubated at 25 °C for 150 min. About 0.5 ml aliquot of the incubated sample is mixed with 0.5 ml of Griess reagent and the absorbance of the formed chromophore was measured at 546 nm using ascorbic acid as standard.

The percent NO scavenging activity was calculated as follows:

$$\text{\% NO scavenging activity} = [(Ac - As) / Ac] \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of test/standard.

In-vivo Antiulcer Activity (Pylorus ligation Induced Ulcer Model):

Experimental Animals: Wistar rats of either sex, weighing 200-250 gm, were used. The experimental protocol was approved by the Institutional Animal Ethics Committee. The rats were quarantined for 7 days and evaluated for weight change and any sign of injury before the study begins. They were kept in 12 hour light and 12 h dark cycle at a temperature of 25 ± 2 ºC and provided with standard diet and water *ad libitum*.

Experimental Design: Rats were divided into five groups, containing six animals each. The group treated with vehicle alone (Group I) was considered as normal control. The animals of group II were considered as experimental control or pylorus ligated. Animals of group III were treated with standard drug ranitidine (50mg/kg). Animals of Groups IV and V were given a hydroalcoholic extract of *Scindapsus officinalis* fruits at 250 mg/kg and 500 mg/kg, respectively. After 1 hr of the respective treatment, pylorus of each rat was tied under light ether anesthesia and the abdominal incision was closed. After 2 h of pylorus ligation, the rats were sacrificed, their stomach was excised and opened along the greater curvature [21]. The mucosal surface of the stomach was observed, and the extent of gastric damage was scored as:

0 = Normal colored stomach
0.5 = Red colouration
1 = Spot ulcers
1.5 = Haemorrhagic streak
2 = Ulcers
3 = Perforation

Mean ulcer score for the test groups were expressed as the ulcer index and was compared with that of the positive control group to determine the percent protection.

Free Acidity and pH of Gastric Content: Gastric juice, collected from the pylorus-ligated rats, was centrifuged, and its volume and pH were measured. Free acidity was measured according to the method used by Dashputre and Naikwade [22] and Singh *et al.* [23] using Topfer's reagent.

Total Acidity of Gastric Content: An aliquot of 1ml gastric juice diluted with 1ml of distilled water was titrated with 0.01N NaOH, using phenolphthalein as indicator, until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted.[22] The total acidity was expressed as mEq/L and calculated using the equation:

$$\text{Total acidity} = [\text{Vol. of NaOH} \times N \times 100] / 0.1$$

Biochemical Estimation:

1. **MDA:** Levels of thiobarbituric acid reactive substances (TBARS) were estimated using the method given by Ohkawa *et al.* [24] Briefly, the tissue homogenate, 0.2 ml of SDS (8.1%), 1.5ml of acetic acid (20%) and 1.5ml of TBA (0.8%) were mixed, and the mixture was made up to 4 ml with distilled water and then heated at 90 ºC for 60 min. After cooling the reaction mixture, 1 ml of water and 5ml n-butanol/pyridine mixture (15:1) was added. The mixture was shaken vigorously, centrifuged at 600 rpm for 10min, and absorbance of the organic layer was measured at 532nm using 1, 1, 3, 3-tetra methoxy propane as the standard. The lipid peroxidation was expressed as nmol MDA/100 mg tissue.

2. **GSH:** Reduced glutathione in tissue homogenate was measured by Ellman's method [25]. Briefly, the tissue homogenate was mixed with 10% TCA and centrifuged at 3000rpm for 10 min. Then, 0.5 ml of supernatant was added with 0.5 ml of phosphate buffer, 0.5 ml of double-distilled water, and 0.5 ml of DTNB. The reaction mixture was incubated for 10 minutes, and absorbance was measured at 412nm against reagent blank. The reduced GSH content was calculated from the standard curve using reduced glutathione and expressed as nM GSH/g of tissue.

3. **Catalase:** The catalase activity in tissue homogenate was estimated using the method of Kaur *et al.* [26] Briefly, 0.1 ml of homogenate was added to 1.9 ml of 50 mM phosphate buffer. To the mixture, 1.0 ml of 30 mM hydrogen peroxide was added, and a change in absorbance was followed for 30 sec at 240 nm at 15-sec intervals. The catalase activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071 mmol cm^{-1}) [27] and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein. Protein content was estimated using Lowry's method [28].

Histopathology Study: Section of gastric tissue was fixed in 10% buffered formalin and was embedded in paraffin blocks. These sections were stained with hematoxylin for the histological evaluation at magnification 40X.

RESULTS:

Preliminary Phytochemical Screening: The phytochemical screening of the plant extract revealed the presence of various bioactive constituents like alkaloid, carbohydrate, phenols, tannins, flavonoids, diterpenes, proteins, and amino acids.

Total Flavonoid and Phenolic Content: The flavonoid content of hydroalcoholic extract of *Scindapsus officinalis* fruits were found to be 37.86 ± 2.75 mg equivalent of quercetin/g of dry extract and the total phenolic content was found to be 25.15 ± 0.668 mg equivalent of tannic acid/g of dry extract.

***In-vitro* Antioxidant Activity:**

Total Antioxidant Capacity: The total antioxidant capacity of plant extract was found to be 67.52 milligram equivalent of ascorbic acid/g of extract.

TABLE 1: DPPH FREE RADICAL SCAVENGING ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* FRUITS

Concentration (µg/ml)	Percentage scavenging	
	Ascorbic acid (Mean ± SD)	HAESO (Mean ± SD)
10	70.926 ± 0.420	65.066 ± 0.191
20	73.110 ± 0.397	67.780 ± 0.312
30	76.966 ± 0.266	69.153 ± 0.200
40	84.193 ± 0.146	69.556 ± 1.382
50	86.280 ± 0.377	71.066 ± 2.053

Values were expressed as Mean ± SD; HAESO = hydroalcoholic extract of *Scindapsus officinalis*

DPPH Radical Scavenging Activity: As presented in **Table 1**, the standard ascorbic acid showed highest DPPH free radical scavenging activity of 86% at 50 µg/ml, however, the HAESO showed highest DPPH radical scavenging activity of 71% at the same conc.

Percentage NO Scavenging: The plant extract showed a good nitric oxide scavenging activity between 50 and 800 µg/ml dose-dependently **Table 2**. However, the effect of ascorbic acid was much less when compared to the extract.

TABLE 2: NO SCAVENGING ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* FRUITS

Concentration (µg/ml)	Percentage scavenging	
	Ascorbic acid (Mean ± SD)	HAESO (Mean ± SD)
50	23.623 ± 3.458	66.163 ± 0.292
100	41.883 ± 10.288	71.690 ± 0.200
200	46.236 ± 6.897	77.200 ± 0.180
400	52.590 ± 3.276	82.793 ± 0.104
800	61.276 ± 3.276	83.360 ± 0.150

Values were expressed as Mean ± SD; HAESO = hydroalcoholic extract of *Scindapsus officinalis*

Antiulcer Activity (Pylorus Ligation Induced Ulcer): As compared to the normal rats, pylorus ligation caused gastric damage with ulcer index of 4.58 ± 0.30 in the experimental control rats. Ranitidine and HAESO were found to produce a significant reduction in the ulcer index in dose-dependent manner as represented in **Table 3**. Ranitidine was found to produce percent protection of 94.32%; however, HAESO 250 mg/kg and HAESO 500 mg/kg showed percent protection of 46.06 and 89.08% respectively. All the aggressive factors, *e.g.* gastric volume, total acidity, and free acidity were decreased, and gastric pH was increased in the ranitidine treated and extract-treated groups, providing evidence of their antiulcer activity **Table 3**.

TABLE 3: EFFECT OF HYDROALCOHOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* FRUITS ON PYLORUS LIGATION INDUCED ULCER MODEL

Groups (n=6)	Gastric volume (ml)	pH	Free acidity (meq/l)	Total acidity (meq/l)	Ulcer index
Normal	2.64±0.17	5.75±0.06	18.36±0.13	33.21±0.73	------
Experimental Control	9.5±0.13	2.54±0.12	47.6±1.25	76.6±1.02	4.58±0.30
Standard (Ranitidine)	6.8±0.07***	5.21±0.21***	25±1.36***	35.5±0.56***	0.26±0.12***
HAESO (250)	8.2±0.08***	3.51±0.08**	42.2±1.25*	60.8±1.37***	2.47±0.27***
HAESO (500)	7.4±0.03***	4.72±0.16***	33.3±1.17***	47.2±0.99***	0.50±0.18***

Results are expressed as mean ± SEM (n=6); ***P<0.001, **P<0.01, *P<0.05 compared with the experimental control group; Data were analyzed by one-way ANOVA followed by Dennett's test; HAESO = hydroalcoholic extract of *Scindapsus officinalis*.

Other Biochemical Parameters: The pylorus ligation was found to cause oxidative stress by increasing lipid peroxidation and decreasing catalase and reduced glutathione in the experimental control group. Treatment with HAESO, at the doses of 250 and 500 mg/kg, significantly reduced lipid peroxidation and increased the activity of antioxidant enzymes, *e.g.*

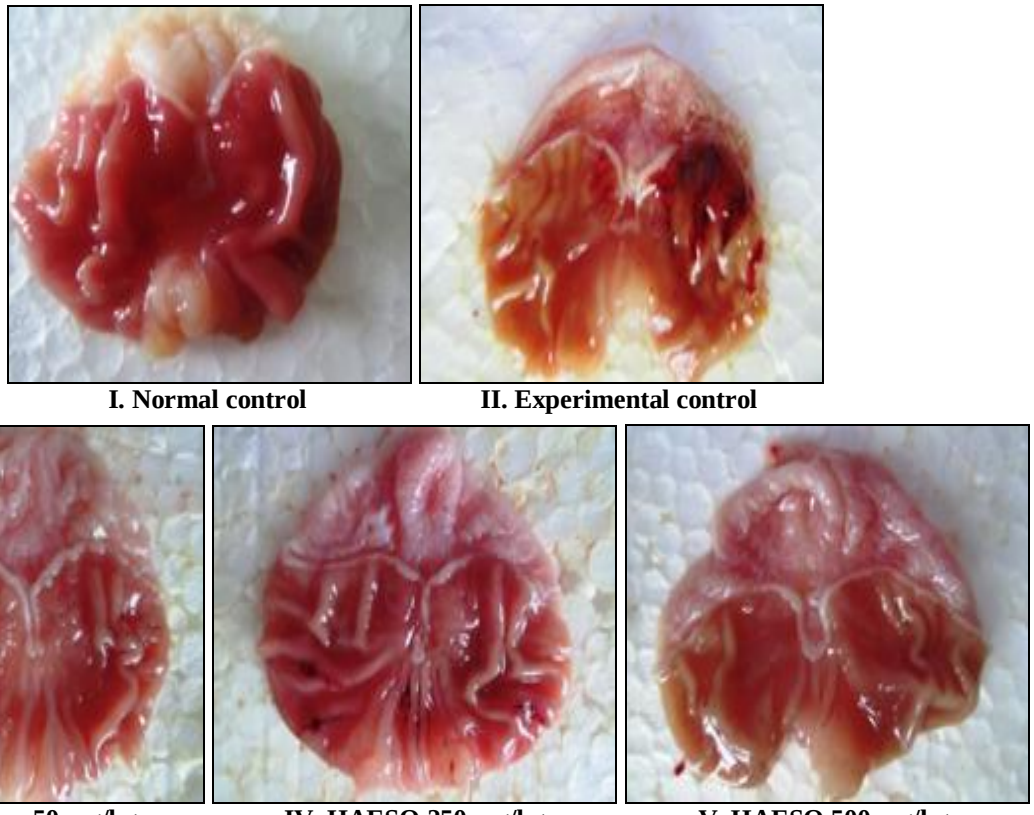
catalase and reduced glutathione. This reduction in lipid peroxidation along with an increase in the antioxidant enzymes, was also observed in ranitidine treated animals **Table 4**.

TABLE 4: EFFECT OF HYDROALCOHOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* FRUITS ON GSH, MDA, AND CAT IN TISSUE HOMOGENATE IN PYLORUS LIGATION INDUCED ULCER MODEL

Groups	GSH nmol/ g of tissue	MDA nmol/ 100mg of tissue	µmol H_2O_2/ min/100mg of tissue
Normal control	74.61 ± 2.01***	3.56 ± 0.81***	19.56 ± 5.92***
Experimental control	55.34 ± 1.63	15.36 ± 0.73	3.51 ± 1.58
Standard (Ranitidine)	74.66 ± 3.39***	4.80 ± 0.89***	16.61 ± 4.99***
HAESO (250)	58.42 ± 1.77ns	10.21 ± 0.68***	11.48 ± 2**
HAESO (500)	72.63 ± 1.58***	6.67 ± 0.38***	14.41 ± 3.09***

Results are expressed in terms of mean ± SD (n=6); ***P<0.001, **P<0.01 when compared with experimental control; Data were analyzed using one way ANOVA followed by Dunnett's test; HAESO = Hydroalcoholic extract of *Scindapsus officinalis*

Morphological Study of the Stomach: In normal group stomach integrity was maintained and appeared normal. In the control group severe bleeding, perforation, spot ulcer, streaks were observed but in the standard group and extract-treated groups, the animal showed extremely less ulceration, and stomach integrity was maintained **Fig. 1**.



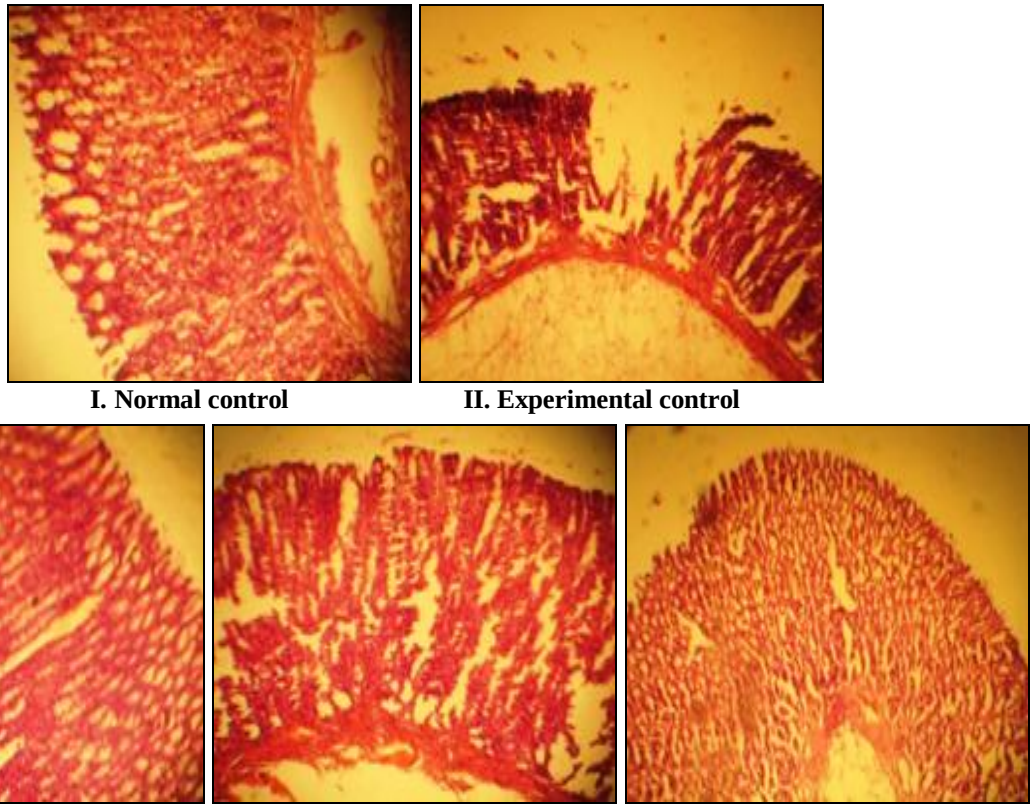
I. Normal control **II. Experimental control**

III. Ranitidine 50 mg/kg **IV. HAESO 250 mg/kg** **V. HAESO 500 mg/kg**

FIG. 1: MORPHOLOGICAL FEATURES OF STOMACH IN PYLORUS LIGATION INDUCED ULCER.

HAESO = hydroalcoholic extract of *Scindapsus officinalis*

Histopathological Study: Histopathological examination of gastric mucosa in the normal control group showed intact gastric mucosa and a continuous epithelial surface **Fig. 2**. Experimental control revealed mucosal ulceration, damaged mucosal epithelium, disrupt the glandular structure, and hemorrhage in gastric mucosa. In ranitidine treated group, the gastric mucosa appeared intact without any inflammatory exudates. Submucosa and deeper mucosa showed no abnormality. In HAESO (250 mg/kg) group, superficial erosions and a few ulcers accompanied by a mild inflammatory infiltrate and congestion in a few areas were observed. Moreover, mild disorganization of mucosa was also observed in a few areas. In HAESO (500mg/kg) group, section revealed intact mucosa with no appreciable inflammation.



I. Normal control **II. Experimental control**

III. Ranitidine 50 mg/kg **IV. HAESO 250 mg/kg** **V. HAESO 500 mg/kg**

FIG. 2: HISTOLOGY OF STOMACH IN PYLORUS LIGATION INDUCED ULCER

HAESO = hydroalcoholic extract of *Scindapsus officinalis*

DISCUSSION: Phytochemical tests on the extract gave positive results for alkaloids, carbohydrates, flavonoids, saponins, phenols, diterpenes, tannins, proteins, and amino acids. The obtained results strongly suggest that phenolics and flavonoids are the major components of the extract, and therefore, some of the pharmacological effects that could be attributed to them.

The antioxidant activity of HAESO fruits was investigated using total antioxidant activity capacity, DPPH free radical scavenging assay, and NO scavenging assay. The free radical DPPH possesses a characteristic absorption at 517 nm. The radical scavengers cause reduction of DPPH by providing proton, which is indicated by color change (from purple to yellow) and a decrease in its absorption. The HAESO proved to be almost equivalent in offering protection against DPPH free radicals as compared to the standard ascorbic acid. NO, and reactive nitrogen species (RNS) have been reported to be involved in the oxidative cellular damage [29]. The major reactive species of NO are nitrous anhydride (N_2O_3) and peroxynitrite ($ONOO^-$). These free radicals are capable of damaging a lot of cellular components such as proteins, lipids, and DNA [30]. Free radicals are involved in the pathogenesis of pylorus ligation induced ulcer [31]. The result indicated that HAESO has a significant effect on scavenging free radicals. Therefore, the protective effect of HAESO against ulcer induced by pylorus ligation could arise from the ability to scavenge free radicals.

The antiulcer activity of the HAESO was evaluated against gastric lesions induced by pylorus ligation. Ulcers caused by pylorus ligation are due to increased accumulation of gastric acid and pepsin, leading to the autodigestion of gastric mucosa [32]. Due to the surgery, the stomach gets larger; the pressure on sensitive receptors in the antral gastric mucosa increases and activates the vagus-vagal reflex, causing increased gastric secretion.

Ulcer index is the measure of the magnitude of ulceration produced in the animals. It is determined from various morphological changes in the gastric mucosa such as spot ulcers, hemorrhagic streaks, perforation, and red coloration. It is evident from the result that pretreatment with hydroalcoholic extract of *Scindapsus officinalis* fruits significantly reduced the ulcer index in pylorus ligation induced

ulcer model as compared to the experimental control animals. The ability to the extract to protect the gastric mucosa from the damage induced by pylorus ligation was expressed in terms of percentage protection.

The extract provides considerable percentage of protection against pylorus ligation induced ulceration. Moreover, animals treated with ranitidine and HAESO showed they decreased gastric acidity and increased pH of gastric content. The volume of gastric content was also reduced in these groups representing a possible anti-secretory activity of the ranitidine and extract.

Inside an organism, there are various antioxidant enzymes, which protect the body from the deleterious effect of reactive oxygen species generated through normal metabolic processes. Any imbalance in the activity of these enzymes leads to accumulation of free radicals which cause oxidation of tissues leading to lipid peroxidation and tissue damage [33]. As shown in the result, a marked decrease in the antioxidant enzymes like GSH and CAT, and an increase in the lipid peroxidation by-product (malondialdehyde) were observed in the experimental control group.

Such results can be correlated to the pathogenesis of inflammation and cytotoxicity as observed in the pylorus ligation induced ulcer model. Treatment with ranitidine and HAESO resulted in a significant increase in the antioxidant enzymes and decrease in the lipid peroxidation, providing evidence of their efficacy in preventing the intensity of reactive oxygen species induced damage.

CONCLUSION: The results obtained in this study displayed that the hydroalcoholic extract of *Scindapsus officinalis* can be a potential therapeutic option in the effective management of ulcer because of the presence of phytoconstituents like flavonoids, phenolics, alkaloids, saponins, tannins, etc, and its antioxidant effect. Further investigations are required to isolate and characterize the active components of the plant extract. There is also the scope to find out the exact mechanism responsible for the anti-ulcer activity.

ACKNOWLEDGEMENT: This research was partially financed by Lovely Professional University (India). The authors are thankful to Dr. Monica Gulati, Sr. Dean, Lovely Faculty of Applied Medical Sciences, Lovely Professional University (INDIA) for providing helpful critique and suggestions.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Choudhary A, Singh A, Duggal N and Kumar B: Fruits of *Scindapsus officinalis* attenuates pylorus ligation induced ulcer in rats. Int J Pharm Sci & Res 2014; 5(10): 4351-58. doi: 10.13040/IJPSR.0975-8232.5(10).4351-58.