Original Article

Evaluation of acute toxicity and antiulcer activity of Pepgard tablet: An Ayurvedic formulation

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Abstract

Background: In traditional Indian medicine, several plants have been used to treat gastrointestinal disorders, including gastric ulcers. Pepgard tablet is an Ayurvedic compound formulation widely used in clinical practice as an antacid for treating nonulcer dyspepsia, gastroesophageal reflux, and drug-induced gastritis. **Aim:** Evaluation of acute toxicity of Pepgard tablet and antiulcer activity against gastric ulcer induced by aspirin plus pyloric ligation in albino rats. **Materials and methods:** Acute toxicity was studied as per OECD 425 guideline at a limit dose of 2000 mg/kg in female albino rats. Antiulcer activity was assessed by aspirin plus pyloric ligation model at two dose levels (90 and 180 mg/kg, po). Gastric juice parameters, stomach tissue parameters, and histopathological study along with an assessment of ulcer index were assessed. **Results:** Pepgard did not produce any toxicity or lethality at a limit dose of 2000 mg/kg and was found safe in female albino rats. Pepgard at both dose levels showed an antiulcer effect as evidenced by an increase in pH value, decreased acidity, and peptic activity of gastric juice along with a decrease in ulcer index and increased antioxidant status of damaged gastric mucosa as revealed by an increase in catalase, glutathione, etc., in stomach homogenate of albino rats. **Conclusion:** The present study of Pepgard tablet revealed its safety in acute toxicity studies and can be categorized as substances with low health hazard potential. Pepgard has been shown to be effective as an antacid, anti-ulcer, and to have gastroprotective effects against experimentally-induced ulcerogenesis in albino rats.

Keywords: Acute toxicity, antioxidants, antiulcer, aspirin, mucin, Pepgard tablet

Introduction

The gastrointestinal tract is one of the important systems in the body. Processes such as ingestion, digestion of food, absorption of digested food, and excretion of undigested food are involved in obtaining nutrition. Being an organ for digestion and a major drug administration route, continuous assault on this system leads to diseases and toxic effects. Hyperacidity, gastric ulcer, and gastritis can be considered important stomach diseases.[1] Considering the several side effects of modern medicine, indigenous drugs possessing fewer side effects should be considered a better alternative for treating peptic ulcer.[2]

In traditional Indian medicine, several plants have been used to treat gastrointestinal disorders, including gastric ulcer.[3] Pepgard tablet is one such proprietary compound formulation widely used in clinical practice as antacid for treating nonulcer dyspepsia, gastroesophageal reflux, and drug-induced gastritis. It contains many potential drugs, derived from plant sources such as *Guduchi* (*Tinospora cordifolia* [Willd.] Miers ex Hook. F. and Thoms.),[4] *Amla* (*Embelica officinalis* Gaertn.),[5] *Shatavari* (*Asparagus racemosus* Willd.),[6] *Kadalipatra* (*Musa sapientum* Linn.),[7] *Kapurkachli* (*Hedychium spicatum* Linn.),[8] and *Shankha Bhasma* (calcified conch shell)[9] and is well known for its antacid and gastroprotective effects.

The Pepgard is a combination of herbal and *Bhasma* preparations prepared by considering the Ayurvedic principles in mind and well established in clinical practices. Therefore, it was thought worth to undertake a pharmacological study of compound formulation in the experimental protocol to substantiate the safety and efficacy claims made on it. The present study was aimed to evaluate Pepgard for acute toxicity

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as per OECD 425 guideline and antiulcer activity against gastric ulcer induced by aspirin plus pyloric ligation in albino rats.

Materials and methods

Animal selection

Wistar strain albino rats weighing 200 ± 20 g of either sex were used for the present study. The animals were obtained from the animal house attached to Pharmacology laboratory of Institute of Teaching and Research in Ayurveda, Jamnagar. The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50%–70%, and the ambient temperature during the period of experimentation was 22°C ± 3°C. Animals were fed with VRK brand rat pellet feed supplied by Keval Sales Corporation, Vadodara, and provided with drinking water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/26/2020/12) in accordance with the guideline formulated by the Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

Drug and chemicals

Pepgard tablet is an Ayurvedic formulation supplied by Vital Care Pvt. Ltd., Vadodara (Batch no. 1100 and mfg. date March-2020). Names of ingredients, Latin names, family, part used, and quantity of each drug are given in Table 1. Omeprazole was used as a standard drug (Zydus Healthcare Limited, Ahmedabad, India, Batch no. V900403, August 2019). Aspirin (Reckitt Benkiser [India] Pvt. Ltd., Batch no. JH339, January 2020) was used as a toxicant to induce ulcers along with pyloric ligation.

Dose

The dose of the test drug was calculated by extrapolating the human dose to the animal dose, based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964).[10] The human dose for Pepgard tablet is one or two tablets twice a day (two or four tablets per day, the weight of each tablet is 500 mg). Thus, the lower dose (LD) was calculated for animals as 90 mg/kg, and the higher dose (HD) was 180 mg/kg body weight of albino rats.

Acute oral toxicity study

The study was conducted as per OECD 425 guideline (Limit test) (OECD, 425).[11] Healthy young adult female Wistar albino rats weighing between 200 ± 20 g were used. Pepgard in a dose of 2000 mg/kg (limit dose) was administered to female albino rats in a sequential manner as per OECD guidelines for the limit test. The rats were observed individually once during the first 30 min after dosing and periodically for the 1st 24 h. Special attention was given for the first 8 h and thereafter once daily during the entire period of study of 14 days.

Individual weights of albino rats were recorded before the test drug was administered and weekly thereafter. Animals were also examined for physical and behavioral changes, signs of toxicity, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, and if any animal died during the study, were subjected to gross necropsy and histopathology.

Antiulcer activity

Total 24 animals of either sex weighing between 200 ± 20 g were divided into 4 groups (6 animals in each group), namely, (I) control group, (II) Pepgard LD, (III) Pepgard HD, and (IV) standard group. Animals of the control group (I) received distilled water (10 ml/kg). Pepgard-treated groups (II and III) received drug at a dose of 90 and 180 mg/kg, respectively, and the standard group (IV) received omeprazole (20 mg/kg) orally.

The test drug was administered orally once daily for seven consecutive days to the respective groups and water to the control group. Gastric ulceration in rats was induced as described by Nariya *et al.* (2013).[12] Aspirin suspension in 1% (Na-CMC) sodium-carboxy methyl cellulose in water was administered 1 h after each of drug administration in a dose of 200 mg/kg, orally once daily for the last 3 days. The aspirin and omeprazole administration started from the 5th day of drug administration. Animals were transferred to single metabolic cages to prevent coprophagy.

On the 7th day, 1 h after aspirin administration, pylorus was ligated as per the method of Shay *et al*.[13] The animals were deprived of both food and water during the postoperative period and were sacrificed at the end of 6 h after pyloric ligation. The gastric contents were drained carefully into tubes

Table 1: The ingredients of Pepgard tablet (each 500 mg)

Ingredients	Latin name	Family	Part used	Quantity (mg)
Sajjikhara	*Astoneman indicum* Linn.	*Dipterocarpaceae*	Whole plant	125
Shankhabhasma	Calcified Conch shell	-	-	125
Yashtimadhu	*Glycyrrhiza glabra* Linn.	*Fabaceae*	Root	25
Amalaki	*Embelica officinalis* Gaertn.	*Euphorbiaceae*	Fruit	25
Guduchi	*Tinospora cordifolia* (Willd.) Miers ex Hook.F. and Thoms.	*Menispermaceae*	Root	25
Pippalimoola	*Piper longum* Linn.	*Piperaceae*	Root	25
Shatavari	*Asparagus racemosus* Willd.	*Liliaceae*	Root	25
Kadali	*Musa sapientum* Linn.	*Musaceae*	Fruit	50
Kapurkachali	*Hydechium spicatum* Linn.	*Zingiberaceae*	Rhizome	30
Excipients				45

and centrifuged at 3000 rpm for 15 min. Volume and pH of gastric juice was noted and used for biochemical estimation. The gastric juice was estimated for its volume, pH, acidity,[14] total carbohydrate (TC) (hexose,[15] fucose,[16] hexosamine,[17] and sialic acid[18]), total protein (TP),[19] mucin activity (TC: TP ratio),[20] and peptic activity.[21]

For the assessment of ulcer index, severity of ulcer and total number of ulcers in each rat were recorded. The stomach was excised, cleaned, and opened along its greater curvature and examined for ulceration.[22] After assessment of the ulcer index, the glandular portion of the stomach was used for the estimation of various biochemical parameters such as TC (hexose,[15] fucose,[16] hexosamine,[17] and sialic acid[18]), TP,[19] mucin activity (TC: TP ratio),[20] catalase,[23] lipid peroxidation (LPO),[24] total glutathione (GSH),[25] GSH peroxidase (GPx),[26] myeloperoxidase (MPO),[27] and nucleic acid content[28] (DNA and RNA). For the histopathological study, a portion of the stomach was fixed in 10% buffered formalin solution prior to dehydrating, wax embedding, sectioning, and staining with hematoxylin and eosin, for histological evaluation of gastric damage by light microscopy.[29]

Statistical analysis

The data were expressed as the mean standard error of the mean for six rats per experimental group. One-way analysis of variance was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple "*t*-test and Student's "*t*-test using Sigma Stat software to determine significant differences between groups at $P < 0.05$.

Results

Acute toxicity

In the acute toxicity study, no significant gross behavioral changes were seen in Pepgard-treated group during the experimental study period. Further, the drug did not show any observable signs and symptoms of toxicity when given orally at a dose of 2000 mg/kg in a sequential manner in female Wistar albino rats. No any mortality was observed in the Pepgard-treated group and all female rats were survived and found healthy during 14 days of the observation period.

Antiulcer activity

Pretreatment with Pepgard showed a statistically non-significant decrease in ulcer index, while the non-significant increase in volume and pH of gastric juice was in comparison to the control group. Omeprazole-treated group showed a significant decrease in ulcer index and volume of gastric juice, while there was an increase in pH when compared to the control group. There was a significant decrease in total acidity in Pepgard at both dose levels and in omeprazole-treated groups in comparison to the control group.

TP and Peptic activity were also observed to be significantly decreased in drug-treated groups, while a non-significant decrease was seen in the omeprazole-treated group as compared to the control group. TC content was significantly decreased in Pepgard HD, while LD and the standard drug produced a non-significant increase in comparison with the control group. Pepgard at a HD and omeprazole showed a non-significant increase, whereas Pepgard LD produced a significant increase in mucin activity (TC: TP ratio) of gastric juice in comparison with the control group. [Table 2]

Pretreatment with Pepgard (90 and 180 mg/kg) and omeprazole produced a significant increase in hexosamine but had no effects on total hexose, fucose, and sialic acid levels. A non-significant increase in TC level while, a significant increase in mucin activity (TC: TP ratio in gastric tissue of albino rats) was observed in drug-treated groups in comparison with the control group. [Table 3]

Catalase activity was non-significantly increased in drug-treated groups when compared with the control group. LPO and MPO levels showed no significant effect on these parameters in drug-treated groups at both dose levels and omeprazole-treated

Table 2: Effect of test drugs on ulcer index and gastric juice parameters

Parameters	Control	Pepgard, LD	Pepgard, HD	Omeprazole
Ulcer index	8.0±1.125	6.667±1.33	5.50±2.377	1.429±0.528$^{@@}$
Gastric juice parameters				
Volume (mL/100 g/6 h)	5.00±0.69	7.55±1.37	5.75±0.79	2.91±0.35*
pH	2.53±0.19	2.71±0.25	2.70±0.26	4.76±0.29$^{@@}$
Gastric acidity (mEq/L)	60.33±5.09	36.00±2.36	41.00±1.34	34.00±3.50$^{@@}$
Pepsin (µ moles tyrosine released/mL/min)	266.8±39.97	86.92±17.8$^{@@}$	99.90±25.91$^{@@}$	179.48±45.07
Total hexose (µg/mL)	66.89±4.19	90.24±5.33**	91.13±14.46	119.86±9.86$^{@@}$
Total fucose (µg/mL)	155.23±12.6	116.59±13.1	77.75±9.13$^{@@}$	116.35±15.74
Hexosamine (µg/mL)	262.34±43.87	308.02±20.8	200.61±16.21	260.18±48.98
Sialic acid (µg/mL)	17.44±3.13	12.33±1.68	6.88±0.58$^{@}$	17.33±0.91
Total carbohydrates (µg/mL)	501.91±46.71	527.18±22.35	376.39±17.12*	513.73±65.05
TP (µg/mL)	801.55±47.41	488.35±92.28$^{@}$	469.53±51.8$^{@@}$	669.01±45.79
TC:TP ratio	0.586±0.073	1.079±0.137$^{@@}$	0.850±0.098	0.759±0.049

*P<0.05, **P<0.01, when compared with control group (unpaired *t*-test), $^{@}P$<0.05, $^{@@}P$<0.01, when compared with control group (ANNOVA followed by Dunnett's multiple *t*-test). Mean±SEM (n=6). TC: Total carbohydrate, TP: Total protein, LD: Lower dose, HD: Higher dose, SEM: Standard error of mean

group in comparison to the control group. Total GSH, GPx, and nucleic acid contents nonsignificantly increased in drug-treated groups when compared to the control group. [Table 4]

Histopathological studies showed that the stomach tissue from aspirin plus pyloric ligated control rats showed severe epithelial destruction, blood spot, and submucosal edema. Pepgard at both dose levels reduced the severity of adverse changes in the cytoarchitecture of the stomach. The standard drug, omeprazole, showed only epithelial erosion and mild adverse changes as compared to the control group.

Discussion

In an acute toxicity study, Pepgard at the dose level of 2000 mg/kg orally did not produce any mortality in any of the treated rats, which suggests Lethal Dose 50 (LD50, i.e., the dose at which 50% of the population dies) value of the drug to be higher than 2000 mg/kg and is safe for oral administration. Further, the test drug did not produce any behavioral changes or toxic/adverse effects during the entire duration of the study and all animals survived during 14 days of observation. As per UN classification, any substance which has oral LD_{50} of more than 2000 mg/kg is considered low hazard potential (Class 4 of Globally Harmonized system of classification and UN 6. 1 PG III). Thus, as per the above criterion, Pepgard can be categorized as a substance with low health hazard potential.

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms.[12] Many different substances are found to have gastroprotective effects, but few are shown to accelerate ulcer healing.[30] Aspirin is a well-known agent documented with consistent production of ulcers in the stomach of albino rats.[31] Aspirin causes a dose-dependent reduction in mucosal prostaglandins, PGE-2 and PGI-2 accompanied by an increase in the mean area of gastric ulcerations.[32,33] Therefore, in the present study, Pepgard was studied against ulceration induced by aspirin plus pyloric ligation in the albino rat. The effects of Pepgard at two dose levels were assessed on ulcer index, gastric juice, and gastric tissue parameters and histopathological study of stomach tissue.

Ulcer index is an important parameter, which may help assess the antiulcerogenic efficacy of the drugs. As expected standard drug, omeprazole produced significant decrease in the severity of ulceration confirming that the protocol employed for ulcer production has good predictability for antiulcer activity. Treatment with Pepgard decreased the incidence of ulcer in dose-dependent manner; however, the values not reach to significant extent in comparison to the control group. This may reflect the presence of cytoprotective activity in the test formulations.

Pepgard shows increase in gastric volume in comparison to the control group, while standard group shows a significant decrease in gastric volume. This indicates that the test drug did not possess antisecretory activity. Pretreatment with Pepgard at both dose levels showed a statistically nonsignificant elevation in pH of gastric juice. Acidity can be decreased by either antisecretory effect that is to reduce the gastric juice secretion or the drug should neutralize the gastric acidity.[34] The animals have continuous acid secretion, plus aspirin administration, and the ligation of pylorus leads to the accumulation of acid in one place causing

Table 3: Effect of test drugs on gastric tissue homogenate parameters

Groups (μg/g)	Control	Pepgard, LD	Pepgard, HD	Omeprazole
Total hexose	102.19±7.06	108.64±6.7	106.39±7.18	93.82±4.78
Total fucose	20.40±1.23	19.84±2.28	20.13±1.19	19.17±1.24
Hexosamine	98.51±10.92	153.70±13.62$^{@}$	187.03±18.66$^{@@}$	145.67±17.25*
Sialic acid	21.99±3.57	14.55±1.49	17.38±3.62	23.16±3.21
TC	242.91±13.94	296.74±14.33	330.95±19.01	282.13±20.13
TP	63.95±3.40	55.52±3.48	65.69±3.96	51.85±2.19$^{@}$
TC: TP ratio	3.60±0.286	5.49±0.515$^{@}$	5.14±0.409*	5.49±0.504$^{@}$

Mean±SEM (n=6); *P<0.05, when compared with control group (Unpaired t-test), $^{@}P$<0.05, $^{@@}P$<0.01, when compared with control group (ANNOVA followed by Dunnett's multiple t-test). TC: Total carbohydrate, TP: Total protein, LD: Lower dose, HD: Higher dose, SEM: Standard error of mean

Table 4: Effect of test drugs on gastric tissue homogenate parameters

Groups	Control	Pepgard, LD	Pepgard, HD	Omeprazole
Catalase (μmoles H_2O_2 consumed/min/mg protein)	0.733±0.117	1.060±0.149	0.759±0.67	0.622±0.049
LPO (μ mole MDA/g)	5.441±0.542	5.127±0.117	5.618±0.274	5.414±0.398
MPO (Unit/g tissue)	24.51±1.675	24.12±0.961	24.61±0.669	22.45±0.513
GSH (μ moles/g tissue)	422.72±89.33	483.91±110.49	539.53±134.59	550.66±77.13
GPx (μmoles of GSH utilised/min/mg protein)	4.60±0.16	8.62±1.28*	5.32±0.46	5.096±0.37
DNA (μg/g tissue)	217.39±16.89	246.74±37.02	241.30±39.77	227.17±21.91
RNA (μg/g tissue)	3004.32±499.6	3101.5±452.39	3378.56±470.12	2996.1±383.84

Data: mean±SEM (n=6); *P<0.05, when compared with control group (unpaired t-test), LD: Lower dose, HD: Higher dose, LPO: Lipid peroxidation, GSH: Total glutathione, GPx: Glutathione peroxidase, MPO: Myeloperoxidase, SEM: Standard error of mean, MDA: Malondialdehyde

ulcer formation. Thus, the drug may not possess antisecretory activity, but it may have antacid properties in neutralizing the acidity. The results obtained indicate a nonsignificant decrease in the acidity of gastric juice in Pepgard-treated groups and a significant decrease in omeprazole-treated groups in comparison with the control group.

Due to any damage or ulceration in the stomach, leakage of plasma protein into gastric juice is observed[35] and a decrease in the protein content of the gastric juice can be taken as an index of decreased leakage. In the present study, a statistically significant decrease in TP content in gastric juice as well as in stomach homogenate was observed in Pepgard-treated groups in comparison to the control group. Gastric juice is a multicomponent secretion; the peptic activity of gastric juice parallels acid output in the stomach. The inhibition of this activity is of prime importance in the pharmacotherapy of peptic ulcer disease.[36] In this study, a statistically significant decrease in the peptic activity of gastric juice was observed in Pepgard-treated groups at both dose levels.

The status of the mucin secretion was evaluated by quantifying different fractions of mucus substances and determining TC:TP ratio. The glycoproteins are of important for their specific properties such as gel formation and viscosity. TC:TP ratio is considered a reliable marker for cellular mucus secretion status.[7] Pretreatment with Pepgard at a HD showed a nonsignificant increase, while a LD produced a significant increase in TC:TP ratio of gastric juice and thus more mucin activity in comparison with the control group. The observed effects contribute to the antiulcer activity of Pepgard tablet.

Free radicals are detrimental to the integrity of biological tissue and mediate their injury. Oxidative damage of the gastric mucosal cell membrane by reactive oxygen species (ROS) is the major contributing factor to gastric ulceration. Increased production of ROS causes a decrease in membrane permeability, activities of enzymes and receptors, and activation of cells.[37] Catalase is one such free-radical scavenging enzyme that scavenges the ROS.[38] Catalase activity was increased nonsignificantly in Pepgard-treated groups, indicating the cytoprotection nature of drug.

The previously published literature data indicate that there is an important relationship between gastric GSH levels and ulcer severity. In tissue, GSH and GSH-related enzymes are accepted as important protective agents due to their antioxidant properties,[39] and both have a protective function against hydrogen peroxide and all lipid peroxidase which damage the gastric mucosa.[40] Lipid peroxidase is an enzyme that oxidizes the polyunsaturated fatty acids which are normally constituted of cellular and subcellular membranes, thus making them susceptible to the preoxidative attack, which leads to degradation and loss of structural and functional integrity of cell membrane.[41]

MPO is an enzyme whose activity is considered a quantitative measure of neutrophil inflammatory response in a variety of clinical and experimental studies.[27] The present study discloses that Pepgard tablet did not have any influence on the evaluation of LPO and MPO levels in gastric tissue homogenate of rats, whereas on assessment of GSH and GPx levels, it showed a non-significant elevation in the gastric tissue homogenate of Pepgard-treated and standard groups.

The assessment of the amount of DNA and RNA in the gastric wall mucosa indicates the increase or decrease in the life span of mucosal cells;[7] therefore, a non-significant increase in DNA content of Pepgard-treated groups and that of RNA content in the HD-treated group of it indicates decreased cell shedding and increased life span of cells, which may corroborate with the histopathological study of stomach tissue that reveals the cytoprotective activity of Pepgard tablet in albino rats.

Most of the drugs of Pepgard tablets are possessing free-radical scavenging activity and restoring the antioxidant level may be due to the presence of phytoconstituents such as phenolic, glycoside, tannin, and alkaloid. The phytoconstituents such as glycyrrhizic acid present in *Yashtimadhu (Glycyrrhiza glabra* Linn.*)* are proven for its ulcer healing property, which is an important ingredient of Pepgard.[42] *Kadali (Musa sapientum* Linn.*),* a major constituent from Pepgard contains pectin and phosphatidylcholine along with other chemicals constituents and is reported for strengthening the mucous phospholipid bilayer that protects the gastric mucosa from the ulcer.[43] It is also reported that a natural flavonoid from unripe banana pulp, leucocyanidin, protects the gastric mucosa from erosions.[44] The calcium content in the form of *Shankha Bhasma* present in the formulation is responsible for gastric stimulation[45] and may be the cause for the increase in pH value.

In addition, the saponins and tannins, the active constituents of Pepgard, are known to affect the integrity of the mucus membrane. Tannins with their protein precipitating and vasoconstriction effects could be advantageous in preventing ulcer development. Tannins also being astringent may have precipitated microproteins on the site of the ulcer thereby forming an impervious protective pellicle over the lining to prevent toxic substances and resist the attack of proteolytic enzymes.[46]

Conclusion

Pepgard tablet has significant antacid, antiulcer, and gastroprotective activity in gastric ulcers-induced by aspirin plus pyloric ligation in albino rats. Pepgard can be categorized as substances with low health hazard potential and is having antacid and antiulcer activity. The observed cytoprotective activity may be due to presence of various phytoconstituents in Pepgard tablet such as phenols, glycoside, tannin, and alkaloid.

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Conflicts of interest

There are no conflicts of interest.

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