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Extraction and Screening of Gastroprotective Activity of Morus Alba Linn.

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ABSTRACT- The purpose of the present study was to evaluate the anti-ulcer effect of Morus alba Linn. The alcoholic extract of Morus alba Linn. leaves were evaluated for its anti-ulcer activity against aspirin plus pylorus ligation induced gastric ulcer in rats, water immersion stress induced ulcer models in rats and HCl-ethanol induced ulcer in mice at two different doses viz. 100 and 200 mg/kg. Biochemical parameters like volume of gastric secretion, pH, free acidity, total acidity and ulcer index were studied. The alcoholic extract of Morus alba Linn. leaves showed significant reduction in gastric volume, pH, free acidity, total acidity and ulcer index at concentration of 100 & 200 mg/kg. The plant extract at concentration of 200mg/kg showed significant gastro protective activity 82.36%, compared with standard drug sucralfate showed 91.18% and protection index in water immersion stress induced ulcer showed 92.14% compared with standard drug omeprazole showed protection index 99.58%. The results suggest that the alcoholic extract of Morus alba Linn. leaves possesses anti-ulcer effect. The observed effect may be due to the presence of bioactive constituents.

KEYWORDS: Anti-ulcer, *Morus alba*, Methanol, Saponin.

INTRODUCTION: An ulcer is a crater-like lesion in a membrane; ulcers that develop in areas of the GIT exposed to acidic gastric juice are called peptic ulcers. „peptic" refers to pepsin, a stomach enzyme that breaks down proteins. A peptic ulcer is defined as disruption of the mucosal integrity of the stomach and duodenum leading to a local defect or excavation due to active inflammation 9. They may arise in the form of single or multiple lesions. Oxidative stress is believed to be important in initiating and aggravating peptic ulcer disease. One of the common denominators for the genesis of this disease is the involvement of free radicals. Reactive oxygen species (ROS) are generated through numerous normal metabolic processes. Mulberry leaves contains various phytochemicals such as alkaloids, anthocyanins, flavonoids, stilbenes, triterpenoids saponin (lupeol), steroidal saponin (β- sitosterol), coumarins and phenolic acids, anthocyanin, stilbenes [17] and glycosides, benzofuran derivatives, anthroquinones, morusimic acid, oleanolic acid [18, 19, 20, 21]. Antiulcer activity of *M. alba* leaf extract has been previously reported. The results of the present study suggest a direct protective effect of *M. alba* extracts on gastric mucosal damage and that the gastroprotective action of this plant may be due to its anti- oxidant properties [22]. Doi K *et al.* reported that l-butanol extract of mulberry leaves scavenged the DPPH radical and inhibited the oxidative modification of rabbit and human LDL. However, antiulcer activity of the saponin-rich fraction of M. alba leaves has not been previously reported.

PLANT PROFILE-

Morus alba linn

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Rosales
Family: Moraceae
Genus: Morus
Species: Morus alba L.

Popular names: Toota, Tut, Tula, Chinni, Ambat, Shetur, Reshme chattu, Musukette, Hipnerle, Tuticoli, Mulberry.

Morus alba is a fast-growing shrub or medium-sized tree with a straight, cylindrical trunk that measures 1.8 m in circumference without buttresses. The bark is dark grayish brown in color, with longitudinal cracks and a rough surface, while the latex is white or yellowish white. The stem is lateral, scaly, and coral, with two rows of oval or nearly oval leaves, and a simple trilobal, dentate, and palm with three veins at the base.

MATERIALS AND METHODS-

Phytochemical studies

M. alba fruits were purchased from local market and identified before extraction of plant materials. The plant material was powdered and extracted with petroleum ether for defatting. The defatted materials were extracted with ethyl alcohol using a soxhlet apparatus at 70-80 ^{0}C up to complete extraction (Lodhi et al., 2010). The ethanol extract was concentrated and dried under reduced pressure and yield was calculated. Extract was investigated for qualitative chemical test for different chemical constituents.

Ethanol induced ulcer

Wistar albino rats (180-200g) of either sex were selected for antiulcer activity. They were housed and divided into six groups containing each group of six animals in polypropylene cages, under standard laboratory conditions of temperature (25±2ºC). The animals were allowed to free access standard food (Brooke Bond-Lipton, India) and water. The animals were acclimatized for minimum 7 days before experiment. All

experimental protocol was approved by the Institutional Animals Ethics Committee as per CPCSEA guidelines. The control group was given only vehicle (sodium carboxymethyl cellulose, 0.5 % p.o.), test group II and III were given ethanol extract with 200 and 300 mg/kg, p.o., respectively. Standard group IV was given standard Omeprazole (20 mg/kg, p.o.).

The ulcer was induced using 1 ml of 80 % ethyl alcohol administered orally to each animal. After 1 hr the rats were anesthetized and die by cervical dislocation. The macroscopic observation of stomach was recorded for ulcer scoring after removal of stomach. Whole stomachs were gently rinsed with water to remove the gastric contents and blood clots. Stomach was examined for lesions in the four stomach portion for indexed according to severity (Jhariya et al., 2015; Jain and Bhandarkar, 2016). Mean ulcer score for each animal was expressed as ulcer index.

$$\text{Ulcer Index (UI)} = \frac{\text{Number of ulcer in control - Number of ulcer in test}}{\text{Number of animals}}$$

$$\text{Percent Inhibition (\% I)} = \frac{\text{UI of control - UI of test}}{\text{UI of control}} X100$$

Gasric contents from each stomach were collected and volume was measured. The pH of the gastric secretion was recorded and determines total acidity of the gastric juice by titration with 0.01 N NaOH and phenolphthalein as indicator. The total acidity is expressed as mEq/l using the following formula:

Total/free acidity = n×0.01×40×1000

Determination of antioxidants levels

The tissues were collected from stomachs were tested for antioxidants assay. Reduced glutathione (GSH) level was determined by the method of Moron (Moron et al., 1979). Superoxide dismutase (SOD) was assayed (Misra and Fridovich, 1972) based on the inhibition of epinephrine autoxidation by the enzyme. Catalase was estimated following the breakdown of hydrogen peroxide (Beers and Sizer, 1952). Skin homogenates were immediately precipitated with 0.1 ml of 25% TCA and removed after centrifugation. Free-SH groups were assayed in a total 3 ml volume by the addition of 2 ml of 0.6 mM DTNB and 0.9 ml 0.2 mM sodium phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using UV spectrophotometer.

Statistical Analysis

Pharmacological data were represented as the mean ±S.D. for six rats. All data were statistically evaluated using the Tukey test. Values of P <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION-

Phytochemical studies

Phytochemical screening reveals the presence of flavonoids, amino acids, proteins, carbohydrates and glycosides in ethanol extract. Practical yield of ethanol extract found 5.3 w/w%. The ethanol extract was reported safe dose up to a dose of 2000 mg/kg body weight. The extract dose of 200 and 300 mg/kg p.o. was selected for the gastroprotective study.

Ethanol induced ulcer

Administration of ethanol in higher doses resulted in the production of gastric mucosal damage. The ulcer index of control group was found 26.21±2.45. Ethanol extract at 300 mg/kg showed 12.62± 1.02 inhibition of the ulcer index which were significantly reduced. The reduction in ulcer index by alcoholic extracts at 200

mg/kg was 39.18%. Standard Omeprazole, showed anti-ulcer effect with 68.44% ulcer index inhibition (Table 1). Total gastric acidity of control group of animals was found 524.4±42.38. Ethanol extract at 300 mg/kg was showed significant decrease in total acidity 398.2±30.71 mEq/l, compared to control group (Table 2).

Table 1. Effect of ethanol extract of *M. alba* fruits on ulcer index in ethanol induced model

Treatment groups	Doses	Ulcer Index	% Inhibition
Control	5 ml/kg	26.21±2.45	-
Omeprazole	20mg/kg	8.27±0.52*	68.44± 3.28
EEMA	200 mg/kg	15.94± 1.37	39.18± 2.17
EEMA	300 mg/kg	12.62± 1.02*	51.85± 3.15

MAEE: Ethanol extract of *M. alba;* Values are mean ± SD; $N = 6$ in each group; $^{*}P < 0.01$, when experimental groups compared with control

Table 2. Effect of ethanol extract of *M. alba* fruits on ulcer parameters in ethanol induced model in rats

Experimental groups	Doses	Gastric volume (ml)	pH	Total acidity (mEq/l)
Control	5 ml / kg	6.52±0.48	1.8±0.74	524.4±42.38
Omeprazole	20 mg/kg	3.74±0.35*	3.8±0.45*	253.8±21.53*
EEMA	200 mg/kg	4.35±0.54	3.2±0.35	328.6±28.62
EEMA	300mg/kg	4.86±0.65*	4.2±0.15*	398.2±30.71*

MAEE: Ethanol extract of *M. alba;* Values are mean ± SD; $N = 6$ in each group; $^{*}P < 0.01$, when experimental groups compared with control

Table 3. Effect of ethanol extract of *M. alba* fruits on antioxidants level in stomach tissues

xperimental groups	Doses	Antioxidants		
		SOD	GPX	CAT
Control	5 ml / kg	11.87±0.57	18.62±0.82	10.92±0.29
Omeprazole	20 mg/kg	25.31±0.47*	31.28±1.62 *	22.30±1.05*
EEMA	200 mg/kg	17.29±0.29	19.62±0.85	15.62±0.56
EEMA	300mg/kg	22.60±0.95*	26.72±1.65*	19.67±1.76*

MAEE: Ethanol extract of *M. alba;* Values are mean ± SD; $N = 6$ in each group; $^{*}P < 0.01$, when experimental groups compared with control

The macroscopic observation of stomach showed that control group of animals found necrosis and hemorrhage in gastric lesions predominant over vast surface area. It showed perforations with complete mucosal destruction. The animals treated with dose of 300 mg/kg ethanol extract and standard drug were not found any necrosis and hemorrhage that reflects the protective effect of extract and drug.

The anti-ulcer activity was tested against gastric lesions induced by ethanol, the experimental models related to lesion pathogenesis with production of reactive species. Ethanol extract of *M. alba* found to increase the pH and decrease the gastric acid content and total acidity of gastric fluid. Alcohol rapidly penetrates the gastric mucosa which can cause cell membrane damage and leading to increased intra cellular membrane permeability to sodium and water. The enormous intracellular gathering of calcium represents a major step in the induction of gastric mucosal injury. This may support to cell death and exfoliation of epithelium surface (Raju, 2009). The antioxidant effects of *M. alba* fruits extract was already reported that can support for gastroprotective effect of *M. alba*. Some oxygen free radicals and causing agents have been implicated in the pathogenesis of ethanol-induced gastric ulcers (Shardul and Gangadhar, 2010).

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