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GASTROPROTECTIVE AND DIGESTIVE STIMULANT ACTION OF FORMULATED ENZYME RICH SUPPLEMENT (CARMOZYME) IN ANIMALS

Pharmacology

Anjan Adhikari Department of Pharmacology, R. G. Kar Medical College & Hospital, Kolkata 700004, India

Tapas Kumar Sur* Department of Pharmacology, Institute of Post Graduate Medical Education & Research & SSKM Hospital, Kolkata 700020, India *Corresponding Author

ABSTRACT

Carmozyme, a natural formulated enzyme rich supplement was prepared for dyspepsia, anorexia, gastric ailments, and other GI problems. The present study was design to find out the digestive stimulant actions of Carmozyme on food propulsion, gastric functions and intestinal enzymes secretions in animals. A significant dose dependent (1ml/kg, 2 ml/kg and 3 ml/kg body weight) inhibitory effect on reducing gastric volume, acidity and ulcer index were observed in Carmozyme treated animals with compared to control animals. Carmozyme also enhances the mucin content in gastric juice. Moreover, Carmozyme produced a dose-dependent effect in reducing intestinal motility and gastric emptying time in rats. It also significantly enhanced the enzymatic activities of gastric pepsin and intestinal amylase and lipase. These findings suggest that the use of enzyme rich supplements in treating hyperacidity, indigestion and abdominal discomfort. Thus, it is concluded that Carmozyme improves digestion and reduce digestive ailments.

KEYWORDS

Digestion, Dyspepsia, Gastric function, Intestinal motility, Amylase, Lipase

INTRODUCTION

Indigestion or dyspepsia is a common suffering in every day's life. Proper digestion is not only required for supplying essential energy to the body but also to overcome some deadly gastrointestinal (GI) disorders. GI disorders affects millions of people throughout the world.[1-3] Gastro-esophageal reflux, gastric and peptic ulcers, *Helicobacter pylori* infection and colorectal cancer referred as organic GI diseases, whilst dyspepsia, anorexia, nausea, constipation and irritable bowel syndrome are mentioned as functional digestive disorders.[4-5] Moreover, overproduction of acid is an attempt of stomach to enhance the activation of pepsin for digestion of proteins. This formation of peptic ulcers depends on imbalance between acid and pepsin secretion and also mucosal defense factors.[6] When acid production is in excess, a healthy alternative approach is to improve the diet to make it more digestible, and to improve the digestive process.[7] However, the formations and secretions of important digestive enzymes usually reduced nearly 10% in every decade in adult life. Hence, older people have improper digestive functions due to lack of enzymes and prone to dyspepsia, anorexia, flatulence, heartburn and reflux.[8]

Inadequate secretion of digestive enzymes and hydrochloric acid can be addressed through the use of a multi-digestive enzyme supplement.[9] Searching of new effective supplemental digestive enzymes to enhance the digestive process is a matter of research. Carmozyme is a liquid formulation prepared with natural ingredients like, hydrolyzed casein, fungal diastase, herbal papain, *nux vomica* and cardamom tincture for dyspepsia, anorexia, gastric ailments, food intoxication, intestinal disorders and other dyspeptic syndromes. Previous literatures suggested that casein has protective role in bacteremia and tumor growth;[10-11] diastase, starch splitting enzyme, has reported for the treatment of dyspepsia, epigastric distress and flatulence;[12-13] Papain, protease enzyme (MW of 23,406 DA) has effective in protein indigestion, bowel inflammation, parasitic worms and diarrhea;[14-15] *nux-vomica* (or *Strychnosnux-vomica)* has therapeutic application in constipation, anorexia, gastric dysfunction, hepatic diseases, and diarrhea; [16-18] Cardamom contains essential oils like α-terpineol, myrcene, limonene, menthone, β-phellandrene and has digestive stimulant properties.[19] The role of natural ingredients, like foods, spices, herbs, microbial metabolites in digestion is not limited to a single effect, but is a combination of their influences on gastric and intestinal secretions and the terminal digestive enzymes present on the mucosa of small intestine. In this context, the digestive stimulant action of Carmozyme was studied in food propulsion, gastric functions and intestinal enzymes secretions in animals.

EXPERIMENTAL

Animals− Adult female Swiss albino mice (25-30 g body weight) and adult male Wistar rats (150-175 g body weight) were procured from registered animal vendors M/s Chakraborty Enterprise, Kolkata (Registration number: 1143/PO/b/11/CPCSEA). Animals were allowed to acclimatization for 15 days to laboratory conditions prior to the initiation of treatments. 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 one 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, (Registration number: R/N 959/C/06/CPCSEA) prior to the commencement of experiments.

Test formulation− The test formulation, Carmozyme was obtained from Mendine Pharmaceuticals Pvt. Limited, Kolkata (Mfg. Lic No. DL-396MB, manufacturing date: Jan 15/2018). Carmozyme was prepared with natural ingredients in syrup form. Each 5 ml of Carmozyme syrup contains papain (2.5 mg), hydrolyzed casein (225 mg), diastase (fungal 1:800, 50 mg), *nux vomica* 30% tincture (0.0125 ml) and cardamom 30% tincture (0.075 ml).

Acute toxicity in mice− Carmozyme was given to the 16 h fasted mice (n=3) at three different doses, *i.e.,* 1 ml/kg, 2.5 ml/kg and 5 ml/kg in progressive manner by oral route in a single dose and observed for 3 consecutive days (OECD No. 423).[20] The rate of mortality up to 72h was recorded for the selection of 50% lethal dose of Carmozyme.

Animal grouping and treatments− Adult male Wistar rats (150-175 g body weight) were 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 Carmozyme (1, 2 and 3 ml/kg/day) for five days. The doses were selected on the basis of pilot studies.

Gastric acidity in rats− Gastric acidity in rats was performed by pylorus ligature model described by Shay and his colleagues (1945)[21] with slight modification (Sur et al., 2013).[22] Briefly, after the last treatment schedule as described earlier, rats from all the groups were starved for a period of sixteen hours and anesthetized (thiopental sodium 40 mg/kg, i.p). A midline abdominal incision was made to open abdomen, pyloric end of the stomach was ligated and abdominal wall was closed by sutures. After four hours of pyloric ligation, all the rats were sacrificed using deep anesthesia (thiopental sodium 80 mg/kg, i.p). The abdomen was cut opened and another ligature was placed around the esophagus close to the diaphragm. The stomach was dissected out and its contents were drained into a glass 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.

Estimation of ulcer formation. The stomach was opened along the greater curvature and examined for ulcer. The ulcer formation in the gastric mucosal layer was counted and ulcer index was calculated. The gastric lesions were counted and the mean ulcerative index was calculated as follows: I, presence of edema, hyperemia and single sub-mucosal punctiform hemorrhages; II, presence of hemorrhagic lesions with small erosions; III, presence of deep ulcer with erosions and invasive lesions.[23]

Ulcer Index= (number of lesion I)+ (number of lesion II) X 2 + (number of lesion I) X 3

Estimation of free and total acid. From the supernatant, 10 µl of gastric juice was pipetted into a 100 ml conical flask. Topfer's reagent was added (2-3 drops) and titrated with 0.01N sodium hydroxide until all traces of red color disappeared and the colour of the solution became yellowish-orange. The volume of alkali added was noted for free acidity. Furthermore, 2-3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appeared. The total volume of alkali added was noted for total acidity.[24] The acid formation in 4 hour was calculated by equivalent per 100 g rat.

Estimation of mucin. The averted stomachs were soaked for two hours in 0.1% alcian blue solution prepared by using 0.16M sucrose buffered with 0.05M sodium acetate. Thje uncomplexed dye was removed by two successive washes of 15 and 45 min in 0.25 M sucrose solution. The dye complex with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 hours. 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.[25] The mucin content of the samples were calculated from standard curve and expressed as µg/g of wet tissues.

Estimation of pepsin. Aliquots of 20 µl of gastric content were incubated with 0.5 ml of albumin solution (5 mg/ml in 0.06 N HCl) at 37ºC for 10 min. The reaction was stopped with 0.2 ml of 10% trichloroacetic acid and centrifuged at 1500 g for 20 min. The supernatant was alkalinized with 2.5 ml of 0.55 M sodium carbonate. Thereafter, 0.4 ml of 1.0 N Folin's reagent was added to the tubes and incubated for 30 min at room temperature. The optical density was measured at 660 nm using spectrophotometer.[26] The pepsin content was calculated as unit activity of pepsin.

Determination of intestinal transit time−Adult male Wistar rats (150-175 g body weight) were sub grouped into four (N=6). Three groups were treated with graded oral doses of Carmozyme (1, 2 and 3 ml/kg/day) for five consecutive days, while, control animals received normal saline (5 ml/kg/day, orally). The food was withdrawn 16 hours before test. At day 6, 1 ml castor oil was given orally to all animals to produce intestinal motility. After 1 hour interval, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) through oral gavage. Water was given *ad libitum*. All the animals were sacrificed 1 hour after the charcoal meal given and dissected. The distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as percentage of distance moved. The peristaltic index (PI) was calculated as follows:[27]

Peristaltic index (PI) = [Total length of the small intestine / distance travelled by the charcoal meal] X 100

Determination of gastric emptying− This test was done according to the method of Droppleman *et al.* (1980).[28] Treatment groups and schedule was same as before. Fasting rats from each group were received 3 ml of test meal containing 2% carboxymethyl cellulose (CMC) solution by gavage. All the animals were sacrificed 1 hour 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− Adult male Wistar rats (150-175 g body weight) were grouped as earlier and treated for 2 weeks. At the end of the treatment, the animals were sacrificed under deep anesthesia (thiopental sodium 80 mg/kg, i.p). Intestinal segments were cut open longitudinally and the duodenal contents were collected from first part of the small intestine in ice-cold conditions.[29] The volume of the intestinal contents was measured and used for biochemical estimations of interstitial enzymes, α-amylase and lipase using commercially available enzymatic assay kits (Coral Clinical Systems, Goa, India) and according to the instructions manuals. In brief, α-amylase catalyses the hydrolysis of a 2-chloro-4-nitro phenol salt to chloro nitrophenol and the rate of hydrolysis was measured as an increase in absorbance due to the formation of chloro nitrophenol proportional to the α-amylase activity in the sample. Pancreatic lipase catalyses the hydrolysis of triolein in the presence of colipase to form monoglycerides and fatty acids and the rate of decrease in turbidity measured at 340 nm was proportional to the lipase activity.[30-31]

Statistical analysis− Data were expressed as mean ± SE. Statistical significance was assessed determined by one-way analysis of variance (ANOVA) followed by Dunnett's test using software (SPSS v.20, IBM, Chicago, USA). *P* value less than 0.05 was considered as significant.

RESULT AND DISCUSSION

Carmozyme, an enzyme rich supplement prepared with casein, diastase, papain, *nux vomica* and cardamom tincture was studied in laboratory animals for digestive stimulant and GI problems. Carmozyme did not showed any signs of toxicity or mortality up to 5 ml/kg oral dose in mice. Therefore, 50% lethal dose of Carmozyme was not determined. The dose up to 5 ml/kg orally in mice is safe and practically non-toxic.

GI tract is divided into four distinct parts: esophagus, stomach, small intestine, and large intestine (colon). Each part of the GI tract has a unique function to perform in digestion, and as a result each part has a distinct type of motility and sensation. 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 the digestive enzymes that are secreted into the intestine.[27] GI motility is an integrated process including myoelectrical activity, contractile activity, tone, compliance and transit. Abnormal motility or abnormal sensitivity in any part of GI tract can cause abdominal pain, heartburn, nausea, vomiting, constipation and diarrhea.[32] The time it takes for ingested food to travel through the gut – also called transit time – affects the amount of harmful degradation products produced along the way. This means that transit time is a key factor in a healthy digestive system. It is well evident that castor oil produces hyper motility of GI tract due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion.[33] In the present study, Carmozyme significantly inhibited excessive intestinal motility caused by castor oil. Carmozyme at the dose of 1 ml/kg lowered 26.84% intestinal motility as reflected by Peristaltic Index, whereas, 2 ml/kg lowered 40.31% and 3 ml/kg up to 55.14% (Table 2). The significance of this evaluation is that, Carmozyme not only control the irregular intestinal movements caused by castor oils but also helpful in restore digestion and absorption of food.

The effect of accumulated gastric juice in the induction of gastric/peptic ulcers is well documented in the pylorus-ligation model.[22] In this study, the gastric juice volume was 3.39 ml, pH was highly acidic *i.e.,* 1.72, free acid concentration was 44.33 mEq/100g/4h and total acid concentration was 467.33 mEq/100g/4h (Table 1). Carmozyme significantly and dose dependently lowered the acid concentrations in gastric juice in rats (20-50%). Gastric mucosal cells help to secret hydrochloric acid in the gastric juice for activation of pepsinogen to formation of pepsin, a protease. Excess acid secretion not only intensify gastric lesion but also promotes other GI disorders like, dyspepsia, heart burn, gastroesophageal reflux disease, peptic ulcers etc.[6,26] Carmozyme showed effectiveness in acidity related common GI complications.

Furthermore, the formation of ulcers in control animals was scored 27 within 4 h. The gastric ulcer formation is directly related with the aggregation of acid secretion by gastric cells. Carmozyme pretreatment significantly reduced the gastric ulcers formation in rats from 40.12 to 56.18%. Gastric ulcer index is the single most important factor for assessing pharmacologically active components for acidity related GI problems.

Gastric mucin is a large glycoprotein which is thought to play a major role in the protection of the gastrointestinal tract from acid, proteases, pathogenic microorganisms and mechanical trauma.[25] Carmozyme dose dependently enhanced the mucin content in gastric juice.

Pepsin is an enzyme that works in an acidic environment and is the active form of pepsinogen. Carmozyme significantly enhanced the concentration of pepsin in ligated rats. One of the important ingredients of Carmozyme is papain, a plant derived protease and it may be influenced on enhancing protein digestive activities.

Bowel habits vary daily, especially in patients with functional GI disorders. Epidemiological findings suggest that women are more constipated than are men and that may be due to hormonal cause, especially in women of childbearing age.[34] Carmozyme significantly and dose dependently enhanced the gastric emptying from 44.93 to 67.84% (Table 3). Acidic solutions slow gastric emptying, so, earlier experiments corroborate this finding.

Although dyspepsia doesn't usually have serious complications, it can affect quality of life by making feel uncomfortable.[4] A number of pharmacological preparations available to correct digestive disorders employ certain foods and spices besides other plant substances. There has been a renewed interest on their role in aiding digestion through a stimulatory influence on the activities of enzymes responsible for digestion.[35]

Amylase is a digestive enzyme that acts on starch in food, breaking it down into smaller carbohydrate molecules. Pancreatic amylase completes digestion of carbohydrate, producing glucose, a small molecule that is absorbed into blood and carried throughout body.[36] In the present study, Carmozyme significantly and dose dependently enhances the intestinal amylase enzyme in rats (Table 4). It was increases up to 54.30% after 2 week treatment.

Most of the lipids in diet are in the form of triacylglycerols (TAG). In the digestive tract, TAG is hydrolyzed by pancreatic lipase to release free fatty acids and monoglycerides. Lipid digestion is greatly aided by emulsification with bile salts and phospholipids. Pancreatic lipase acts on these fat globules, converting them into fatty acids and glycerol.[36] In the present study, Carmozyme significantly enhanced the concentration of pancreatic lipase in the rat intestine (Table 4). Intestinal lipase activity was increases up to 37.71% after 2 week treatment. Earlier in ligated model, Carmozyme exhibited pepsin enhancing activity. Hence, it may be inferred that Carmozyme has digestive stimulant actions and may be helpful in dyspepsia and dyspeptic syndromes.

From the above discussion, it may be concluded that enzyme rich formulation, Carmozyme has digestive stimulant and gastroprotective actions and therefore, may be useful in the treatment of dyspepsia, flatulence and other GI functional dysfunctions.

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Table 1. Effect of Carmozyme on gastric acidity in Wistar rats

	Control	Carmozyme (1 ml/kg)	Carmozyme (2 ml/kg)	Carmozyme (3 ml/kg)
Body weight (g)	165±8.41	165.33±7.52	165.16±7.38	165.83±7.13
Ulcer Index	27±1.41	16.1±0.98* [-40.12]	15.1±0.99* [43.85]	11.8±1.32* [56.18]
Volume (ml)	3.39±0.10	2.88±0.07* [-15.05]	2.45±0.13* [-27.72]	1.79±0.15* [-47.19]
pH	1.72±0.11	2.76±0.14* [60.46]	3.52±0.13* [104.65]	4.13±0.07* [140.12]
Free Acid (mEq/100 g/4 h)	44.33±3.50	35.16±3.11* [-20.68]	23.83±3.92* [-46.24]	19.50±2.88* [-56.01]
Total Acid (mEq/100 g/4 h)	467.33±1 9.72	369.66±6.21* [-20.89]	316.66±10.29* [-32.24]	263.33±7.20* [-43.65]
Mucin (µg/g wet tissue)	195.83±6. 55	228.33±4.84* [16.56]	245±4.73* [25.10]	264.5±6.12* [35.57]
Pepsin (U activity)	3.76±0.51	4.90±0.23* [30.32]	5.70±0.28* [51.59]	6.83±0.21* [81.65]

Table 2. Effect of Carmozyme on food transit in Wistar rats

	Control	Carmozyme (1 ml/kg)	Carmozyme (2 ml/kg)	Carmozyme (3 ml/kg)
Body Weight	160.3±3.72	157.6±4.48	159.6±6.65	158.3±4.22
Peristaltic Index	69.62±1.31	50.93±1.82* [-26.84]	41.55±1.43* [-40.31]	31.23±1.44* [- 55.14]

N=6; Wistar Rats; Mean ± SEM; ANOVA followed by Dunnett's test; Data in parenthesis indicate % change when compared to control; * indicate p<0.05 significant

Table 3. Effect of Carmozyme on gastric emptying in Wistar rats

	Control	Carmozyme (1 ml/kg)	Carmozyme (2 ml/kg)	Carmozyme (3 ml/kg)
Body Weight	161.5±4.37	160.5±4.72	161.8±2.14	161.1±2.65
Gastric empty (g)	2.27±0.14	1.25±0.09* [-44.93]	0.89±0.07* [-60.79]	0.73±0.10* [-67.84]

N=6; Wistar Rats; Mean ± SEM; ANOVA followed by Dunnett's test; Data in parenthesis indicate % change when compared to control; * indicate p<0.05 significant

Table 4. Effect of Carmozyme on intestinal digestive enzymes in Wistar rats

	Control	Carmozyme (1 ml/kg)	Carmozyme (2 ml/kg)	Carmozyme (3 ml/kg)
Body Weight	161.6±4.72	160.6±5.43	163.8±7.70	160.6±5.78
α-Amylase	46.6±4.23	58.8±2.31* [26.08]	66.1±1.94* [41.79]	72±2.61* [54.30]
Lipase	114.8±7.62	130.6±5.35* [14.61]	141.1±2.71* [23.82]	157±7.21* [37.71]

N=6; Wistar Rats; Mean ± SEM; ANOVA followed by Dunnett's test; Data in parenthesis indicate % change when compared to control; * indicate p<0.05 significant

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