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Antiulcer Potential of Extracts of *Urena lobata* Plant Leaves

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Abstract

The plant belonging to the family *Malvaceae*, the scanty availability of information on this plant facilitates the study on it. Entire plants of *Urena lobata* were collected from India. The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use. The phytoconstituents were extracted by using different solvent of increasing polarity like chloroform, ethyl acetate, n-hexane, ethanol and water. The acute toxicity test aims an establishing the therapeutic index, i.e. the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species (LD_{50}/ED_{50}). In any screening program, acute toxicity on mice/rat is usually performed first. Acute oral toxicity was performed used the Up and down method, OECD Guideline 425 (Revised: 17 December 2001) The single dose of aqueous extract of leaves of *Urena lobata* was used for the acute oral toxicological study for appreciating the usefulness of the chosen plant ant in understanding the effects of the same in relation to the gastric ulcer. LD_{50} / tolerable dose are estimated in rats. We were found that the animals were safe and no mortality was observed up to 2000mg/kg body weight. It records that LD_{50} determination of the aqueous extract of leaves of *Urena lobata* are > 2000mg/kg body weight. Extract of leaves of *Urena lobata* on alcohol-induced gastric ulceration in rats on different dose were tested (10, 15 and 20mg/kg body weight) the % protection range was observed from 26.33 to 71.14 (standard drugs omeprazole),. 54.18 observed on 20mg/kg dose.

Keywords: *Urena lobata,* Acute oral toxicity, alcohol- induced gastric ulceration, anti-ulcer

INTRODUCTION

Herbs are defined in several ways depending on the context, which the world is used. In the field of medicine, they are most accurately defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health. Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluidextracts, extracts, or the like, are known as phytomedicinals. Herbs are used as medicine by about 80% of the world population, mainly in the developing countries, for primary healthcare because of better cultural acceptability, better compatibility with the human body and lesser side effects. India is one of the countries in the world today where ancient system of medicine, such as Ayurveda, Siddha, Unani, Tribal medicine and Naturopathy have been in practice for several years [1].

International regulations on traditional medicine based on inadequate research investments:

Globally, most of the pharmacopoeia on THS products has been restricted to single drugs or single plant extracts or at the most extracts of five herbs as fixed combinations. This is because not enough investment has been put into designing appropriate tools and methods to rapidly assess the quality of poly- herbals [2]. While with respect to safety, traditional remedies will definitely need to be assessed within the same parameters now used for modern pharmaceutical, nutraceutical and cosmeceutical products, WHO has accepted that traditional medicines may need less rigorous preclinical toxicological evaluations since their safety of use has been documented historically [3].

MATERIALS AND METHODS:

Plant Selection: The process typically begins with a botanist, ethno botanist, ethnopharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxon collected randomly for a large screening program. On the basis of intensive literature survey; *Urena lobata* was selected for present study.

METHODS:

Preparation of extracts

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios [4] The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with n-hexane, chloroform, ethyl acetate, alcohol and water using a Soxhlet apparatus. The yield of the plant extracts ethanol (70%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The

standard extracts obtained from *Urena lobata* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening [5].

Maintenance of animals and Exposure Conditions

Earlier to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (conditions (temp: 20–25^{0}C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rat feed for 1 week in order to adapt to the laboratory conditions and water *ad libitum.* They were fasted overnight (12 h) before experiments, but were allowed free access to water [6]. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee.

Acute toxicity study

(a) Principle

Toxicity studies involved a test in which single dose of the drug used in each animal on the occasion only for the determination of LD_{50} or median lethal dose [7], i.e. the dose which kills 50% animals of a particular species.

(b) LD_{50} & ED_{50} / Tolerance dose determination

The determination of ED_{50} values helps in ascertaining the potency of the drug in terms of a reference standard. The calculation of the ED_{50} values is done when a drug is showing graded response. But, when the response is quantal or all or none, the ED_{50} value becomes ED_{50}. Both these values of ED50 and LD_{50} are important for knowing the safety of the drug.

The ratio between LD_{50} and ED_{50} represent the therapeutic index. Greater the therapeutic index, safer the drug [8].

(c) Experimental protocol

Animal:	Wistar rats
Number of animal:	5
Sex:	Female
Age & Body weight:	10 weeks, 175-225gm
Sample:	#extract of leaves of *Urena lobata*
Dose:	2000mg/kg body weight
Route of administration:	Oral
Duration observation:	14 days

***In-vitro* Anti-ulcer activity by Broth Dilution Method (Minimum inhibitory concentration of the leaf extracts):**

Microorganism:

The *Helicobacter pylori* strain used was ATCC 43504 (vacA and cagA positives), procured by Fundac¸ ão Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil. Stock cultures were maintained frozen at −30 ◦ C in Skim Milk broth. For reactivation, *Helicobacter pylori* was inoculated on selective Belo Horizonte medium (BHM), a BHI broth (Newprov, Brazil) supplemented with 10% fetal calf serum (Cultilab, Brazil) and incubated at 37 ◦ C [9] under microaerophilia in an atmosphere of 5–15% O2 and 5–10% CO_2, for 2–4 days.

Determination of anti-Helicobacter pylori activity by broth microdilution assay:

The broth microdilution assay allows the determination of the Minimum Inhibitory Concentration (MIC). 100 µL of BHI broth supplemented with 10% fetal calf serum inoculated with 6 × 108 Helicobacter pylori (McFarland turbidity standard 2) and 100 µL of serial dilutions of HECb, and all extracts (*n-hexane, chloroform, ethyl acetate, ethanol and aqueous)* individually dissolved in 2% Tween (v/v) was added to each well in the microplate, to reach final concentrations of 50; 100; 200 and 400 µg/mL. The standard drug, Clarithromycin, was diluted to the same concentrations. The microplate was incubated at 37 ◦ C under microaerophilic conditions in an atmosphere of 5–15% O_2 and 5–10% CO_2, for 48–72 h. After incubation [10], the plates were visually examined, the optical density was determinated at 450 nm and each well was replicated in blood agar (Mueller–Hinton agar with 5% sheep blood), to determine whether growth had occurred, as well as the MIC.

Pylorus ligation induced gastric ulcers:

The rats were starved for thirty six hours prior to study providing sufficient drinking water by laying them in individual crate to prevent them from cannibalism and coprophagy. During the starvation period all the animals received saline orally (1mL/rat). With 5% isoflurane anesthesia the abdominal cavity was afforded by midline slit just beneath the xiphoid and the stomach was divulged out [11]. The pyloric end of the stomach was slimly raised and ligated, extreme care taken to evade damaging of its blood supply. The different leaf fractions of all extracts at a dose of 200 mpk or Omeprazole at a dose of 2 mpk formulated in 0.5% CMC in water were dispensed intra-duodenally instantly after pylorus ligation. The stomach was stationed back cautiously and the abdominal walls were sutured. Postoperatively the rats were stripped of food and water. Six hours later rats were terminated by carbon-di-oxide anesthesia. Stomach's were excised and the contents were gathered and centrifuged. The gastric juice volume was assessed and was employed for determining free acidity, total acidity and total proteins. Stomach samples were opened on the greater deflection and flashed by a scanner. The total ulcerated and mucosal area were assessed by employing Scion image software [12] to determine the ulcer index as mentioned above.

Ethanol induced gastric ulcers:

In this model the gastric ulcers in rats were induced by ethanol administration. The rats were starved for thirty six hours with water adlibitum before administering ethanol. Misoprostol (100µg/kg, p.o.) /the active fractions of *Urena lobata* (200 mpk, p.o.) suspended in 0.5% CMC [13] were dosed orally to their respective groups one hour prior to ethanol administration. All the rats received ethanol (1mL/rat) and 60 minutes later all the rats were terminated by carbon-di-oxide anesthesia. The stomach's were isolated and opened on the greater deflection to spread on a transparent sheet which aids for easy scanning of the images using computer scanner [14]. The total ulcerated and mucosal area was assessed by employing Scion image software to determine the ulcer index as cited earlier.

Statistical analysis

The data were expressed as mean ± SEM. All the data were analyzed by one way analysis of variance (ANOVA) [15, 16] followed by "Dunnet's t-test" [17]. *p*-value less than 0.05 were considered as statistically significant.

RESULT AND DISCUSSION:

Extractive Values:

The phytoconstituents were extracted by using different solvent of increasing polarity like chloroform, ethyl acetate, n-hexane, ethanol and water. The extractive values of various extract are expressed in table and figure 1.

Table 1: The extractive values of various extract of leaves of *Urena lobata*

S. No.	Extracts	Estimated percentage	Colour of extract
1.	Chloroform	4.52 % w/w	Dark Yellow
2.	Ethyl acetate	4.02 % w/w	Light Brown
3.	n-hexane	3.73 % w/w	Greenish brown
4.	Ethanol (70%)	9.45 % w/w	Dark green
5.	Water	11.21% w/w	Dark Brown

We were found that the animals were safe and no mortality was observed up to 2000mg/kg body weight. It records that LD50 determination of the aqueous extract of leaves of *Urena lobata* are > 2000mg/kg body weight. The acute oral toxicological study has not shown any deviation from the normal behavior of the Wistar rat during the entire period of study. So, there was no acute toxicological changes were observed for aqueous extract of leaves of *Urena lobata* up to 2000mg/kg body weight of the animals. Hence 1/10th of the dose was taken as effective dose that was 200mg/kg body weight.

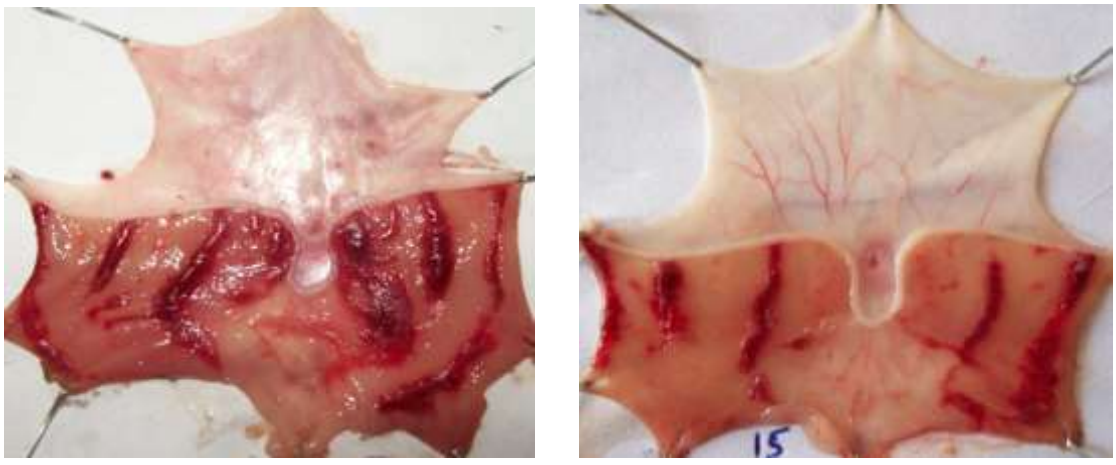
Table- 2: Acute oral toxicity study of different extracts of *Urena lobata*

Group	Dose (mg/kg)	Route	Death/Total in Ethanolic extract	Death %	Death/Total in Aqueous extract	Death %
I	500	Oral	00/10	0	00/10	0
II	1000	Oral	00/10	0	00/10	0
III	1500	Oral	00/10	0	00/10	0
IV	2000	Oral	00/10	0	00/10	0
V	3000	Oral	00/10	0	00/10	0
VI	4000	Oral	00/10	0	00/10	0

Result: Mortalities after 72hrs were recorded as shown in the Table.

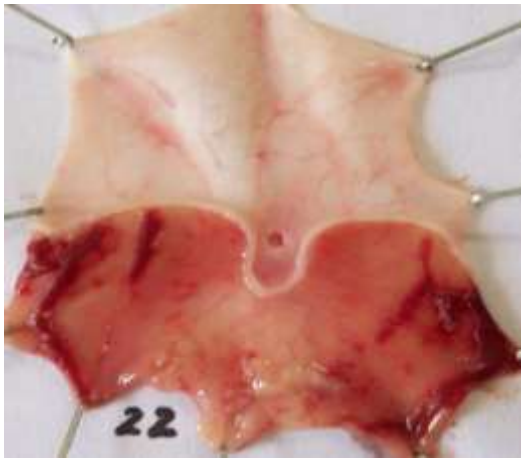
ANTI-ULCER ACTIVITY:

Ethanol Induced Ulcer

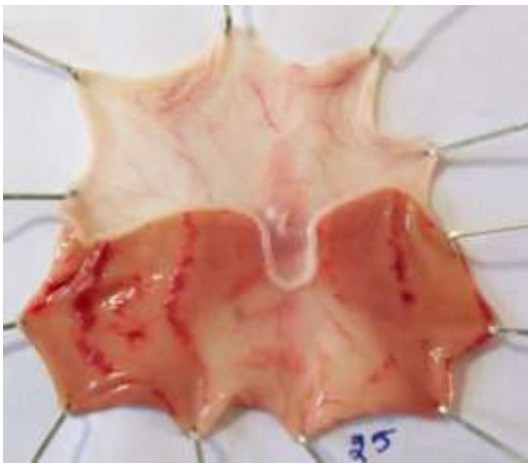


G-I G-II

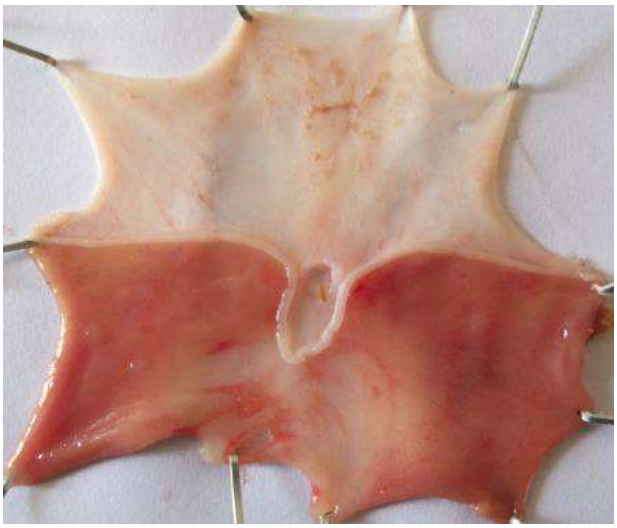
Figure 1(A): Ethanol Induced Ulcer



G-III



G-IV



G-V

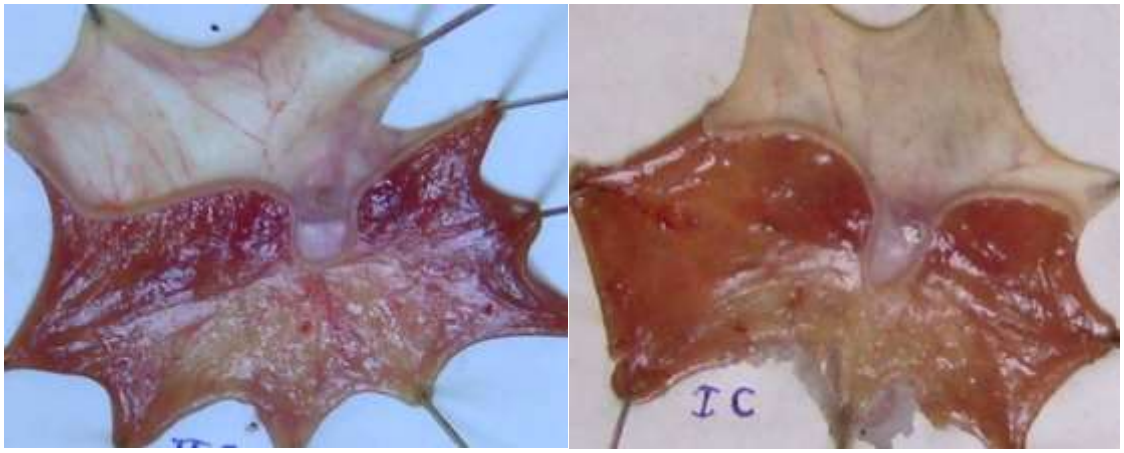
Figure 1(B): Ethanol Induced Ulcer

Table 3: Effect of isolated compounds of leaves of *Urena lobata* on alcohol- induced gastric ulceration in rats

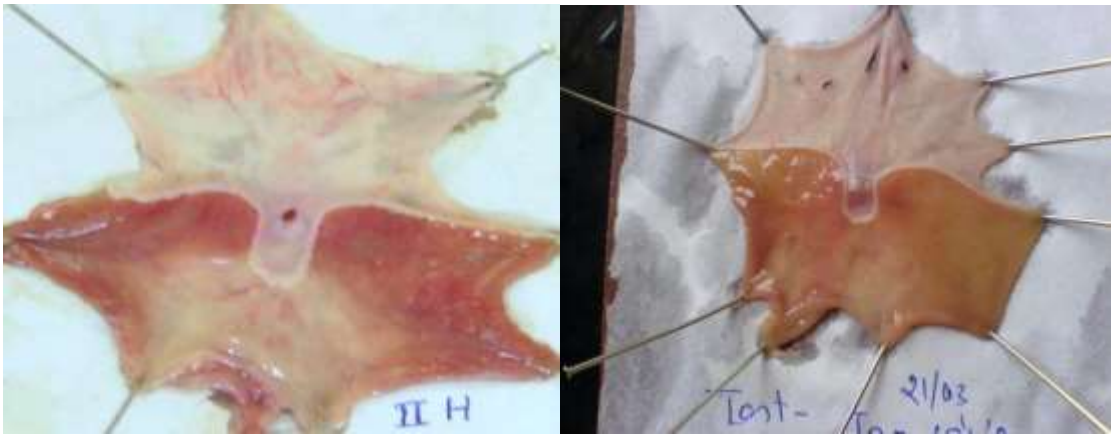
Group	Treatment	Ulcerated area Mean ± SEM (mm2)	Ulcer index	% Protection
I	Normal saline	105 ± 6.5	1.162 ± 0.517	-
II	isolated compound A of leaves of *Urena lobata* (10mg/kg body weight)	92.50 ± 4.5	0.845 ± 0.125	26.33
III	isolated compound A of leaves of *Urena lobata* (15mg/kg body weight)	75.50 ± 3.7	0.734 ± 0.174**	38.10
IV	isolated compound A of leaves of *Urena lobata* (20mg/kg body weight)	49.70 ± 4.0	0.538 ±0.103***	54.18
V	Omeprazole 20 mg/kg b.w. (Standard)	34.40 ± 3.0	0.345 ±0.104***	71.14

Mean ±SEM; N=6 in each group) ** p<0.01, ***p<0.001 (VS Diseased control) respectively. The results were expressed as Mean ± S.E.M. and statistical significance by means of Oneway ANOVA followed by Tukey-Kramer multiple comparisons test.

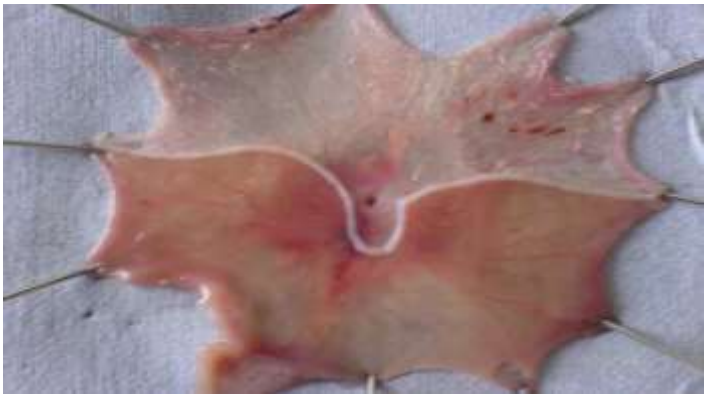
Pylorus ligation induced gastric ulcers:



G-I G-II



G-III G-IV



G-V

Figure 2: Pylorus ligation induced Ulcer

Table 4: Histopathology of alcohol-induced ulcer of rat's stomach

Group	Treatment	Histopathological findings
I	Normal saline	Ulcers (Grade III) present along with inflammatory changes including sub-mucosal oedema (Grade II).
II	isolated compound A of leaves of *Urena lobata* (10mg/kg body weight)	Ulcers (Grade III) present along with inflammatory changes including severe submucosal oedema (Grade III).
III	isolated compound A of leaves of *Urena lobata* (15mg/kg body weight)	Ulcers (Grade I) of minimal intensity with congestion and mononuclear cell infiltration were observed. Sub-mucosal oedema of Grade II was observed.
IV	isolated compound A of leaves of *Urena lobata* (20mg/kg body weight)	No ulcers were observed. Mild erosions and submucosal oedema (Grade I) were observed.
V	Omeprazole 20 mg/kg b.w. (Standard)	Ulcers of minimal intensity (Grade I), deep congestion of sub-mucosal blood vessels and oedema of sub-mucosa were observed.

Table 5: Histopathology of Pylorus ligation induced gastric ulcers

Group	Treatment	Histopathological findings
I	Normal saline	Mild erosions with moderate inflammatory changes were observed. Sub-mucosal oedema of (Grade I) was found.
II	isolated compound A of leaves of *Urena lobata* (10mg/kg body weight)	Ulcers of minimal intensity (Grade I) along with inflammatory changes were observed.
III	isolated compound A of leaves of *Urena lobata* (15mg/kg body weight)	Ulcers of minimal intensity (Grade I) along with inflammatory changes were observed.
IV	isolated compound A of leaves of *Urena lobata* (20mg/kg body weight)	Ulcers of moderate intensity (Grade II) along with inflammatory changes were observed.
V	Omeprazole 20 mg/kg b.w. (Standard)	No ulcers were observed.

CONCLUSION:

The plant *Urena lobata* commonly is an indigenous herb which was chosen for this study. The plant belonging to the family Malvaceae, the scanty availability of information on this plant facilitates the study on it. Entire plants of *Urena lobata* were collected from Indore India. The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use. The leaves of *Urena lobata* plant was characterized by its morphological features like colour, shape, size and surface characteristics has been studies. The acute toxicity test aims an establishing the therapeutic index, i.e. the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species (LD50/ED50). In any screening program, acute toxicity on mice/rat is usually performed first. Acute oral toxicity: Up and down method, OECD Guideline 425 (Revised: 17 December 2001) The single dose of aqueous extract of leaves of *Urena lobata* was used for the acute oral toxicological study for appreciating the usefulness of the chosen plant ant in understanding the effects of the same in relation to the gastric ulcer. LD50 / tolerable dose are estimated in rats. We were found that the animals were safe and no mortality was observed up to 2000mg/kg body weight. It records that LD50 determination of the aqueous extract of leaves of *Urena lobata* are > 2000mg/kg body weight. Effect of isolated compounds of leaves of *Urena lobata* on alcohol- induced gastric ulceration in rats on different dose were tested (10, 15 and 20mg/kg body weight) the % protection range was observed from 26.33 to 71.14 (standard drugs omeprazole),. 54.18 observed on 20mg/kg dose. Histopathological findings for alcohol-induced ulcer of rat's stomach was found No ulcers were observed. Mild erosions and submucosal oedema (Grade I) were observed for high dose of test compounds as 20mg/kg dose. Histopathology of Pylorus ligation induced gastric ulcers was found Ulcers of moderate intensity (Grade II) alongwith inflammatory changes were observed for high dose of test compounds as 20mg/kg dose.

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