ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 3, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

TERATOGENICITY STUDY OF OLEANOLIC ACID (PENTACYCLIC TRITERPENOID) EXTRACTED FROM ROOT OF *LANTANA CAMARA* IN WISTAR RATS

RITESH KUMAR, ANU T SINGH

Department of Toxicology, Dabur Research Foundation, Ghaziabad, Uttar Pradesh, India.
***Corresponding author: Ritesh Kumar; Email: riteshhamdard@gmail.com**

Received: 03 November 2025, Revised and Accepted: 12 January 2026

ABSTRACT

Objectives: The aim of the present study was to observe any teratological effects in the fetus parameters anogenital distance (AGD), crown to rump length (CRL), body weight, visceral as well as skeletal structure and dams fertility parameters due to repeated dose administration of oleanolic acid extracted from root of *Lantana camara* atthe different dose level of 250, 500 and 1000 mg/kg body weight in pregnant dams from implantation day to day before parturition. Furthermore, the aim of this study was to determine the no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL).

Methods: Oleanolic acid was extracted from the roots of *L. Camara* by the extraction and isolation process. Three treatment groups at the dose level of 250, 500 and 1000 mg prepared in 0.1% between 80 and 0.5% CMC in milli Q water. Doses were administered once daily to pregnant rats from gestation day 5 to GD 19, and on GD 20, rats were terminally sacrificed. Biochemistry, hematology, body weight, feed intake, dam, and fetus parameters were observed.

Results: No test item-related significant changes observed in the evaluation of body weight, food, necropsy findings, organ weight, hematology, and biochemistry dam and fetus parameters (AGD, body weight, CRL, visceral and skeletal examination) at the maximum dose level of 1000 mg/kg bwt.

Conclusion: There were significant differences observed in the biochemical parameters, such as albumin, alkaline phosphatase, total bilirubin, total cholesterol, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, total protein, and hematology parameters such as hemoglobin, hematocrit, and mean corpuscular volume. These changes are not uniform, dose-dependent, minimal in magnitude, and not correlated with the histopathological findings of the respective organs. On theobservation of results of clinical signs, clinical pathology, histopathological findings, dams and fetus parameters, it may be concluded that NOAEL/LOAEL of test item oleanolic acid extracted from *L. camara* roots at the highest dose level of 1000 mg/kg body weight when administered orally once daily from gestation day 5 to gestation day 19.

Keywords: Dam, Fetus, Teratology, Visceral, Skeleton, No-observed-adverse-effect level, Lowest-observed-adverse-effect level.

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i3.57361. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Lantana camara is an invasive shrub found largely in tropical and subtropical regions worldwide, especially in Asia, Africa, and Australia. This plant, distinguished by its hardy nature and vibrant flowers, largely grows in disturbed soils, forests, and pastures [1,2]. *L. camara* is a beautiful plant, and it is found throughout India, mostly in hilly areas. This herb is widely used in traditional medicinal therapy throughout the world, and is also used as an ornamental plant. While this plant has a long history of use in traditional medicine, studies have often reported it as a noxious weed owing to its aggressive growth and ecological impact. However, studies have revealed that this plant shows toxic effects, particularly in cattle, where its consumption results in photosensitization and hepatic damage [3]. Various parts of the plant, such as the leaves, roots, and flowers, are traditionally used to treat diseases such as skin infections, respiratory problems, wounds, and gastrointestinal disturbances [4]. On this account, several studies have been carried out in recent years to isolate the various phytoconstituents present in *L. camara* [5]. The anticancer, anti-osteoporosis, anti-obesity, anti-diabetic, lipid-lowering, anti-inflammatory, antioxidant, immune-regulatory, chronic illness, and hepatoprotective properties of oleanolic acid and its derivatives are investigated [6,12-14]. Among these, oleanolic acid, a key bioactive component that is found in abundance in the roots and leaves of the plant, has been identified to contribute to its therapeutic as well as toxic properties [7].

The aim of teratogenicity toxicity was for the evaluation of any abnormality in external, vicera and skeletal development in pups during fetal development in pregnant female Wistar rats by dosing with oleanolic acid isolated from *L. camara* roots. As a traditional medicine, it might be taken by pregnant women. Evaluations such as these ensure that drugs and herbal products serve the intended purpose of promoting public health while observing regulatory compliance [8]. Considering the widespread traditional use of *L. camara*, it is necessary to analyze its effects on developmental toxicity in order to establish safety in this population. As recommended by the OECD 414 guideline, the study should be conducted with three treatment groups and a vehicle control group [23]; hence, the experiment was designed at dose levels of 250, 500, and the highest dose, 1000 mg/kg body weight, along with a vehicle control group. 1000 mg/kg is sufficiently high to detect relevant hazards for human health, as doses higher than this are often not practically or ethically justified.

METHODS

The roots of *L. camara* were collected from the local hilly area of Raisen district, geographical coordinates around 23°20' N latitude and 77°48' E longitude, Bhopal, Madhya Pradesh, India. The roots were collected in the morning time and the roots were cut into small pieces. The roots were washed and allowed to dry in the open air for a few days. After drying, the roots were crushed and formed into a granular powder for the extraction process. 500-g crude drug form of *L. camara* roots was extracted with petroleum ether at room temperature overnight. The crude extract was filtered and concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 45°C to

prevent thermal degradation of active constituents. The solvent was removed under vacuum, and the crude extract was dissolved in $CHCl_3$ and left overnight for precipitation. The precipitate so obtained was crystallized with methanol. Precipitation and crystallization processes were repeated 4 times, which gave oleanolic acid crystals [25].

The isolated oleanolic acid sample was subjected to various analytical techniques to assess its identity and purity. These included Fourier-transform infrared spectroscopy, differential scanning calorimetry (DSC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS) [9].

The isolated sample of oleanolic acid from *L. camara* roots contains 91.9% purity with very few impurities.

Animal husbandry

Healthy adult Wistar rats (*Rattus norvegicus*) were used for the study. Animals were purchased from a CPCSEA-registered animal breeder (Gentox Bio Services Pvt Ltd., Hyderabad). Animals received 10-week-old adult 50 male and 100 female in the facility. Animals were housed in "C" type cages of polycarbonate with a solid bottom (size 421×290×190 mm length, width, height, respectively) and stainless-steel top grills having facilities for holding feed and water. Autoclaved cage, water bottle, and corn cob were changed twice a week. The animal room was maintained under controlled conditions of temperature (22±2°C), relative humidity (55±10%), and a 12 h light–dark cycle [20]. Artificial controlled fluorescent light was provided to the animals. Standard laboratory rat feed, phytoestrogen-free feed, was provided to animals and RO water *ad libitum*.

After receiving the animal from the breeder, animals were quarantined for 7 days. During the quarantine period, animals were thoroughly observed for clinical signs, mortality, and morbidity. After completion of the quarantine period, animals were acclimatized for 10 days in a different room with the same environmental conditions. Feed and water were provided *ad libitum* during the quarantine and acclimatization period.

Institutional animal ethics committee (IAEC) approval

The study was conducted in compliance with institutional ethical standards. Approval was obtained from the IAEC, following the guidelines set by the Committee for the control and supervision of experiments on animals (CCSEA), Government of India. Animals for the experiment were approved by the IAEC of Dabur Research Foundation, Ghaziabad, IAEC No. IAEC/64/1252. The animal facility of Dabur Research Foundation was used for the animal experiment, CCSEA registration no. 64/PO/RcBi/s/99. The study was strictly adhered to the OECD 414 guideline. Throughout the experiment period, all ethical principles were followed during the handling of animals.

Experiment design

Animals' mating procedure

For mating purpose male and female rats were house together in evening and after mating in morning male and female rats were houses separately. Every morning, vaginal smears/vaginal plugs were prepared on a slide by pipette and evaluated microscopically for the presence of sperm and the estrus cycle [30]. After positive vaginal smear observations/vaginal plug, female/s were removed from the cage of respective male/s and were distributed serially to each study group with respective identification number in new cages. Female which was found negative during smear examination/vaginal plug were kept again for mating in the same male cage until the desired number of positive females were found for the study. The day the female tested positive on vaginal smear/plug was considered day 0 of gestation (GD 0). After getting the required numbers of mated females for the study, male/s and remaining females were handed over to the animal facility without any further examination.

Instrument: During the experiment, many instruments were used for the data generation, including a weighing balance (Sartorius), Stereo Microscope (Nikon), Vernier calipers (Mititoyo), Biochemical analyzer (Aggape), Hematology analyzer (Mindray), enzyme-linked immunosorbent assay analyzer (Biotek), HPLC (Agilent Technology), FTR (Agilent Technology), DSC (Mettler Toledo).

Grouping description

Pregnant dams were randomly distributed in the different groups based on the mean body weight. The study comprised four experimental groups (G1–G4), each group consisting of 20 pregnant dams. As OECD guideline 414 recommends that at least 16 pregnant animals should be present in each group for the validity of the study, 20 pregnant animals per group were selected for the study to reach at least the desired number of animals per group.

- Group 1 (G1): Control group receiving only vehicle (mixture of 0.1% Tween 80 and 0.5% carboxy methylcellulose in Milli-Q Water)
- Group 2 (G2): Received 250 mg/kg body weight of oleanolic acid
- Group 3 (G3): Received 500 mg/kg body weight of oleanolic acid
- Group 4 (G4): Received 1000 mg/kg body weight of oleanolic acid.

Dose formulation for oleanolic acid was made by dissolving in a mixture of 0.1% Tween 80 and 0.5% carboxy methylcellulose in Milli-Q Water. Pregnant animals were divided into three treatments and one vehicle control group. The dose regimen once daily was determined to cover the period of organogenesis from gestation day 5 to day 19. Treatment groups of low, mid, and high dose received the doses 250, 500, and 1000 mg/kg body weight of oleanolic acid, respectively, whereas the control group animals received vehicle only mixture of 0.1% Tween 80 and 0.5% carboxy methyl cellulose in Milli Q water. The doses were administered by oral route using a disposable syringe attached with 16 gauze stainless steel oral gavage cannula. The dose volume of 10 mL/kg body weight was maintained throughout the dosing period. Same dosing time (Morning) was maintainedthroughout the dosing period.

Observations

Clinical signs, mortality, and morbidity

After 2–3 h of dosing, animals were observed once daily for clinical signs such as changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, and lethargy during gestation days 5–19. Animals were observed twice daily, morning and evening, for the mortality/morbidity in the dosed animals.

Body weight and feed consumption

Body weight for the confirmed pregnant female rats was observed on gestation day 0 (GD 0), on GD5, 1st day of dosing, thereafter on GD8, GD11, GD14, GD17, and GD20.

During the acclimatization period, *ad libitum* feed was provided; however, during the dosing period, from GD5 to GD19, weight feed was provided. Feed intake was measured on GD 5–8, GD 8–11, GD 11–14, GD 14–17, and GD 17–20. RO water for drinking purposes was provided *ad libitum* throughout the experiment period.

Clinical chemistry analysis

For the biochemistry and hematology parameter analysis, blood was collected from the dams at the end of the study through the retro-orbital plexus. Prior to blood collection, local anesthesia, 0.5% paracain eye drop, was instilled into the eye. For the hematology parameters analysis, blood was collected in the K2EDTA tube, and for the biochemistry parameters analysis, blood was collected in the heparin tube. Hormones T3, T4, thyroid-stimulating hormone (TSH) were analyzed after serum separation from the ratsblood by the ELISA kit method.

Necropsy and gross pathological examination

On day 20, prior to the day before parturition, all animals of the treatment groups as well as the control group were terminally sacrificed by carbon dioxide asphyxiation method. Animals were examined externally; cranial, thoracic, and visceral cavities were opened and examined macroscopically. The entire uterus of all the females was transferred into a freshly prepared aqueous 10% ammonium sulphide solution for a minimum period of 10 min to identify post-implantation loss, which appeared as fine black spots. All live fetuses were sacrificed by intraperitoneal injection of sodium thiopentone (~0.25 mL of 100 mg/mL solution) after weighing and were allotted for further examination.

Tissue/fetus collection and weights

On completion of gross pathology examination, the gravid uterus with cervix of pregnant females was collected and weighed, thyroid gland was collected and weighed. Vital organs such as the heart, liver, kidney, spleen, adrenal gland, brain, and thymus were weighed and collected for histopathological examination. The fetuses were taken out, and a gross evaluation of the placenta was done.

The following observations were made and recorded for females:

a. Number of live and dead fetuses
b. The number of early resorption and late resorption. If any
c. The number of corpora lutea
d. Number of implantation sites
e. Gravid uterus weight
f. Pup's body weight, anogenital distance (AGD), crown to rump length (CRL), and sex ratio measurements
g. Visceral examination: Visceral examination was performed via serial sectioning under a stereomicroscope. Major internal organs such as the heart, brain, kidneys, liver, and lungs were inspected for anomalies, and any deviations were recorded. The head razor observation was performed by Wilson's method after fixation in formaldehyde and the thoracic and abdominal organs were examined by Nishimura's micro dissection method under a stereomicroscope.
h. Skeletal examination: Pups were eviscerated for the skeletal examination. The eviscerated fetuses were placed in a 2% KOH solution to clear the non-qualified tissue. Fetuses chosen for skeletal analysis were subjected to double staining with Alizarin Red S and Alcian Blue to visualize ossified bones and cartilage, respectively. Skeletal structures such as the skull, vertebrae, cervical, thoracic, lumbar, sacral, caudal, sternal, ribs, pectoral girdle alignment (clavicle, scapula, humerus, radius, ulna, metacarpals, phalanges (fore limb), ileum, ischium, femur, pubis, tibia, fibula, phalanges (hind limb) bones were examined for ossification defects, abnormal fusions, and malformation.

Data analysis

As per the OEDCD 414 guideline for the validity of a study, at least 16 pregnant female rats per group should be present. Data were analyzed using appropriate statistical methods. Body weight, feed consumption, gravid uterus weight, organ weights, hematological and biochemical parameters were subjected to descriptive statistical analysis, followed by assessment of data normality using the Shapiro–Wilk test. Then, the data were analyzed by Analysis of variance (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 using the Kruskal–Wallis test followed by Dunn's *post hoc* test to compare test item-treated groups with control groups. In addition, categorical data, including the incidence of malformations, were analysed using the Chi-square test to evaluate differences among groups. A $p<0.05$ was considered statistically significant for all analyses.

RESULTS

Clinical signs, mortality/morbidity

All animals of the treatment and control groups were found to be normal throughout the experiment period (Table 1).

No mortality/morbidity was observed in any group during the entire course of study (Table 2).

Body weight

Progressive body weight increase was observed in the treatment as well as the control group animals from Gestation Day 0 (GD0) to GD20 (Table 3 and Fig. 1).

No statistically significant differences ($p>0.05$) were observed between any treatment group (G2: 250 mg/kg, G3: 500 mg/kg, G4: 1000 mg/kg) and the vehicle control group (G1) at any time point, as determined by repeated measures ANOVA followed by Dunnett's test.

Feed consumption

Feed in consumption in the treatment groups was found to be non-significant as compared to control group from GD5 to GD20 (Table 4 and Fig. 2). Statistically significant differences ($p>0.05$) were

Table 1: Clinical signs

Group	Dose (mg/kg b.wt)	GD Days 5–19
G1	0	Normal
G2	250	Normal
G3	500	Normal
G4	1000	Normal

Animals were observed once daily for clinical signs throughout the experiment period

Table 2: Mortality and morbidity

Group	Dose (mg/kg b.wt)	GD Days 5–19
G1	0	0
G2	250	0
G3	500	0
G4	1000	0

Animals were observed twice daily for mortality and morbidity throughout the experiment period

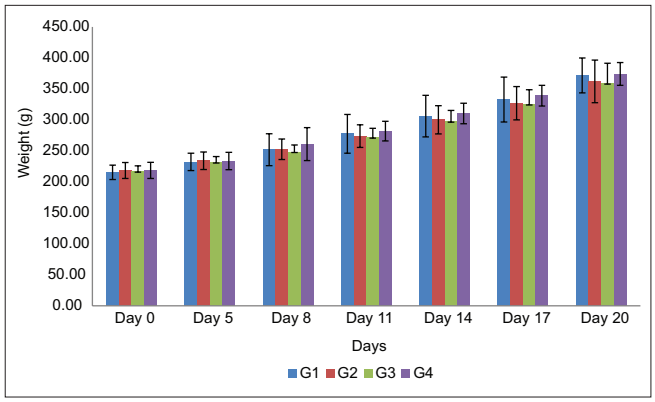

Fig. 1: Body weight

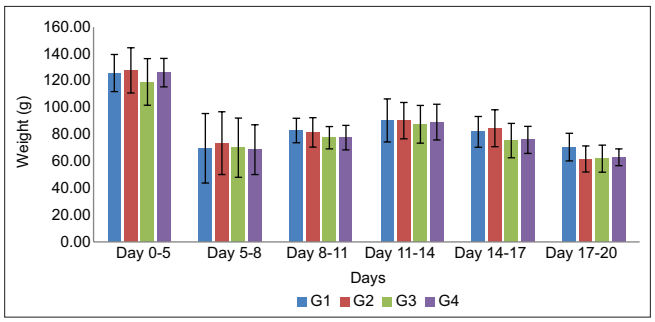

Fig. 2: Feed consumption

observed in treatment group (G2: 250 mg/kg and G3: 500 mg/kg body weight) as compared with vehicle control group (G1) at any time point, these differences are incidental and not dose dependent because at high dose no statistically significant diffences was observed as compared with vehicle control group as determined by repeated measures ANOVA followed by Dunnett's test.

Organ and gravid uterus weight

Statistically non-significant changes in the organ and gravid uterus weight were observed in the treatment groups as compared to the control group.

Clinical chemistry parameters

Hematology

Significant statistical changes were observed in the hemoglobin, hematocrit, and mean corpuscular volume in the treatment groups as compared with the control group (Table 5). These differences are minimal in magnitude, irregular, and not dose dependent; it may be considered due to biological variation.

Biochemistry

The statistically significant difference observed in the biochemical parameters of Albumin, alkaline phosphate, total bilirubin, total cholesterol, Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and total protein was found not to be dose dependent pattern and irregular. These findings are also not correlated with the histopathological observations; hence, they are consider incidental findings due to biological variation. There was no abnormalityobserved in the histopathology of the liver of the dam, hence liver-related biochemical parameters such as SGOT, SGPT, and alkaline phosphatase findings are incidentalnot dose dependent.

No statistical differences were observed in the T3, T4, and TSH parameters in the treatment groups as compared to the control groups (Table 6).

Gross pathological findings

There was no test item-related abnormality observed in the internal and external gross pathological examinations of treatment groups as well as control group dams (Table 7).

External observations of pups

External investigations of all fetuses showed that nearly all individuals from each group displayed no external abnormalities. Across groups, a small number of cases of hematoma were noted. More specifically, one fetus from the control group (G1) had a hematoma, as well as one fetus each from the Low Dose (G2) and High Dose (G4) groups. No external deformities were noted from mid dose (G3) group. Because of the very low prevalence and lack of consistent dose-related trends, statistical evaluation was not conducted for external deformities, which pointed toward no definite teratogenic modification of external structure under the conditions tested (Table 8).

Fetus parameters

Fetus body weight

Statistical analysis was performed for sex wise male and female pups separately. No statistically significant difference in body weight was observed in the treatment group as compared to the control group pups in both sexes (Figs. 3 and 4).

AGD: The AGD measurement is the most dependable way to determine sex. The distance from the anus to the genitals is longer in males than in females. Statistical analysis was performed for sex wise male and female pups separately. No statistically significant difference in AGD

Table 3: Summary of body weight (Mean±SD) across groups

Day	Group G1 (Mean±SD)	Group G2 (Mean±SD)	Group G3 (Mean±SD)	Group G4 (Mean±SD)
Day 0	215.27±11.49	218.32±12.81	216.72±8.99	218.34±13.03
Day 5	230.98±15.51	232.22±14.05	231.23±9.38	233.58±14.12
Day 8	251.80±25.84	252.48±16.45	248.29±11.20	260.83±26.66
Day 11	277.40±31.11	273.76±18.25	271.52±14.66	281.69±15.91
Day 14	305.92±33.56	300.09±22.81	297.31±17.75	310.28±16.55
Day 17	332.68±36.17	326.72±26.88	325.07±23.36	338.84±16.73
Day 20	371.63±28.15	361.95±34.39	358.68±32.70	374.08±18.44

N (Number)=20, N (Number)=20, Mean±standard deviation (n=X dams/group). p<0.05compared to the vehicle control group (G1) using one-way ANOVA followed by Dunnett's test (or Kruskal-Wallis with Dunn's test).

Table 4: Summary of feed consumption across groups

Parameter	Group 1 (Mean±SD)	Group 2 (Mean±SD)	Group 3 (Mean±SD)	Group 4 (Mean±SD)
Feed consumption (g)				
0–5	125.79±13.81	127.83±16.87	119.18±17.42	126.12±10.62
5–8	69.72±25.89	73.60±23.39	70.21±22.10	68.70±18.53
8–11	83.02±9.17	81.59±10.93	77.65±8.29	77.69±9.09
11–14	90.51±16.06	90.31±13.51	87.63±13.99	89.29±13.33
14–17	81.94±11.53	84.69±13.82	75.48±12.75	76.05±10.06
17–20	70.66±10.29	61.81±9.66↓	62.01±10.09↓	63.04±6.30

N (Number)=20, N (Number)=20, Mean±standard deviation (n=X dams/group). p <0.05compared to the vehicle control group (G1) using one-way ANOVA followed by Dunnett's test (or Kruskal-Wallis with Dunn's test)

Table 5: Hematology parameters

Parameters	G1 (Control	G2 (250 mg/kg b.wt)	G3 (500 mg/kg b.wt)	G4 (1000 mg/kg b.wt)
Hemoglobin (g/dL)	11.88±2.18	13.08±1.90	14.37+1.38	15.48±2.48↑*
Hematocrit (%)	34.05±4.57	34.73±4.93	40.37±4.86↑**	39.86±4.90 ↑**
MCV (fL)	48.93±5,97	51.85±2.67	51.99±2.96	52.31+1,43↑**

N (Number)=20, N (Number)=20, Mean±Standard Deviation (n=X dams/group). p <0.05compared to the vehicle control group (G1) using one-way ANOVA followed by Dunnett's test (or Kruskal–Wallis with Dunn's test)

Table 6: Biochemistry parameters

Parameters	G1 (Control	G2 (250 mg/kg b.wt)	G3 (500 mg/kg b.wt)	G4 (1000 mg/kg b.wt)
Albumin (g/dL)	3.36±0.31	3.13±0.26	2.93±0.23↓**	3.26±0.73
Alkaline phosphate (IU/L)	94.17±71.51	106.75±45.32	140.24±28.09↑**	114.73±34.56↑**
Total bilirubin (mg/dL)	0.11±0.04	↓*0.07±0.02	0.11±0.03	0.08±0.02
Total cholesterol (mg/dL)	89.13±12.36	83.60±5,82	75.61±13.70↓**	82.28±6.97
SGOT (IU/L)	136.34±35.25	107.05±18.68↓*	133.40±23.34	119.81±26.92
SGPT (IU/L)	44.83±15.57	57.12±12.97	46.91±20.89	62.36±16.25↑**
Total protein (g/dL)	6.33±0.65	5.75±0.42	6.05±0.81	5.59±0.67↓**

N (Number)=20, Mean±Standard Deviation (n=X dams/group). *P*<0.05compared to the vehicle control group (G1) using one-way ANOVA followed by Dunnett's test (or Kruskal-Wallis with Dunn's test). SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase

Table 7: Dams' fertility parameters

Dam parameters	G1	G2	G3	G4
No. of pregnant rats	20	19	19	20
Non-pregnant rats	0	1	1	0
Total No. of corpora lutea/L	11.35	10.53	9.53	9.95
Implantation index (%)	100	100	100	100
Pre-implantation loss (%)	0	0	0	0
Post-implantation loss (%)	0.87	0.44	0	0
Live fetus/L (%)	99.13	99.56	100	100
Dead fetus/L (%)	0	0	0	0
Total early resorption/L (%)	0.87	0.44	0	0
Total late resorption/L (%)	0	0	0	0

"Implantation index (%)=(Number of implantation sites/Number of corpora lutea)×100. Pre-implantation loss=Number of corpora lutea - Number of implantation sites. Post-implantation loss=(Number of implantation sites - Number of live fetuses)/Number of implantation sites×100

Table 8: Incidence of external malformations (hematoma) across groups

Group	Dose (mg/kg b.wt)	Total fetuses examined (Number)	Number with hematoma	Incidence (%)
Control (G1)	0	107	1	0.93
Low dose (G2)	250	198	1	0.51
Mid dose (G3)	500	170	0	0
High dose (G4)	1000	199	1	0.50

Incidence is calculated as (Number of fetuses with hematoma/total number of fetuses examined)×100. No statistically significant difference was found between groups using the Chi-square test (p>0.05). The findings were considered incidental due to low incidence and lack of a dose-response relationship

was observed in the treatment group as compared to the control group pups in both sexes (Figs. 3 and 4).

CRL: Statistical analysis was performed for sex wise male and female pups separately. No statistically significant difference in body weight was observed in the treatment group as compared to the control group pups in both sexes (Figs. 3 and 4).

Sex ratio: No statistically significant difference was observed in the sex ratio of treatment group animals as compared to control group animals.

Visceral examinations

All examined fetuses showed no visceral malformations in any of the study groups and controls, as well as all treated with oleanolic acid. All internal organs were perfectly structured, and there were no abnormalities in all doses.

Skeletal examination

Based on the skeletal examination, none of the treatment groups showed any significant malformations as compared to the control group. The occurrence of minor skeletal anomalies, such as rib abnormalities and differences in the degree of ossification, was noted in all groups, including the control group, but no consistent correlation with dose level was observed. These changes were only observed sporadically (Table 9).

Histopathology

Infiltration of inflammatory cells, basophilic tubules and tubular dilation in both medullary and cortex regions of kidneys (G1: 2/20, G4: 1/20), perivascular and periportal infiltration of inflammatory cells, degeneration andcytoplasmic vacuolation in liver G1: 2/10, G4: 2/20) along with infiltration of inflammatory cells in heart (G1: 1/20, G4: 2/20), multibranchial cyst, present in thyroid gland (G1: 1/20).

The rates of occurrence of all findings in the high dose group were either very low or comparable tothe concurrent control group. Hence, all these findings should be considered spontaneous or incidental in

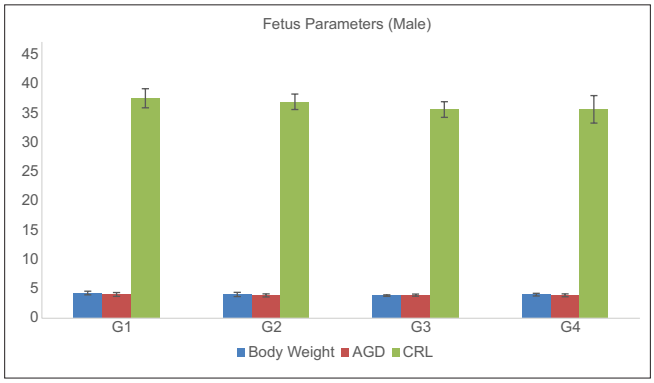

Fig. 3: Fetus parameter. AGD: Anogenital distance, CRL: Crown rump length

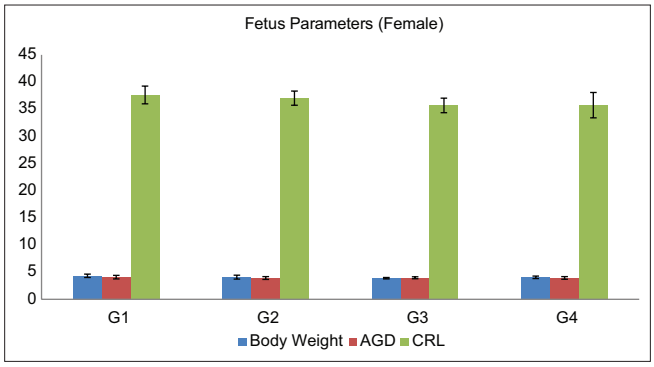

Fig. 4: Fetus parameter. AGD: Anogenital distance, CRL: Crown rump length

nature, representing the normal physiological/metabolic or congenital changes encountered in rats of this age kept under laboratory conditions.

Table 9: Skeletal malformations and variations observed across groups

Group (Dose)	Malformations	Variations (e.g., fused/absent ribs, ossification delays)
G1 (0 mg/kg)	None	Minor rib variations (e.g., 9th/11th rib variations) in a few cases (<5%)
G2 (250 mg/kg)	None	Mostly normal; very few cases with minor rib variations (similar to control)
G3 (500 mg/kg b.wt)	None	Mostly normal; minor variations likely in phalanges and ribs, no severe malformations reported
G4 (1000 mg/kg b.wt)	None	Mostly normal; minor variations likely in phalanges and ribs, no severe malformations reported

All fetuses were examined for skeletal malformations and variations after staining with Alizarin Red S and Alcian Blue. Variations were minor (e.g., rudimentary rib, slight delay in ossification) and their incidence was comparable to the concurrent control group, indicating they were not treatment-related

DISCUSSION

Study: Teratogenic evaluation of oleanolicacid in Wistar rats extracted from roots of *L. camara was* performed in pregnant rats. No compound-related adverse effects were observed in any of the treatment groups. Similar types of results were observed in 15-day subchronic developmental toxicity studies of Ursolic acid in Wistar rats [25].

On the basis of study results, oleanolic acid can be used as a medicine for antioxidant and anti-inflammatory, hepatoprotective, and many other treatments. Oleanolic acid cannot be directly use to the pregnant women for the treatment of such health-related issues. Further studies still need to be performed for the evaluation of test item-related effects in pups post-parturition and clinical trials. In addition, the current database included more detailed information on maternal toxicity, animal-to-human exposure margin, mechanism of action, and therapeutic class. These data can be used to proceed with further studies prior to use for human health.

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 [6]

The results suggest that oleanolic acid at all the dose levels was well tolerated and did not cause maternal toxicity or structural malformations, did not lead to substantial, dose-dependent effects in fetal body weight, crown-rump length, and AGD, indicating possible antenatal growth retardation, body weight, organ weights, food consumption, gross pathology, histopathological, and clinical pathology parameters.

Progressive weight gain was observed in all treatment group rats as compared to control group rats.

The findings in clinical chemistry parameters at the dose levels of 250 mg/kg b.wt, 500 mg/kg body weight, and 1000 mg/kg body weight show statistical differences that are not dose dependent, minimal in magnitude, and spontaneous due to biological variation, also not correlated with histopathological findings of the respective organs. Findings in the histopathology at high doses are either spontaneous or age-related. No teratology study has ever been conducted for oleanolic acid in accordance with OECD 414 guidelines, with extra factors that are not addressed in the guidelines.

CONCLUSION

The use of experimental animals suitable for the teratology studies, as recommended by the guideline, based on the feed consumption, organ weight, clinical chemistry, fertility parameters, gross and histopathology of dams and fetus findings, CRL, fetus weight, sex ratio, AGD visceral and skeletal examination, concluded that at the highest dose level of 1000 mg/kg b.wt there were no adverse effects observed in dams as well as the fetus. Hence, the NOAEL of oleanolic acid for the conducted study would be more than 1000 mg/kg b.wt, and it is safe up to 1000 mg/kg b.wt. Hence, this study indicates that oral dosing with oleanolic acid is safe for adult rats and their offspring, and the no observed adverse effect level for oleanolic acid is likely higher than 1000 mg/kg/day. NOAEL of oleanolic acid for the conducted study would be more than 1000 mg/kg b.wt.

ACKNOWLEDGMENT

I would like to thank Mr. Vijay K. Naidu and Dr. Surjit Singh for their assistance during the research work, and the management of the Dabur Research Foundation provided the facility for the research work.

AUTHOR CONTRIBUTION

The authors' contributions included conceptualization, design, planning, test material preparation, data creation and collection, analysis, and result interpretation. Ritesh Kumar wrote the first draft, and Anu T. Singh reviewed the final version. The final manuscript was reviewed by both authors and approved.

CONFLICT OF INTEREST

Regarding the publication of this research effort document, the author has no conflicts of interest

FUNDING

DRF has provided open access of facilities to perform the study, and remainingparts funded by the authors themselves.

REFERENCES

1. Taylor S, Kumar L, Reid N, Kriticos DJ. Climate change and the potential distribution of an invasive shrub, *Lantana camara* L. PloS One. 2012 Apr 19;7(4):e35565. doi: 10.1371/journal.pone.0035565, PMID 22536408
2. Gonvlaves E, Herrera I, Duarte M, Bustamante RO, Lampo M, Velasquez G, *et al*. Global invasion of *Lantana camara*: Has the climatic niche been conserved across continents? Plos One. 2014 Oct 24;9:e111468.
3. Kumar R, Katiyar R, Singh V, Kumar S, Kumar T. *Lantana camara*: An alien weed, its impact on animal health and strategies to control. J Exp Biol Agri Sci. 2016 May 21;4(3S):321-37. doi: 10.18006/2016.4(3S).321.337
4. Nawaz A, Ayub MA, Nadeem F, Al-Sabahi JN. Lantana (*Lantana camara*): A medicinal plant having high therapeutic potentials-a comprehensive review. Int J Chem Biochem Sci. 2016;10: 52-9.
5. Oladoye SO, Falade VA, Adepoju AJ, Ibikunle GJ. *Lantana camara*: Phyto-constituents and antimicrobial activity study. Pan Afr J Life Sci. 2021: 5(2):289-98.
6. Chao L, Xiaoan W, Hongbin S. Oleanolic acid derivatives for pharmaceutical use: A patent review. Expert Opin Ther Pat. 2016 Jun;26(6):643-55.
7. Kumar R, Guleria N, Deeksha MG, Kumari N, Kumar R, Jha AK, *et al*. From an invasive weed to an insecticidal agent: Exploring the potential of *Lantana camara* in insect management strategies-a review. Int J Mol Sci. 2024;25(23):12788. doi: 10.3390/ijms252312788, PMID 39684506
8. Ross EJ, Graham DL, Money KM, Stanwood GD. Developmental consequences of fetal exposure to drugs: What we know and what we still must learn. Neuropsychopharmacology. 2015;40(1):61-87. doi: 10.1038/npp.2014.147, PMID 24938210
9. Vyas N, Argal A. Isolation and characterization of oleanolic acid from roots of *Lantana camara*. Asian J Pharm Clin Res. 2014;7(2):189-91.
10. Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN, *et al*. Phytochemical characterization and assessment of crude extracts from *Lantana camara* L. for antioxidant and antimicrobial activity. Front Agron. 2020;2:582268. doi: 10.3389/fagro.2020.582268
11. Furtado NA, Pirson L, Edelberg H, Miranda LM, Loira-Pastoriza C, Preat V, *et al*. Pentacyclic triterpene bioavailability: An overview of

in vitro and *in vivo* studies. Molecules. 2017;22(3):400. doi: 10.3390/molecules22030400, PMID 28273859
12. Liu J, Lu YF, Wu Q, Xu SF, Shi FG, Klaassen CD. Oleanolic acid reprograms the liver to protect against hepatotoxicants, but is hepatotoxic at high doses. Liver Int. 2019;39(3):427-39. doi: 10.1111/liv.13940, PMID 30079536
13. Hu J, Liu W, Zou Y, Jiao C, Zhu J, Xu Q, *et al*. Allosterically activating SHP2 by oleanolic acid inhibits STAT3-Th17 axis for ameliorating colitis. Acta Pharm Sin B. 2024;14(6):2598-612. doi: 10.1016/j.apsb.2024.03.017, PMID 38828149
14. Li Y, Wang J, Li L, Song W, Li M, Hua X, *et al*. Natural products of pentacyclic triterpenoids: From discovery to heterologous biosynthesis. Nat Prod Rep. 2023;40(8):1303-53. doi: 10.1039/d2np00063f, PMID 36454108
15. Castellano JM, Ramos-Romero S, Perona JS. Oleanolic acid: Extraction, characterization and biological activity. Nutrients. 2022;14(3):623. doi: 10.3390/nu14030623, PMID 35276982
16. Silva AM, Alvarado HL, Abrego G, Martins-Gomes C, Garduño-Ramirez ML, García ML, *et al*. *In vitro* cytotoxicity of oleanolic/ursolic acids-loaded in PLGA nanoparticles in different cell lines. Pharmaceutics. 2019;11(8):362. doi: 10.3390/pharmaceutics11080362, PMID 31344882.
17. Hao J, Liu J, Wen X, Sun H. Synthesis and cytotoxicity evaluation of oleanolic acid derivatives. Bioorg Med Chem Lett. 2013;23(7):2074-7. doi: 10.1016/j.bmcl.2013.01.129, PMID 23434227
18. Fernández-Aparicio Á, Correa-Rodríguez M, Castellano JM, Schmidt-RioValle J, Perona JS, González-Jiménez E. Potential molecular targets of oleanolic acid in insulin resistance and underlying oxidative stress: A systematic review. Antioxidants (Basel). 2022;11(8):1517. doi: 10.3390/antiox11081517, PMID 36009236
19. Peng HB, Wang RX, Deng HJ, Wang YH, Tang JD, Cao FY, *et al*. Protective effects of oleanolic acid on oxidative stress and the expression of cytokines and collagen by the AKT/NF-κB pathway in silicotic rats. Mol Med Rep. 2017;15(5):3121-8. doi: 10.3892/mmr.2017.6402, PMID 28350071
20. Government of India. Committee for Control and Supervision of Experimental on Animals (CCSEA) by Ministry of Fisheries, Animal Husbandry and Dairying. New Delhi: Government of India.
21. Srivastava SK, Khan M, Khanuja SP. Extraction Process for the Isolation of Oleanolic Acid from the Roots of *Lantana camara*. Vol. 4. Virginia: U.S. Patent applications publication; 2004. p. 15.
22. Wang X, Ye XL, Liu R, Chen HL, Bai H, Liang X, *et al*. Antioxidant activities of oleanolic acid *in vitro*: Possible role of Nrf2 and MAP kinases. Chem-Biol Interact. 2010;184(3):328-37. doi: 10.1016/j.cbi.2010.01.034, PMID 20100471
23. OECD. Test No. 414: Prenatal Developmental Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2018.
24. Navika G, Anu ST. Toxicological evaluation of oleanolic acid (pentacyclic triterpenoid) extracted from *Lantana camara* roots following oral exposure in wistar rats. Asian J Pharm Clin Res. 2024;17:2455-3891.
25. Geerlofs L, He Z, Xiao S, Xiao ZC. Repeated dose (90 days) oral toxicity study of ursolic acid in han-wistar rats. Toxicol Rep. 2020;7:610-23. doi: 10.1016/j.toxrep.2020.04.005, PMID 32435599
26. Geerlofs L, He Z, Xiao S, Xiao ZC. 15-day subchronic developmental toxicity studies of ursolic acid in rats. Food Chem Toxicol. 2020 Jul 7;144:111537. doi: 10.1016/j.fct.2020.111537, PMID 32649969
27. Gupta N, Chandra S, Singh AT, Jaggi M. Characterization of *Lantana camara* roots (pentacyclic triterpenoid) and mutagenicity testing of extracted oleanolic acid using *Salmonella Typhimurium*. Arch Clin Microbiol. 2022;1(1):20-30.
28. Nistor M, Rugina D, Diaconeasa Z, Socaciu C, Socaciu MA. Pentacyclic triterpenoid phytochemicals with anticancer activity: Updated studies on mechanisms and targeted delivery. Int J Mol Sci. 2023;24(16):12923. doi: 10.3390/ijms241612923, PMID 37629103
29. Wasim M, Bergonzi MC. Unlocking the potential of oleanolic acid: Integrating pharmacological insights and advancements in delivery systems. Pharmaceutics. 2024;16(6):692. doi: 10.3390/pharmaceutics16060692, PMID 38931816
30. Upendra B, Navin RB, Vijaykumar MB, Lakshmi GN, Reshma S, Kumar SV. Extended one generation reproductive toxicity study of Thiamethoxam technical in rats. Int J Curr Res 2023 Dec;15(12):26565-72.