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RESEARCH ARTICLE

Gastroprotective and digestive potential of an Ayurvedic asava–arishta preparation

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Abstract
Asavas and arishtas are considered as important Ayurvedic self-fermented dosage forms which are being used widely to promote health and well-being and for management of digestive and metabolic disorders. The present investigation was carried out to study the potential of an Ayurvedic polyherbal asava–arishta preparation (ZP) in gastroprotection, intestinal motility and gastric dyspepsia in experimental rodent models. The gastroprotective effect of ZP at doses 1.5, 3 and 6 ml/kg of body weight was studied using battery of animal models such as pyloric ligation in rats, intestinal transit time using charcoal meal test, gastric emptying and digestive stimulation action in rats. In addition, anti-spasmodic activity was studied using isolated guinea pig's ileum. The role digestive enzymes such as pepsin, α-amylase and lipase were accessed by various enzymatic assays. A dose-dependent and statistically significant ($p < 0.01$) inhibitory effect on reducing gastric volume, free acidity, total acidity and ulcer index were observed in ZP treated animals as compared to the control. ZP also enhanced the mucin content of the gastric juice. Pre-treatment with ZP produced a dose-dependent effect in reducing intestinal motility, intestinal spasms and increased the gastric emptying time in rats. These observations validated the age old claims of ZP for its use as digestive tonic. Thus, results concluded that ZP is having potential to improve digestion and reduces recurrent digestive ailments.

Keywords Asava–arishta · Zandu Pancharishta · Hyperacidity · Ulcer index · Gastroprotection · Digestive juice

Introduction

In Ayurveda, medicinal plants and certain types of minerals are extensively used for the preparation of various types of formulations including arishtas (fermented decoctions) and asavas (fermented infusions) (Sekar 2007; Khan et al. 2017). Arishtas are made with decoctions of herbs in boiling water while asavas are prepared by directly using fresh herbal juices followed by fermentation with the addition of sugar and dried flowers of *Woodfordia fruticosa* (Khan et al. 2017; Sekar and Mariappan 2008; Sushruta and Anubha 2011). Asava and arishta are the unique liquid beverages that contain self-generated alcohol (not more than 12% by volume) and are also known as medicinal wine (Sekar and Mariappan 2008; Sushruta and Anubha 2011). These liquid dosage forms are having high palatability and stability due to its fermented form (Khan et al. 2017; Sushruta and Anubha 2011). These self-generated alcoholic preparations having several advantages over tinctures or other liquid beverages, such as improvement in the efficiency of extraction of several phytochemicals (both alcohol and water soluble) from the crude herbs, conversion of phytochemicals to less toxic and more potent drugs, and more importantly, improvement in the absorption and drug delivery at the site of action (Sekar and Mariappan 2008; Mishra et al. 2010). Asavas and arishtas are highly potent classical Ayurvedic preparations typically used for digestive and cardiac health purposes (Pillai and Pandita 2016). Because of their therapeutic property,

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sweet taste and easy availability people are inclined more to consume higher doses of these medications for longer periods. However, the Ayurvedic practitioners recommended the use of asavas or arishtas after examining the patient's health condition (Weerasooriya et al. 2006).

The preparation (Coded as ZP) is an Ayurvedic polyherbal formulation prepared by amalgamation of both asavas and arishtas. A total of thirty-five different herbs generally mentioned in Ayurvedic classical texts namely, *Aegle marmelos*, *Clerodendrum phlomidis*, *Oroxylum indicum*, *Gmelina arborea*, *Stereospermum suaveolens*, *Tribulus terrestris*, *Desmodium gangeticum*, *Solanum indicum*, *Withania somnifera*, *Asparagus racemosus*, *Tinospora cordifolia*, *Sida cordifolia*, *Glycyrrhiza glabra*, *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Rubia cordifolia*, *Symplocos racemosa*, *Terminalia arjuna*, *Aloe barbadensis*, *Vitis vinifera, Woodfordia fruticosa, Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Cinnamomum zeylanicum*, *Cinnamomum tamala*, *Amomum subulatum*, *Coriandrum sativum*, *Cuminum cyminum*, *Curcuma longa*, *Hedychium spicatum*, *Trachyspermum ammi*, *Syzygium aromaticum* and *Saccharum officinarum*, are used to prepared the polyherbal formulation. The polyherbal formulation (ZP) is used as digestive tonic to promote digestive stimulant, mild laxative, sialogogue and carminative actions. Furthermore, it is also used to treat different digestive problems like hyperacidity, indigestion, flatulence and constipation (Khan et al. 2017).

The heterogeneous nature of Ayurvedic polyherbal arishtas or asavas required continuous monitoring for quality, efficacy and safety. Due to increasing interest and popularity in traditionally fermented formulations, proper scientific validation is needed to verify the traditional claims and the efficacy of polyherbal arishta formulation. Khan and his co-workers have already illustrated the comparative metabolic profiling of its component with a scientific correlation among metabolites and the steps involved in its preparation (Khan et al. 2017).

Critical analysis of currently available information on quality, efficacy, safety and metabolic profiling of the arishtas and asavas suggest that appropriately processed and standardize techniques opt for this polyherbal preparation could be beneficial and is therapeutically effective against different digestive problems commonly associated with indigestion and ulcer. Therefore, we became interested in experimentally verify the therapeutic claims of ZP for its effectiveness in digestion and controlling acidity in experimental rodent models. The results of our experiment suggesting such possibilities are described and discussed in this communication. The proposition of the reported observations for better understanding of the pharmacological principles behind traditionally known medicinal uses of the arishtas and asavas for their therapeutic purposes are also pointed out.

Materials and methods

Animals

Male Wistar rats (150–200 g body weight) and guinea pig (400–450 g body weight) used in this study were procured from Central Animal House of R. G. Kar Medical College and Hospital, Kolkata (Registration number: R/N 959/C/06/CPCSEA). They were randomly selected and group-housed (six animals per cage) in polypropylene cages provided with husk bed at an ambient temperature (25 ± 1 °C) and relative humidity (50 ± 10%) with a 12:12 h light/dark cycle. All the animals were acclimatized to laboratory conditions for at least 1 week before the start of the experiments. They were always fed with standard rodent diet and water *ad libitum*. The waste in the cages was removed daily to ensure hygienic condition and maximum comfort for animals. Ethical clearance for animal experimental work was obtained from the Institutional Animal Ethical Committee of the R. G. Kar Medical College and Hospital, Kolkata prior to the commencement of experiments. All the experimental groups were always tested in parallel (i.e. on the same day of the experiment), and handled, weighed, and observed by a single blinded observer and using the same laboratory environment.

Sample formulation (ZP) and chemicals

The sample formulation (ZP) was obtained from Emami Limited, Kolkata of the commercial batch (Batch No HK0092, manufacturing date: 07/2016), prepared at production unit of Emami Limited (Hemma Herbals Pvt Ltd, Solan, India). The specimen of the formulation was retained at real-time stability study storage chamber at Research & Development Center, Emami Limited, Kolkata, India. The ZP was prepared in airtight fermenter by anaerobic digestions of herbal decoction. Briefly, the powdered raw materials were boiled together with water in a vessel, thereafter the collected herbal decoction was fermented with sugar and *Dhataki* (*Woodfordia fruticosa*) flowers. The anaerobic fermentation was initiated by the *Yeast* and continued for 6–10 days until the desired percentage of alcohol (not more than 12% by volume) was formed. The fermentation was stopped by heating the vessels to 60 ± 2 °C and cooled immediately to room temperature. Finally, the liquid preparation was filtered and packed in the amber coloured glass bottles. The analytical procedures opt for metabolic profiling is described elsewhere in details (Khan et al. 2017).

All other chemicals and reagents used were from other laboratory suppliers and of highest analytical quality available in India.

HPTLC analysis of ZP

500 ml separating funnel (Borosil), rotary vacuum evaporator (Buchi-R300), water bath, sonicator (Trans O Sonic), weighing balance (Sartorius) were used for general experiments. CAMAG Linomat 5 sample applicator, CAMAG TLC Scanner 4 and CAMAG Photodocument chamber were used for HPTLC (High Performance Thin Layer Chromatography) analysis. Solvents such as *n*-hexane, ethyl acetate, *n*-butanol and pre-coated aluminum silica gel $60F_{254}$ TLC plates were procured from Merck specialties private limited. Anisaldehyde reagent was procured from Sigma Aldrich. Reference marker compounds were isolated and identified by Phytochemistry department, R&D Healthcare, Emami Ltd., Kolkata, India.

200 ml of Pancharishta was concentrated in rotavapor at 60 °C. The mass was dissolved in water (150 ml). It was partitioned subsequently in *n*-hexane, ethyl acetate and *n*-butanol. The aqueous solution was partitioned in *n*-hexane (150 ml, 3 times) in separating funnel. The hexane fraction obtained 60 mg. The aqueous portion was fractionated in ethyl acetate (150 ml, three times) and 294.5 mg of ethyl acetate fraction was obtained. Further the aqueous portion was partitioned with *n*-butanol (150 ml, 3 times). 7.402 g of *n*-butanol fraction was obtained. 6-gingerol, Glabridin, Quercetin, Shatavarin IV, Withaferin A, Piperine, Arjunetin, and Glycirrhizic acid are used as phyto-marker.

The chromatographic estimation was performed by spotting standard and fractions of Pancharishta on pre-coated aluminum TLC plates of silica Gel $60F_{254}$ (20 cm × 10 cm, E. Merck, Darmstadt, Germany) using a Linomat V sample applicator and a 100 ml syringe. The samples, in the form of bands of length 8 mm were spotted at a constant application rate of 80 nl/s using nitrogen aspirator. Plates were developed using mobile phase chloroform: methanol: water (20: 7: 1, v/v/v). Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer for 5 min. Then the spots were visualized by dipping the plate in anisaldehyde-sulphuric acid reagent and subsequent heating at 105 °C for 5 min in hot air oven. The slit dimension settings of length 6.00 × 0.30 mm and a scanning rate of 20 mm/s was employed. Densitometric scanning was performed on Camag TLC scanner 4 in absorbance mode at 366 nm and operated by win CATS planar chromatography version 1.4.9.

Animal grouping and treatments

Experimentally naive male Wistar rats are randomly assigned into four groups of six animals each. Group I animals were served as control and was daily treated with normal saline (5 ml/kg/day, orally) for five consecutive days and the remaining three groups were similarly treated with graded oral doses of test formulation (1.5, 3 and 6 ml/kg/day) for 5 days. During the course of an experiment, all animals were closely observed for apparent behavioural abnormalities. On all observational days, the body weights of the animals were recorded 1 h before the oral administration.

Gastric acidity in rats

Gastric acidity in rats was performed by pylorus ligature model described by Shay et al. (1945) with slight modification (Shay et al. 1945; Sur et al. 2013). Following the last treatment schedule, rats from all the groups were starved for a period of 16 h. Individual rat from each group was taken in the dissection room and a midline abdominal incision was made under light ether anaesthesia. The abdomen was opened and the pyloric end of the stomach was ligated with a thread, and the abdominal wall was closed by sutures. Appropriate care has been taken so that no damage to the blood supply or traction on the pylorus occurs. Immediately thereafter, the rats were placed back in their home cages and allowed them to recover from the anaesthesia. After 4 h of pyloric ligation, all the rats were sacrificed using deep anaesthesia. The abdomen was cut opened and another ligature was placed around the oesophagus close to the diaphragm. The stomach was dissected out and its contents were drained into a graduated centrifuge tube and centrifuged at 2000 g. The supernatant obtained was used to access the volume of the gastric content, pH, free and total acid concentration.

Ulcer index

The ulcer index of gastric mucosal lesions was evaluated by the scoring system described elsewhere (Szelenyi and Thiemer 1978). Briefly, after collecting gastric juice, the stomach was opened along the greater curvature and rinsed with 0.1 mol/l ice-cold PBS. The stomach was then examined with the aid of hand lenses (10×) to observe ulcer lesions or erosions. The gastric erosions were counted and the mean ulcerative index was calculated as follows:

A. Petechiae and erosions of < 1 mm size
B. Presence of submucosal hemorrhagic lesions with erosions of 1 to 3 mm size
C. Presence of deep ulcer with erosions of > 3 mm size

$$\text{Ulcer Index} = [A + (B \times 2) + (C \times 3)] \div N$$

N total number of animals

Free and total acidity

From the supernatant 10 µl of gastric juice was pipetted into a 100 ml conical flask. Two to three drops of Topfer's reagent was added and this was titrated with 0.01 N sodium

hydroxide (NaOH) until all traces of red colour disappeared and the colour of the solution became yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Further, two to three drops of phenolphthalein solution were added and titration was continued until a definite red tinge appeared. The total volume of alkali added was noted for total acidity (Dhuley 1999).

Biochemical Estimations The supernatant volume of the gastric content was subjected to biochemical estimation as follows:

Estimation of mucin activity

The glandular portion of the stomach was excised and opened down along the lesser curvature. The averted stomachs were soaked for 2 h in 0.1% alcian blue solution prepared by using 0.16 M sucrose buffered with 0.05 M sodium acetate. The pH of this solution was adjusted with hydrochloric acid. The uncomplexed dye was removed by two successive washes of 15 and 45 min in 0.25 M sucrose solution. The dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The resulting blue solutions were shaken briefly with an equal volume of diethyl ether and the optical density of the aqueous phase was measured at 605 nm using spectrophotometer (Srivastava et al. 2010). The mucin content of the sample was determined from the standard curve of mucin (µg/g of wet gland tissue).

Estimation of pepsin activity

Aliquots of 20 µl of the gastric content were incubated with 0.5 ml of albumin solution (5 mg/ml in 0.06 N hydrochloric acid) at 37 °C for 10 min. The reaction was stopped with 0.2 ml of 10% trichloroacetic acid and the reaction mixtures were centrifuged at 1500 *g* for 20 min. The supernatant was alkalinized with 2.5 ml of 0.55 M sodium carbonate and 0.4 ml of 1.0 N Folin's reagent was added to the tubes, which were incubated for 30 min at room temperature. The absorbance of the samples was measured at 660 nm using spectrophotometer (Srivastava et al. 2010). The concentration of pepsin is determined by a standard curve.

Determination of anti-spasmodic activity in isolated guinea pig's ileum

The anti-spasmodic action of the test formulation was assessed in guinea pig's ileum against histamine. The male guinea pig was anaesthetized and sacrificed by cervical displacement followed by exsanguination. The proximal ileum was removed, washed and placed in a petri dish containing Krebs solution (NaCl 118 mM; $NaHCO_3$ 25 mM; KCl 4.75 mM; KH_2PO_4 1.25 mM; $CaCl_2$ 2.5 mM; $MgSO_4$ 0.108 mM and glucose 12.5 mM). Ileum segments of 2–3 cm were placed in a 15 ml organ bath containing Krebs solution. The bath temperature was maintained at 37 °C and gassed with carbogen (95% O_2 and 5% CO_2). This preparation was set up with an initial tension of 1 g and allowed to stabilize for 30 min before drug application (Samuelson 1991). The efficacy of test formulation was evaluated in three-point assay curve. After the initial equilibration period, histamine (10^{-5} M) was added to the organ bath and the control cumulative concentration-response was noted. Each time the added concentration of the histamine was left in contact with the tissues for 30 s before adding the next concentration. Then the tissue was washed two times with Krebs solution at the interval of 10 min and then allowed to resume its normal position. After a stabilized regular contraction, the tested formulation was added to the bath in a volume of 100 µl at different concentrations (12.5, 25, 50 µl/ml) along with histamine. All the responses were recorded and concentration curve was plotted. The antispasmodic activity of the test formulation was assessed by its ability to prevent the contractions induced by a submaximal concentration (experimentally determined) of histamine (1×10^{-5} M) (van Rossum and van den Brink 1963).

Determination of intestinal transit time

For this test, selected male Wistar rats were divided into four groups of six rats in each. Among four groups, three groups were treated with graded oral doses of test formulation (1.5, 3 and 6 ml/kg/day) for 5 days, similarly control group animals were treated with normal saline (5 ml/kg/day, orally). The food was withdrawn 24 h before measurement of small intestine transit. At first, 1 ml castor oil was given orally in every rat of each group to produce intestinal motility. After 1 h, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) through oral gavage. All the animals were sacrificed 1 h after the charcoal meal administration, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of distance moved. The peristaltic index (PI) was calculated, expressed as percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine (Mittelstadt et al. 2005).

Determination of gastric emptying

This test was done according to the method of Droppleman et al. (1980) with little modification (Droppleman et al. 1980). As described in the previous two rat experiments, similar treatment groups and treatment schedule was maintained for this test. The food was withdrawn for at least 24 h after the last treatment schedule. Rats from each group were received 3 ml of semi-solid test meal containing

methylcellulose by stomach tube. All the animals were sacrificed 1 h after the methylcellulose meal administration and the stomachs removed. The full stomachs were weighed on an analytical balance; they were then cut open and rinsed under running water. Excess moisture was removed by gentle pressing with tissue paper immediately before weighing each empty stomach. The difference between the weight of the full and empty stomach, which is indicative of the amount of meal remaining in the stomach, was subtracted from the weight of 3 ml of test meal to yield the quantity emptied from the stomach during the test period.

Determination of digestive stimulation action

In this test, the experimentally inexperienced rats were maintained in four groups and with similar treatments as described in other rat experiments. The only difference was made in the treatment schedule, where the treatment with test formulation was continued for 2 weeks. At the end of the treatment, the animals were sacrificed under light ether anaesthesia. Intestinal segments were cut open longitudinally and the duodenal contents were collected from first part of the small intestine in ice-cold conditions. The volume of the intestinal contents was measured and used for various enzyme assays (Platel et al. 2002). Standard methods were employed for determining the activities of α-amylase and lipase using commercially available enzymatic assay kits (Coral Clinical Systems, Goa, India) and according to the instructions manual of the test kits.

Data and statistical analysis

Data were expressed as mean and standard error of the mean. Statistical significance between the animals treated with test and control was determined by using paired or unpaired Student's *t*-test or analysis of variance for repeated measures as appropriate. GraphPad Prism-5 (GraphPad Software Inc., California, USA) software was used for statistical analysis. A p value less than 0.05 was considered as statistically significant.

Results

HPTLC fingerprinting of ZP

In total eight major chemical markers have been checked in various fractions of Pancharishta. The hexane fraction consisted of glabridin (*G. glabra*), 6-gingerol (*Z. officinalis*) and piperine (Piper species) as major markers (Fig. 1a). The ethyl acetate fraction consisted of quercetrin and piperine, Shatavarin IV (*A.recemosus*) and glycirrhizic acid (*G. glabra*) are obtained as major compounds in *n*-butanol fraction (Fig. 1b).

Body weight

Mean body weights of different groups of rats recorded are summarized in Table 1. In the test groups, daily oral treatments with the three tested oral doses of test formulation ZP have steadily maintained their body weights during the course of the experiments. These observations were quite analogous to those of the saline treated control animals. However, the tested daily oral doses did not induce body weight gains or losses as compared to their initial body weights.

Gastric ulcers

The calculated gastric ulcer index of the experimental groups is summarized in Table 1. Submucosal hemorrhagic lesions with gastric erosions are observed in the stomachs of control group animals, which are indicative of the gastric ulcers. The gastric ulceration induced by pyloric ligation and physical stress were not observed with the test formulation ZP treated groups and was less severe than the saline treated control animals. The mean ulcer index of the test formulation ZP treated groups was significantly lower than the saline treated control group (ca. 53.3% protection with 6 ml/kg/day dose).

Estimation of pH, free acidity, total acidity and volume of gastric juice

Results summarized in Fig. 2. The analysis of results showed that, the test formulation ZP pre-treatment with a dose of 6 ml/kg caused a decrease in the volume of gastric juice by 44.8%, free acidity 52.2% and total acidity 38.9% when compared to control, which is statistically significant. Pre-treatment with three graded oral doses (1.5, 3 and 6 ml/kg) of ZP showed a dose dependent reduction in the secretion of gastric juice and also free and total acid concentrations. The results are statistically significant by ANOVA test. A significant increase in pH was observed with the ZP treated groups when compared with the control group.

Estimation of mucin and pepsin activity

Results summarized in Fig. 3 shows that pyloric ligation caused increase in gastric juice pepsin activity as well as reduction in mucin content of gastric juice in ulcerated control rats. Pre-treatment with test formulation ZP produce a significant effect in reducing pepsin activity as well as increase in mucin content as compared to control group.

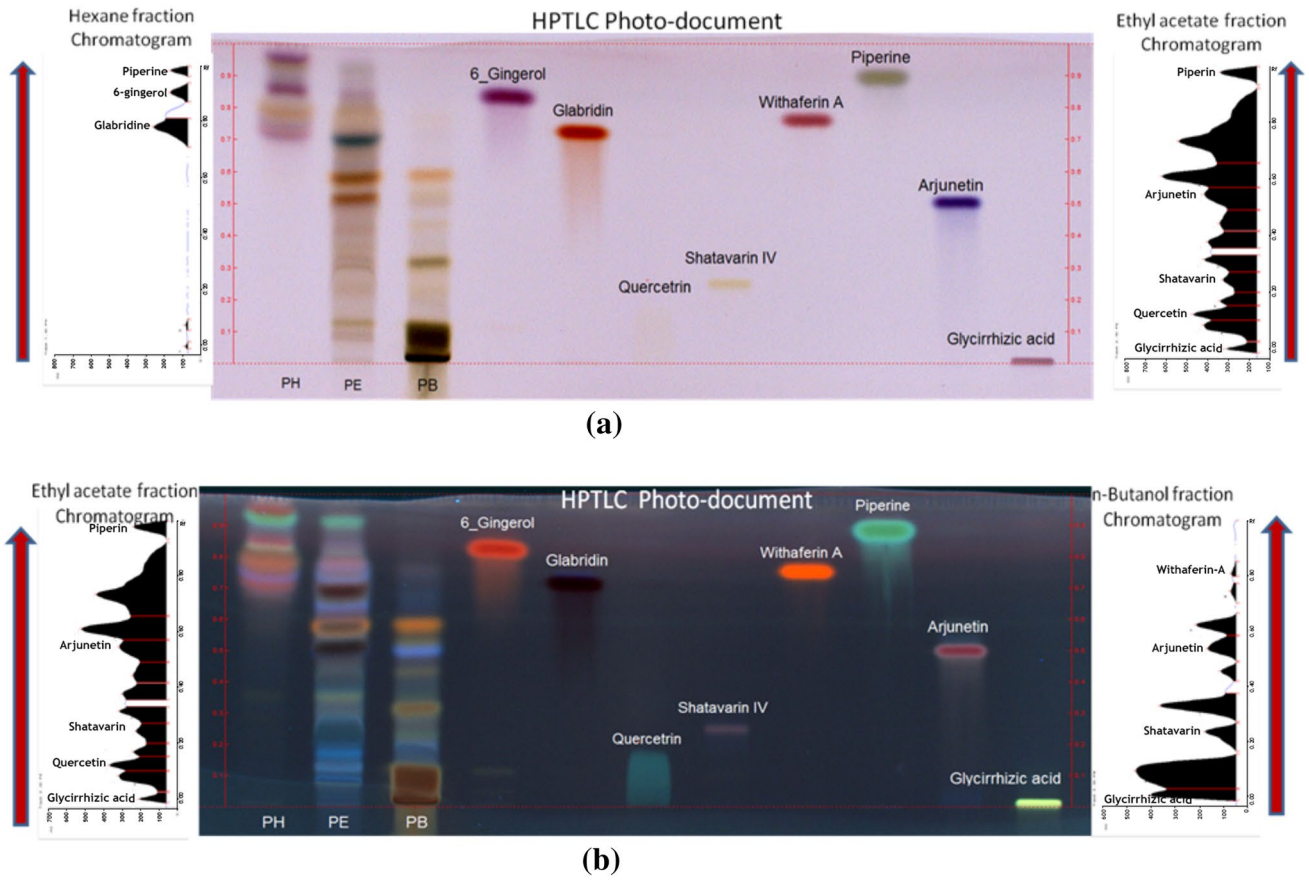

Fig. 1 HPTLC fingerprinting of ZP. The hexane fraction consisted of glabridin (*G. glabra*), 6-gingerol (*Z. officinalis*) and piperine (Piper species) as major markers (**a**). The ethyl acetate fraction consisted of quercetrin and piperine, shatavarin IV (*A.recemosus*) and glycirrhizic acid (*G.glabra*) are obtained as major compounds in *n*-butanol fraction (**b**)

Table 1 Effect of test formulation (ZP) on the body weights and stomach ulceration in male Wistar rats

Treatment groups	Body weight (g)	Ulcer index	Percentage reduction
Control normal saline (5 ml/kg/day)	165.3 ± 2.60	27 ± 0.63	–
ZP (1.5 ml/kg/day)	165.3 ± 4.86	17.3 ± 0.66***	35.9%
ZP (3 ml/kg/day)	166.1 ± 3.62	16.1 ± 0.65***	40.3%
ZP (6 ml/kg/day)	165.6 ± 2.90	12.6 ± 0.49***	53.3%

Values are mean ± SEM (n = 6). Statistically significant difference was denoted with ***$p < 0.001$ versus control group

Anti-spasmodic activity in isolated guinea pig ileum

Results summarized in the Table 2 has revealed the antispasmodic activity of test formulation ZP with respect to guinea-pig ileum contractions induced by histamine. It is evident from the observed effect that histamine acts as an agonist for ileum contraction and the tested formulation (12.5–50 µl/ml) produced a concentration dependent inhibition of the ileum contractions induced by histamine.

Intestinal transit time by charcoal meal

Effect of test formulation ZP on peristaltic index and the percentage reduction in peristaltic movement are summarized in Fig. 4. The percent intestinal transit covered by charcoal meal was increased with castor oil (1 ml) administration in the control group. Such effect was reversed by the pre-treatment with three graded oral doses (1.5, 3 and 6 ml/kg/day) of test formulation ZP and a significant reduction in the gastrointestinal distance travelled by the charcoal meal was observed in all the treatment groups.

Gastric emptying

In this test, pre-treatment with test formulation ZP produced significant and dose dependent increase in gastric emptying as compared to control rats, indicating its therapeutic

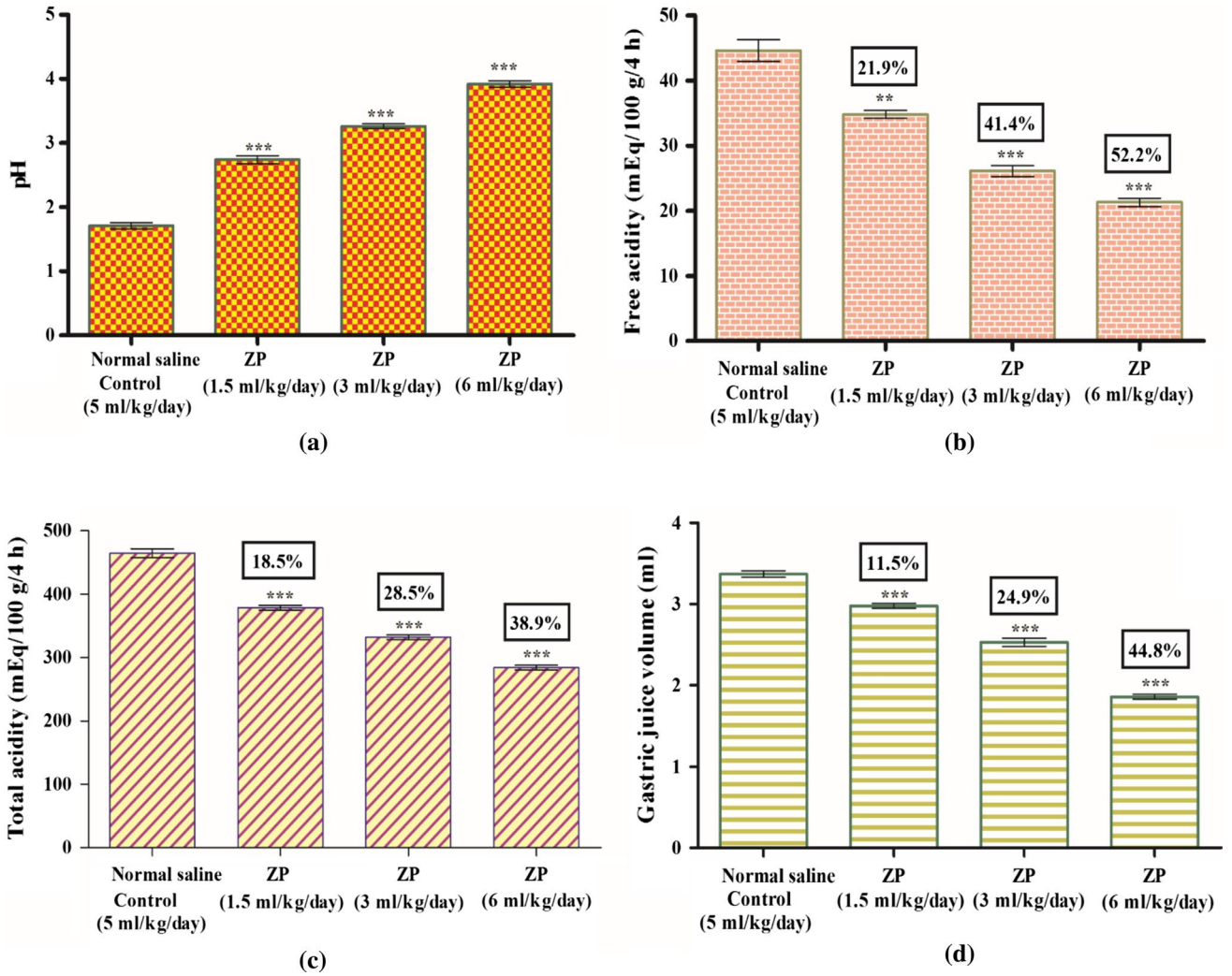

Fig. 2 Effect of test formulation (ZP) on the gastric **a** pH, **b** free acidity, **c** total acidity and **d** volume of gastric juice. Values are mean ± SEM (n = 6). Values denoted in brackets are the percentage reduction effect in comparison to control group. Statistically significant difference was denoted with $^{**}p < 0.01$ and $^{***}p < 0.001$ versus control group

potentials on stomach upsets and pain generally associated with gastroparesis. The percentage gastric emptying of the rats pre-treated with test formulation ZP at doses 1.5, 3 and 6 ml/kg was found to be 34.9, 48.8 and 60.9% respectively (Fig. 4). These values are significantly more as compared to control group.

Stimulatory action on digestive enzymes

In the present study, the influence of test formulation ZP on digestive enzymes of small intestine is presented in Fig. 5. The analysis of results showed that, repeated daily treatment with oral doses (3 or 6 ml/kg) of test formulation ZP caused an increase in α-amylase enzyme activity in the small intestine by 33 and 42.6% respectively as compared to control. It is also observed that, treatment with three graded oral doses (1.5, 3 and 6 ml/kg) of test formulation ZP showed a dose dependent increase in the lipase activity in small intestine.

Discussion

In our present study, the significant reduction in gastric acid secretion and inhibition of ulcers by the polyherbal asava–arishta formulation (ZP) after pylorus ligation suggests that its cytoprotective mechanism of action on the gastric mucosa may be responsible for the direct reduction of gastric acid secretion. Gastric ulcer is a recurrent disease resulting from an imbalance between aggressive factors (hyperacid secretion) and the cytoprotective defence mechanism (Pandit et al. 2000) generally associated *Helicobacter pylori* infection and increased use of ulcer causing

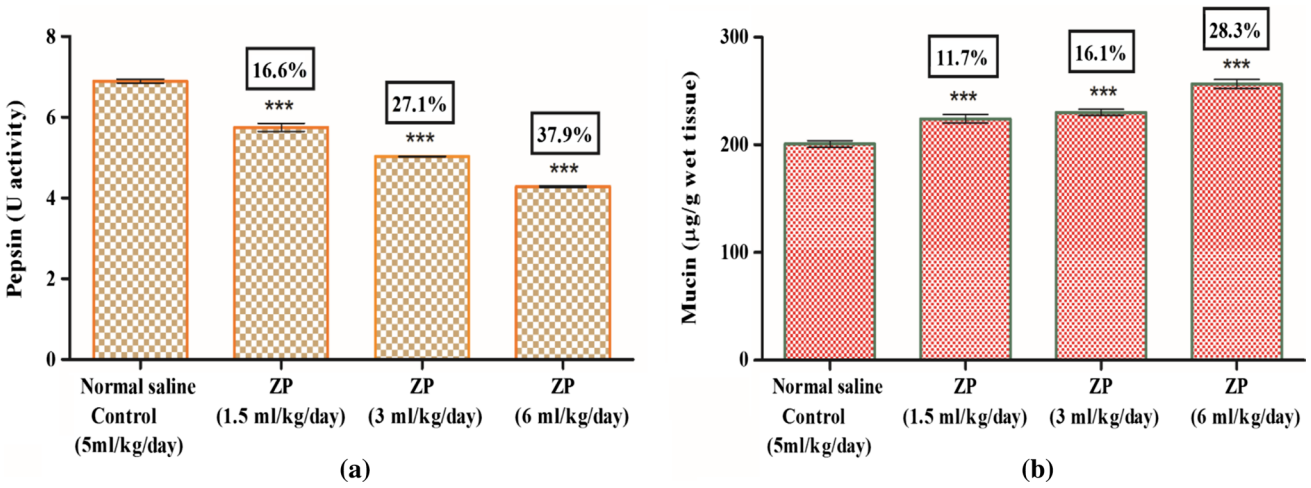

Fig. 3 Effect of test formulation (ZP) on the **a** pepsin and **b** mucin content activity in the gastric juice. Values are mean ± SEM ($n = 6$). Values denoted in brackets are the percentage reduction (Pepsin) and percentage increase (Mucin) effect in comparison to control group. Statistically significant difference was denoted with ***$p < 0.001$ versus control group

Table 2 Antispasmodic activity of test formulation (ZP) with respect to guinea-pig ileum contractions induced by histamine

Treatment groups	Histamine control (10^{-5} M)	ZP (12.5 µl/ml)	ZP (25 µl/ml)	ZP (50 µl/ml)
Percentage inhibition of tissue contraction	100	9.03 ± 0.17***	15.28 ± 0.19***	32.15 ± 0.19***

Values are mean ± SEM (n = 3). Statistically significant difference was denoted with ***$p < 0.001$ versus contractions induced in the presence of histamine (10^{-5} M)

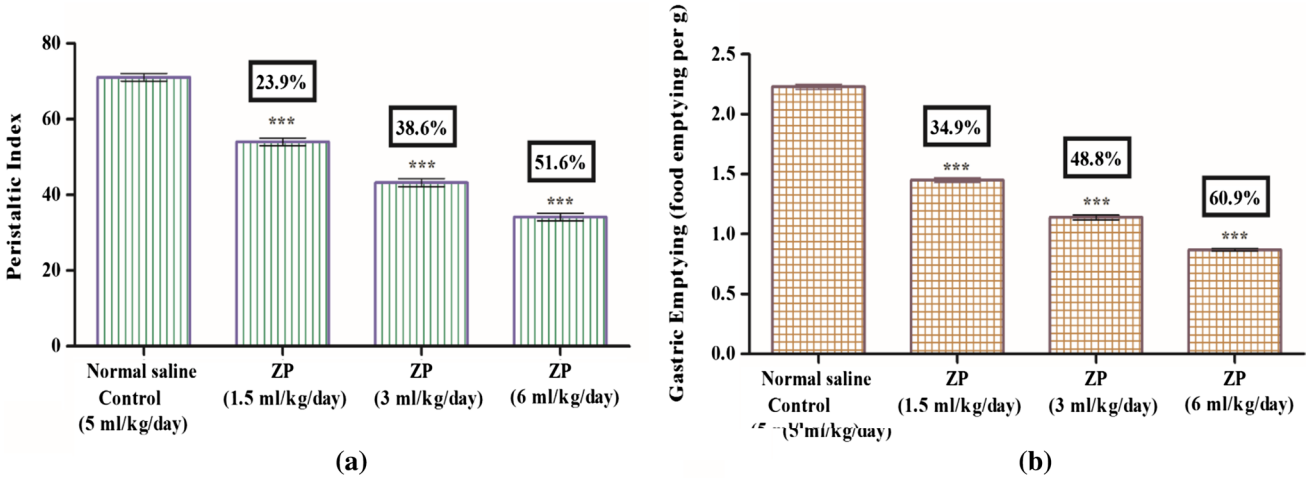

Fig. 4 Effect of test formulation (ZP) treatment on small intestinal transition [peristaltic index (**a**)] and gastric emptying rate (**b**) in male Wistar rats. Values are mean ± SEM (n = 6). Statistically significant difference was denoted with ***$p < 0.001$ versus control group

medication such as non-steroidal anti-inflammatory drugs (Srivastava et al. 2010). Gastric hydrochloric acid has been known for a long time to be a key factor in normal upper gastrointestinal capacities, including protein assimilation, calcium and iron absorption, and in addition, giving some protection against bacterial diseases. However, uncontrollable secretion of gastric acid triggers the prevalence of gastric ulcer (de-Faria et al. 2012). Our results showed the inhibitory effect of the pre-treatment of the drug on gastric juice and acid secretion suggesting a direct inhibition of either H^+/K^+-ATPase pump or histamine-2 receptor or both. This action is attributable to the presence of gastric acid lowering

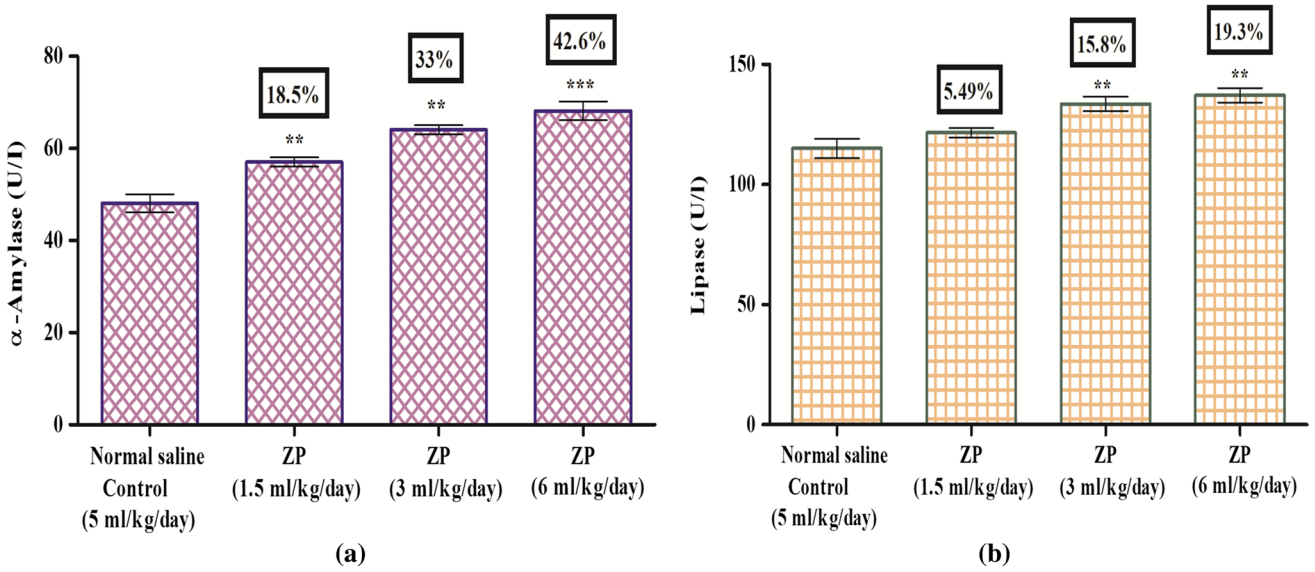

Fig. 5 Effect of test formulation (ZP) treatment on digestive enzymes [**a** α-amylase and **b** lipase] of small intestine. Values are mean ± SEM (n = 6). Statistically significant difference was denoted with $^{**}p < 0.01$ and $^{***}p < 0.001$ versus control group

herbs such as *Asparagus racemosus* (*Shatavari*), *Glycyrrhiza glabra* (*Yasti*), *Coriandrum sativum* (*Dhanyaka*) and *Cuminum cyminum* (*Svetajiraka*) in this blend of asava and arishta (Khan et al. 2017). Our findings are in agreement with the earlier reports that the presence of these herbs not only inhibit gastric acid secretion but also reduced the ulcer index in rats (Jalilzadeh-Amin et al. 2015; Singh and Singh 1986; Al Mofleh et al. 2008).

Further, pre-treatment with the test formulation ZP have increased the pH level and mucin concentration in the gastric juice of pylorus-ligated rats, which indicates its cytoprotective action through mucus production in the gastric mucosa. The mucus consists of mucin-type glycoproteins and can be identified by amounts of alcian blue binding (Corne and Woods 1974). These observations clearly demonstrated that ZP protects the gastric mucosal layer and strengthen the gastric mucosal resistance. Moreover, the progression of peptic ulcer is reduced to a greater extent after its daily use, and it also reduced in gastro-oesophageal reflux and hyperacidity. Due to the fermentation of thirty-five medicinally important herbs (Khan et al. 2017) and presence of self-generated alcohol, the bioactive metabolites present in polyherbal preparation absorbs quickly from the gastro-intestinal tract (Sekar and Mariappan 2008; Mishra et al. 2010), which could be a reason for its quick action.

Additionally, ZP inhibits the intestinal spasm observed in guinea-pig ileum contractions induced by histamine. Abdominal pain and intestinal spasms are common symptoms which are associated with functional gastrointestinal disorders or inflammatory bowel diseases (Makharia 2011). Antispasmodics are generally used for smooth muscle relaxation and to prevent spasms of the stomach and intestine. It is interesting to note that, traditionally used Ayurvedic medicinal herbs such as *Vitis vinifera* (*Draksa*), *Tribulus terrestris* (*Goksura*), *Ailanthus excelsa* (*Aralu*), *Trachyspermum ammi* (*Yavani*), *Piper nigrum* (*Marica*), *Curcuma longa* (*Haridra*) *and Zingiber officinale* (*Shunthi*) are useful in abdominal pain, are also present in ZP. The current data clearly demonstrated that presence of these herbs and their flavonoid and phenolic metabolites (Khan et al. 2017) inhibited the intestinal smooth muscle contractility through inhibition of histamine receptors or other neurotransmitters. It is also claimed that, carminative agents also act by relieving spasms in the intestinal tract. Thus the presence of carminative spices such as *Tvak* (*Cinnamomum zeylanicum*), *Tvakpatra* (*Cinnamomum tamala*) *Lavanga* (*Syzygium aromaticum*), and *Dhanyaka* (*Coriandrum sativum*) in ZP formulation additionally benefits in patients suffering from abdominal spasms and bloating generally associated with digestive dysfunction.

The inhibitory activity of the momentum of a charcoal meal in the rats as well as the effects on the peristaltic index showed by ZP indicates its action on delayed food transit time in the small intestine. During and after a meal, the intestine normally shows very irregular or unsynchronized contractions which move the food content back and forth and mix it with digestive enzymes that are secreted into the intestine (Guyton and Hall 2000). Pre-treatment with ZP showed inhibitory action on the small intestinal motility suggests that the fermented preparation is helpful to hold food content in the small intestine for prolonged enzymatic degradation (digestion) and maximum absorption of nutrients from

food. On the other hand, the reduction in food transit time produced by the test formulation appears to be associated with its beneficial influence either on digestive enzymes or on bile secretion (Guyton and Hall 2000). Thus, the test formulation improves the digestion process and reduces digestive ailments such as acidity, gas, flatulence, and abdominal distension generally associated with indigestion of foods. In addition, pre-treatment with ZP also increases the gastric emptying in rats. In the stomach, foods are grind down to smaller particles and mixed with digestive juices so that food nutrients can be absorbed when it reaches the small intestine (Guyton and Hall 2000). Delay in gastric emptying is generally associated with post-prandial fullness, early satiety and epigastric pain or burning, which are the common symptoms of dyspepsia. Our present study suggests that the test formulation enhances the gastric emptying in a dose dependent and statistically significant manner, indicating its therapeutic action on stomach upsets which are associated with dyspepsia.

Glabridin, 6-gingerol, piperine, quercetrin, Shatavarin IV and glycirrhizic acid phyto-markers has been found in chromatographic separation of ZP. HPTLC analysis of ZP reveals that major compounds present in formulation is saponins (steroidal glycosides). Therapeutic action of ZP may be the presence of these phytochemicals. The role of ZP in digestion is not limited to a single effect but is a combination of their influences on gastric, biliary and pancreatic secretions and the terminal digestive enzymes present on the mucosa of the small intestine. In the present study, 2-week treatment with ZP exhibited stimulatory and secretory actions on intestinal amylase and lipase in rats thus plays a major role in digestion. Amylase is a digestive enzyme that acts on starch in food, breaking it down into smaller carbohydrate molecules, whereas, lipase is an enzyme that breaks down dietary fats into smaller molecules called fatty acids and glycerol.

Conclusion

From the above study, it was found that when compared with control the Ayurvedic asava–arishta preparation showed an effective role in reducing acidity, pepsin secretion and ulcers formation in ulcerative rat models. The polyherbal fermented preparation has the ability to regulate intestinal motilities in isolated intestinal tissues and may be useful in digestive motility related disorders. It also potentiates the gastric emptying and stimulates the digestive enzymes time that may be beneficial for treating indigestion, acidity and abdominal discomfort. As per above analysis of property and benefits of ingredients, the Ayurvedic polyherbal preparation (ZP) is beneficial in controlling the secretion of acid in the stomach, increase digestion process, provide strength to the gastric mucosa and gives relief from digestive ailments. Its effective therapeutic doses need to be adjusted according to the pre-existing allostatic load of patients.

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Compliance with ethical standards

Ethical statement Ethical clearance for animal experimental work was obtained from the Institutional Animal Ethical Committee of the R.G. Kar medical College and Hospital, Kolkata prior to the commencement of experiments (Registration number: R/N 959/C/06/CPCSEA).

Conflict of interest This manuscript described has not been published before; not under consideration for publication anywhere else; and has been approved by all co-authors.

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