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Pharmacognostical, Phytochemical and Pharmacological Evaluation for the Antiulcer Activity of Polyherbal Suspension

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ABSTRACT

Objective: From the past few years there is increasing research interest on the natural compounds, due to their wide variety of pharmacological activities. The main aim of the present study was to conduct the evaluation of the Poly herbal suspension (PHS) which includes the preliminary phytochemical screening, standardisation and experimental antiulcer activity.

Methods: In this study we conduct the phytochemical screening, standardisation using the high performance thin layer chromatography (HPTLC) & infra-red spectroscopy (IR). Also, the PHS was evaluated for the acute toxicity and antiulcer activity at 400 mg/kg and 800 mg/kg in adult albino wistar rats induced by the ethanol (1ml/kg), indomethacin (40 mg/kg) and aspirin (150 mg/kg) ulcer models.

Results: After phytochemical screening, the PHS showed the presence of the terpenoids, flavonoids and tannins which produces antiulcer activity. The therapeutic efficacy achieved at both dose levels of the PHS when compared with the standard sucralfate (100 mg/kg) showed a significant ($P<0.05$) antiulcer activity in ethanol induced model, PHS shown a significant reduction ($P<0.05$) of ulceration when compared with the indomethacin induced control group as that of misoprostol (100 mcg/kg) and same as the above it shown the anti-ulcerogenic protection in aspirin induced model, here the standard drug was omeprazole (10mg/kg). The highly significant antiulcer activity was exhibited at dose level of 800mg/kg.

Conclusion: The results of the present study showed the pronounced anti-ulcerogenic activity of the PHS at the dose of 800mg/kg after the standard phytochemical and pharmacognostical study.

Keywords: Antiulcer Activity, Aspirin, Ethanol Induced Ulcer, Indomethacin, and Polyherbal Suspension.

1. Introduction

Peptic ulcer is an excorticated area of the gastric duodenal mucosa associated with the action of the gastric juice exhibited by the gastric hyperacidity [1]. It is commonly regarded as imbalance between the aggressive and mucosal defensive factors associated with erosions, type C gastritis, petechiae, and ulceration [2]. The etiology of peptic ulcers are associated with the various aggressive & defensive factors like acid-pepsin secretion, parietal cell, blood flow, mucosal barrier, mucus secretion, cellular regeneration and endogenous defensive agents involved in protection such as prostaglandins and epidermic growth factors [3]. Along with these some other etiological factors are dietary habits like western style of food habits, stress, excessive ingestion of non-steroidal anti-inflammatory agents (NSAIDs), hereditary predisposition and helicobacter pylori (*H. pylori*). Uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa by the H^+K^+ -ATPase pump involves hypersecretion of gastric acids from the parietal cells also one of the pathophysiological event leads to gastric ulcers [4]. Now-a-days different therapeutic strategies are used are in the management of gastric ulcers such as inhibition of gastric ulcers, protection of gastric mucosa by the cytoprotective agents, inhibition of apoptosis, and stimulation of gastric epithelial cells along with the conventional methods. On the contrary, plant drugs are now-a-days gains the more extensive attention for various therapeutic strategies, because they reduce the offensive factors, better tolerability and globally competitive [5].

The present study was aimed to evaluate the antiulcer activity of PHS containing the alcoholic extract of *Allophylus serratus* (Roxb.) Kurz, *Psidium guajava*, *Adhatoda vasica* L., *Eclipta alba* L., *Embllica officinalis* L. From the ancient days onwards compound suspensions were used to treat the diseases of human beings based on the concept of compound suspensions are having the synergistic effect than individual preparations and to limits their individual adverse effects of the primer drug [6].

2. Materials and methods

2.1 Collection and identification of plant materials

The leaves of *Allophylus serratus* (Roxb.) Kurz (Sapindaceae) (Ref No: PARC/2012/1122), *Psidium guajava* L. (Myrtaceae) (Ref No: PARC/2012/1121), *Adhatoda vasica* L. (Acanthaceae), *Eclipta alba* L. (Asteraceae) and *Embllica officinalis* L. (Euphorbiaceae) were collected from Tirumala hills, Tirupati, (A.P.), India. The taxonomical identification and authentication of the plant was done by Dr. P. Jayaraman, Director, National Institute of Herbal Medicine, Plant Anatomy Research Centre, Chennai. The voucher specimen is preserved in laboratory, department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy for further reference.

Table 1: Composition of Polyherbal Suspension

S. No	Name of the plant	For 100ml
1	<i>Allophylus serratus</i> (Roxb.)Kurz	1.6g
2	<i>Psidium guajava</i> L.	0.8 g
3	<i>Adathoda vasica</i> L.	1.0 g
4	<i>Eclipta alba</i> L.	2 g
5	<i>Embllica officinalis</i> L.	2 g
6	Tween 80	0.1%
7	Sodium CMC	2 g
8	Sucrose	10 g
9	Sorbitol	5 g
10	Methyl paraben	0.20%
11	Strawberry Flavour	q. s.
12	Purified water q. s.	100 ml

2.4 Experimental animals

Adult albino wistar rats of both sexes weighing 170–190 g were used in the experiments and were procured from animal house, Sree Vidyanikethan College of Pharmacy. They were housed in standard polypropylene cages at room temperature (20-25 °C) and 12-12hr dark and light cycles were maintained. They were provided with a standard pellet feed procured from Hindustan Ltd., Bangalore, and water *ad libitum*. Twenty four hours before each experiment, the animals were deprived of food but not of water. All the experiments involving animals have been performed after obtaining clearance from institutional animal ethics committee (IAEC) of CPCSEA, Sree Vidyanikethan college of Pharmacy (SVCP/IAEC/I-016/2011-2012).

2.5 Experimental protocol

Group 1: Normal control group received normal saline water p.o.
 Group 2: Negative control group received normal saline water p.o.
 Group 3: Positive control group received Standard drug, Sucralfate 100mg/kg p.o. for Ethanol (1ml) induced method, Misoprostol 100 mcg/kg p.o. for Indomethacin (40mg/kg p.o.) induced method and Omeprazole (10 mg/kg p.o.) for Aspirin

2.2 Drugs and Chemicals

Aspirin, omeprazole, and misoprostol were procured from the Sigma-Aldrich, Bangalore and all the chemicals were used in the phytochemical analysis were of analytical grade procured from the local companies.

2.3 Preparation of Polyherbal Suspension

Poly herbal suspension was prepared by using various alcoholic extracts of *Allophylus serratus* (Roxb.) Kurz (Sapindaceae), *Psidium guajava* L. (Myrtaceae), *Adhatoda vasica* L. (Acanthaceae), *Eclipta alba* L. (Asteraceae) and *Embllica officinalis* L. (Euphorbiaceae) with a suitable suspending agent. The PHS containing the following composition (Table 1). Required quantity of sucrose was taken to a clean mortar and triturates it into powder form and it was dissolved in 50 ml of water. The extracts, tween 80 and sodium CMC in respective quantities were taken and was made into a homogeneous mixture. This mixture was added to 50 ml of sucrose solution. Then the excipients like sorbitol, flavouring agent and preservative were added and it was made into a homogeneous mixture finally the volume was made upto 100ml.

(150mg/kg p.o.) induced model.

Group 4: Received PHS 400mg/kg p.o.

Group 5: Received PHS 800mg/kg p.o.

2.6 Preliminary phytochemical screening

For the estimation of physiochemical parameters like moisture content, ash values, extractive values, and phytochemical screening was performed by using the standard methods.[7] The fluorescence analysis was performed by the Infra-red spectroscopy and finger printing of the suspension was performed by the high performance thin layer chromatography (HPTLC).

2.7 HPTLC finger printing of *Psidium guajava* L. Extract

In pre-coated aluminium TLC plates of 3µl and 6µl of *Psidium guajava* L. extract, 3µl and 6µl of standard quercetin were applied as 6mm band using Linomat V applicator with Hamilton syringe. Applied plate was developed in a twin trough chamber containing Chloroform: Acetone: Formic acid (75: 16.5: 8.5) as mobile phase. The plate was developed for a migration distance of 75 mm. It was then scanned under 254 nm and visible light and photo documented using Camag Reprostar 3. The R_f value and peak area of the

extracts and standard were interpreted by using the software.

2.8 HPTLC finger printing of *Allophylus serratus* (Roxb.) Kurz Extract

In precoated aluminium TLC plate 3 μ l and 6 μ l of *Allophylus serratus* (Roxb.) Kurz extract, 6 μ l and 9 μ l of standard β -sitosterol were applied as 6mm band using Linomat V applicator with Hamilton syringe. Applied plate was developed in a twin trough chamber containing Petroleum ether: ethyl acetate (8:2) as mobile phase. The plate was developed for a migration distance of 75mm. Developed plate was then derivatised with liebermann-burchard reagent by dipping in it and dried at 110 °C in a hot air oven for 10 min. It was then scanned under all the three wavelengths using Deuterium, Mercury and Tungsten lamps respectively and photo documented using Camag Reprostar 3. The R_f value and peak area of the extracts and standard were interpreted by using the software.

2.9 Acute Toxicity Studies

Acute toxicity study of PHS was carried out in the adult wistar rats following OECD guidelines 423. A single oral dose of the PHS was administered at the dose of 2000 mg/kg, p.o body weight respectively to the three rats and observed for the any signs of toxicological symptoms, behavioural changes, locomotion, convulsions and mortality for 1, 2, 4, 8, and 24hr and further followed for 14 days period. During this period, their behaviour and activity patterns were meticulously watched, there is no onset and signs of toxicity were observed [8].

2.10 Anti-Ulcer activity

2.10.1 Ethanol Induced Ulcer

Adult albino wistar rats, 6 per group divided randomly and were used for the study. Gastric ulceration was induced in 48 hours starved rats using the method of Hara and Okabe (1985). Thirty minutes after poly herbal suspension administration, each rat was given orally 1.0 ml of HCl-ethanol mixture (0.3 M solution of HCl in 60 % (v/v) ethanol). The animals were sacrificed one hour later. The stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcer score was determined. The tissues were subjected to histo-pathological studies. Ulcer index was estimated by calculating the ulcer score [9, 10].

2.10.2 Aspirin Induced Ulcer

Aspirin (150mg/kg) was administered orally to the animals at a dose of 150 mg/kg, b.w after 45 min of poly herbal suspension and

omeprazole treatment. After 5hrs, the animals were sacrificed and lesions in the gastric mucosa were scored. The tissues were subjected to histopathological studies. Ulcer index was estimated by calculating the ulcer score [11].

2.10.3 Indomethacin Induced Ulcer

Indomethacin (40mg/kg) suspended in 0.5% carboxymethyl cellulose (CMC) was given as a single oral dose to induce gastric ulcers after 30 min of test or standard drug treatment. After 5hrs, the animals were sacrificed and lesions in the gastric mucosa were scored. The tissues were subjected to histopathological studies. Ulcer index was estimated by calculating the ulcer score [12,13].

2.10.3.1 Calculation of ulcer index [14]:

Ulcer index=UA+US+UP/10.,

Where, UA=Average number of ulcers per animal, US=Ulcer severity score, UP=Percentage of animals with ulcers. UP=Total ulcers in a group/total number of animals x 100.

2.10.3.1 Percentage ulcer inhibition was calculated by the formula: [15]

Percentage inhibition=UIC-UIT/UIC X 100.

Where UIC=Ulcer index of control group, UIT= Ulcer index of test group.

2.11 Histopathology

After collecting the gastric contents small pieces of stomachs from each group were embedded in paraffin wax. Sections of 5 μ m thick were cut in a microtome and mounted on glass slides using standard techniques. After staining the tissues with hematoxylin-eosin stain (H&E), the slides were viewed under a light microscope equipped for photography.

2.12 Statistical Analysis

The results were reported as Mean \pm SEM. Results were analysed by using one way ANOVA followed by Tukey's Kramer multiple comparison tests. $P < 0.05$ was used to indicate statistical significance.

3. Results

3.1 Phytochemical screening

Preliminary phytochemical screening of the PHS revealed the presence of alkaloids, glycosides, tannins, saponins, flavonoids, steroids, and terpenoids (Table 2).

Table 2: Phytochemical Screening of Polyherbal Suspension

Phytoconstituents	<i>A. serratus</i> (Ethanol)	<i>E. officinalis</i> (Methanol)	<i>P. gujava</i> (Ethanol)	<i>A. vasica</i> (Methanol)	<i>E. alba</i> (Water)
Alkaloids	-	+	-	+	-
Glycosides	+	-	-	-	+
Resins	-	-	-	+	-
Tannins	-	+	+	-	-
Carbohydrates	-	+	+	+	+
Proteins	-	+	-	-	+
Flavonoids	+	+	+	+	-
Steroids	+	-	-	-	-
Saponins	-	-	+	-	-
Terpenoids	-	-	+	-	-

+ = indicates presence, - = indicates absence.

3.2 HPTLC finger printing of *Psidium guajava* L. Extract and *Allophylus serratus* (Roxb.) Kurz Extract

The extracts of *Psidium guajava* L. and *Allophylus serratus* (Roxb.) Kurz were subjected to HPTLC analysis by using specific solvent systems like Chloroform: Acetone: Formic acid (75: 16.5: 8.5), Petroleum ether: ethyl acetate (8:2) respectively and detected under UV at different wave lengths. The HPTLC images indicate that all sample constituents were clearly separated without any tailing and diffuseness. The extract of *Psidium guajava* has shown four different well defined peaks indicating the occurrence of at least four different components. Standard quercetin was detected at R_f 0.59 and the same spot was revealed by methanol extract of

Psidium guajava L. The standard and sample spots were clear under 254 nm and visible light. This confirms the presence of quercetin in the extract. At the same time *Allophylus serratus* (Roxb.) Kurz has also shown 7 different well defined peaks indicating the presence of at least 7 different components in the methanol extract. β -sitosterol the biomarker has shown the R_f value at 0.53 and the same spot was revealed by methanol extract of *Allophylus serratus* (Roxb.) Kurz after derivatization. This confirms the presence of β -sitosterol in the extract. The standard spot was not pronounced under 254 nm and 366 nm. Under 366 nm the spots has shown magenta and violet coloured fluorescence (Figure 1 & 3).

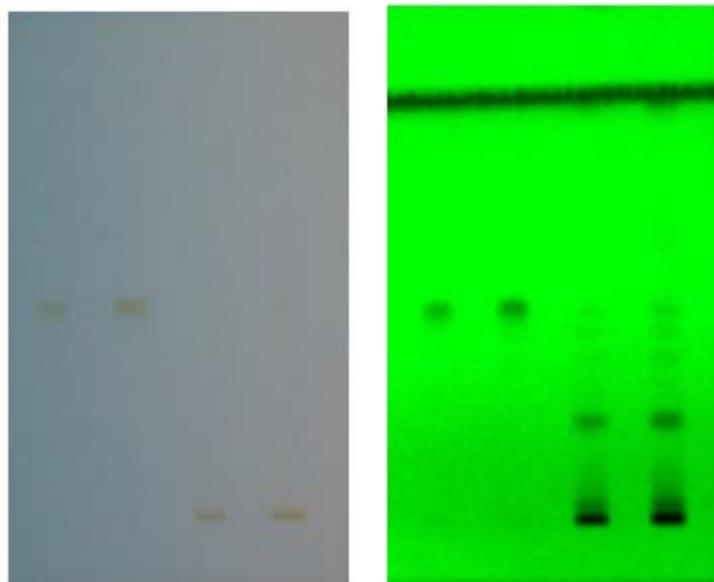


Fig 1: High Performance Thin Layer Chromatographic characterization of *Psidium guajava* L. ethanolic extract and Quercetin under visible & 254 nm

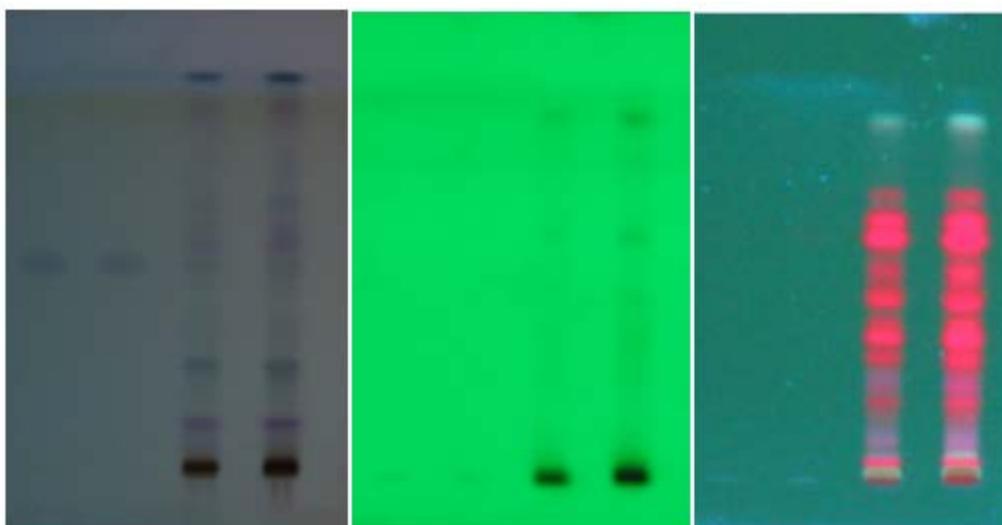


Fig 3: High Performance Thin Layer Chromatographic characterization of *Allophylus serratus* (Roxb.) kurz ethanolic extract and Beta-sitosterol under visible, 254 nm & 366

3.3 FTIR Analysis

The peak corresponding to Ar-OH stretching in guava extract showed a peak at 3365.57 cm^{-1} indicating the presence of OH group, but the peak has been shifted to 3257.76 cm^{-1} in poly herbal suspension. The peak at 1623.93 cm^{-1} of the guava extract indicating the presence of C=O functional group has been shifted to

1620.28 cm^{-1} in poly herbal suspension. Peak at 3675.75 cm^{-1} of guava extract represents the C—H stretching vibrations. But the peak has been shifted to 3711.96 cm^{-1} . Similarly C—H stretching in *Allophylus* extract showed a peak at 2942.33 cm^{-1} representing the presence of CH group, but the peak has been shifted to 3257.76 cm^{-1} in poly herbal suspension. *Allophylus* also showed a peak at

3520.76 cm^{-1} which indicate the presence of OH group. The peak at 1388.85 cm^{-1} of the guava extract indicating the presence of C—C functional group has been shifted to 997.59 cm^{-1} in poly herbal suspension. Similarly C—C stretching in *Allophylus* extract also

showed a peak at 1059.75 cm^{-1} . No additional peaks were seen in the spectrum of poly herbal suspension proving no sign of formation of new chemical compounds indicating no chemical interactions [Figure 2 & 4].

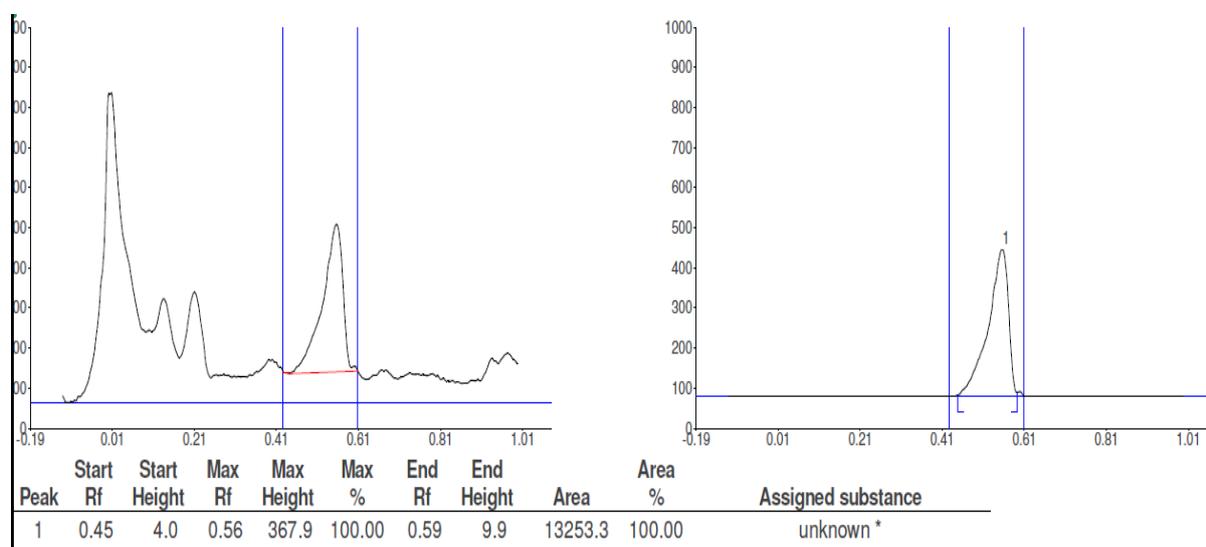


Fig 2: Chromatogram showing Quercetin in ethanolic extract of *Psidium guajava* L.

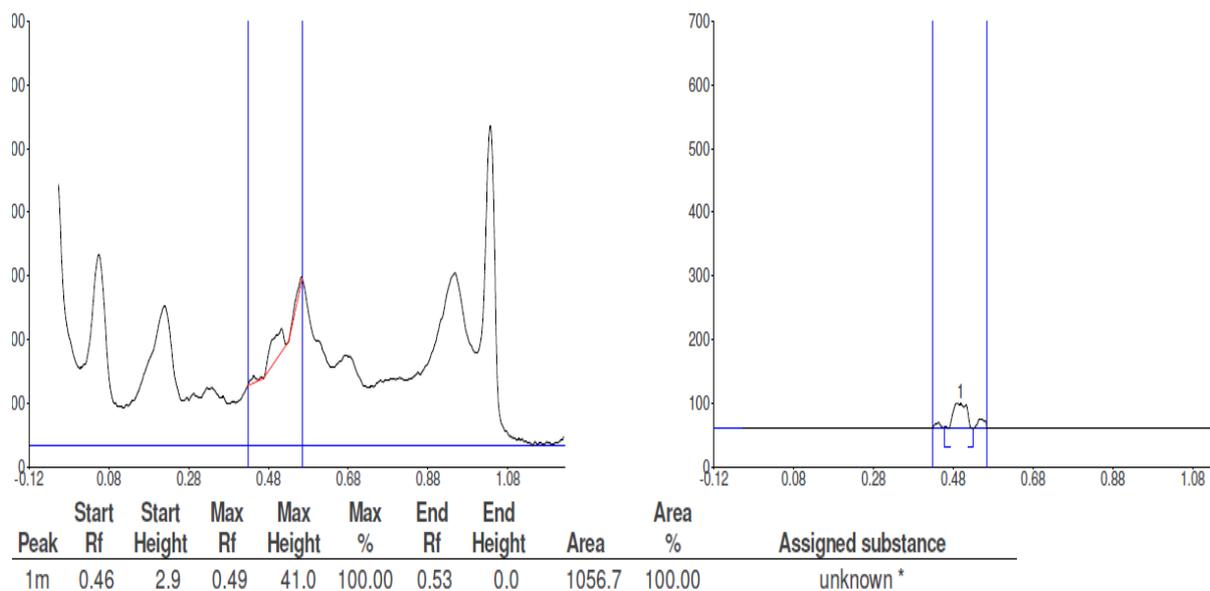


Fig 4: Chromatogram showing Beta-sitosterol in ethanolic extract of *Allophylus serratus* (Roxb.) kurz.

3.4 Pharmacological Activities

3.4.1 Acute Toxicity Studies

No signs & symptoms of toxicity were observed during the acute toxicity study of PHS after oral administration of dose upto 2000 mg/kg.

3.4.2 Ethanol Induced Model

Mean ulcer score was reduced from 22.42 for control to 5.917 for the group which received 800 mg/kg with a significant value ($P < 0.05$). The lower dose of suspension also showed significant protection from ulcers. Sucralfate (100 mg/kg p.o) significantly ($P < 0.05$) inhibited the ulcer formation. Percentage ulcer protection for suspension at 800 mg/kg was found to be 73.60 % and standard drug showed 61.15 % protection. The results were comparable with the standard group.

3.4.3 Indomethacin Induced Model

Pretreatment of poly herbal suspension at different concentration showed reduction in the ulcer score on compared to negative control group where only inducing agent was given. Suspension at 400 mg/kg b.w showed a protective response upto 37.13% but higher concentration at 800 mg/kg b.w showed better ulcer protection of 56.25% with a highly significant value. In this model the standard misoprostol (100 mcg/kg, p.o) has shown better ulcer protection than poly herbal suspension.

3.4.4 Aspirin Induced Model

Oral treatment of poly herbal suspension at different dose levels inhibited the appearance of gastric lesions in a dose dependent manner. Higher dose of poly herbal suspension at 800 mg/kg b.w has shown 52.87% ulcer protection when compared to negative

control group. Increase in ulcer protection was observed with higher dose. Similarly the standard drug omeprazole (10 mg/kg,

p.o) also protected ulcer production significantly ($P < 0.05$) [Table 3] [Figure 5].

Table 3: Effect of Polyherbal Suspension on Ethanol, Indomethacin and Aspirin induced ulcer models

Groups	Parameter	Negative Control	Standard Control	PHF 400mg/kg	PHF 800mg/kg
Ethanol	Ulcer Index	22.42±0.05	8.708±0.174*	13.80±0.036*	5.917±0.238*
	% Ulcer Inhibition	-	61.15	38.44	73.60
Indomethacin	Ulcer Index	22±0.577	7.95±0.289*	13.83±0.208*	9.625±0.272*
	% Ulcer Inhibition	-	63.86	37.13	56.25
Aspirin	Ulcer Index	20.25 ±0.381	7.583±0.238*	14.79±0.208*	9.542±0.245*
	% Ulcer Inhibition	-	62.55	26.96	52.87

PHS: Poly herbal Suspension; * $p < 0.05$ compared to control

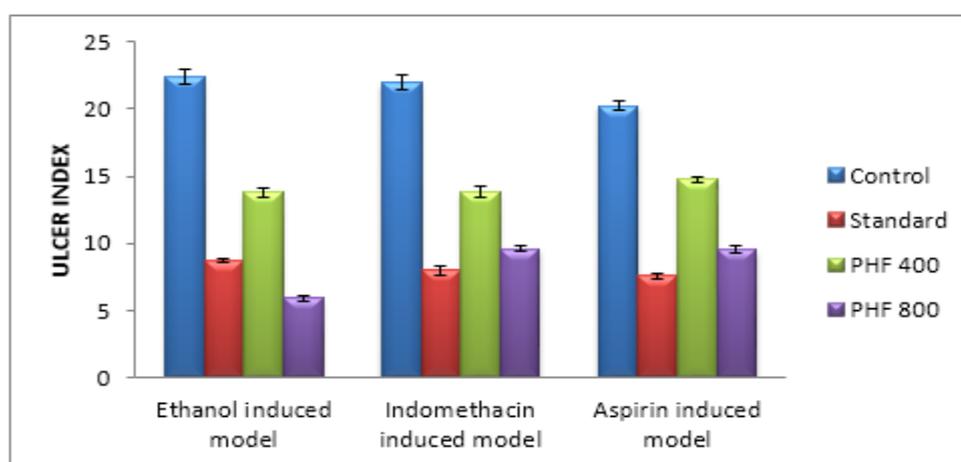


Fig 5: Anti-ulcer activity of Poly herbal formulation on various ulcer induced models

3.5 Histopathological Analysis

Histopathological analysis of rat stomach samples further confirmed the antiulcer activity of PHS by decreasing the congestion, oedema, haemorrhage in gastric mucosa (Figure 6).

4. Discussion

Previous studies have shown that *Allophylus serratus* [15] *Emblca officinalis* L [16] have been reported to possess antiulcer activity. Now-a-days, plant based natural products are able to produce the antiulcer activity in various animal experimentation models [17].

Ethanol induced ulcers were predominant in the glandular part of the stomach contributes significant production of ulcers by the formation of leukotriene C₄ (LTC₄), mast cell secretory products and free radical species results in insult of rat gastric mucosa [18-20]. NSAID's like aspirin known to produce ulcers by acting on cyclooxygenase enzyme, leading to decreased production of prostaglandins, increased acid secretion, and by denaturing the mucous glycoproteins along with free radical formation [21, 22]. Free radicals are mainly responsible for the alteration of epithelial cells integrity, there by contributes for the production ulcers and modulation of pain [23].

Poonam Dharmani *et al.*, reported the antiulcerogenic potential of crude ethanolic extract of *Allophylus serratus* in aspirin, alcohol induced models mainly by regulating the antisecretory and

cytoprotective activities [15]. Al-Rehaily *et al.*, reported the antiulcer activity of *Emblca officinalis* L. In various *in-vivo* test models profoundly acts through the protection of stomach mucosa reduction in non-protein sulfhydryl concentration [24]. In this, present study PHS was found to possess antiulcer activity, probably due to the presence of flavonoid i.e. quercetin & β -sitosterol having the antioxidant activity. PHS shown the significant gastro-protection of ulceration in ethanol, indomethacin, and aspirin induced ulcer models at doses of 400 mg/kg and 800 mg/kg. More significant protection ulceration was observed in ethanol induced model at 800mg/kg with ulcer inhibition of upto 73.60 %.The gastric mucosa tissue protection was further confirmed by the histopathological analysis.

The other plant ingredients present in the PHS have been shown to possess antioxidant activity e.g. *Psidium guajava* L has been observed to decrease lipid peroxidation, due to the presence of phenolic components like quercetin, β -sitosterol [25]. The phytoconstituents of *Eclipta prostrata* L., documented to produce the antioxidant in the treatment of hepatitis & hyperacidity activity due to the presence of stigmasterol, wedelolactone and anticancer activity [26, 27]. Bhattacharya *et al.*, reported the antioxidant activity of *Emblca officinalis* [28], gastroprotective activity by the Al-Rehaily *et al.* [24] Hence the antioxidant and gastroprotective effects of PHS may be attributed to the antiulcer activity.

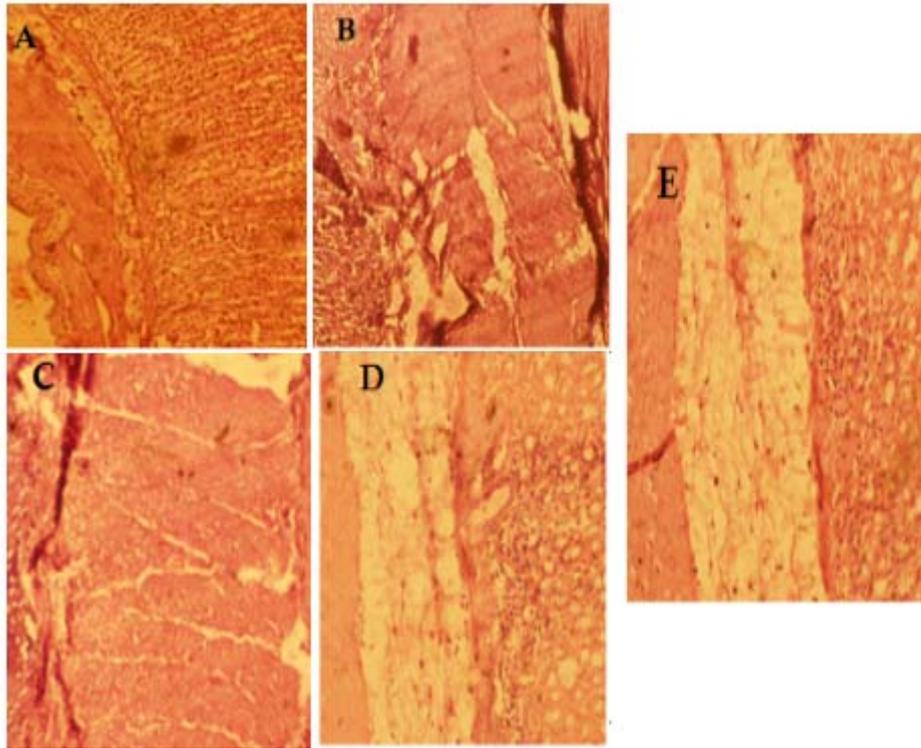


Fig 6: Histopathology of rat gastric mucosa samples in ethanol induced model

- a) Negative Control showing normal architecture
- b) Positive Control showing the edema, congestion, and haemorrhage
- c) Standard control (Sucralfate 100mg/kg)
- d) PHS (400 mg/kg) showing the normal architecture
- e) PHS (800 mg/kg) showing the normal architecture

5. Conclusion

Finally, peptic ulcers were mainly attributed due to the imbalance between defensive and aggressive factors. The results have shown the promising therapeutic beneficial of PHS in different ulcer induced models probable due to the antioxidant and cytoprotective effects of its ingredients. Further, extensive research work needed to elucidate the actual mechanism involved in the antiulcer activity of PHS.

6. Acknowledgements:

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7. Conflict of interest:

We declare that we have no conflict of interest.

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