Indian Journal of Traditional Knowledge
Vol 22(2), April 2023, pp 381-389
DOI: 10.56042/ijtk.v22i2.36141

Gastroprotective effect of *Indukanta ghritam* in Aspirin plus Pylorus ligation induced gastric ulcers in Wistar Albino rats – An experimental evaluation

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Received 05 June 2020; revised 05 April 2023; accepted 09 June 2023

Among various gastric diseases, peptic ulcer is commonly reported in practice. The exact aetiology is unknown however, offensive and defensive factors and no acid no ulcer theories are nearer to its aetiology. *Helicobacter pylori* (*H. pylori*) infection is one of the causative factors, responsible for Peptic Ulcer Disease (PUD). Various treatment modalities along with antacids, proton pump inhibitors, and H_2-receptor antagonists etc. are available in conventional medical science but they have certain limitations as well as side effects. On the other hand, the traditional system of medicine may have the upper hand in such conditions. Various herbs, herbo-mineral and herbal formulations are described in Ayurveda for these conditions. *Indukanta ghritam* is one among the herbal formulations, which is mentioned in scheduled books of Drugs & Cosmetics act, 1940 and *Sahasrayogam*. It is useful in various gastric diseases and its clinical efficacy is proven. However, experimental data on its gastro-protective activity is not available till date. Thus, the present study was conducted to assess its gastroprotective activity at two different dose levels (4.5 g/kg and 9.0 g/kg) in aspirin plus pylorus ligation-induced gastric ulcer model in wistar albino rats. The efficacy of the formulation was determined on the basis of ulcer index, gastric volume, pH of gastric juice, anti-oxidant parameters, and histopathology of the stomach. *Indukanta ghritam* at double therapeutic dose showed comparatively better results in comparison to standard treatment and therapeutic dose group inferring the therapeutic values of the *Ghritam* in cases of PUD.

Keywords: Antioxidant activity, Gastric ulcer, Gastroprotective activity, *Indukanta ghritam*, Pylorus ligation, Ulcer index

IPC Code: Int Cl.[23]: A61K 36/00, A61K 45/00

Peptic ulcer is a gastro-intestinal disease that is a global health problem[1]. Acid Peptic Disorders (APD) are generally reported in clinical practices. An epidemiological study of 30,216 patients (41.7±12.7 years; 66% males) suggests that Peptic Ulcer Disease (PUD) has a prevalence of 7.8% in India, while the prevalence of Gastro-Esophageal Reflux Disease (GERD) from south-west India is around 28%[2,3]. The exact aetiology of the disease is unknown but still no acid no ulcer[4], and an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors are two theories that are responsible for the disease[5].

Helicobacter pylori (*H. pylori*) infection is one of the causative factors, responsible for Peptic Ulcer Disease (PUD) which is found in about 90% of duodenal and 60% of gastric ulcer patients[6]. The treatment modality for gastric ulcers includes antacids, anticholinergics, proton pump inhibitors, and H_2-receptor antagonists[6].

Though conventional medical science has ample drugs for such manifestations like APD and GERD, they are known for certain limitations. These conventional molecules are also familiar for side effects like headache, bowel upsets, disorientation etc[7].

Ayurveda has a huge number of time-tested formulations, which can be used in such manifestations. *Indukanta ghritam* is one such compound herbal formulation, widely practiced in Indian scenario with proven clinical efficacy in peptic and duodenal ulcers[8-10]. Though, it is an important formulation with proven clinical efficacy, its pre-clinical efficacy has not yet been reported. Considering this, the current study is planned.

Materials and Methods

Authentication

All the ingredients of *Indukanta ghritam* were authenticated at CSIR-NIScPR, New Delhi for their authenticity. The ingredients were cleaned to remove physical impurities and allowed to dry completely in the shade.

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Plant material

Indukanta ghritam was prepared by following guidelines as described in the Ayurvedic Pharmacopoeia of India[11] by using authentic raw material in the department of Rasashastra and Bhaishajya Kalpana, All India Institute of Ayurveda, New Delhi. It is made up of; *Kalka dravyas*, *Drava dravyas, Sneha dravya* as mentioned in Table 1. The prepared *Ghrita* was labeled as *Indukanta ghritam*.

Other drugs used for the Study

Aspirin: Tablets IP - ECOSPRIN - 75, Batch number 52000989, Mfg. date: 09/2019, Exp. date 09/2021.

Ranitidine: Rantac-150, Batch No. KR319167, Mfg. date: Jul/2019, Exp. Date: June/2021.

Other reagents used for standard protocol were Ketamine and Xylazine.

Dose fixation

The experimental study dose was calculated by extrapolating the human dose to the animal dose based on the body surface area ratio[12]. The human dose for *Indukanta ghritam* is 48 g/day. The Therapeutic Equivalent Dose (TED) for rats was calculated as 4.32 g/kg (considered as 4.5 g/kg) while in the double Therapeutic Equivalent Dose (TED x 2) group, 9 g/kg was administered.

Animals

Male Wistar albino rats were used in this experimental study. Rats of 12-15 weeks old, with body weight in a range of 170±30 g were obtained from APT Testing and Research Pvt. Ltd., Pune. Animals were acclimatized for five days in the experimental room after the veterinary examination. Animals were placed in the room with a temperature maintained between at 22±3°C and 50-60% relative humidity. The illumination cycle was set to 12 h of artificial fluorescent light and 12 h of dark. Groups of three animals were kept in polypropylene cages with stainless steel grill tops, facilities for food and water bottle, and bedding of clean paddy husk. Pelleted feed supplied by Amrut feed, Chakan, Pune. Potable water passed through a reverse osmosis filtration system was provided ad libitum in glass bottles with stainless steel sipper tubes. The study was approved by the Institutional Animal Ethics Committee (IAEC) for APT Testing and Research Pvt. Ltd. through protocol

Table 1 — Formulation Composition of *Indukanta ghritam*

	Ingredients	Latin name	Part Used	Proportion
Kalka dravya				
1.	*Pippali*	*Piper longum* Linn.	Dried Fr.	48 g
2.	*Pippalimula*	*Piper longum* Linn.	Dried Rt.	48 g
3.	*Chavya*	*Piper chaba* Linn.	Dried St.	48 g
4.	*Chitraka*	*Plumbago zeylanica* Linn.	Dried Rt.	48 g
5.	*Shunthi*	*Zingiber officinale* Roscoe.	Dried Rz.	48 g
6.	*Yavaksara*	*Hordeum vulgare* Linn.	Water soluble ash of whole plant	48 g
Kwatha dravya				
7.	*Putika*	*Holoptelea integrifolia* Planch.	Dried St. Bk.	256 g
8.	*Devadaru*	*Cedrus deodara* (Roxb. ex D. Don) G. Don	Dried Ht. Wd.	256 g
9.	*Bilva*	*Aegle marmelos* (L.) Correa.	Dried St. Bk.	25.6 g
10.	*Agnimantha*	*Premna integrifolia* Willd.	Dried St. Bk.	25.6 g
11.	*Shyonaka*	*Oroxylum indicum* (L.) Kurz.	Dried St. Bk.	25.6 g
12.	*Patala*	*Stereospermum suveolance* (Roxb.) DC.	Dried St. Bk.	25.6 g
13.	*Gambhari*	*Gmelina arborea* Roxb.	Dried St. Bk.	25.6 g
14.	*Shalaparni*	*Desmodium gangeticum* (L.) DC.	Dried Pl.	25.6 g
15.	*Prshniparni*	*Uraria picta* (Jacq.) DC.	Dried Pl.	25.6 g
16.	*Brihati*	*Solanum indicum* Linn.	Dried Pl.	25.6 g
17.	*Kantakari*	*Solanum xanthocarpum* Schard. & H. Wendl.	Dried Pl.	25.6 g
18.	*Gokshura*	*Tribulus terrestris* Linn.	Dried Pl.	25.6 g
	Jala for decoction	Potable water	-	12288 mL
	reduced to	-	-	1/4 part (3072 mL)
19.	*Kshira* (*Go dugdha*)	Cow's milk	-	768 mL
Sneha dravya				
20.	*Ghrita* (*Go ghrita*)	Clarified butter from cow's milk	-	768 g

*Fr. - Fruit, St. - Stem, Rt. - Root, Rz. - Rhizome, St. Bk. - Stem bark, Ht. Wd. - Heart wood, Pl. – Plant

no. 28/1920 and executed by following the CCSEA guidelines.

Animal grouping and treatment regimen

Wistar albino rats of the male sex were randomly divided into six groups, each group comprised of six rats. The grouping and treatment regimen is mentioned in Table 2.

Assessment of gastric ulcer activity

Aspirin Plus Pylorus ligation induced Gastric Ulcer

Selected animals were randomly divided into different groups a day prior to the dosing. The test drugs were administered orally once daily for seven consecutive days to respective groups and vehicle to the control group. Gastric ulceration in rats was induced following standard protocol[13].

Aspirin suspension prepared in 1% Carboxy Methyl Cellulose (CMC) in water was administrated in a dose of 200 mg/kg, orally once daily for three days starting from the fifth day, one hour after drug administration. In reference standard group, ranitidine administration was started on the fifth day of drug administration. One hour after aspirin administration, pylorus was ligated as per the standard method[14] on the seventh day.

The rats were housed in individual metabolic cages to prevent coprophagia. The animals were fasted overnight (18 hours) with *ad libitum* access to water. Rats were anesthetized with ketamine: xylazine (1:1) and a portion of the abdomen was incised through a small midline incision just below and adjacent to the xiphoid process. The pylorus of the stomach was raised slightly to avoid stressing the pylorus or compromising its blood supply. The pylorus was ligated with cotton thread and the stomach was carefully inserted. The incision was closed with intermittent sutures. Animals were deprived of both food and water during the post-operative period and sacrificed by ether overdose four hours after pyloric ligation. The abdominal cavity was carefully reopened and the stomach was removed after ligating the distal esophagus to avoid loss of gastric contents during dissection. The gastric contents were drained into test tubes and centrifuged at 2500 rpm for 20 min. Gastric juice volume was recorded and used for biochemical evaluation. Gastric biopsies were performed to assess gastric ulcer, histopathologic, and antioxidant parameters.

Assessment of stomach ulcer

The stomach was dissected, cleaned, and opened along its greater curvature, and the inner surface was gently washed with cold saline and examined for ulceration with a magnifying lens. The ulcer severity and the total number of ulcers in each rat were recorded to calculate the ulcer index[15]. The number of ulcers was counted with magnifying lenses. Each ulcer was measured with a Vernier caliper to determine its diameter[16]. The percentage protection was calculated[15].

Gastric acid secretion

The gastric content obtained from the pylorus was centrifuged at 2500 rpm for 20 min at 4°C; the volume (mL) and the pH value were measured[17].

Measurement of pH of gastric juice

A drop of gastric juice was taken with a glass rod and placed on a strip of narrow-range pH paper. The color change was matched with the standard chart and the reading was noted.

Study of antioxidant activity

Stomach homogenate was prepared in Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 × g at 4°C for 20 min, using Remi C-24 high-speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content)[18], catalase (CAT)[19] and reduced glutathione (GSH)[20,21].

Histopathology study

The stomach was stored in 10% formalin solution. Tissue sections of 6 µm thick were cut using a Spencer-type rotary microtome. Slides were prepared by following standard methods, and stained with hematoxylin and eosin. Histopathological changes such as hyperaemia, bleeding, necrosis, inflammation,

Table 2 — Animal grouping and treatment regimen

	Group	Treatment
Group 1	Normal Control (NC)	No treatment
Group 2	Disease Control (DC)	Aspirin (200 mg/kg) + Pyloric ligation
Group 3	Vehicle Control (VC)	Cow ghee (4.5 g/kg) for seven days + Aspirin (200 mg/kg) + Pyloric ligation
Group 4	Standard Group (STD)	Ranitidine (10 mg/kg) for seven days + Aspirin (200 mg/kg) + Pyloric ligation
Group 5	Therapeutic Equivalent Dose of test drug (TED)	*Indukanta ghritam* (4.5 g/kg) for seven days + Aspirin (200 mg/kg) + Pyloric ligation
Group 6	Double Therapeutic Equivalent Dose of test drug (TED x 2)	*Indukanta ghritam* (9 g/kg) for seven days + Aspirin (200 mg/kg) + Pyloric ligation

infiltration, erosion, and ulceration were examined under the microscope (40x magnification)[22].

Statistical analysis

All values were expressed as mean ± SEM (Standard Error of Mean). The data of animal weight was analysed by paired 't' test and all other data was analysed by unpaired modified 't' test and analysis of variance (ANOVA) followed by Dunnett's 't' test for all the treated groups. A level of $p<0.05$ was considered statistically significant. The level of significance was noted and interpreted accordingly.

Results

Effect of *Indukanta ghritam* on ulcer index, the volume of gastric contents, and pH

A significant increase in ulcer index was observed in the disease control group in comparison to the normal control group. In the vehicle control group and *Indukanta ghritam* (TED) group, a statistically significant decrease in ulcer index was observed when compared with the disease control group (Table 3).

Gastric contents were significantly increased in the disease control group in comparison with the normal control group. A highly significant decrease in gastric content was observed in all drug-treated groups when compared with the disease-control group. pH in gastric juice was non-significantly decreased in the disease control group in comparison to the normal control group. A significant reversal of pH was observed in the vehicle control group while a non-significant increase was observed with the administration of *Indukanta ghritam,* in both the dose levels in comparison with the disease control group (Table 3).

Effect of *Indukanta ghritam* on antioxidant parameters

Highly significant decrease in catalase activity was observed in all treated groups in comparison to the normal control group. Highly significant increase was observed in catalase activity in all drug-treated groups in comparison to the disease control group.

Highly significant increase in lipid peroxidation in stomach homogenate was observed in the disease control group in comparison to the normal control group. In all drug-treated groups when compared with the disease control group, highly significant reversal in lipid peroxidation content was observed.

There was a significant increase observed in reduced glutathione activity in all treated groups when compared with the disease control group. In all drug-treated groups, highly significant reversal was observed except the therapeutic dose of *Indukanta ghritam* treated group in which statistically significant reversal was observed in comparison with the disease control group (Table 4).

Table 3 — Effect of *Indukanta ghritam* on ulcer index, the volume of gastric contents, and pH in gastric juice

Groups	Ulcer index	% change	Volume of gastric contents (mL/100 g body wt./4 h.)	% change	pH	% change
NC	3.00±0.577	--	1.18±0.031	--	5.72±0.095	--
DC	10.17±2.868$^{@\beta}$	239 ↑	3.17±0.156$^{@@@\beta}$	168.64 ↑	5.15±0.300	9.96 ↓
STD	5.83±1.249	42.67 ↓	2.24±0.068***$^{\beta\&}$	29.33 ↓	5.03±0.105	2.33 ↓
VC	2.00±0.258*$^{\&}$	80.33 ↓	2.10±0.058***$^{\beta\&}$	33.75 ↓	5.93±0.184$^{\&}$	15.14 ↑
TED	2.00±0.577*$^{\&}$	80.33 ↓	1.70±0.037***$^{\beta\&}$	46.37 ↓	5.53±0.225	7.37 ↑
TED x 2	5.33±1.520	47.59 ↓	1.47±0.033***$^{\beta\&}$	53.62 ↓	5.45±0.167	5.82 ↑

Data: Mean ± SEM; ↑- Increase, ↓- Decrease; $^{@}p<0.05$, $^{@@@}p<0.001$, compared to normal control group (Unpaired 't' test); *$p<0.05$, ***$p<0.001$, compared to disease control group (Unpaired 't' test); $^{\beta}p<0.01$, compared to normal control group and $^{\&}p<0.01$, compared to disease control group (Anova followed by Dunnett's multiple 't' test).

Table 4 — Effect of *Indukanta ghritam* on antioxidant parameters in stomach homogenate obtained from Aspirin Plus Pylorus ligation induced Gastric Ulcer in rats

Groups	Catalase activity (units/ml enzyme/mg solid/mL)	Lipid peroxidation (µmole MDA/g tissue)	Reduced glutathione (ΔOD_{412nm}/min)
NC	271.00±14.530	0.22±0.005	6.21±0.179
DC	120.26±4.403$^{@@@\beta}$	0.25±0.005$^{@@@\beta}$	6.96±0.186$^{@\beta}$
STD	206.98±4.850***$^{\beta\&}$	0.22±0.006**$^{\&}$	6.22±0.129**$^{\&}$
VC	210.03±18.492***$^{\beta\&}$	0.21±0.006**$^{\&}$	5.82±0.134***$^{\&}$
TED	243.56±17.338***$^{\beta\&}$	0.22±0.007**$^{\&}$	6.32±0.146*$^{\&}$
TED x 2	206.30±13.682***$^{\beta\&}$	0.23±0.003**	5.99±0.042***$^{\&}$

Data: Mean ± SEM;$^{@}p<0.05$, $^{@@@}p<0.001$, compared to the normal control group (Unpaired 't' test); *$p<0.05$, **$p<0.01$, ***$p<0.001$, compared to the disease control group (Unpaired 't' test); $^{\beta}p<0.01$, compared to the normal control group and $^{\&}p<0.01$, compared to the disease control group (Anova followed by Dunnett's multiple 't' test)

Histopathology

Normal histopathological features of stomach cells were found in the normal and vehicle control groups. In the disease control group, degenerative changes of glandular and uniform layers of the mucosal epithelium with focal loss were noted due to degenerative and necrotic changes with loss of mucosal cells occasionally.

Hyperplastic changes of mucosa were noted occasionally. Acute reactive inflammatory status with congested blood vessels was noted. Focal vacuolar changes of mucosal epithelium and focal congestion of blood vessels were noted in the standard treatment group. In both the groups of *Indukanta ghritam,* glandular and non-glandular regions of the stomach were seen, which showed normal histomorphology of mucosal layers. No inflammatory changes in the mucosa and submucosa were found in both groups as depicted in Fig. 1-10.

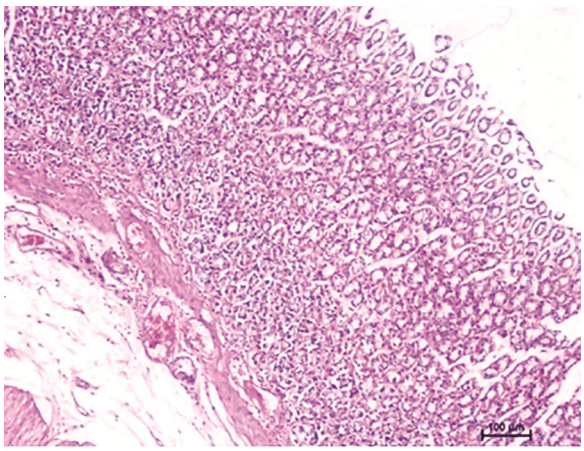

Fig. 1 — Glandular and non-glandular regions of stomach which showed normal histomorphology of mucosal layers in NC

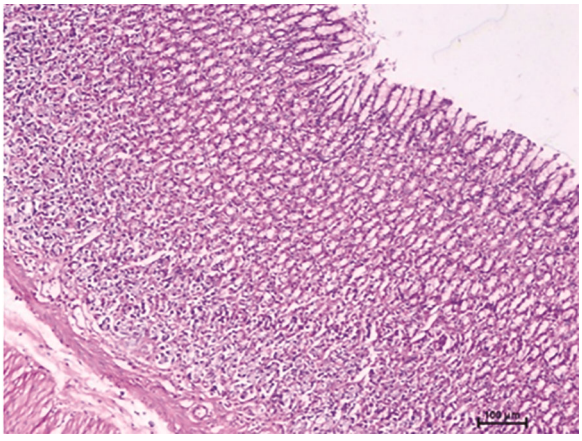

Fig. 2 — Glandular and non-glandular regions of stomach which showed normal histomorphology of mucosal layers in VC

Discussion

It is reported that accumulation of gastric juice and interference with gastric blood circulation are responsible for the induction of gastric ulcer[23,24]. Break down in gastric mucosa or gastric ulcer takes place due to increased acid-pepsin, which leads to

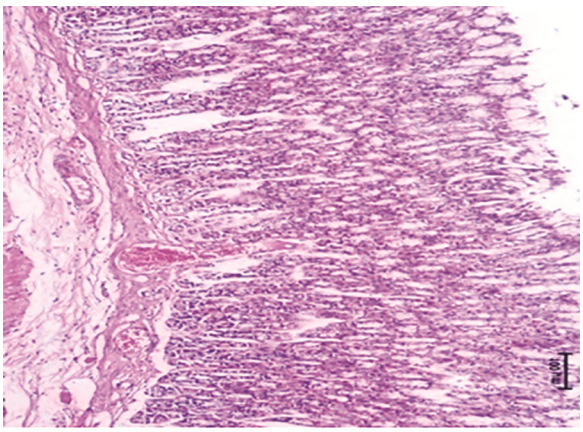

Fig. 3 — Acute reactive inflammatory status with congested blood vessels DC-a

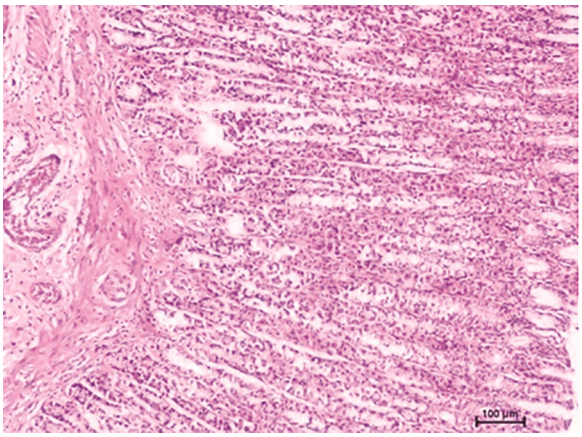

Fig. 4 — Acute reactive inflammatory status with congested blood vessels DC-b

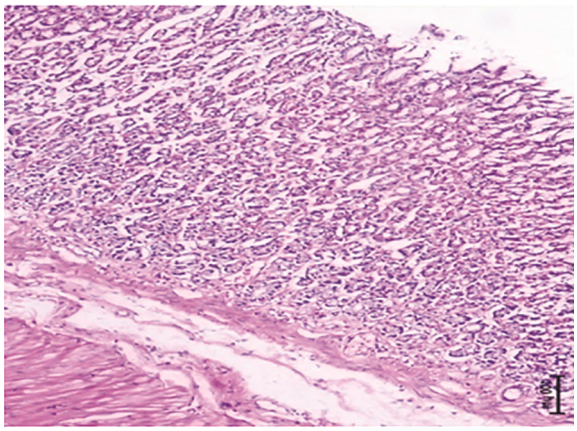

Fig. 5 — Focal vacuolar changes of mucosal epithelium and focal congestion of blood vessel STD-a

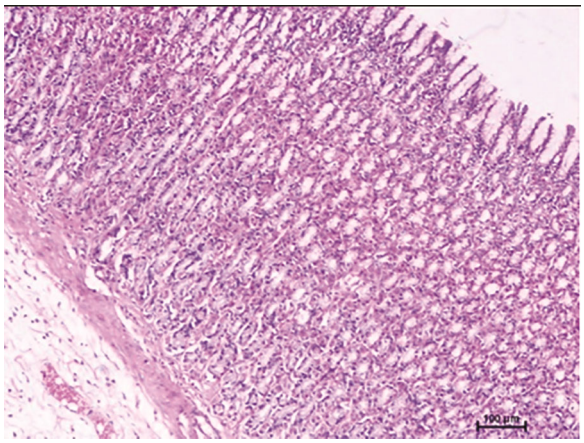

Fig. 6 — Glandular and non-glandular regions of the stomach which showed normal histomorphology of mucosal layers STD-b

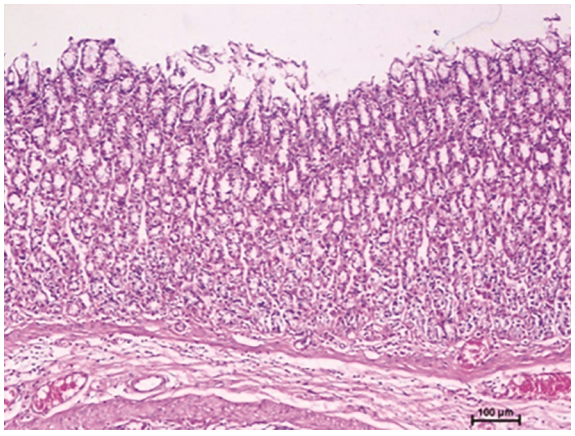

Fig. 7 — Focal vacuolar changes of mucosal epithelium and focal congestion of blood vessel TED-a

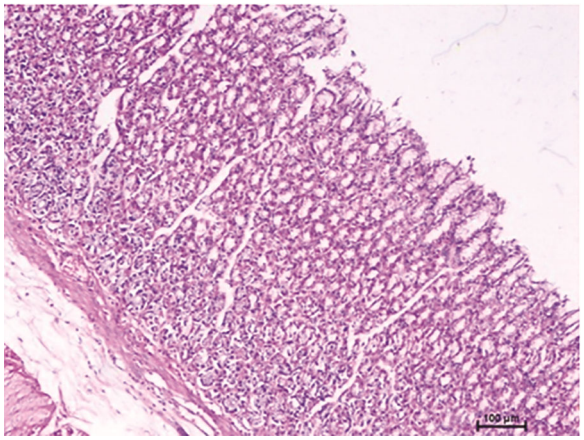

Fig. 8 — Glandular and non-glandular regions of the stomach which showed normal histomorphology of mucosal layers TED-b

auto-digestion of gastric mucosa[25]. Pepsin secretion with or without acid is also responsible for the progress of gastric ulcers[26]. Prostaglandins synthesis, mucus, and bicarbonate secretion, surface epithelial hydrophobicity, and mucosal blood flow are

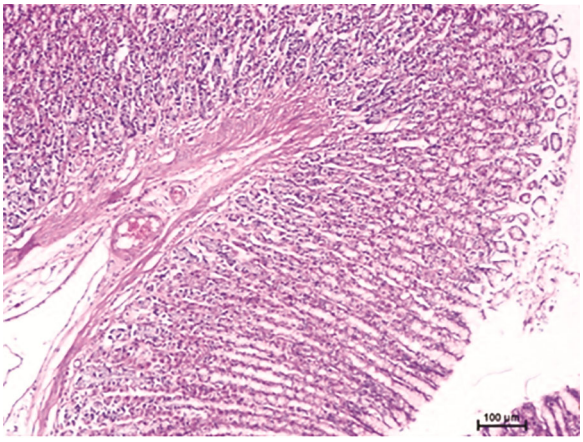

Fig. 9 — Glandular and non-glandular regions of the stomach which showed normal histomorphology of mucosal layers. Absence of any inflammatory changes in the mucosa and submucosa TEDx2-a

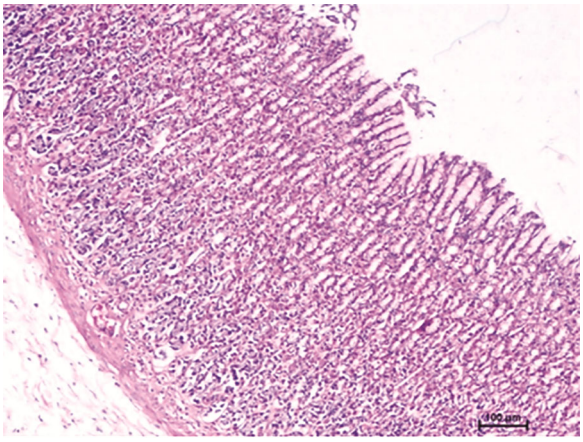

Fig. 10 — Glandular and non-glandular regions of the stomach which showed normal histomorphology of mucosal layers. Absence of any inflammatory changes in the mucosa and submucosa TEDx2-b

responsible for the protective mechanism of gastric mucosa which is altered due to Aspirin[27,28]. These changes promote the diffusion of acid through the breached surface to destroy cells, capillaries, and veins causing hemorrhagic ulcers[29].

Aspirin was administered to pyloric ligated rats; in this procedure, aspirin further aggravates the acidity and attenuates the resistance of the gastric mucosa by causing extensive damage to the glandular region of the stomach[30]. The ulcer index is an important parameter that may help to assess the anti-ulcerogenic efficacy of the treatments. In the present study, a significant decrease in ulcer index was observed in the vehicle control group and *Indukanta ghritam* treated animals at a therapeutic dose level. The gastroprotective activity of the individual ingredients of *Indukanta ghritam i.e.*, *Pippali*[31], *Chavya*[32],

Shunthi[33], *Bilva*[34], *Devadaru*[35], *Shyonaka*[36], *Patala*[37], *Gambhari*[38] and *Shalaparni*[39] may also be imbibed by *ghritam* due to *Sansakaranuvartana* property[40]. While in Ranitidine and *Indukanta ghritam* (TEDx2) treated groups, a non-significant decrease was observed in ulcer index when compared with the disease control group. Ranitidine is a selective and competitive histamine H_2-receptor antagonist established for its effectiveness in gastric and duodenal ulcers[41].

A highly significant decrease in gastric content was observed in all the drug-treated groups when compared with the disease-control group. In Ayurveda, gastric secretions can be comparable with *Pachaka pitta*[42]. *Ghrita* has the *Pitta-anilahara* property[43]. Thus, it helps in reducing the *Pitta* aggravation that may be the reason for the quantitative decrease of gastric secretions in the treatment groups. The decreased volume can be attributed to the anti-secretory activity. This anti-secretory activity along with pH elevation, which may perhaps indicate elevated bi-carbonate secretion, may contribute to the observed anti-ulcer activity of reference standard.

A significant reversal of pH was observed in the vehicle control group while a non-significant increase was observed in *Indukanta ghritam* (both dose levels) treated groups in comparison with the disease control group.

Lipid peroxidation is one common factor that is responsible for cell death. The excessive oxidation of lipids is also responsible for damage to the cell membrane[44]. Highly significant reversal (increase) in lipid peroxidation content was observed in all drug-treated groups in comparison to the disease control group. Increased elevation in lipid peroxidation is one of the main contributing factors in the severe ulceration observed in this group. This enhanced lipid peroxidation was found to be significantly antagonized by all the tested drugs and the reference standard. This effect may be responsible for the observed significant anti-ulcer effect.

A drug-producing cytoprotection especially against cell injury due to the generation of free radicals is expected to elevate the activity of enzyme. Catalase activity was found to be increased to highly significant levels in all the drug-treated groups in comparison to the disease control group. Hence it can be suggested that catalase activity enhancement is one of the mechanisms of the observed anti-ulcer activity of all treated groups.

Glutathione (GSH) is an important component of the intracellular protective mechanisms against oxidative stress. In addition, GSH scavenges superoxide anion and protects the thiol group of protein from oxidation[45]. The redox state of the cell is controlled by the tripeptide glutathione which is a good intracellular antioxidant. In the present study, a significant increase was observed in reduced glutathione activity in all the drug-treated groups when compared with the disease control group, which may be responsible for the gastro-protective activity[46]. Highly significant reversal was observed in all drug-treated groups except in *Indukanta ghritam* (therapeutic dose) treated group. Histopathology of the stomach also confirms the results obtained in the experimental study Fig. (1-10).

Conclusions

On the basis of overall results, it can be concluded that *Indukanta ghritam* at both dose levels has anti-ulcer activity against Aspirin plus Pylorus Ligation induced Gastric Ulcers model in wistar albino rats. However, *Indukanta ghritam* at double therapeutic equivalent dose showed much better results in comparison to the standard treatment and therapeutic equivalent dose group of *Indukanta ghritam.* The study has generated evidence against the therapeutic use of the trial drug in various forms of gastro-intestinal ulcers.

Acknowledgement

The authors are thankful to APT research foundation, Pune for the conduction of the experimental study. Dr. Vishal Kumar and Dr. Vijay Kumar for guidance in the research study.

Conflict of Interest

We declare no Conflict of Interest.

Authors' Contributions

Each author has contributed sufficiently to the research and made significant intellectual contributions, including the conceptualization, design, data collection as well as the analysis and interpretation of the data. In addition, each author assisted with the article's development and critical revision. As a result, they all satisfy the requirements for authorship of the manuscript.

References

1 Klopell, F C, Lemos, M & Sousa, J P B, Nerolidol, An antiulcer constituent from the essential oil of Baccharis dracunculifolia DC (Asteraceae), *Z Naturforsch C J Biosci*, 62 (7-8) (2007) 537-542.

2 https://www.ncbi.nlm.nih.gov/pubmed/22766645accessed on 07.04.2020 at 2.45 PM.

3 https://www.ncbi.nlm.nih.gov/pubmed/26557556accessed on 07.04.2020 at 2.45 PM.

4 Munjal Y P, *API Textbook of Medicine Vol. 1st*, 6th Chapter, (The Association of Physicians of India, Jaypee Brothers Medical Publishers (P. ltd.), 2015, 1063.

5 W A Hooderwerf & P J Pasricha, Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease, in Goodman and Gilman's Therapeutic Reference, L. Brunton, (2006) 104-121.

6 Bhattamisra S K, Yan Y, Lee K, Hui K C, *et al,.* Protective activity of geraniol against acetic acid and Helicobacter pylori - induced gastric ulcers in rats, *J Tradit Complement Med*, 9 (3) (2019) 206-214.

7 Tripathi K D, *Essentials of Medical Pharmacology*, 7th Ed., JayPee Brothers Medical Publishers Pvt ltd., New Delhi, 2013, 649-655.

8 Warrier P K, Bhattathiri P P N, Radhakrishnan P & Balachandran P, Comparative clinical study of lndukanta Ghrita and Mahatiktaka Ghrita in Parinamasula (Peptic Ulcer), *J Res Ayurvedic Sci*, 10 (1-2) (1998) 15-29.

9 Devidas K V, Radhakrishnan P & Warrier P K, Effect of Indukanta Ghritam and Mahatiktaka Ghritam in Parinamasula (Duodenal Ulcer), *J Res Ayurvedic Sci*, 19 (3-4) (1998) 98-106.

10 P K Warrier, Bhattathiri P P N & Bhaskaran K P, Effect of Indukanta Ghrta in Parinamasula, *J Res Ayurvedic Sci*, (1980).

11 Anonymous, *Ayurvedic Pharmacopoeia of India-Part-II Vol –II*, Ministry of Health and Family Welfare, Govt. of India, New Delhi, (2008) 96-98.

12 Paget, G E & Barnes, J M, Evaluation of drug activities, In: Pharmacometrics, eds. Laurence, D.R. and Bacharach, A.L., *Academic press New York*, 1 (1964) p. 161.

13 Goel R K, Chakrabarti A & Sanyal A K, The effect of biological variables on the anti ulcerogenic effect of vegetable plantain banana, *Planta Med,* 2 (1985) 85-88.

14 Shay, H, Komarov, S A, Fels, S E, Meraze, D, Gruenstein, M, *et al*., A simple method for the uniform production of gastric ulceration in rat, *Gastroenterology*, 5 (1945) 43-61.

15 Suzuki Y, Hayashi M, Ito M & Yamagami J, Antiulcer effect of 4'-(2-carboethyl) phenyltrans-4-amino methyl cyclohexane carboxylate hydrochloride (Cetraxate) on various experimental ulcers in rats, *Japanese J Pharmacol*, 26 (1976) 471.

16 Deoda R S, Kumar D & Bhujbal S S, Gastroprotective effect of Rubia cordifolia linn on aspirin plus pyloric ligated ulcer, *Evid-Based Complement Altern Med*, (2011) 1-6.

17 Yesilada E, Takaishi Y, Fujita T & Sezik E, Antiulcerogenic effects of *Spartium junceum* flowers on in vivo test models in rats, *J Ethnopharmacol*, 70 (3) (2000) 219-226.

18 Okhawa H, Ohishi M & Yagi K, Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analyt Biochem*, 95 (1979) 351.

19 https://www.sigmaaldrich.com/technical-documents /protocols/biology/enzymatic-assay-of-catalase.html assessed on 19.04.2020 at 8.30 PM.

20 Akerboom T P & Sies H, Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples, *Methods Enzymol*, 77 (1981) 373-382.

21 Eyer P & Podhradsky D, Evaluation of the micro method for determination of glutathione using enzymatic cycling and ELLman's reagent, *Anal Biochem*, 153 (1986) 57-66.

22 Raghuramulu N, Nair K M & Kalyanasundaram S, A manual of laboratory techniques. Hyderabad, *Nat Inst Nutr*, (1983) 246-53.

23 Brodie D A, The mechanism of gastric hyperacidity produced by pyloric ligation in the rat, *Am J Digest Dis*, 11 (1966) 231-241.

24 Patel A V, Santani D D & Goyal R K, Anti-ulcer activity and the mechanism of action of magaldrate in gastric ulceration models of rat, *Indian J Physiol Pharmacol*, 44 (2000) 350-354.

25 Goel R K & Bhattacharya S K, Gastro-duodenal mucosal defense and mucosal protective agents, *Indian J Exp Biol*, 29 (1991) 701-714.

26 Anup A & Jegadeesan M, Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R Br var. *indicus*, *J Ethnopharmacol*, 84 (2-3) (2003) 149-156.

27 Langman M J S, Brok P, Hawkey C J, Silverstein F & Yeomans N, Non-steroidal anti-inflammatory drug associated ulcer - epidemiology, causation and treatment, *J Gastroenterol Hepatol*, 6 (1991) 442-448.

28 Rao C V, Sairam K & Goel R K, Experimental evaluation of Bacopamonniera on rat gastric ulceration and secretion, *Indian J Physiol Pharmacol*, 44 (2000) 35-41.

29 Ajaikumar K B, Asheef M, Babu B H & Padikkala J, The inhibition of gastric mucosal injury by *Punica granatum* L. (Pomegranate) methanolic extract, *J Ethnopharmacol*, 96 (2005) 171-176.

30 Sanmugapriya E & Venkataraman S, Anti ulcerogenic potential of *Strychnos potatorum* Linn. seeds on aspirin plus pyloric ligation-induced ulcers in experimental rats, *Phytomedicine*, 14 (2007) 360-365.

31 Agarwal A K, Rao C V, K Sairam, *et al*., Effect of *Piper longum* Linn, *Zingiber officianalis* Linn, and *Ferula* species on gastric ulceration and secretion in rats, *Indian J Exp Biol*, 38 (2000) 994-998.

32 Morikawa T, *et al*., New Amides and gastroprotective constituents from the fruit of *Piper chaba*, Heruntergeladen von: University of Florida, *Urheberrechtlich geschützt*, 70 (2004) 152-159.

33 Nanjundaiah S M, Annaiah H N M & Dharmesh S M, Gastroprotective effect of ginger rhizome (*Zingiber officinale*) extract: Role of gallic acid and cinnamic acid in H+, K+-ATPase/*H. pylori* Inhibition and anti-oxidative mechanism, *Evid Based Complement Altern Med, Volume*, (2011) 13.

34 Das S K & Roy C, The protective role of aegle marmelos on aspirin–induced gastro-duodenal ulceration in albino rat model: A possible involvement of antioxidants, *Saudi J Gastroenterol*, 18 (3) 2012 188-194.

35 Kumar A, Singh V & Chaudhary A K, Gastric anti secretory and antiulcer activities of *Cedrus deodara* (Roxb.) Loud. in Wistar rats, *J Ethnopharmacol*, 134 (2011) 294-297.

36 Arul V, Miyazaki S & Dhananjayan R, Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr., *J Ethnopharmacol*, 96 (2005) 159-163.

37 Chatopadhyay P, Gupta V & Gogoi H K, Antiulcer and gastroprotective potential of *Stereospermum suaveolens* in Wistar Rats, *J Pharmacol Pharmacother*, 2 (2) 2011.

38 Lawrence L, Menon S, Vincent S, *et al*., Radical scavenging and gastroprotective activity of methanolic extract of *Gmelina arborea* stem bark, *J Ayurveda Integr Med,* 7 (2016) 78-82.

39 Mahesh A, Jeyachandran R, Rao D M, *et al.*, Gastroprotective effect of *Desmodium gangeticum* roots on gastric ulcer mouse models, *Rev Bras Farmacogn*, 22 (5) (2012) 1085-1091.

40 Acharya Y T editor, *Charaka Samhita,* Sutra Sthana,13th Chapter, 13th *Sloka*, (Varanasi: Chaukhambha Orientalia), 2011, 82.

41 Jha S K, Karki R, Puttegowda V D & Ghosh A, Pharmacodynamics and pharmacokinetics evaluation of ranitidine micro emulsion on experimental animals, *Adv Pharm*, (2014) 1-6.

42 Acharya Y T editor, *Charaka Samhita,* Sutra Sthana, 1st Chapter, 60th *Sloka*, (Varanasi: Chaukhambha Orientalia), 2011, 36.

43 Kunte A M, Navare K S & Shastri H S editor, *Ashtanga Hridayam* with commentary, Sutra Sthana, 12th Chapter, 10-13th *Sloka*, (Varanasi: Chaukhambha Samskrita Samsthana), 2017, 193.

44 Gaschler M M & Stockwell B R, Lipid peroxidation in cell death, *Biochem Biophys Res Commun,* 482 (3) (2017) 419-425.

45 Villegas I, Lastra C A, Casa L A, Motilva V & Martin M J, Effect of food intake and oxidative stress on intestinal lesion caused by meloxicam and piroxicam in rats, *Eur J Pharmacol,* 414 (2001) 79-86.

46 https://www.sciencedirect.com/science/article/pii/S2225411018301032 assessed on 28.03.2020 at 8:45 PM.