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# Evaluation of acute oral toxicity of a herbal estrus inducer

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**Abstract**
Maintaining fertility is essential for successful dairy enterprise. Fertility is widely challenged by a number of problems like anovulation, delayed ovulation, silent estrus, and anestrus. AV/OIP/22 (M/s Ayurvet Limited, India) is a polyherbal estrus inducer that helps to maintain and restore better reproductive efficiency. A study was conducted to assess the acute oral toxicity of AV/OIP/22 according to OECD 423 guidelines. Nine adult female Swiss albino mice, weighing 25-28 g, were used for the study. Following the oral administration of AV/OIP/22, the animals were observed for the manifestation of toxic effects and mortality. Toxicity was evaluated on the basis of changes in weight, signs of toxicity, histology of important organs (heart, liver, kidney, and lungs), and blood biochemical parameters (AST, ALT, ALP and creatinine). No toxic effects or mortalities were noticed until day 14 and AV/OIP/22 was found safe for oral consumption.

**Keywords:** Acute oral toxicity, AV/OIP/22, estrus inducer, herbal, OECD 423, post-partum anestrus

## Introduction
Dairy herd profitability is influenced largely by reproductive success. A key cause of lower production efficiency in dairy farms is the failure to achieve optimum fertility levels. Anovulation, delayed ovulation, and postpartum anestrus are causes of reduced fertility, which are widespread and have a large financial impact on dairy farmers (Ravinder *et al.*, 2016; Rajkumar *et al.*, 2021) [13, 12]. The inability of dominant follicles to ovulate during the post-partum period is associated with irregular luteinizing hormone (LH) pulses; both suckling and low levels of nutrition are associated in the prolonged inhibition of LH pulses in the lack of progesterone (Crowe, 2014) [5].

AV/OIP/22 (M/s Ayurvet Limited, India), a scientific combination of potent medicinal herbs, which, helps in the timely onset of estrus and ovulation, and increases the chances of conception, thereby, improving the reproductive efficiency of cows and buffaloes (Hadiya *et al.*, 2017; Jyothi *et al.*, 2020) [6, 7]. AV/OIP/22 is a herbal heat inducer that helps in inducing ovulatory estrus by the stimulating release of gonadotropins and mimics the action of follicle stimulating hormone (FSH) and LH (Bhageerathi *et al.*, 2009) [3]. Its key ingredients, which include *Citrullus colocynthis*, *Piper longum*, and *Zingiber officinale*, help in follicular development, onset of ovulation, and improve estrus response and reproductive health (Sarwar *et al.*, 2014; Yilmaz *et al.*, 2018; Kapoor *et al.*, 2020) [14, 15, 8]. The current study was undertaken to evaluate the acute oral toxicity of a herbal estrus inducer.

## Materials and Methods
The study was performed at the Department of Veterinary Pharmacology and Toxicology at the Post-Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, India and the Institutional Animal Ethics Committee (IAEC) of PGIVAS, Akola, approved the trial protocol (approval number 312/4/14/2000/20; dated 06.03.2020).

Nine adult female Swiss albino mice, weighing 25-28 g, were used. The animals were obtained from the animal resource section, Department of Pharmacology, PGIVAS, Akola. IAEC SOPs and CPCSEA regulations were followed for all animals. Picric acid staining was used for animal identification. The number of animals per cage was limited to three for ease of monitoring. The animals were reared in a 12 h light and dark cycle with constant temperature (25±2 °C) and relative humidity (70%). The animals were fed a regular pelleted feed and had access to ample water (OECD, 2001) [10].

Animals were housed in cages for five days to adapt to the experimental settings. Thereafter, the animals were fasted for three to four hours. After fasting, the animals were weighed and the test substance was administered orally. Three mice in Group I were administered the test substance at a dose of 300 mg/Kg body weight. When no evidence of toxicity was seen in Group I, the remaining six mice of Group II were administered the maximum dose of the test substance, which was 2000 mg/Kg of body weight. In both groups, feeding was withheld for 1-2 h after the test substance was administered.

Animals were monitored continuously for toxic effects and mortality for at least 30 minutes, frequently for the first 24 h, and, then, intermittently for a total of 14 days. Changes occurring in the eyes, skin, and coat, in the respiratory, circulatory, and central nervous systems, as well as autonomic and somatic activity were observed. Muscle tremors, lethargy, drowsiness, diarrhoea, salivation, convulsions, and coma, if any, were recorded. After 14 days of observation, animals were euthanized, and necropsy was performed, followed by histological examinations of the heart, liver, lungs, and kidneys. Biochemical markers *viz.* aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and creatinine were measured in the blood serum. The biochemical parameters were statistically examined using one-way ANOVA.

## Results and Discussion

The body weights of mice were recorded separately on days 0, 7, and 14 of the study. During the study period, body weights of both groups I and II continued to increase (Table 1). After oral administration of AV/OIP/22 at 300 and 2000 mg/Kg body weight to groups I and II, respectively, no mortality or abnormal signs were observed. The $LD_{50}$ of AV/OIP/22 was greater than 2000 mg/Kg as no mortality was noticed at this limit dose.

The heart, liver, lungs, and kidneys did not show any significant changes in appearance at necropsy after 14 days. Similarly, no gross or histological abnormalities of the heart, liver, lungs, and kidneys were noticed in any of the mice (Figure 1). While the mean values of ALT, AST, ALP, and creatinine differed significantly between both groups (Table 2), all of these analytes were well within their normal ranges in both the groups, indicating no damage to any of these vital organs.

**Table 1:** Individual weekly body weights and mortality of experimental mice administered AV/OIP/22

| Group | Animal No. | Body Weight (g) on day | | | Mortality |
|---|---|---|---|---|---|
| | | 0 | 7 | 14 | |
| I | 1 | 25 | 26 | 27 | No |
| | 2 | 27 | 28 | 28 | No |
| | 3 | 27 | 28 | 29 | No |
| | Mean ± SD | 26.33 ± 0.943 | 27.33 ± 0.943 | 28 ± 0.816 | - |
| II | 1 | 26 | 26 | 27 | No |
| | 2 | 28 | 29 | 30 | No |
| | 3 | 27 | 28 | 28 | No |
| | 4 | 26 | 27 | 27 | No |
| | 5 | 25 | 26 | 27 | No |
| | 6 | 28 | 28 | 29 | No |
| | Mean ± SD | 26.67 ± 1.105 | 27.33 ± 1.105 | 28 ± 1.154 | - |

**Table 2:** Mean ± SD values of AST, ALT, ALP and creatinine values in experimental mice

| Group | AST (U/L) | ALT (U/L) | ALP (U/L) | Creatinine (mg/dL) |
|---|---|---|---|---|
| I | $50.39 \pm 0.39^{b}$ | $41.67 \pm 0.85^{b}$ | $118.28 \pm 1.47^{b}$ | $0.46 \pm 0.012^{b}$ |
| II | $54.85 \pm 0.25^{a}$ | $46.38 \pm 0.40^{a}$ | $125.54 \pm 1.74^{a}$ | $0.55 \pm 0.008^{a}$ |

$^{a-b}$ values bearing different superscripts differ significantly within columns ($p< 0.05$)

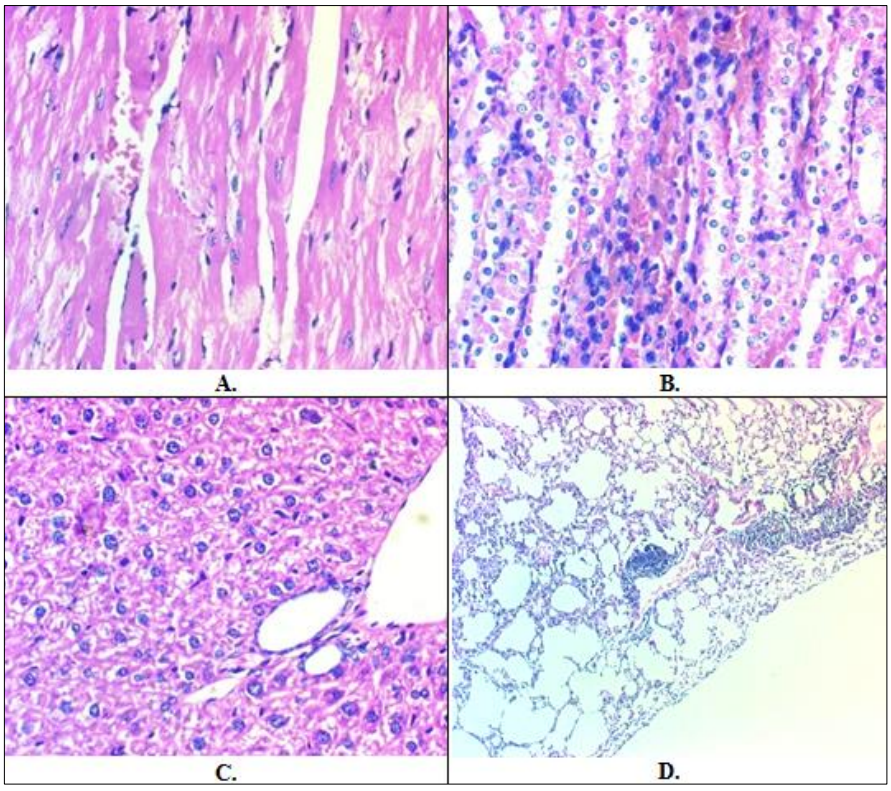


**Fig 1:** Histological appearances of A. heart, B. kidneys, C. liver and D. lungs of mice receiving AV/OIP/22 at 2000 mg/Kg of b.wt.

AV/OIP/22 is a mixture of different herbs like *Citrullus colocynthis*, *Piper longum* and *Zingiber officinale* which belong to the Generally Regarded as Safe (GRAS) category. *Citrullus colocynthis* is a rich source of flavonoids, polyphenols, terpenoids, glycosides, alkaloids, caffiec acid, steroids, tannins, and saponins (Ahmed *et al.*, 2019; Bhasin *et al.*, 2020; Kapoor *et al.*, 2020; Meybodi, 2020) [1, 4, 8, 9]. Cucurbitans, a phytochemical present in *Citrullus colocynthus*, is known to possess significant anti-inflammatory property (Peters *et al.*, 1997) [11]. *Zingiber officinale* is known to possess anti-oxidant property and may aid in onset of ovulation in cows suffering from postpartum anestrus (Amin *et al.*, 2022) [2]. Hadiya *et al.* (2017) [6] have already reported a significant increase in the number of cows showing estrus and a significantly higher conception rate in the AV/OIP/22 treated group compared to control group without any adverse effects on the health of the animals. Similarly, Jyothi *et al.* (2020) [7] could also report the best estrus response and highest conception rate without any noxious effects in the water buffaloes that received treatment with a combination of herbal uterine cleanser, mineral supplement, and AV/OIP/22. Hence, our results uphold that AV/OIP/22 can be used as an estrus inducer in livestock without exerting any toxic effects.

## Conclusion

The present study revealed that AV/OIP/22 did not produce acute oral toxicity, even when administered at the maximum limit dose (2000 mg/Kg) in mice, as evident from the absence of death, any clinical toxicity, and gross or histological alterations. Based on these findings, it could be concluded that AV/OIP/22 is safe for oral use.

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