ACUTE ORAL TOXICITY AND ANTI ULCER ACTIVITY OF LIPOSOMAL FORMULATION UTLIZING INDOMETHACIN INDUCED ULCER MODEL

Shakti Jaiswal[1], Alok Kumar dash[2] *Jhansee Mishra[3]
[123]*Institute of Pharmacy V.B.S. PurvanchalUniversity,Jaunpur,Uttarpradesh,India*
**Corresponding Author*
Dr.Jhansee Mishra,Assistant Professor,Institute of Pharmacy
V.B.S. Purvanchal University, Jaunpur,Uttarpradesh,India
Email: alokjina@gmail.com Mob:9648349477

ABSTRACT
The study investigates the acute oral toxicity and anti-ulcer efficacy of a novel liposomal formulation in an indomethacin-induced ulcer model. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), is known to cause gastrointestinal ulcers, serving as a reliable model for testing anti-ulcer agents. The liposomal formulation was designed to enhance the bioavailability and therapeutic efficacy of the encapsulated drug while minimizing systemic toxicity. In the acute oral toxicity study, the liposomal formulation was administered to Wistar rats at varying doses, and the animals were monitored for 14 days. The results indicated that the formulation was well-tolerated up to the highest tested dose, with no significant toxicological effects observed. For the anti-ulcer activity assessment, rats were pretreated with the liposomal formulation before being administered indomethacin to induce ulcers. The extent of ulceration was evaluated macroscopically and microscopically. Key parameters such as ulcer index, percentage inhibition of ulceration, and histological changes in gastric tissues were measured. The liposomal formulation demonstrated significant anti-ulcer activity, evidenced by a marked reduction in ulcer index and histopathological improvements compared to the control group. These findings suggest that the liposomal formulation not only has a favorable safety profile but also possesses potent anti-ulcer properties, making it a promising candidate for further development as a therapeutic agent for NSAID-induced gastric ulcers.

Keywords: Acute oral toxicity, Liposomal formulation, Indomethacin, Anti-ulcer activity, NSAID-induced ulcers

1. INTRODUCTION

Pharmacology has undergone a revolution with the invention of liposomal drug delivery systems, which present a viable means of improving the safety and therapeutic efficacy profiles of different pharmacological agents. The ability of liposomes, spherical vesicles made of phospholipid bilayers, to encapsulate drugs—both hydrophilic and lipophilic—to increase bioavailability and decrease side effects has been the subject of much research **(Akbarzadeh et al., 2013)**. NSAIDs, or non-steroidal anti-inflammatory medicines, are commonly used to treat inflammation, fever, and pain. These medications do, however, have certain adverse effects, particularly on the digestive system. Lately, it has also been demonstrated that reactive oxygen species (ROS) are essential for the pathogenesis of acute experimental stomach lesions brought on by ethanol, stress, and NSAIDs **(Banarjee, 1990, Das et al., 1997, Miura et al., 2002)**. Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin are commonly used because of their strong analgesic, antipyretic, and anti-inflammatory effects. But serious gastrointestinal (GI) adverse effects like ulceration, bleeding, and perforation frequently prevent it from being used clinically **(Hawkey, 1999).** These negative effects are mainly

ascribed to the suppression of cyclooxygenase (COX) enzymes, which causes the stomach mucosa's protective prostaglandins to diminish **(Wallace, 2008)**. As a state of unfavorable effects brought about by the interaction of toxicants with cells, toxicity is an expression of being poisonous. This interaction can happen at the extracellular matrix, on the cell surface, inside the cell body, or in the tissues underneath, and it can vary based on the chemical characteristics of the toxicants and the cell membrane. Before the poisonants connect to important organs like the liver and kidneys, there may be harmful effects. Because exposure to chemicals can be dangerous and have negative effects on humans, it is imperative to evaluate a substance's toxic qualities when considering public health protection. Evaluations in practice usually take into account consequences that are carcinogenic, reproductive, subchronic, and chronic **(Asante, 2002).** Many approaches of medication distribution have been investigated in an effort to lessen these GI adverse effects. Among these, liposomal formulations with sustained release, targeted distribution, and less systemic toxicity have demonstrated a great deal of promise in improving the therapeutic index of NSAIDs **(Allen & Cullis, 2013).** Using an indomethacin-induced ulcer model in rats, this study examines the acute oral toxicity and anti-ulcer activity of a liposomal formulation containing the drug. Evaluating the liposomal formulation's protective effects on the gastric mucosa and ability to reduce stomach damage caused by indomethacin is the main goal.

2. MATERIAL AND METHOD

2.1 Animal

Institutional Animal Ethics Committee (IAEC) approval was obtained for all animal research under the approval no (PBRI/IAEC/15-12-23/011). The animals employed were 150–250 grams in weight and of either sex. At a regulated temperature of 22 ± 2°C, they were housed in different cages. Every animal received a standard food (golden feed, New Delhi) and regular access to water.

2.2 Acute oral toxicity study

Three animals of the same sex are used in each phase of the step-by-step process for the acute toxic class technique outlined in the guidelines. An assessment of the test substance's acute toxicity may need, on average, two to four phases, and contingent on the animals' mortality and/or moribund state. One of the specified doses of the material is given orally to a group of experimental animals. Three animals of the same sex are used in each phase of the methodical testing process for the chemical. The next course of action, which entails treating three more animals at the same dose and three more animals at the next higher or lower dose level, will depend on whether compound-related mortality of the animals dosed at one step is present or absent. For every step, three animals are utilized. A starting dose of one of four preset dose levels—5, 50, 300, or 2000 mg/kg body weight—must be chosen.

Parameters: Body weight, mortality and behavior changes.

2.3 Anti-ulcer activity

The albino rats of either sex weighing between 180 and 200 g were divided into six groups of six animals each and fasted for 24 h with water *ad libitum* prior to experiment. The animals of group I were treated with vehicle Normal saline (5 ml/kg p.o) daily, for 5 days and the animals of groups II, III, IV were treated with indomethacin sodium (30 mg/kg p. o.) once, on day 1. Animals of group III were treated with ranitidine 50 mg/kg thrice a day in water, for 5 days before the induction of ulcer. Similarly, the animals of group IV treated with Kaempferol (50 mg/kg bw), group V treated with Mesalamine (150 mg/kg bw) and group VI were treated with Liposomal formulation for 5 days respectively, before the induction of ulcer. Indomethacin (30 mg/kg p.o.) was administered to the animals of groups II–VI on day 6. On 6th day, the animals were sacrificed by cervical dislocation after 12 h of the last dose. The stomach was taken out

and cut open along the greater curvature of the stomach. The number of ulcers per stomach was noted, and severity of the ulcers was observed microscopically and scoring was done.

2.4 Parameters assessed for anti-ulcer activity

2.4.1 Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. After making an incision along the larger curvature of the stomachs, the contents were rinsed out with regular saline, and the formation of ulcers was inspected with a 10x magnifying lens. The Kulkarni technique (0 = no ulcer, 0.5 = red coloring, 1 = spot ulcers, 2 = hemorrhagic streaks, 3 = ulcers > 3 but < 5 and 5 = ulcers > 5) was used to rate the number of ulcers.

The ulcer Index and percentage of ulcer inhibition were determined as follows:

$$\textbf{Ulcer index (UI) = UN + US + UP} \times \textbf{10}^{-1}$$

Where,

UN = Average number of ulcers per animal,

US = Average of severity score,

UP = Percentage of animals with ulcers

2.4.2 Collection of gastric juice:

Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10min.

2.4.3 Volume of gastric juice:

The volume of the centrifuged sample was expressed as ml/100 g body weight.

2.4.5 PH of gastric juice

A pH meter is used for determining pH after diluting 1 ml of gastric juice aliquot with 1 ml of distilled water.

4.2.5 Total Acidity and Free acidity

Gastric juice (1ml) was pipette into a 100ml conical flask and diluted with 9ml distilled water. Two or three drops of Toepfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red color disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein will be then added and the titration was continued until a definite red ting appeared; the volume of alkali added was noted. The volume corresponds to total acidity. Acidity was expressed in terms of mEq/L.

2.4.6 Histopathology

The stomach was immersed in 10% formalin solution for histopathological examination. These tissues were processed, dehydrated in different grades of alcohol, cleared in toluene, and impregnated in molten paraffin wax for specified periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Sections were at 3 μ and dried on a hot plate for 15 min and stained with hematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam.Sections were viewed microscopically using ×10 and ×40 objective lenses. Stained sections were examined under microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcer.

2.4.7 Statistical Analysis

Results are provided as Mean±SD (n=6). Results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Bonferroni t-test. $P < 0.05$ was considered as level of significance while comparison of two groups.

3. RESULTS & DISCUSSION

3.1. Acute toxicity study

Table 1: Toxicity parameters after dosing of 5, 50, 300 and 2000 mg/kg

Sr. No.	Toxicological parameters	Observations of administrated liposomes			
		(5 mg/kg)	(50 mg/kg)	(300 mg/kg)	(2000 mg/kg)
1	Eyes	Normal	Normal	Normal	Normal
2	Mucous membranes	Normal	Normal	Normal	Normal
3	Salivation	Normal	Normal	Normal	Normal
4	Stool	Normal	Normal	Normal	Normal
5	Diarrhoea	No	No	No	No
6	Sleeping pattern	Normal	Normal	Normal	Normal
7	Mortality (14 days)	No	No	No	No

In this study, the rats received liposomal formulation and animals were observed for 7 days, to detect the peak of systemic toxic effects and 14 days, as recommended by the OECD guideline. Behavioral modifications, body weight, and mortality were evaluated during the experiment of acute toxicity study. The weight variation is calculated by the difference between the final and initial weight of the animals. After 7 and 14 days, the rats administered with liposomal formulation was found to be safe at a dose 2000 mg/kg bw. No sign of any toxicity or behavior changes were observed.

3.2. Antiulcer activity

Comparative analysis of UI data revealed significant decrease ($p<0.05$) in UI intreated group as compared to indomethacin sodium treated group. In Indomethacin treated group, the volume of gastric content, pH, total acidity and free acidity was significantly different ($p<0.05$) as compared to Normal Control (NC) group. In standard ranitidine and test samples (Kaempferol, Mesalamine, liposomal formulation) treated groups, the volume of acid secretion, total acidity was decreased significantly ($p<0.05$) and pH of the gastric juice was increased significantly ($p<0.05$) as compared to Indomethacin controlgroup.

The Liposomal formulation exhibited an inhibition percentage of 72.22respectively; and, the standard drug, ranitidine, exhibited an inhibition percentage of 87.96 at 50 mg/kg bw (Table: 1).

Table 2: Effect of samples Kaempferol, Measlamine and Liposomal formulationon ulcer index in indomethacin induced ulcer

Group No.	Treatment	Ulcer Index	%
I	Normal Control (NC) (Placebo treated)	0.00±0.00	-
II	Indomethacin sodium (30 mg/kg bw) p.o.	1.08±0.075	-
III	Standard (Ranitidine) (50 mg/kg bw, p.o.)	0.13±0.163**	87.96
IV	Kaempferol (50 mg/kg bw, p.o.)	0.57±0.081	47.22
V	Mesalamine (150 mg/kg bw, p.o.)	0.48±0.271	55.55
VI	Liposomal Formulation	0.30±0.236**	72.22

Values Provided as Mean± SD (n=6), *P<0.05 as compared to indomethacin induced

From biostatistical data analysis, theindomethacin sodium treated group was found statistically significant (P<0.05) when compared to Standard (Ranitidine) and Liposomal formulation. In this data Standard ranitidine and Liposomal formulation was found highly significant when compared to indomethacin sodium group also better results were seen in other drugs treated groups (Kaempferol and Mesalamine) but non-significant.

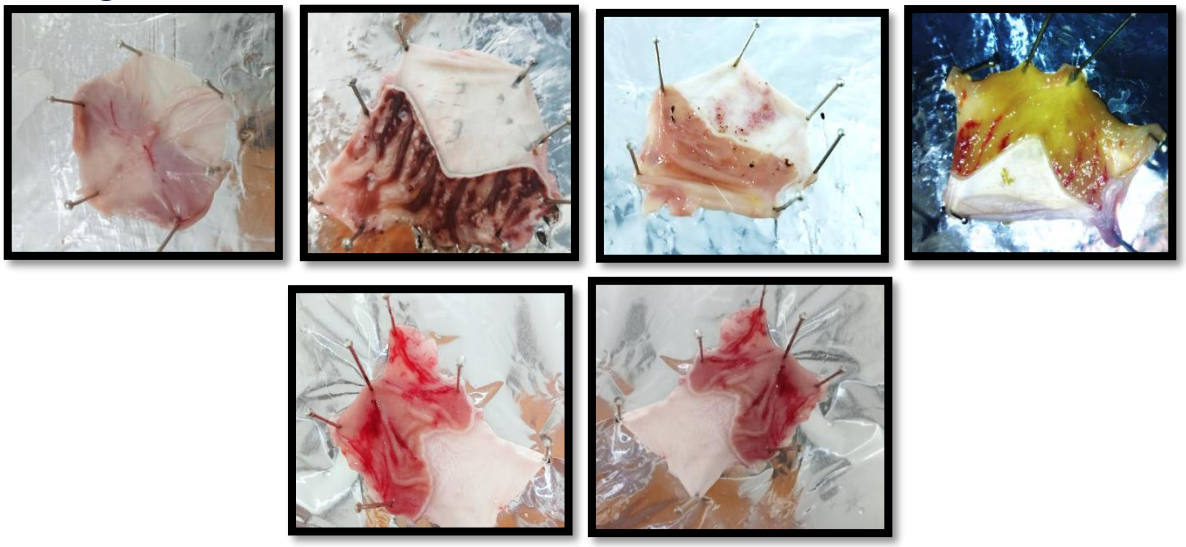
Table 3: pH, volume of gastric contents, free acidity, and total acidity of the test and control groups in the indomethacin-induced ulcer model

Group No.	Treatment	pH	Total acidity (mEq/lt)	Free acidity (mEq/lt)	Gastric juice (ml)
I	Normal Control (NC) (Placebo treated)	6.43±0.340	37.62±6.234	26.55±3.112	5.19±0.156
II	Indomethacin sodium (30 mg/kg bw) p.o.	3.23±0.537	127.74±9.216	89.64±8.389	10.10±0.632
III	Standard (Ranitidine) (50 mg/kg bw, p.o.)	5.83±0.254**	49.54±8.370**	41.13±6.241**	5.72±0.203**
IV	Kaempferol (50 mg/kg bw, p.o.)	4.16±0.205	84.07±6.876*	76.77±6.201*	7.90±0.302*
V	Mesalamine (150 mg/kg bw, p.o.)	5.87±0.276**	80.24±6.241**	56.28±7.681**	6.59±0.506**
VI	Liposomal Formulation	6.00±0.179**	65.83±5.913**	51.66±2.338**	6.16±0.492**

Values Provided as Mean ± SD (n=6), *P<0.05 as compared to indomethacin induced

From biostatistical data analysis, Inindomethacin control group (Group II), the volume of gastric juice, pH, total acidity and free acidity was significantly different (p<0.05) as compared to Normal Control (NC) group (Group I). In Test Sample (Standard, Kaempferol, Mesalamine and Liposomal formulation), the volume of gastric juice, total acidity was decreased significantly (p<0.05) and pH of the gastric juice was increased significantly (p<0.05) as compared to indomethacin control (Group II). When Standard (Ranitidine) treated group (Group IV) was compared with liposomal formulation treated group (Group VI), they showed highly significant variation as compared to Indomethacin group (Group II).

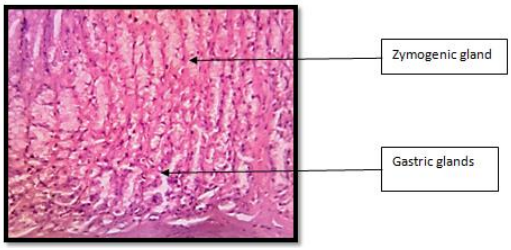
3.3 Images of Ulcer



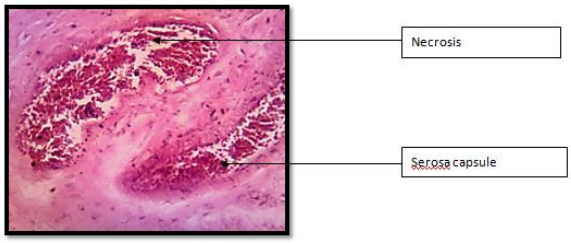
Group I: Normal Control, Group II: Indomethacin, Group III: Ranitidine, Group IV: Kaempferol, Group V: Mesalamine, Group VI: Liposomal formulation

3.4 Histopathology

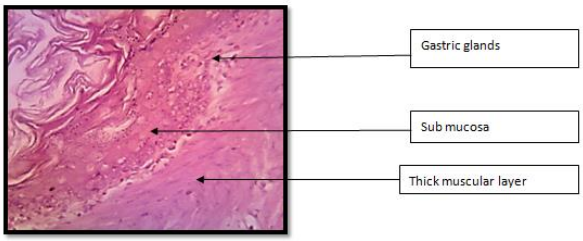
Group I: Normal control



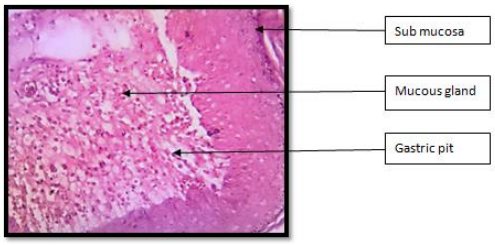
Group II: Indomethacin sodium treated



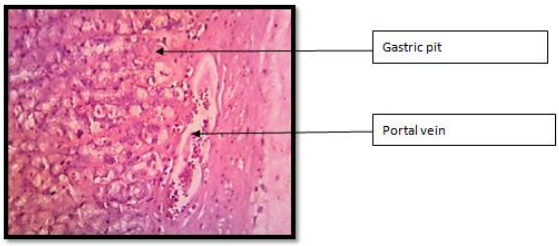
Group III: Ranitidine (50 mg/kg bw p.o.)



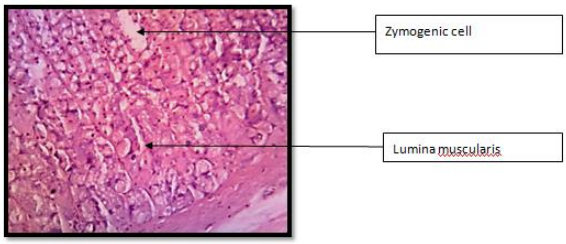
Group IV: Kaempferol (50 mg/kg bw, p.o.)



Group V: Mesalamine (150 mg/kg bw, p.o.) treated rats



Group VI: Liposomal formulation



Group I: Normal control group showed the normal submucosa, gastric glands and outer longitudinal muscle. Group II: Indomethacin treated rats showed inflammation, necrosis, and severe effect on mucosa with leucocyte infiltration and cellular debris. Group III: Standard Ranitidine treated with normal appearance of Gland cell and sub mucosal cell. Group IV: Kaempferol (5 mg/kg bw) treated rats showed muscularis mucosa (MM), vacoulation, Submucosa (SM) and blood in mucosa cells. Group V: Mesalamine (150 mg/kg bw, p.o.)

treated rats showed mild inflammation, muscularis mucosa (MM), vacoulation, Submucosa (SM) and blood in mucosa cells. Group VI: Liposomal formulation rats showed longitudnal muscle, mucosal layer, gastric glands, lumina muscularis, zymogenic cells

4. CONCLUSION

The study on the acute oral toxicity and anti-ulcer activity of the liposomal formulation utilizing indomethacin-induced ulcer model demonstrated significant findings. The liposomal formulation exhibited a favorable safety profile, as evidenced by the absence of acute toxicity symptoms and mortality in the tested subjects. Furthermore, the anti-ulcer activity was markedly enhanced in the liposomal group compared to the control, indicating the potential of liposomal encapsulation in mitigating indomethacin-induced gastric ulcers. These results suggest that the liposomal formulation not only improves the therapeutic efficacy but also minimizes the gastrointestinal side effects associated with indomethacin, offering a promising alternative for safer and more effective ulcer treatment. Future studies focusing on long-term efficacy and detailed mechanistic insights will further elucidate the potential clinical applications of this liposomal formulation.

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