FORM 2

THE PATENTS ACT 1970
(Act 39 of 1970)
&
THE PATENTS RULE, 2003

COMPLETE SPECIFICATION

(Section 10 and Rule 13)

TITLE OF THE INVENTION

"Floating Bilayer Tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits"

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THE FOLLOWING SPECIFICATION DESCRIBES THE NATURE OF THE INVENTION AND THE MANNER IN WHICH IT IS TO BE PERFORMED

TECHNICAL FIELD OF INVENTION:

The present invention relates to the Floating Bilayer Tablet of Amoxicillin trihydrate and the aqueous extract of *Benincasa hispida* fruits and preparation method thereof and more particularly to the bilayer floating tablet of Amoxicillin trihydrate and the aqueous extract of *Benincasa hispida* fruits for the treatment of peptic ulcers.

BACKGROUND

Ulcers are perforations in the lining of the upper gastrointestinal tract (GI tract). Peptic ulcer is a sore in the lining of stomach or duodenum due to attack by acid & pepsin. While acid is still thought to have a role in ulcer formation, the most common cause of ulcer illness is now thought to be a stomach infection caused by the bacteria *Helicobacter pylori*.

Various types of treatments are available in the market for treating peptic ulcer in the form of oral medications. Traditional oral dosage forms, such as tablets and capsules, provide a specific drug concentration in systemic circulation that does not release at a constant rate for a long time and does not account for site- specific absorption rates within the gastrointestinal tract. As a result, there is a need to develop dosage forms that release the drug at the right time and at the desired rate at the right site.

Floating Drug Delivery System is considered suitable for treating different types of gastrointestinal tract infections as it overcomes the difficulties like gagging or choking while swallowing medicinal pills. Bilayer floating tablet remains a novel approach designed for the effective advance of controlled delivery design laterally through numerous sorts to deliver an approach of efficacious drug transport system. Bilayer floating tablet has advantages like Enhanced bioavailability, Targeted therapy for ailments in the upper GIT, Reduces fluctuation of drug concentration, Minimise adverse activity at the colon site.

Few patent and non-patent literature has been retrieved that discloses bilayer tablet for the treatment of peptic ulcers includes: Indian patent application no. 2117/MUM/2014 discloses floating dosage form containing Ciprofloxacin (Antibacterial) & Misoprostol (Synthetic Prostaglandin E1 (PGE1) Analog) to serve as antibacterial and also prevent gastric ulcer.
AAPS PharmSciTech. 2012 Dec; 13(4): 1518–1523; Arati N. Ranade, Sonali S. Wankhede, Nisharani S. Ranpise, and Mayur S. Mundada discloses development of a bilayer floating tablet of amoxicillin and Aloe vera gel powder for the treatment of peptic ulcer. In Manish A.

Rachchh et al (2008) The antiulcer activity of *Benincasa hispida* (Thunb.) fruits is evaluated in rats against ethanol-induced gastric mucosal damage, pylorus ligated (PL) gastric ulcers, and cold restraint-stress (CRS)-induced gastric ulcer models.

However, no attempt is made of combining amoxicillin and aqueous extract of *Benincasa hispida* fruits in the form of a bilayer tablet.

OBJECTS OF THE INVENTION

Some of the objects of the presently disclosed invention, of which at the minimum one object is fulfilled by at least one embodiment disclosed herein are as follow:

a) An object of the present invention is to develop a floating bilayer tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits.
b) Another object of the present invention is usage of floating bilayer tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits for treating peptic ulcer.

SUMMARY OF THE INVENTION

The present invention relates to the Floating Bilayer Tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits and preparation method for the treatment of peptic ulcer. The bilayer floating tablet comprises of Amoxicillin trihydrate, aqueous extract of Benincasa *hispida* fruits, a polymer, effervescent agent, lubricant, glidant and compression aid element.

Polymer is selected from the group containing Hydroxypropyl methylcellulose (HPMC), sodium alginate, gelatin, chitosan, cellulose derivatives, semisynthetic polymers, synthetic polymers.

Effervescent agents selected from the group sodium bicarbonate, sodium carbonate, adipic acid, malic acid, tartaric acid, ascorbic acid, fumaric acid, maleic acid, succinic acid, or citric acid.

Lubricant is selected from talc, silica, fats, e.g. vegetable stearin, magnesium stearate or stearic acid.

Glidant is selected from talc, colloidal silicon dioxide, magnesium stearate, and silica.

Binders are selected from the group Avicel PH 102, cellulose, gelatin, cellulose derivatives, polyvinyl pyrrolidone, starch, sucrose, mannitol, polyethylene glycol, and liquid glucose.

Process for preparation of floating bilayer tablet includes: preparation of aqueous extract of *Benincasa hispida* fruits; pre-formulation studies of aqueous extract of *Benincasa hispida* fruits with respect to determination of extraction yield, organoleptic properties, phytoconstituents and acid neutralizing capacity; evaluation of formulation of Bilayer floating tablet.

Preparation of Floating Bilayer Tablet: Round shaped flat punch with diameter 1.5 cm is used to punch the tablets. Preparation of powder blend includes:
First layer: Direct compression method is used to prepare bilayer tablet. Amoxicillin, HPMC K4M, HPMC K100M, $NaHCO_3$, Citric acid are passed through sieve no #40. All the ingredients are geometrically mixed in mortar to obtain a homogenous blend. Magnesium stearate and talc are added to the above mixture. The blend 1 is compressed at low pressure on tableting machine to form first layer with amoxicillin trihydrate drug.
Second layer: Aqueous extract of *Benincasa hispida* fruits and Avicel PH 102 are mixed together in mortar and pestle to form homogeneous mixture. Then the blend 2 is compressed on blend 1 to form the bilayer tablet.

Various evaluation parameters are studied for the characterization of floating bilayer tablets. The floating bilayer tablet is optimized on the basis of floating lag time and total floating time. The formulation containing HPMC K4M and HPMC K100M of 85:15 and effervescent agent sodium bicarbonate and citric acid 4:1 is found to give satisfactory release. It shows 89.36% drug release within 8 hours. It is subjected to mathematical model fittings to find out the drug release mechanism. On the basis of coefficient regression value, 0.9578, Higuchi model is the best fit model for the formulation. ‘n’ value was found to be 0.518 which signifies that it shows release pattern of non Fickian type (i.e., the release pattern was govern by more than one process). In weight gain and water uptake study it has been observed that swelling increased up to 4-5 hours but after that it decreased. In first 4-5 hours water is absorbed by the polymer and 17% weight gain by tablet is seen.

From in vivo study it is concluded that powdered aqueous extract of *Benincasa hispida* fruits and amoxicillin trihydrate have gastric acid inhibitory properties in addition to gastroprotective activity. Antiulcer activity is studied separately only for aqueous extract of *Benincasa hispida* fruits which shows it has anti-inflammatory activity along with anti-ulcer activity which suggests its usage in peptic ulcer treatment.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWING

The present invention will now be described with the help of the accompanying drawings, in which:

FIG 1: illustrates Pictorial Representation of the tablet

FIG 2: illustrates Pictorial presentation of Stomach tissue of rat of group I to VI

FIG 3: illustrates Histopathological studies of rat stomach: Group I (a-g), Group II (b-h), Group III (c-i), Group IV (d-J), Group V (e-k) and Group VI (f-l)

DETAIL DESCRIPTION OF THE INVENTION

The present invention relates to the Floating Bilayer Tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits and preparation method for the treatment of peptic ulcer. The bilayer floating tablet comprises of Amoxicillin trihydrate, aqueous extract of Benincasa *hispida* fruits, a polymer, effervescent agent, lubricant, glidant and compression aid element.

Polymer is selected from the group containing Hydroxypropyl methylcellulose (HPMC), sodium alginate, gelatin, chitosan, cellulose derivatives, semisynthetic polymers, synthetic polymers.

Effervescent agents selected from the group sodium bicarbonate, sodium carbonate, adipic acid, malic acid, tartaric acid, ascorbic acid, fumaric acid, maleic acid, succinic acid, or citric acid.

Lubricant is selected from talc, silica, fats, e.g. vegetable stearin, magnesium stearate or stearic acid.

Glidant is selected from talc, colloidal silicon dioxide, magnesium stearate, and silica

Binders are selected from the group Avicel PH 102, cellulose, gelatin, cellulose derivatives, polyvinyl pyrrolidone, starch, sucrose, mannitol, polyethylene glycol, and liquid glucose.

Preparation of Aqueous extract of *Benincasa hispida* fruits:

As the first step cuticle of the *Benincasa hispida* fruits is removed and seeds are separated. The pulp is grinded by an electric mixer. Further, the pulp is dried under shade for 1 day and at room temperature for 1 day to obtain in powder form. The powder is first defatted with 250 ml petroleum ether for 50 mg of powder by the soxhlet apparatus for 24 h. The defatted powder is then dried and extracted with 250 ml of ethyl acetate for 24 h and thereafter with 250 ml methanol for 24 h successively. The remaining powder is dried and extracted with 250 ml distilled water for 24 h to give aqueous extract. Further to get the dry form (powder) of aqueous extract it is dried by rota evaporator to remove excess water. The dried fractions are stored in a refrigerator at 4°C throughout the study.

Pre-formulation study of Aqueous extract of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits*:*

Organoleptic Properties:

The sample of amoxicillin trihydrate is found to be white to off-white crystalline powder. It has a characteristic odor and bitter taste. The sample of aqueous extract of *Benincasa hispida* fruits is found to be brown in color and it has aromatic odor.

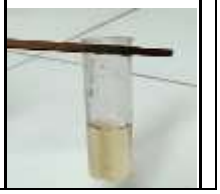
Extraction yield: The extraction yield of aqueous extract of *Benincasa hispida* was found to be 5.4%.

Preliminary Phytoconstituent Test:

Test for Flavonoid:

Table 1. Phytochemical test for flavonoid

Phytochemical test	**Observation**	**Inference**	**Pictorial presentation**
1) To the small quantity residue, add lead acetate solution	Yellow color precipitate is formed	Flavonoid Present	

2) Add NaOH to residue yellow color forms which decolorizes after addition of acid	Yellow color formed and decolorized after addition of acid	Flavonoid present	

A preliminary phytoconstituent test for flavonoids and phenolic compounds reveals their presence in the aqueous extract of *Benincasa hispida* fruits.

Table 2. Phytochemical test for phenolic compound.

Phytochemical test	Observation	Inference	Pictorial presentation
1) 5 % $FeCl_3$ Solution	Deep blue-black color	Phenolic compound present	
2) Acetic acid solution	Red colored formed	Phenolic compound present	
3) Dil. HNO_3	Reddish to Yellow colored formed	Phenolic compound present	

In Vitro Acid Neutralizing Capacity (ANC)

The ANC value obtained for the aqueous extract of *Benincasa hispida* fruits (100, 150, 200, 250 and 300 mg/ 5 ml) is compared with the standard antacid's mixture. ANC value of aqueous extract of *Benincasa hispida* at concentration 300 mg was found to be near standard mixture ANC value i.e. 6.9. Therefore, 300 mg concentration of aqueous extract of *Benincasa hispida* fruits is used for further study.

Preparation of Floating Bilayer Tablet:

Round shaped flat punch with diameter 1.5 cm is used to punch the tablets.

Preparation of powder blend includes:

First layer: Direct compression method is used to prepare bilayer tablet. Amoxicillin, HPMC K4M, HPMC K100M, $NaHCO_3$, Citric acid are passed through sieve no #40. All the ingredients are geometrically mixed in mortar to obtain a homogenous blend. Magnesium stearate and talc are added to the above mixture. The blend 1 is then subjected for evaluation of flow characteristics. Further, the blend 1 is compressed at low pressure on tableting machine to form first layer with amoxicillin trihydrate drug.
Second layer: Aqueous extract of *Benincasa hispida* fruits and Avicel PH 102 are mixed together in mortar and pestle to form homogeneous mixture. Then the blend 2 is compressed on blend 1 to form the bilayer tablet.

Formulation of the Bilayer tablet : A bilayer tablet was prepared using HPMC polymer, sodium bicarbonate, citric acid, Avicel PH 102, magnesium stearate and talc as the first layer and the second layer contained aqueous extract of *Benincasa hispida* fruits and Avicel PH 102.
Evaluation Floating Bilayer Tablet: (Post compression studies)

General appearance
After compression various quality control tests were carried out, which demonstrates following organoleptic properties viz. colour, odour and shape. The formulation was found to be white for first layer and brown for second layer. Floating characteristics like floating lag time and total floating time were studied. The tablet did not stick to the dissolution vessel or the shaft. It is found to float freely in the vessel without being hindered by the shaft movement. The formulation showed 80.09% drug release at the end of 8 hours. While 52% drug release at 4 h. Thus, it did not meet the criteria of controlled release matrix, which requires a drug release of 50% at the end of 3 hour. The release pattern was found to be Higuchi diffusion kinetic, in which R^2 was found to be 0.9578**.**

In Vivo study:
The in vivo study for checking the effect of amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits in combination for ulcer therapy is carried out by pylorus ligation method in rats. Omeprazole, amoxicillin, and aqueous extract of *Benincasa hispida* fruits, bilayer tablet powder suspensions were prepared by mixing with gum acacia powder. Groups of animals were divided such as first group as normal control only for histopathological studies, second as gastric ulcer control (vehicle solution without drug), third as treated with

standard omeprazole (20 mg/kg), fourth group received marketed amoxicillin (50 mg/kg), fifth group treated with bilayer tablet of amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits (50 and 300 mg/kg) and sixth group received only aqueous extract of *Benincasa hispida* fruits (300 mg/kg). Wistar rats were anaesthetized with ketamine (20 mg/kg) and xylazine (13 mg/kg) a portion of the abdomen is opened by a small midline incision below the xiphoid process. The pylorus portion of the stomach is lifted and ligated. The stomach is then sealed up by interrupted sutures while precautions are made to prevent traction to the pylorus or harm to its blood supply. 30 minutes prior to the ligation, the appropriate medication solutions are given orally. Animals in all the 6 groups after 5 hours of ligation are sacrificed and the stomachs are removed, cut along the greater curvature, and examined for ulcer

Parameters estimated are ulcer index and % inhibition of ulceration

Table 3. Groups of animals

Group Name	Dose	Route of administration	No. of animals
I	Control	Oral	6
II	Gastric Ulcer Control	Oral	6
III	Standard Omeprazole (20mg/kg)	Oral	6
IV	Marketed Amoxicillin (50mg/kg)	Oral	6
V	Bilayer tablet (50mg/kg and 300mg)	Oral	6
VI	Aqueous extract of *Benincasa hispida* (300mg)	Oral	6

Table 4. Effect of treatment on pylorus ligation induced gastric ulcer in rats

Groups	Dose (mg/kg)	Ulcer index	% Inhibition of ulceration
Ulcer control	-	15.78±0.2387	-
Standard omeprazole	20mg/kg	6.12±0.2134	61.21±0.5643
Marketed Amoxicillin	50mg/kg	12.0987±0.3241	23.33±0.7657
Bilayer Tablet	50mg/kg and 300mg	4.6538±0.1665	70.50±0.3421
Aqueous extract of *Benincasa hispida* fruits	300mg	5.9876±0.2721	62.29±0.8769

Results of group 2 which is gastric ulcer control group shows ulcer while results of group 3, 4, 5, 6 shows reduction in ulceration after 5 hours of the treatment. The values of ulcer index and % inhibition of ulceration of group 3, 4, 5 and 6 are given in Table 4 above. Reduction in ulceration of for pure amoxicillin trihydrate is found to be 23.33% but in case of the floating bilayer tablet of amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits shows

70.50% of ulcer inhibition. In case of only aqueous extract of *Benincasa hispida* fruits shows 62.29% ulcer inhibition. The combination of aqueous extract of *Benincasa hispida* fruits along with amoxicillin trihydrate having gastric acid inhibitory properties in addition to gastroprotective activity of aqueous extract of *Benincasa hispida* fruits at 300 mg concentration indicating its usage in peptic ulcer treatment.

Histopathological Evaluation:

The stomach samples from the pylorus ligated treated groups are preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using a rotary microtome, sections of thickness of about 5 mm are cut and stained with hematoxylin and eosin. These are examined under the microscope for histopathological changes such as degeneration, hemorrhage, edematous appearance, erosion, and necrosis are compared with normal histopathology of rat stomach of normal control

Histopathological studies for the ulcer control group in Figure 3 shows disruption in intact mucosa with inflammatory cells when compared to normal rat stomach (Figure3: a,g,b,h). However, animals treated with omeprazole shows almost normal pattern (Figure 3: c-i). In case of amoxicillin treated animals (Figure 3: d-J) the microphotographs show superficial ulcer with inflammatory cells the base of ulcerated area indicated by arrows. Further groups which are animals treated with bilayer tablets (Figure 3: e-k) and aqueous extract *of Benincasa hispida* fruits. (Figure 3: f-l) shows no ulcer and retains normal stomach physiology. Histopathological evaluation of group VI it indicates that the aqueous extract of *Benincasa hispida* fruits have anti- inflammatory activity along with antiulcer activity.

We Claim,

1) A Floating Bilayer Tablet comprises of Amoxicillin Trihydrate, Aqueous extract of *Benincasa hispida* fruits, polymers, effervescent agents, lubricating agent, binding agent and glidant.
2) The Floating Bilayer Tablet as claimed in claim 1, wherein dose of Amoxicillin Trihydrate in bilayer tablet is 50mg/kg and dose of Aqueous extract of *Benincasa hispida* fruits is 300 mg/kg.
3) The Floating Bilayer Tablet as claimed in claim 1, wherein polymers are selected from the group containing Hydroxypropyl methylcellulose (HPMC), sodium alginate, gelatin, chitosan, cellulose derivatives, semisynthetic polymers, synthetic polymers.
4) The Floating Bilayer Tablet as claimed in claim 1, wherein HPMC K4M and HPMC K100M polymers are in the ratio of 85:15.
5) The Floating Bilayer Tablet as claimed in claim 1, wherein effervescent agents are selected from the group sodium bicarbonate, sodium carbonate, adipic acid, malic acid, tartaric acid, ascorbic acid, fumaric acid, maleic acid, succinic acid, or citric acid.
6) The Floating Bilayer Tablet as claimed in claim 1, wherein effervescent agent sodium carbonate and citric acid are in the ratio 1:4.
7) The Floating Bilayer Tablet as claimed in claim 1, wherein lubricant is selected from the group containing talc, silica, fats, like vegetable stearin, magnesium stearate or stearic acid.
8) The Floating Bilayer Tablet as claimed in claim 1, wherein glidant is selected from talc, colloidal silicon dioxide, magnesium stearate, and silica; and binding agents selected from the group Avicel PH 102, cellulose, gelatin, cellulose derivatives, polyvinyl pyrrolidone, starch, sucrose, mannitol, polyethylene glycol, and liquid glucose.
9) The Floating Bilayer Tablet as claimed in claim 1 is used for the treatments of peptic ulcers.
10) A method of preparation of floating bilayer tablet comprises of;
    - Preparation of aqueous extract of *Benincasa hispida* fruits
    - Evaluation of aqueous extract of *Benincasa hispida* fruits
    - Preparation of Floating Bilayer Tablet
    - Evaluation of Floating Bilayer Tablet

- In vitro study, In vivo study and histopathological evaluation of the batch formulation

Dated this 30th day of September 2022

For Applicants:

(Signed) _____

Mahesh Madanrao Jadhav

Indian Patent Agent (IN/PA/2087)

To

The Controller of Patents,

The Patent Office Branch, Mumbai

“Floating Bilayer Tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits”

ABSTRACT

The present invention relates to the Floating Bilayer Tablet of Amoxicillin trihydrate and aqueous extract of *Benincasa hispida* fruits and preparation method for the treatment of peptic ulcer. The bilayer floating tablet comprises of Amoxicillin trihydrate, aqueous extract of *Benincasa hispida* fruits, polymers HPMC K4M and HPMC K100M in the ratios of 85:15, sodium bicarbonate and citric acid in the ratio 1:4 as effervescent agents, Avicel PH 102, Magnesium stearate and talc. Aqueous extract of *Benincasa hispida* fruits shows anti-inflammatory activity along with antiulcer activity.

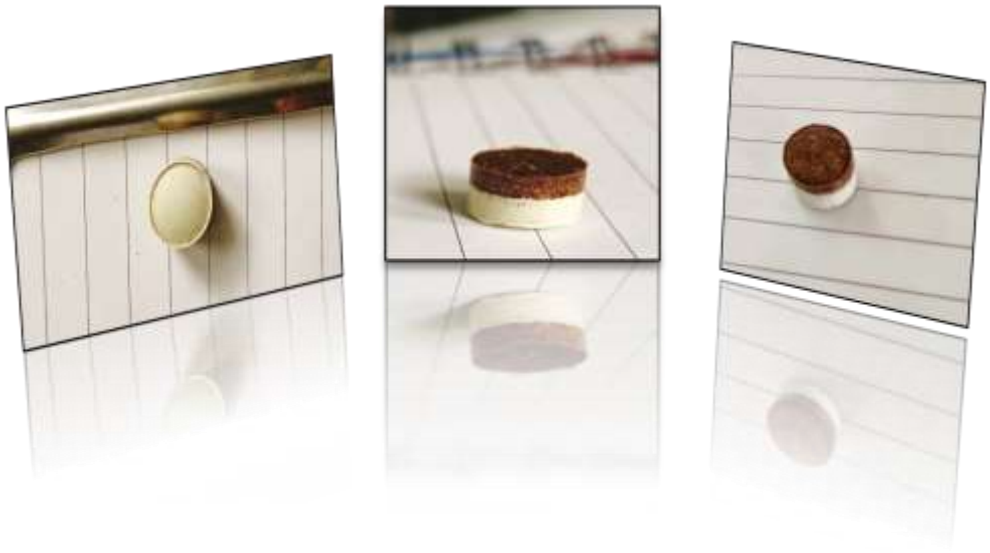


Figure 1: Pictorial Representation of the tablet

Dated this 30th day of September 2022

For Applicants:

(Signed) _

Mahesh Madanrao Jadhav

Indian Patent Agent (IN/PA/2087)

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I. II. III. IV. V. VI

Figure 2. Pictorial presentation of Stomach tissue of rat of group I to VI

Dated this 30th day of September 2022

For Applicants:

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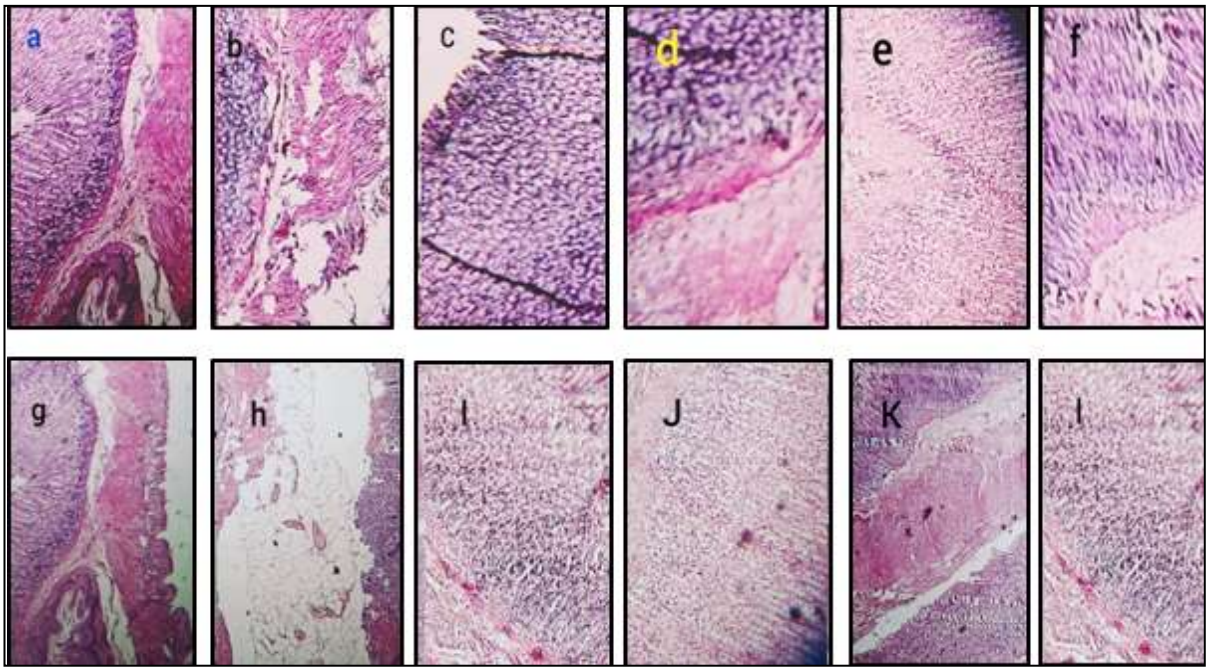

Figure 3: Histopathological studies of rat stomach: Group I (a-g), Group II (b-h), Group III (c-i), Group IV (d-J), Group V (e-k) and Group VI (f-l)

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