

TOXICOLOGICAL EVALUATION OF OLEANOLIC ACID (PENTACYCLIC TRITERPENOID) EXTRACTED FROM *LANTANA CAMARA* ROOTS FOLLOWING ORAL EXPOSURE IN WISTAR RATS

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ABSTRACT

Objective: The aim of the present study was to perform an acute toxicity study to obtain information on the possible adverse effects from a single oral administration of Oleanolic acid in Wistar rats as the onset of toxicity, and to determine the range of exposure (to the LD50 cut-off criteria). Following a sub-chronic 90-days Repeated toxicity study by oral route to determine any potential indication of its dose response relationship and determine no observed effect level (NOEL)/ no-observed adverse effect level (NOAEL)/ low observed effect level (LOEL)/ low observed adverse effect level (LOAEL).

Methods: A dose level of 2000mg/kg body weight was employed as step one for single acute study. Based on the survival pattern of the previously dosed animals after 48 hours, same dose 2000 mg/kg body weight) was repeated for Step 2 as a confirmatory test. For the 90-day toxicity study, the highest dose was determined as 1000mg/kg Body weight, and the middle and lower doses were 500 and 250mg/kg Body weight respectively. The rat group was held for a 14-day recovery period after the last dose administration, to observe for any persistence or reversal of toxic effects.

Results: Results of the acute toxicity study showed no mortality on the dose level of 2000mg/kg body weight with no significant clinical and body weight changes. During the 90-day Repeated dose oral sub-chronic toxicity study, no rats died. There were no significant clinical changes related to the test item in terms of functional evaluation, body weight, food and water consumption, ophthalmological tests, urine analysis, necropsy findings, or organ weight, Hematology, and biochemistry at the highest dose level of 1000 mg/kg bwt.

Conclusion: It is concluded that LD₅₀ Cut-off Value of "Oleanolic acid" in from acute oral toxicity study in Wistar Rats is 2000 mg/kg b.wt. In addition, 90 days study have showed no significant changes with respect to any hematological or blood chemical analyses in 1000, 500 and 250 mg/kg bwt groups. Based on histopathological findings, clinical signs and other parameters, it may be concluded that upon repeated once oral administration for consecutive 90 days, the Oleanolic acid (Pentacyclic Triterpenoid) extracted from *Lantana camara* roots at the dose level of 1000 mg/kg body weight have caused no adverse effect in both sexes of Wistar Rats.

Keywords: Oleanolic acid, *Lantana camara*, Pentacyclic Triterpenoid, NOAEL.

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INTRODUCTION

India is a country rich in flora and fauna, with a wide range of trees, flowers, and fruits found in diverse coastal, eastern, and estuarine regions of the Indian Ocean. Fruits have been an important source of traditional medicines due to their pharmaceutical and therapeutic significance. In general, medicinal plants also cure ailments with their highly complex bioactive entities. In recent decades, research has demonstrated the usefulness of medicinal plants in the treatment of a wide range of human diseases [1]. Many countries have been conducting extensive research on medicinal plants for medical advancement. Nevertheless, at times, these herbs and medicinal plants can be hazardous or non-toxic, depending on the dose and frequency of the dose administered. Scientific literature has published very thorough descriptions of the uses of herbs and many other plants that can support in healing many diseases in the past few decades.

The present studies were aimed at a short and sub-chronic safety assessment of oleanolic acid (pentacyclic triterpenoid) extracted from *Lantana camara* roots. *L. camara* is high in bioactive content and has been utilized in traditional medicine for long time [2]. The roots of *L. camara* have large quantities of triterpenoid oleanolic acid [3]. Many major biological effects of oleanolic acid have been reported, including anti-inflammatory [4], anti-hyperlipidemic [5], anti-ulcer [6], antioxidant activity [7], and hepatoprotective capabilities [8-10]. This chemical has recently been recognized for its antitumor-promotion function [11].

Extraction is the first and most critical process in recovering and purifying active compounds from plant materials. Many procedures have been devised to extract terpenoids from ginseng roots, the most prevalent of which include reflux, cooking [12], and solid-liquid extraction [13].

Despite having positive anti-inflammatory, anti-hyperlipidemic, anti-ulcer, antioxidant, and hepatoprotective properties, the safety (toxicity) profile of oleanolic acid is not well understood. Consequently, the current study was designed to evaluate the acute single dose, MTD, and 90-day oral toxicity study as repeated dosages.

METHODS

Collection, authentication, and extraction of the test item roots of *L. camara* Linn. were gathered in hilly terrain near Bhopal, Madhya Pradesh, India. Plant stuff was removed in the early morning. Then, the roots were ripped apart and left to dry in the open air to obtain granular powder for the studies.

Before adopting the plant for the herbarium, the Department of Botany, University of Lucknow, India, confirmed its authenticity.

In brief, 500 g of powdered crude drug was thoroughly extracted with petroleum ether over the course of an overnight period at room temperature and then 4 times thoroughly extracted with ethanol. The crude extract was dissolved in CHCl₃ and left to stand overnight for the

solvent to be taken out under vacuum before precipitating. The precipitate that was created in this way was crystallized using methanol. Crystals of oleanolic acid were created following four cycles of condensation and precipitation. The obtained oleanolic acid from the roots of *L. camara* was chemically analyzed by DSC, HPLC, and FTIR techniques [14].

Characterization of test item

The FTIR spectra that were obtained revealed several groups in the following spectrum regions: 3434 (OH), 2861 (CH₂), 1690 (C=O), 1462 (OH), and 1363. (CH₃). Oleanolic acid's IR spectrum exhibits an adsorption ribbon that originates from an OH group in the 3434 cm⁻¹ range. The symmetric vibrations of the CH₂ cm⁻¹ group produce a very strong absorption ribbon at 2861 cm⁻¹. A distinctive ribbon of the carbonyl group (C=O) occurs in the region of 1690 cm⁻¹. At 1462 cm⁻¹, a planar distortion-induced absorption ribbon may be seen. A distinctive ribbon from the CH₃ group may be seen in the region of 1361 cm⁻¹.

Based on the acquired IR spectra, it is possible to identify the several functional (Fig. 1) groups of phenolics, which are supported by the oleanolic acid structure [15,16].

Animal and housing

For both studies, Wistar rats were employed for toxicity assessment, obtained from Rodent Research India. In the acute Study, 12 female and 3 female Wistar rats (6–8 weeks) per group were kept in a polycarbonate cage.

A maximum of three Wistar rats (6–8 weeks) were housed for 90 studies in each traditional polycarbonate cage, which measured 421 × 290 × 190 mm. The experimental animals were fed UV-irradiated regular commercial pelleted rat chow (Altromin Spezialfutter GmbH and Co.Kg), and their housing was kept at a constant temperature of 22°F (3°C) and relative humidity of 50–60%. In polycarbonate water bottles with stainless steel nozzles, RO-treated water was made available whenever needed.

Ethics

The combined protocol No. IAEC/64/1237 was approved by the Institutional Animal Ethics Committee (IAEC) of the Dabur Research

Foundation (DRF) (Registration No. 64/PO/RcBi/s/99/Committee for Control and Supervision of Experimental Animals [CCSEA]) for the acute and 90-day repeated dose oral toxicity studies, respectively. These studies were carried out strictly in accordance with the recommendations of the CCSEA, guidelines for laboratory animal facilities [17] and as per OECD guidelines 423,408 [18,19], and the drug and clinical trial rule [20]. All efforts were taken to follow humane endpoints and reduce pain when the animals were sacrificed via carbon dioxide asphyxiation.

Experimental design for acute toxicity study

Dosing

The test item was freshly prepared in 0.5% CMC+ tween 20 (vehicle) and administered every day step-by-step (Table 1).

Acute Toxicity Study: 2000 mg (test item) was mixed with vehicle (0.5% CMC+ tween 20) with the help of a mortar and pestle, transferred into the volumetric flask, and made up to the final volume of 10 mL. The test item formulation was prepared freshly before oral administration. Feed was withdrawn for 17–18 h before dosing [21], and water was provided before dosing and 3–4 h post-dosing.

The formulated test item was orally administered to animals of the respective steps (Set I and Set II) by oral gavage using stainless-steel ball-tipped oral intubation needles attached to a calibrated syringe to different groups as a single dose using disposable syringes. The starting dose of 2000 mg/kg body weight of the test item was administered to the Set I and Set II animals' step wise.

Clinical observation

For the acute study, animals were observed for general clinical signs of toxicity on the day of dosing at 30 to 40 min and approximately at 1 h (+10 min), 2 h (+10 min), and 4 h (+10 min) post-dosing and once daily thereafter till experiment completion (Tables 2 and 3).

The mortality and morbidity of all the animals were observed twice daily: once in the morning and once in the afternoon or evening throughout the observation period (Table 4).

Table 1: Dose group for acute study step-by-step method

Group	Test item	Dose in mg/kg body weight	Sex	No. of animals in each step
Step I	0.5% CMC+tween 20+Oleanolic acid	2000	Female	3
Step II		2000	Female	3

Table 2: For step 1 at dose level of 2000 mg/kg bwt

Animal No.	Day																		
	0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	30 min	1 h	2 h	3 h	4 h														
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1=normal

Table 3: For step 2 at dose level of 2000 mg/kg bwt

Animal No.	Day																		
	0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	30 min	1 h	2 h	3 h	4 h														
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1=Normal

Table 4: Individual animal mortality/morbidity details

Step and dose	Animal No.	Day	Mortality/morbidity
2000 mg/kg b. wt.	1	0-14	Nil
	2	0-14	Nil
	3	0-14	Nil
2000 mg/kg b. wt.	4	0-14	Nil
	5	0-14	Nil
	6	0-14	Nil

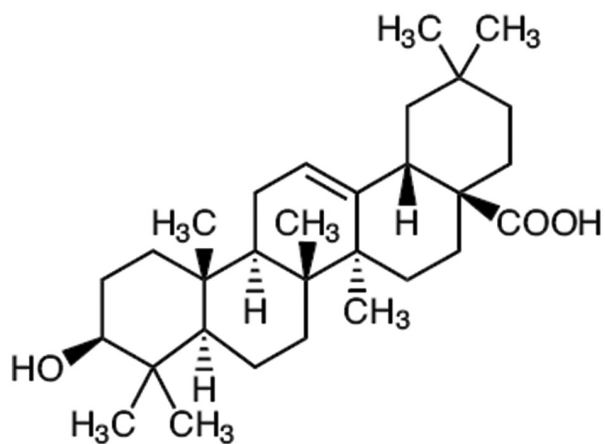


Fig. 1: Structure of oleanolic acid

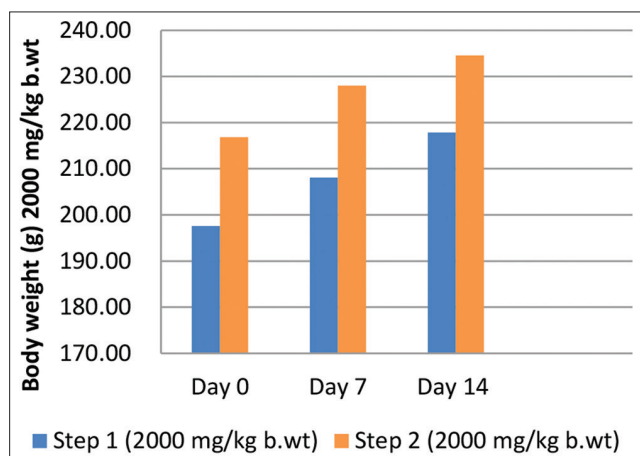


Fig. 2: Body weight female

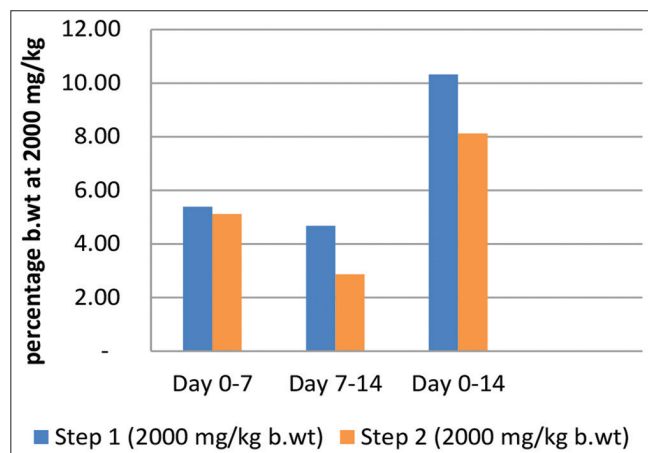


Fig. 3: Percentage body weight female

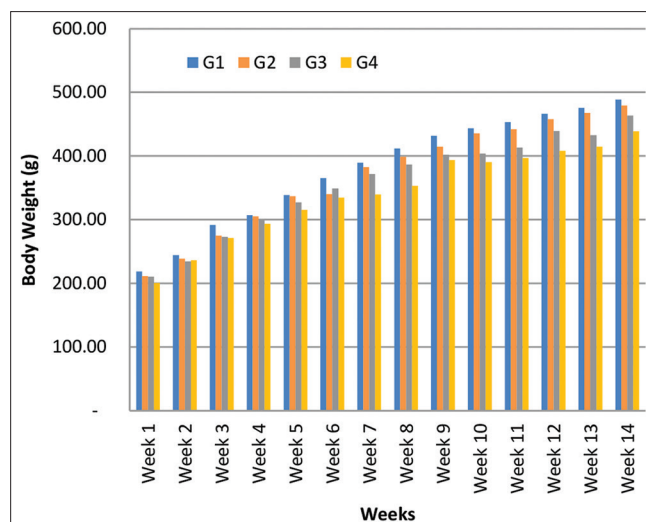


Fig. 4: Body weight male

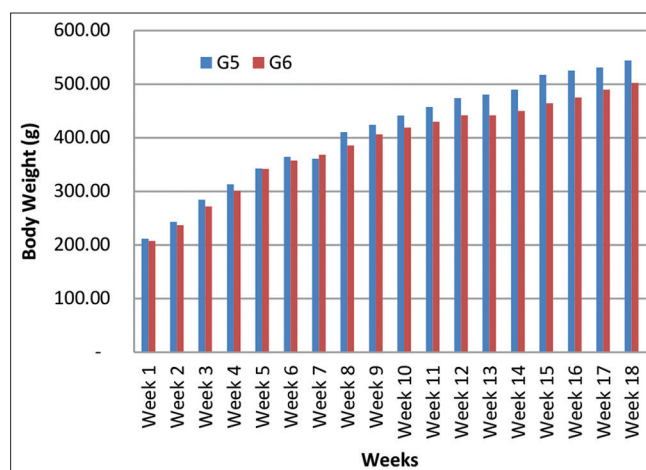


Fig. 5: Body weight male recovery

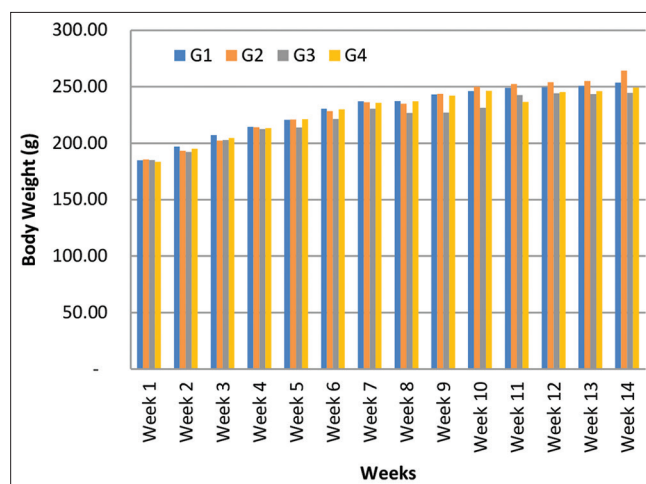


Fig. 6: Body weight female

Body weight and Percentage body weight

The body weight of all the animals was recorded on the day of receipt, at the time of animal selection, day 0 (before dosing), day 7, and day 14, and the percentage body weight was calculated from days 0 to 7, 7 to 14, and day 0 to 14 (Figs. 2 and 3).

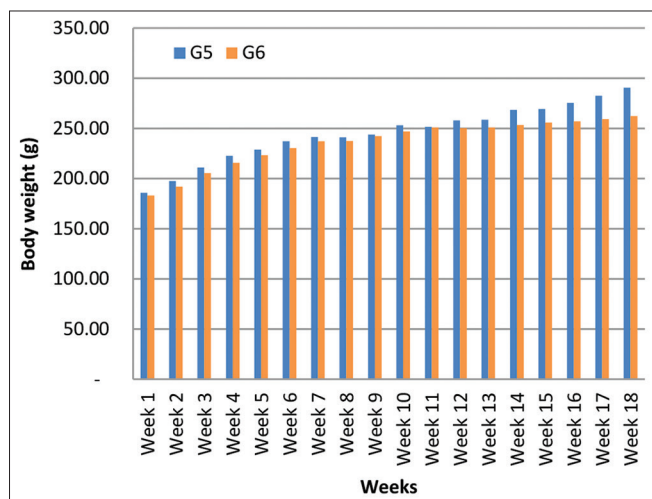


Fig. 7: Body weight female recovery

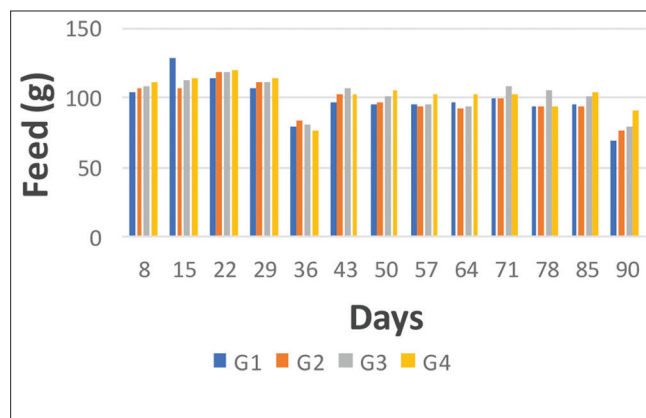


Fig. 10: Feed consumption female

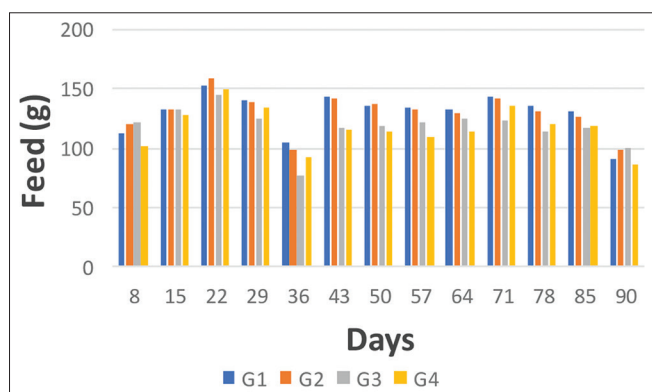


Fig. 8: Feed consumption male

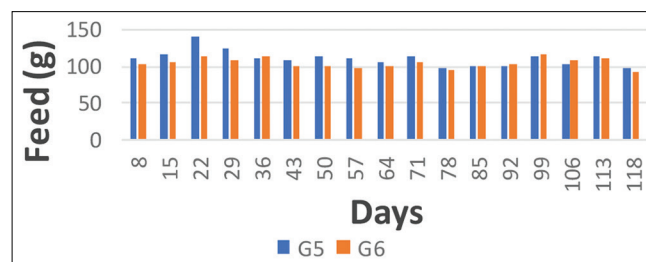


Fig. 11: Feed consumption female recovery

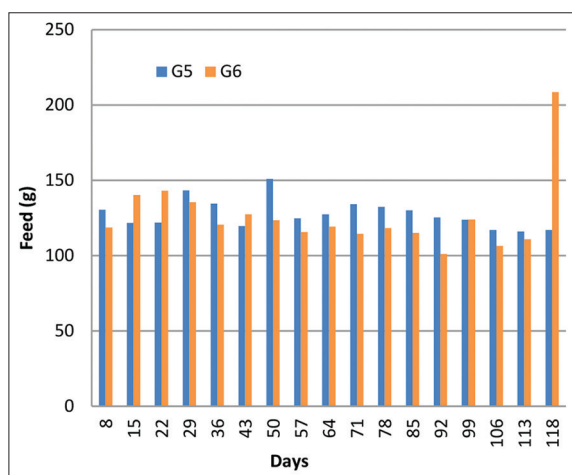


Fig. 9: Feed consumption male recovery

Table 5: Individual animal gross pathological findings. Sex: Female

Group	Dose (mg/kg b. wt.)	Animal No.	External examination	Internal examination
Step 1	2000	1	NAD	NAD
		2	NAD	NAD
		3	NAD	NAD
Step 2	2000	4	NAD	NAD
		5	NAD	NAD
		6	NAD	NAD

Keys: NAD: No abnormalities detected

using disposable syringes with stainless steel ball-tipped oral gavage for 90 consecutive days. The control group animals were administered with the vehicle once daily for 90 days in the same manner, with the maximum dose volume maintained at 10 mL/kg body weight. The animal was dosed at the same time each day (varying by + 1 h from the 1st day of dosing).

Clinical observation

For a total of 90 days, clinical symptoms and mortality were monitored twice a day (morning and afternoon) in all treated control rats. Every rat had its body weight measured on the 1st day of the study, the 1st week after that, and at the end of the 90-day experiment.

Body weight and food intake

We computed group mean body weights and the amount of food consumed by each rat.

The average weekly consumption was calculated after keeping track of the cage starting on the 1st day of treatment. Food intake for the rats in the recovery group was tracked weekly during the post-treatment period.

Gross pathology

All the surviving animals were sacrificed by CO₂ asphyxiation, and gross pathology was carried out for external and internal examination on day 14 (Table 5).

Experimental design for a 90-day sub-chronic toxicity study

The test item was freshly prepared in 0.5% CMC+ tween 20 (vehicle) daily administration. The test item was orally administered once daily

Neurobehavioral assessment

The neurobehavioral assessment was carried out before termination during the last week for the main group and on the last week for the recovery satellite group. Sensory reactivity to stimuli of different types (approach response, touch response, tail pinch response, air righting reflex, grip strength, and motor activity assessment) was conducted. At the end of treatment, all surviving animals were fasted overnight with water given *ad libitum*.

Ophthalmological examination

An ophthalmic examination was performed on all the treated animals, as well as the control animals, before the first treatment, and before study termination.

Hematology and clinical chemistry

Blood samples were collected in EDTA tubes for the determination of hematological parameters and in heparin tubes for clinical chemistry.

Necropsy/gross pathology and organ weight

On completion of 90 days and 118 days in the case of treatment and recovery periods, all the animals in the main treatment groups (G1-G4) were sacrificed on day 91, while the satellite animals were sacrificed after the completion of the 28-day reversal period. A necropsy was performed, including examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents. A gross examination of all the collected organs and tissues was performed.

Histopathology

Histopathology of all the preserved tissues: brain, spinal cord (at three levels: cervical, mid-thoracic, and lumbar), pituitary, thyroid, parathyroid, thymus, esophagus, salivary glands, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, testes/ovary, epididymides/uterus, cervix, vagina, female mammary gland, prostate, urinary bladder, lymph nodes, peripheral nerve, section of bone marrow, skin, and gross lesion organs were collected from all the animals and preserved in 10% neutral buffered formalin with the eyes preserved in Davidson's fluid and processed for paraffin embedding.

Statistical analysis

The data on the parameters, namely, body weight, percent change in body weight, detailed clinical observation (rears, urination, defecation, grip strength, and motor activity assessment), body weights, feed consumption, hematology, biochemistry, and organ weights, were analyzed for differences among the treated and control groups and subjected to statistical analysis using GraphPad Prism software, version 9.2.0 (332),

The results of the statistical analysis were reported in the form of mean, SD, and N. Data were evaluated for normal distribution by the D'Agostino-Pearson omnibus and then homogeneity of variance by the Bartlett's test. Then, the data were analyzed by ANOVA followed by Dunnett's *post hoc* test to compare test item-treated groups with control groups. Where data homogeneity and/or normality showed significance, the data were then analyzed by using the Kruskal-Wallis test followed by Dunn's *post hoc* test to compare test item-treated groups with control groups.

All analyses and comparisons were evaluated at the 95% ($p < 0.05$) significance level.

RESULTS

Acute toxicity study

This study was conducted to provide information on the possible health hazards likely to arise from a single oral administration of oleanolic acid (pentacyclic triterpenoid) extract in Wistar.

Acute toxic class method. This study was not intended to calculate a precise LD_{50} but to determine the range of exposure (to the LD_{50} cut-off criteria) where lethality is expected since the death of a proportion of the animals was the major endpoint of this test.

Clinical signs

No treatment-related clinical signs were observed in all the animals in Step 1 and Step 2 at the dose level of 2000 mg/kg b.wt. throughout the experiment period until day 14. (Tables 2 and 6).

No mortality or morbidity was observed in Step 1 and Step 2 animals at the dose level of 2000 mg/kg body weight (Table 4).

Body weight

The body weight gain and gain in percentage body weight were observed in Step 1 and Step 2 animals on day 7 and day 14 as compared to day 0 (Figs. 2 and 3).

Gross pathology

Gross pathology on Step 1 and Step 2 showed no external and internal finding (Table 5).

90-day sub-chronic toxicity study

The 90-day study was done to determine the assessment and evaluation of the toxic characteristics of a chemical and the determination of chronic toxicity, which need to be carried out after initial information on toxicity has been obtained by acute testing. To provide possible information, health hazards arise from repeated doses by oral administration over a limited route period. Wistar rats were evaluated with 0.5% CMC + tween 20 + Oleanolic acid (Pentacyclic Triterpenoid) by oral administration (G2, G3, G4, and G6) once daily using disposable syringes tipped with an oral gavage for 90 consecutive days. The control group and control recovery (G1 and G5) animals were administered with the vehicle once daily for 90 days in the same manner, and the dosing was stopped after 90 days of oral administration. These control group animals were observed for another 28 days to evaluate the effect during the recovery period (Table 6).

Table 6: Dosage group for repeated dose 90-day toxicity study

Group	Group description	Dose (mg/kg b.wt.)	No. of animals per group	
			Male	Female
G1 (control)	0.5% CMC+tween 20	0	10	10
G2 (Low Dose)	0.5% CMC+tween	250	10	10
G3 (Mid Dose)	20+Oleanolic acid	500	10	10
G4 (High Dose)	1000	1000	10	10
G5 (Control Recovery)	0.5% CMC+tween 20	0	5	5
G6 (High Dose Recovery)	0.5% CMC+tween 20+Oleanolic acid	1000	5	5

Table 7: Summary of mortality

Group	Dose	No. of animal used		Mortality		Total	% Mortality
		Male	Female	Male	Female		
G1	Control	10	10	0	0	0	0
G2	Low	10	10	0	0	0	0
G3	Mid	10	10	0	0	0	0
G4	High	10	10	0	0	0	0
G5	Control recovery	5	5	0	0	0	0
G6	High dose recovery	5	5	0	0	0	0

General clinical observations and mortality

No mortality and no clinical signs of toxicity were observed in any of the treated as well as control group animals (Table 7).

Body weight and feed input/output

Male (Fig. 4) and female (Fig. 6) animals from all the treated groups including the recovery groups (Figs. 5 and 7) exhibited a normal gain in body weight throughout the study period. The mean values of body weight were found to be statistically non-significant ($p < 0.05$) and comparable with the respective control groups.

The feed consumption in male (Fig. 8) and female (Fig. 10) animals, including the recovery groups (Fig. 9. and 11), was found to be statistically non-significant ($p \geq 0.05$) in both sexes when compared with the control group, except for significant ($p < 0.05$) increase in the week 13 G4 group when compared with the control group G1. The increase value was not dose dependent, and the reversible group was found to be statistically non-significant.

Neurobehavioral assessment

No adverse clinical signs were found while performing detailed clinical observations such as home-cage observations (posture, convulsion), handling observations (ease of removing from cage, handling reactivity, skin examination, piloerection, palpebral closure, eye examination, lacrimation, salivation), and open-field observations (arousal, respiration, clonic movement, gait, rearing, urination, defecation, mobility, stereotype, bizarre behavior) in all animals once before the first treatment and once every week thereafter.

Sensory reactivity to stimuli of different types (e.g., auditory, visual, and proprioceptive stimuli, assessment of grip strength, and motor activity assessment were recorded before termination during the 4th week for the main group and on the 6th week for the recovery satellite group). The rearing, urination and defecation, grip strength, and motor activity assessments in male and female rats of the entire treatment group revealed statistically non-significant results, comparable with the control group.

Ophthalmological examination

No treatment-related ophthalmic findings were observed in control or treated animals of both sexes (male and female).

Urinalysis

No abnormalities were detected in all the urine parameters, such as appearance, volume, pH, specific gravity, glucose, protein, and blood, from all the treated and control group animals.

Absolute organ weights and relative organ weights

No statistically significant ($p < 0.05$) difference was observed in the male and female main group and recovery group animals of both sexes in absolute and relative organ weight.

T3, T4, and TSH

No statistically significant ($p < 0.05$) difference was observed in the male and female main groups as well as recovery group animals of both sexes in the calculation of the T3, T4, and TSH parameters through the Elisa kit (Kit Make) method.

Hematology and clinical chemistry

All statistically significant ($p < 0.05$) differences observed in hematology parameters such as MCV, MCH, APTT, PLT, and PT were considered incidental and unrelated to the administration of the test item. These findings were either of minimal magnitude, lacked a clear dose-related pattern, were limited to one sex, or were randomly distributed across groups without consistency between the terminal and recovery phases of the study.

Parameters	Hematology			
	Male		Female	
	↑	↓	↑	↓
MCV	G3**, G4*	-	-	-
MCH	G3**, G4*	-	-	-
APTT	G3**	-	G2**, G3**, G4***	-
PLT	-	-	-	G6* (R)
PT	-	-	-	G6** (R)

R: Reversal Group, ↑: Increase, ↓: Decrease

All statistically significant ($p < 0.05$) differences observed in biochemistry parameters such as albumin, BIL.T, CHO, CRT, glucose, PHO, TGL, TPR, AG ratio, ALP, GGT, SGPT, VLDL, and globulin were considered incidental and unrelated to the administration of the test item. These findings were either of minimal magnitude, lacked a clear dose-related pattern, were limited to one sex, or were randomly distributed across groups without consistency between the terminal and recovery phases of the study. The increased or decreased values were found within the range of historical control data in our laboratory.

Parameters	Biochemistry			
	Male		Female	
	↑	↓	↑	↓
Albumin	G4*	-	-	G2****, G3**, G4**
BIL.T	G2**	-	G4*	-
CHO	G3*, G4****	-	G3**	-
CRT	G4****	-	G6* (R)	-
Glucose	-	G3***, G4***	-	G3**, G4*
PHO	-	G3*	-	G3*
TGL	-	G3***, G4**	-	G3***, G4**, G6** (R)
TPR	-	G2*	-	G2*, G4*
ALP	-	G6* (R)	-	G4*
GGT	-	-	G3***, G4**	G6** (R)
SGPT	-	-	-	G4*, G6* (R)
AG Ratio	-	-	G2*, G4*	-
VLDL	-	-	-	G3***, G4*
Globulin	-	-	-	G4*

R: Reversal group, ↑: Increase, ↓: Decrease

Necropsy/gross pathology

External examination of the terminally sacrificed animals across various experimental groups (G1-G6) did not reveal any abnormality of pathological significance.

Internal examination of the terminally sacrificed animals across various experimental groups (G1-G6) did not reveal any abnormality of pathological significance.

Histopathology

Histopathological examination of organs from the high dose group (1000 mg/kg b.wt.) did not reveal any evidence of test item-related significant toxicological changes upon oral administration of the test item, oleanolic acid (Pentacyclic Triterpenoid) extracted from *L. camara* roots.

Rats. Microscopic examination of various organs and tissues from the terminally sacrificed control (G1) and high dose (G4) groups revealed minimal [22] to mild findings in various tissues such as osseous metaplasia, focal, present (G1 M 1/10, G4 M 1/10), histiocytosis, alveolar, diffuse, and 2+ (G1 M 1/10) in the lungs. Basophilic tubules, cortex, multifocal, 2+, bilateral (G1 M 1/10), basophilic tubules, cortex, multifocal, 1+, bilateral (G4 M 1/10) in kidneys vacuolation, epithelium,

focal, 1+, unilateral (G1 M 1/10), atrophy/degeneration, tubular, focal, 1+, unilateral (G1 M 1/10), atrophy/degeneration, seminiferous tubules, diffuse, 2+, bilateral (G1 M 1/10) in epididymis, dilatation, glandular, mucosa, multifocal 1+ (G1 M 1/10) in stomach, ultimobranchial cyst, present, unilateral (G4 M 1/10) in thyroid, cyst, pars distalis, present (G1 M 1/10) in pituitary in male, and osseous metaplasia with mineralization, multifocal, present (G1 F 1/10) in lungs, basophilic tubules, cortex, focal, 2+, unilateral (G1 F 1/10), basophilic tubules, cortex, focal, 2+, bilateral (G4 F 1/10), dilatation, renal tubules, cortex, multifocal, 1+, bilateral (G1 F 1/10, G4 F 1/10), mineralization, medulla, multifocal, 1+, bilateral (G1 F 1/10) in kidneys, ultimobranchial cyst, present (G1 F 1/10 G4 F 1/10) in thyroid, dilatation, luminal, 2+, bilateral (G4 F 1/10), dilatation, luminal, 3+, bilateral (G1 F 1/10) in the uterus, increased mucification, epithelial, diffuse, 1+ (G1 F 1/10, G4 F 1/10), increased, mucification, epithelial, diffuse, 2+ (G1 F 1/10) in the vagina in females.

Keys: Severity of lesions: 1+ - Minimal (1–5%), 2+ - Mild (6–20%) and 3+ - Moderate (21–35%).

DISCUSSION

The toxicity evaluation of oleanolic acid (pentacyclic triterpenoid) extracted from *L. camara* roots revealed no adverse effects, thereby indicating the safety potential of the test item. The results obtained were concomitant with other compounds of the same class (ursolic acid), which were found to be safe in Han-Wistar rats [23].

Researchers have demonstrated that oleanolic acid could be used as a therapeutic agent because it has long been used in traditional medicine. For instance, in addition to its ecological functions in plants, oleanolic acid has been linked to its pharmacological actions in many disease models, including antioxidant, anti-tumor, anti-inflammatory, anti-diabetic, and anti-microbial properties [24–29]. In China, oleanolic acid has been utilized as a hepatic medication for more than 20 years due to its hepatoprotective properties. Oleanolic acid not only protects against acute chemical liver injury but also provides protection toward liver fibrosis and cirrhosis [16].

The oral administration was well tolerated, according to the findings of our initial investigation regarding the oral acute toxicity experiment. No toxicities, mortality, or aberrant behavior were seen in the rats for up to 14 days of the observation period following the dose of 2000 mg/kg bw. The findings are in accordance with the OECD test guidance for labeling and categorizing acute systemic toxicity of the test item as it was given Class 5 status, indicating that there was a reasonably low risk of acute toxicity and that the oral LD50 was in the 2000–5000 mg/kg range (OECD 423, 2001).

Despite the interest in oleanolic acid, a thorough investigation of its impact on long-term toxicity is not available in the literature, which led to the concept of the present study. This study was able to culminate as to how oleanolic acid has affected various aspects of health over time, including general well-being, organ function, blood chemistry, behavior, and motor skills. This is the first study that examines oleanolic acid's sub-chronic level of toxicity in accordance with OECD recommendations and good laboratory practice norms (OECD 408; 2018).

The results have revealed that for nearly all of the (sub)parameters, there was no statistically significant difference between the control group and various doses of the test groups (250 mg/kg/day, 500 mg/kg/day, 1000 mg/kg/day). The findings of this sub-chronic study encountered during the analysis of hematology and biochemistry parameters were spontaneous, incidental, congenital, physiological, or metabolic in nature, which were encountered in this species (Rat) and strain (Wistar rats) of this age kept under laboratory conditions found in both the control and high dose groups [30]. There was no clinical correlation (hematological, biochemical, and bone marrow examination) between the above histopathological findings across the control and high dose groups (G1 and G4), body weight, feed consumption, or neural behaviors.

Hence, it was confirmed that the above findings were spontaneous, incidental, congenital, physiological, or metabolic in nature. Repeated oral doses of oleanolic acid (pentacyclic triterpenoid) extracted from *L. camara* roots at dose levels of 250, 500, and 1000 mg/kg b.wt in Wistar rats for 90 days resulted in no test item-related changes in body weight, feed consumption, organ weight, sensory function, hematology, urine, biochemistry parameters, and histopathology. All the other changes were considered unrelated to the test item because they lacked microscopic correlations.

CONCLUSION

It can be concluded that based on body weight, food consumption, clinical condition (including neurotoxicity assessment), hematology, coagulation, blood chemistry, or macroscopic and microscopic examinations. The no observed adverse effect level (NOEL) for oleanolic acid (pentacyclic triterpenoid) in this study was >1000 mg/kg/day of body weight.

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AUTHOR CONTRIBUTION

All the authors contributed to the study of conception and design. Material preparation, analysis, data collection, research, and the first draft were drafted by NG, and the final draft was reviewed by ATS. Both the authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors do not have any conflict of interest in the publication of this manuscript.

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