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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF HYDRO ALCOHOLIC EXTRACT OF FRUITS OF *TERMINALIA CHEBULA* ON DIFFERENT ULCER-INDUCED MODELS

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ABSTRACT

The objective of the present study is to study the effect of hydro alcoholic (70%) extract of Fruit of *Terminalia chebula* on gastric ulcers by using different experimental models such as aspirin induced ulcer, ethanol induced ulcers, cold restrain stress induced ulcers and pylorus ligation induced ulcers. In all these models the common parameter determined was ulcer index. The treatment with hydro alcoholic extract of *Terminalia chebula* at (500 mg/kg) significantly inhibited the gastric lesions induced by Aspirin induced gastric ulcer (85%), Ethanol induced gastric ulcer (72%), Cold restraint ulcer (79%), and Pylorus ligation induced ulcer (76%).The extracts (200 mg/kg and 500 mg/kg b.w.) showed a significant effect on ulcer by different ulcer induced models. These results may further suggest that hydro alcoholic extract was found to possess anti-ulcerogenic as well as ulcer healing properties, which might also be due to its antisecretory activity.

Keywords: *Terminalia chebula*; Gastric ulcer; Pylorus ligation; ethanol, Aspirin.

INTRODUCTION

Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastroprotective and aggressive factors is lost. Major aggressive factors are acid, pepsin, *Helicobacter pylori* and bile salts. Defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H^+K^+ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost [1-4].

The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastroprotection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing. Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H_2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAID's and *H. pylori*, but there are reports of adverse effects and relapse in the long run.

In present study; detail investigations of antiulcer activity of *Terminalia chebula* had not been carried out so far. Hence this leads us to study the antiulcer activity of *Terminalia chebula* in different ulcer models [5-8].

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MATERIALS AND METHODS

Material

The fruit of *Terminalia chebula* was collected from Regional Research Institute (RRI), Bhopal, and were authenticated by the Botany Department, Saifia College Bhopal. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2019-286) of fruit has been deposited in museum of Department of pharmacology, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences.

Animals

Wistar albino rats of either sex weighing between 150-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Albino rats were used in this thesis was obtained from the Bioneeds Animal House Dhavas Pet, Tumkur. The animals were housed in Poly propylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium1%, Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

METHODS

Extraction of fruits

The fruits of *Terminalia chebula* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction in batches using petroleum ether, chloroform, and Hydro alcohol (70%) solvents in a soxhelet extractor. The different extracts obtained were evaporated at 45˚C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis, . The percentage yield of hydro alcoholic extract was found to be 32.50%.

Phytochemical analysis of the extracts

The extracts of *Terminalia chebula* were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoid.

Acute toxicity study

The acute oral toxicity study were carried out for hydro alcoholic extract of *Terminalia chebula* using fix dose method according to OECD guideline no.420. Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for study. Animals were divided into four groups of three animals each and kept fasted overnight. The different doses like 5, 50, 300 and 2000 mg/kg b. w. were administered to the Group I, II, III, IV respectively[61]. After administering the hydro alcoholic extract to different groups the behavioral changes like body temperature, CNS activity, micturation, defection etc were observed for 24 h[62].

Evaluation of Antiulcer Activity

Aspirin Induced Ulcer

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received hydro alcoholic (70%) extract of *Terminalia chebula* 200 and 500 mg/kg. Omeprazole (20 mg/kg) b.w. were administered orally for group IV as reference drug. Aspirin in dose of 500 mg/kg was administration to the animals after 45 min of extract and omeprazole treatment. The animals were sacrificed after 4 h and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. A score for the ulcer was made as follows:[9-15]

Red colouration (0.5), Spot ulcer (1), Haemorrhagic streak (1.5), Ulcers (2), Perforation (3)

Mean ulcer score for each animal was expressed as ulcer index. The percentage of

ulcer protection was determined as follows:

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}}$$

Ethanol Induced Ulcers

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received hydro alcoholic (70%) extract of *Terminalia chebula* 200 and 500 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for group IV as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1 ml/200 g b.w.) orally, after 45 min of hydro alcoholic extract and Omeprazole treatment .They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was study similar to Aspirin induced ulcer model.

Cold Restraint Stress Induced Ulcers

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received hydro alcoholic extract of *Terminalia chebula* at a dose of 200 and 500 mg/kg Omeprazole, in the dose of 20 mg/kg were administered orally for Group IV for ulcer protective studies. Animals of different group were subjected to cold stress after 45 min of extract and Omeprazole treatment. Rats were deprived of food, but not water, for about 18h before the experiment. Rats were immobilized by strapping the fore and hind limbs in restraint cage and kept for 2h, at temperature of 4ºC. two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomach was incised along the greater curvature and ulcer was scored. A score for the ulcer was study similar to

Aspirin and ethanol induced models.

Pylorus ligation induced ulcers,

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received hydro alcoholic extract of *Terminalia chebula* in a dose of 200 and 500 mg/kg. Omeprazole, in the dose of 20 mg/kg were administered orally for Group IV for ulcer protective studies. After 45 min of hydro alcoholic extract of *Terminalia chebula* and Omeprazole treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under pentobarbital anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed.

The gastric juice collected was centrifuged for 1000 rpm for 10 min and the volume of gastric juice, pH, total and free acidity was measured [20-22].

Determination of free acidity and total acidity

Reagents

1. Freshly prepared 0.01N oxalic acid solution for standardization of sodium hydroxide
2. Freshly prepared 0.01N NaOH solution
3. Topfer's reagent (Loba chemie, Mumbai, India): It is dimethyl amino benzene 0.5% in absolute ethanol
4. Freshly prepared 1% Phenolphthalein (Nice chemicals, Cochin, India) solution

Procedure

Gastric juice centrifuged at 1000 rpm for 10 min 1 ml of supernatant was diluted with 9 ml of distilled water and was titrated with 0.1N NaOH using 2-3 drops of topfers reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink color was restored. This gives total acidity. Free acidity and total acidity is expressed in terms of 0.1N HCL per 100 gms of gastric contents[66].

Statistical analysis

The data are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of ANOVA, followed by Dunnets t-test where $P<0.05$ was considered statistically different.

RESULTS AND DISCUSSION

Acute Toxicity Studies

The acute toxicity studies of the Hydro alcoholic extract of fruit of *Terminalia chebula* was found to be non-lethal up to dose of 2000 mg /kg body weight of the animals so that 1\10th and 1\4th (*i.e.* 200 mg/kg and 500 mg/kg orally) was selected for different ulcer-induced models.

Aspirin Induced Gastric Ulcer

The effect of the hydroalcoholic extract on Aspirin induced ulceration was studied and the results are tabulated in Table-6. The extract significantly reduced the ulceration produced by Aspirin with a ulcer index of 76% and 85% with the dose of 200 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 87%.

Ethanol-Induced Gastric Ulceration

In the present study, Hydroalcoholic extract of *Terminalia chebula* was evaluated for its antiulcer activity against ethanol-induced gastric ulceration in rats. The results are tabulated in Table-9. Oral administration of ethanol produced severe ulceration. Hydro alcoholic extract of *Terminalia chebula* has shown significant protection index of 60% and 72% with the dose of 200 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 83%.

Cold Restraint Induced Ulcer

The Hydroalcoholic extract of *Terminalia chebula* showed significant protection index of 65% and 79% with the dose of 200 mg/kg and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 83%. the results are tabulated in Table-12.

Pylorous Ligation Induced Gastric Ulcer

In Pylorous ligation induced gastric ulcer the Hydroalcoholic extract of *Terminalia chebula* showed significant reduction in gastric volume, free acidity, total acidity and ulcer score in Table-15. It was showing protection index of 72% and 76% at the dose of 200 and 500 mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 78%.

Preliminary Phytoconstituents

The preliminary phytochemical analysis of petrolium ether, chloroform and hydroalcoholic extract of fruit of *Terminalia chebula* revealed that the presence of various phytoconstituents which are present in Table-1.

Table 1. List of Presence of Various Phytoconstituents

Constituents	Chemical test	Ethanolic extract
	Hager's Test	– ve
	Mayer's Test	– ve

Alkaloids	Dragendroff's Test	– ve
	Wagner's Test	– ve
Carbohydrates	Molish's Test	+ ve
	Fehling's Test	+ ve
	Benedict's Test	+ ve
Cardiac glycosides	Baljet's test	– ve
	Legal's test	– ve
Anthraquinone glycosides	Borntrager's test	+ ve
	Modified Borntrager's test	+ ve
Saponin glycosides	Foam test	+ ve
Fixed oil	Stain Test	– ve
Proteins and Amino acids	Millon's Test	+ ve
	Biuret Test	+ ve
	Ninhydrin Test	+ ve
Triterpenoids	Liebermann-Burchard Test	+ ve
Flavonoids	Shinoda test	+ ve
	Sodium hydroxide test	+ ve
Tannin and Polyphenols	Lead acetate solution	+ ve
	5% $FeCl_3$ solution	+ ve
	Bromine water test	+ ve
	Potassium dichromate test	+ ve

Table 2. Effect of Hydro Alcoholic (70%) Extract of *Terminalia Chebula* on Aspirin Induced Ulcer

Animal	Treatment	Dose	Ulcer Index	Animal	Treatment	Dose	Ulcer Index
1	Distill water	1 ml/100g	62.00	1	Omeprazole	20 mg/kg	6.50
2			44.50	2			12.50
3			32.00	3			1.50
4			54.00	4			6.00
5			60.00	5			5.00
6			40.00	6			7.00
Mean			47.30	**Mean**			6.42
SEM			5.94	**SEM**			1.45

Table 3. Effect of Hydro Alcoholic Extract of *Terminalia Chebula* On Aspirin Induced Ulcer

Animal	Treatment	Dose	Ulcer Index	Animal	Treatment	Dose	Ulcer Index
1	Hydro alcoholic extract	200 mg/kg p.o.	13.5	1	Hydro alcoholic extract	500 mg/kg p.o.	8.50
2			7.00	2			4.00
3			8.50	3			3.00
4			18.50	4			13.00
5			12.00	5			3.50
6			9.00	6			10.50
Mean			11.42	**Mean**			7.08
SEM			1.72	**SEM**			1.71

Table 4. Gastroprotective Activity of Hydroalcoholic Extract of *Terminalia Chebula* On Aspirin Induced Ulcer

Treatment	Dose	Mean ulcer index	% Protection
Vehicle	1ml/100g	47.33 ± 0.94 **	--
Omeprazole	20 mg/kg	6.42 ± 1.45 **	87%
Hydroalcoholic extract of *Terminalia chebula*	200 mg/kg	11.42 ± 1.72**	76%
Hydroalcoholic extract of *Terminalia chebula*	500 mg/kg	7.08 ± 1.71 **	85%

Results are expressed as Mean ± SEM (n = 6) in each group.

Table- 5: Effect Of Hydro Alcoholic Extract Of *Terminalia Chebula* On Ethanol Induced Ulcer

Animal	Treatment	Dose	Ulcer Index	Animal	Treatment	Dose	Ulcer Index
1			28.00	1			4.50
2			18.00	2		20	5.50
3	Distilled	1 ml/100gm	40.00	3		mg/kg	3.50
4	water		28.00	4	Omeprazole		11.00
5			23.00	5			7.50
6			23.00	6			7.50
Mean			26.67	**Mean**			6.58
SEM			3.70	**SEM**			1.10

DISCUSSION

Ulcers develop when the normal defense and repair mechanisms of the lining of the stomach or duodenum are weakened, making the lining more likely to be damaged by stomach acid. A peptic ulcer is a sore on the lining of the stomach, small intestine or esophagus. A peptic ulcer in the stomach is called a gastric ulcer. Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defence mechanism by increasing the mucus production protecting the surface epithelial cells, or interfering with PG synthesis. Gastrointestinal injury is induced by various chemical agents. Thus the present investigation was carried out to evaluate the antiulcer activity of the hydroalcoholic extract of *Terminalia chebula* against different ulcer models.

Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis[67], increasing acid secretion by increasing the H^+ ion transport/backdiffusion of H^+ ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway[68]. The hydroalcoholic extract significantly reduced the ulcer index and afforded significant protection against Aspirin induced ulcers could be due to prevention of direct irritation, increased mucus secretion and due to its 5-lipoxygenase pathway.

Hydro alcoholic extract of *Terminalia chebula* showed the ability to reduce significantly the severity of ulceration of stomach induced by absolute ethanol. The results revealed that the pretreatment with hydro alcoholic extract of *Terminalia chebula* absolutely prevented the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration in the gastric mucosa of rats. The incidence of ethanol-induced ulcers predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products, and reactive oxygen species resulting in the damage of rat gastric mucosa starts with microvascular injury, namely a disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting[70]. In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin. The effectiveness of hydro alcoholic extract of *Terminalia chebula* protection against mucosal damage caused by ethanol is indication of its effect on prostaglandins.

Animals subjected to restraint plus cold for 2 h showed the presence of considerable ulcerogenicity in the form of hemorrhagic mucosal lesions in the stomach, which were confined to the glandular segment only. Peripheral sympathetic activation plays an important role in induction of ulcers by restraint. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion, a reduction in mucous production and generation of free radicals etc.. Increase in gastric motility, vagal overactivity[74], mast cell activation, alterations in prostaglandin generation, cytokine liberation and breakdown of normal cytoprotective mechanism. Incidence of cold restrain ulcer due to increased acid secretion and generation of free radicals.

Ulcers due to cold stress are both due to physiological and psychological factors. The gastroprotective action of hydro alcoholic extract of *Terminalia chebula* against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic and antisecretory effects.

After 45 min of treatment with an hydroalcoholic extract of *Terminalia chebula*, pylorus ligation of rats for 4 h resulted in accumulation of gastric secretory volume, and increase in titrable acidity and ulceration. The cause of gastric ulcer after Pyloric ligation are believed to be due to stress-induced increase in gastric hydrochloric acid secretion and/ or stasis of acid. Pylorus ligation induced gastric ulcers occur because of an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms.

Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa[81]. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion is valuable part of the study to clarify the mechanism of action of drug under trial. In the present

study, hydro alcoholic extract of *Terminalia chebula* has reduced the free acidity by 21% and 24% at a dose level of 200 and 500 mg/kg; Standard drug Omeprazole has reduced the free acidity 55%; whereas total acidity reduction by hydro alcoholic extract of *Terminalia chebula* is 47% and 55% at a dose level of 200 and 500 mg/kg and standard reference drugs Omeprazol reduced the total acidity at 60%.

Overall, Hydro alcoholic extract of *Terminalia chebula* has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of hydro alcoholic extract of *Terminalia chebula* and increase its usage in contemporary medicine [20-23].

CONCLUSION

Phytochemical analysis of the fruit extract revealed that extract contains the Carbohydrates, flavanoid, saponin, tannin/polyphenol and fat, phytosterols and triterpenoids. In the acute toxicity studies of the hydro alcoholic extract *was* found to be non-lethal up to dose of 2000 mg/kg body weight of the animals. so that and 200 mg/kg and 500 mg/kg orally was selected for different ulcer-induced models. The anti-ulcer effect was evaluated using the following ulcer models. Aspirin induced ulcer,Ethanol induced ulcer, Cold restrain induced ulcer, Pylorus ligation induced ulcer.

The hydro alcoholic extracts of *Terminalia chebula* was found to be effective in healing the ulcer induced by different ulcer induced models. In Aspirin induced ulcer the hydro alcoholic extracts of *Terminalia chebula* (200 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg p.o) showed a significant reduction in the ulcer area. This suggests the cytoprotective effect of *Terminalia chebula* extract. In ethanol induced ulcers the hydro alcoholic extracts (200 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg) showed a significant reduction in ulcer index. This suggests that hydro alcoholic extracts of *Terminalia chebula* might have gastric cytoprotection effect. In cold restrain stress-induced ulcers the hydro alcoholic extracts (200 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg .p.o) showed a significant reduction in ulcer index. This suggests that some of the constituents present in the *Terminalia chebula* extract might have central actions, which are helpful in reducing the gastric ulcers. In pylorus ligation induced ulcers the extracts (200 mg/kg and 500 mg/kg body weight) of *Terminalia chebula* and Omeprazole (20 mg/kg) significantly decreased the total acidity and free acidity. ulcers the hydro alcoholic extracts (200 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg) showed a significant reduction in ulcer index. This suggests that hydro alcoholic extracts of *Terminalia chebula* might have gastric cytoprotection effect. present study suggest that the fruits of *Terminalia chebula* showed anti-ulcer activity. The antiulcer effect of hydroalcoholic fruit extracts of *Terminalia chebula* may be due to both reductions in gastric acid secretion and gastric cytoprotection.

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