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SUB-ACUTE ORAL TOXICITY EVALUATION OF GYNOVEDA® JEEHV AYURVEDIC TABLETS IN WISTAR RATS AS PER OECD TG 407

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ABSTRACT

Background: Gynoveda® JEEHV Ayurvedic Tablets are a polyherbal formulation developed to support women's reproductive health and enhance natural fertility. Given the potential for prolonged use in clinical settings, a systematic evaluation of its safety profile is essential. **Objective:** To assess the sub-acute oral toxicity of Gynoveda® JEEHV Ayurvedic Tablets following repeated administration over 28 days in Wistar rats, in accordance with OECD Test Guideline 407. **Methods:** A total of 40 Wistar rats (20 males and 20 females) were randomized into four groups: control, low dose (200 mg/kg/day), medium dose (400 mg/kg/day), and high dose (1000 mg/kg/day). The doses were derived using allometric scaling from the human therapeutic dose. The formulation was administered orally once daily for 28 consecutive days. Clinical observations, body weight, food and water intake were monitored throughout the study. At study termination, haematological and serum biochemical parameters were evaluated, followed by necropsy and histopathological examination of major organs. A satellite group from the high-dose cohort was observed for an additional 14-day recovery period. **Results:** No mortality or treatment-related clinical signs of toxicity were observed in any group. All animals exhibited normal growth patterns with no significant changes in food or water intake. Haematological parameters remained within physiological limits, although a dose-dependent increase in platelet counts was noted. Serum biochemical analysis revealed no significant alterations in liver and kidney function markers across all groups. Elevated triglyceride levels were observed, particularly in female rats; however, these changes were reversible, as evidenced by normalization in the satellite group. Histopathological evaluation of vital organs showed no treatment-related abnormalities. **Conclusion:** Repeated oral administration of Gynoveda® JEEHV Ayurvedic Tablets for 28 days did not produce any significant toxicological effects in Wistar rats, even at doses up to five times the human therapeutic equivalent. The formulation can be considered safe under the conditions of this study, supporting its potential for long-term use.

KEYWORDS: Ayurvedic formulation, sub-acute toxicity, OECD TG 407, Wistar rats, reproductive health, safety evaluation, polyherbal formulation.

INTRODUCTION

The increasing global interest in integrative and traditional systems of medicine has led to a renewed focus on the therapeutic potential of Ayurvedic formulations, particularly in the domain of women's reproductive health,[1] Conditions such as polycystic ovarian syndrome (PCOS), unexplained infertility, and hormonal imbalances are rising in prevalence and often require long-term management strategies.[2] In this context, herbal formulations are frequently preferred due to their holistic approach, perceived safety, and suitability for prolonged use.

Gynoveda® JEEHV Ayurvedic Tablets are a polyherbal formulation designed to support female reproductive health, and enhance fertility outcomes. The formulation is composed of traditionally used Ayurvedic ingredients known for their roles in balancing hormonal function, improving uterine health, and promoting overall well-being. Given its intended use in women attempting

conception, the formulation may be consumed over extended periods, making its safety profile a critical consideration.

Despite the long-standing traditional use of many Ayurvedic ingredients, scientific validation of safety through standardized toxicological studies remains essential for regulatory acceptance and wider clinical application. Toxicity studies, particularly repeated-dose studies, provide crucial insights into the potential adverse effects of a formulation when administered over time. These studies help identify target organ toxicity, establish safe dosage ranges, and evaluate reversibility of any observed effects.[3][4]

The Organisation for Economic Co-operation and Development (OECD) Test Guideline 407 provides a standardized framework for evaluating sub-acute oral toxicity through 28-day repeated dosing in rodents. This model is widely used in regulatory and industrial settings as an initial safety assessment tool. It enables early identification of target organ toxicity, dose-related effects, and cumulative toxicity within a short duration, while recovery groups allow assessment of reversibility. Its efficiency and scientific robustness make it particularly suitable for formulations intended for prolonged human use.[5]

In the present study, the sub-acute oral toxicity of Gynoveda® JEEHV Ayurvedic Tablets was evaluated in Wistar rats following daily oral administration at therapeutic, intermediate, and high dose levels derived through allometric scaling from the human dose. The study aimed.

To.

1. Evaluate systemic toxicity by assessing clinical signs, body weight, and food and water intake in Wistar rats following once-daily oral administration of Gynoveda® JEEHV Ayurvedic Tablets at doses of 200, 400, and 1000 mg/kg/day for 28 consecutive days.
2. Quantify haematological and biochemical changes by measuring parameters such as haemoglobin, RBC, WBC, platelet count, liver enzymes (SGOT, SGPT, ALP), renal markers (BUN, creatinine), lipid profile, and electrolytes at the end of the 28-day treatment period.
3. Identify target organ toxicity through gross necropsy and histopathological examination of major organs, including liver, kidneys, heart, lungs, brain, spleen, adrenals, and reproductive organs, following repeated exposure.

This study seeks to generate scientific evidence supporting the safety of Gynoveda® JEEHV Ayurvedic Tablets, thereby contributing to their rational use in clinical practice and supporting their potential role in long-term management of women's reproductive health.

2. METHODOLOGY

2.1 *Study Design and Objective*

The present study was conducted to evaluate the sub-acute oral toxicity of Gynoveda® JEEHV Ayurvedic Tablets following repeated administration for 28 days in Wistar rats, in accordance with OECD Test Guideline 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents). The formulation is intended for long-term use in women's reproductive health; therefore, its safety under repeated exposure was assessed using an appropriate animal model.

2.2 *Dose Selection and Justification*

The marketed human dose of the formulation is 4 tablets per day (2 tablets twice daily after meals), with each tablet weighing 500 mg, resulting in a total daily dose of 2000 mg. The human equivalent dose (HED) was converted to the animal equivalent dose using standard allometric scaling based on body surface area correction factors (Km method), as per regulatory guidelines.

Rat dose (mg/kg)=Human dose (mg/kg)×Km(human)/Km(rat)

Where: Km factor for human (60 kg) = 37 & Km factor for rat (200 g) = 6.2

The human dose on a mg/kg basis was calculated as: 2000 mg ÷ 60 kg = 33.33 mg/kg/day Rat dose = 33.33 mg/kg/day x 6.2 = 206.65 mg/kg/day (~ 200 mg/kg/day)

Considering, 200 mg/kg/day as therapeutic equivalent dose (x value) for 2000 mg/day human therapeutic dose,

- *Low dose (1x-LD) = 200 mg/kg/day*
- *Medium dose (2x-MD) = 400 mg/kg/day*
- *High dose (5x-HD) = 1000 mg/kg/day*

2.3 *Test Substance and Experimental Animals*

The test substance used in the study was Gynoveda® JEEHV Ayurvedic Tablets. A total of 40 healthy Wistar rats (20 males and 20 females), aged 6–7 weeks and weighing 150–200 g, were used as the experimental animals for the study.

2.4 *Procurement, Acclimatization, and Housing*

Animals were procured and acclimatized for one week prior to the initiation of the study. During the study period, the animals were housed in polypropylene cages under controlled environmental conditions, maintained at a temperature of 22 ± 1°C with relative humidity of 60–70% and a 12-hour light/12-hour dark cycle. Each cage housed five animals, with a total of eight cages used for the study. Male and female rats were housed separately throughout the experimental period.

2.5 *Animal Handling, Randomization, and Husbandry*

he animals were randomly assigned into four groups, each comprising 10 animals (5 males and 5 females), with each group distributed across two cages. Individual animals were identified using non-invasive tail markings (I, II, III, IIII, and unmarked). Animals were handled gently for 3–5 days prior to study initiation to minimize stress and were observed to confirm their health status.

Throughout the study period, animals were provided with standard pellet diet and water ad libitum. Feed and bedding material were also procured. Only healthy animals showing no signs of disease were included in the study, and body weights were recorded at the time of procurement.

2.7 Grouping and Treatment Regimen

Animals were divided into the following groups.

Table 1: Grouping of animals.

Group	Animal Group	Dose (mg/kg/day)	Number of Animals
C	Normal Control	–	10 (5 male, 5 female)
T1	Test Group I – Low Dose (1×)	200	10 (5 male, 5 female)
T2	Test Group II – Medium Dose (2×)	400	10 (5 male, 5 female)
T3	Test Group III – High Dose (5×)	1000	10 (5 male, 5 female)
	Total		40 (20 male & 20 female)

2.8 Preparation and Administration of Dose

For sub-acute oral toxicity, formulation was administered orally at 3 dose levels 200, 400 and 1000 mg/kg. The test formulation tablets were crushed into a fine powder and suspended in distilled water to prepare dosing solutions. The formulation was administered orally once daily for 28 consecutive days using an oral gavage. Prior to dosing, animals were fasted for 3–4 hours. The administered volume did not exceed 1 mL per 100 g body weight. Dose calculations were adjusted weekly based on the average body weight of animals in each group.

Table 2: Dose Calculation for Sub-acute 28-day Study.

Dose (mg/kg /day)	Sex	Day 1–7 Avg. Weight (g)	Dose Given (mg)	Day 8–14 Avg. Weight (g)	Dose Given (mg)	Day 15–21 Avg. Weight (g)	Dose Given (mg)	Day 22–28 Avg. Weight (g)	Dose Given (mg)
200	Male	208	41.6	248	49.6	255	51.0	258	51.6
	Female	192	38.4	199	39.8	206	41.2	212	42.4
400	Male	162	64.8	175	70.0	186	74.4	196	78.4
	Female	198	79.2	202	80.8	198	79.2	200	80.0
1000	Male	167	167	199	199	211	211	230	230
	Female	178	178	185	185	188	188	197	197

2.9 Experimental Procedure

The sub-acute toxicity study was conducted in animals, comprising 5 females and 5 males (n = 10) for each dose level of the formulation. As per OECD TG 407, the Ayurvedic formulation was orally administered at three dose levels (low, medium, and high) over a period of 28 days.

The prepared doses were administered via gavage. Throughout the dosing period, animals were observed daily for 28 days for any signs of toxicity. General clinical observations, body weight, and food and water consumption were recorded on a weekly basis.

At the end of the 28-day treatment period, 4 animals (2 males and 2 females) from each group were selected for necropsy. Retro-orbital blood samples were collected for haematological (CBC) and clinical (serum) biochemical analysis (n = 10; 5 males and 5 females). Animals were then sacrificed, and vital organs—including the brain, heart, lungs, liver, kidneys, spleen, adrenals, testes, ovaries, and uterus—were collected and preserved in 4% neutral buffered formalin (NBF) for histopathological examination using Haematoxylin and Eosin staining.

2.10 Satellite Group (Recovery Study)

Two animals (one male and one female) from the high-dose group were maintained as a satellite (recovery) group and observed for an additional 14 days without treatment to assess the persistence of toxic effects, delayed toxicity, and recovery potential. At the end of the recovery period, these animals were further evaluated for hematological, biochemical, and histopathological parameters.

2.11 Post-Experimental Procedures

Following necropsy, carcasses were stored at −20°C and disposed of in accordance with biomedical waste management guidelines.

2.12 Statistical Analysis

All data were analyzed using GraphPad Prism (version 11.0.0). Statistical analysis
of main study was performed by ordinary one-way ANOVA followed by Dunnett's test and satellite group data was analysed using paired sample t-test. Where, * indicates $p < 0.05$ (significant), ** indicates $p < 0.01$ (moderately significant), *** or **** indicates $p < 0.001$ or < 0.0001 (highly significant) and ns (non-significant) when compared to control.

3. RESULTS

3.1 *Evaluation of Systemic Toxicity (Clinical Signs, Body Weight, Food and Water Intake)*

No mortality was observed in any of the experimental groups throughout the 28-day treatment period. Daily clinical observations revealed no treatment-related signs of toxicity. Animals in all groups appeared normal with respect to skin, fur, eyes, mucous membranes, posture, gait, and behavioural patterns. No abnormalities such as tremors, convulsions, salivation, diarrhoea, lethargy, or changes in autonomic activity were noted.

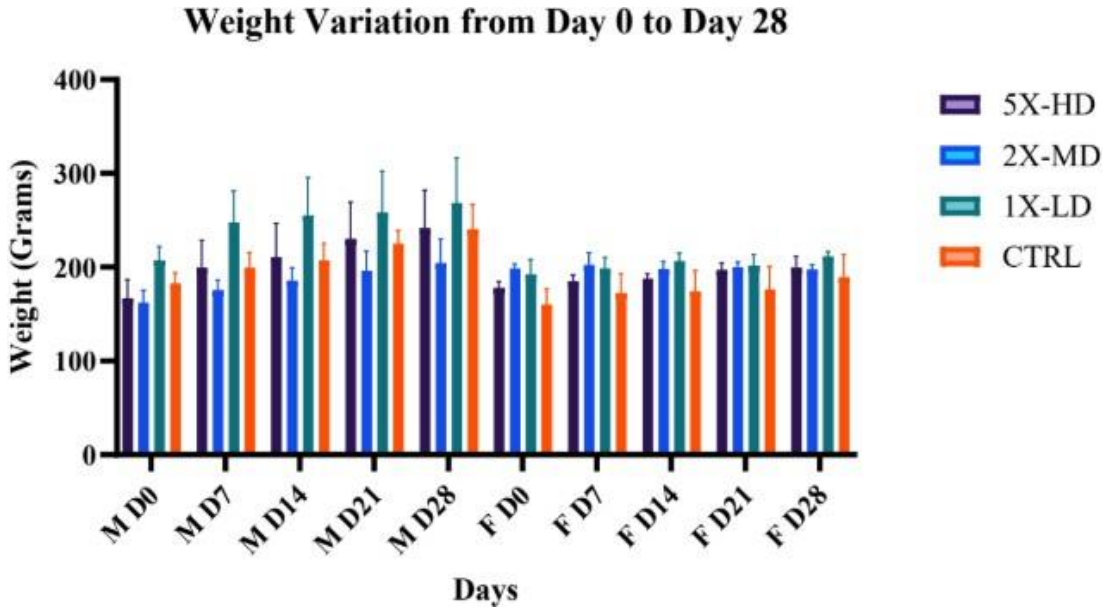

Figure 1: Body weight variation in Wistar rats from Day 0 to Day 28 across control and treatment groups.

Male rats showed relatively higher weight gain compared to females, consistent with physiological norms. No significant changes were observed in food consumption or water intake across any of the treated groups, indicating that the test formulation did not adversely affect general health status or metabolic activity.

3.2 *Haematological and Biochemical Findings*

Haematological parameters in both male and female rats remained within normal physiological ranges across all dose groups. Parameters including haemoglobin concentration, red blood cell count, haematocrit, and differential leukocyte counts did not show biologically significant deviations from control values. Platelet counts were observed to be elevated across all dose groups.

Serum biochemical analysis demonstrated that liver function markers, including total bilirubin, direct bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total protein, and albumin, remained comparable to the control group.

Renal function parameters, including blood urea nitrogen (BUN), creatinine, and uric acid, were within normal physiological ranges in all treated groups, suggesting preserved kidney function. Lipid profile parameters remained largely unaltered, with total cholesterol levels comparable to control across all groups. However, triglyceride levels were elevated at all dose levels, with a more pronounced increase in female rats. Notably, this effect was reversed in the satellite group (5 times therapeutic dose for 28 days followed by a 14-day recovery period), where triglyceride levels returned to levels comparable to control in both sexes. No significant changes were observed in random blood glucose levels across all treatment groups.

3.3 *Target Organ Toxicity (Necropsy and Histopathology)*

Gross necropsy did not reveal any visible abnormalities in major organs across control and treated groups. Histopathological examination of vital organs, including brain, heart, lungs, liver, kidneys, spleen, adrenal glands, and reproductive organs (testes, ovaries, and uterus), showed normal histoarchitecture without any evidence of inflammation, degeneration, necrosis, or other pathological alterations.

4. DISCUSSION

The present study evaluated the sub-acute oral toxicity of Gynoveda® JEEHV Ayurvedic Tablets following repeated administration for 28 days in Wistar rats, in accordance with OECD Test Guideline 407. The findings provide important evidence regarding the systemic safety profile of the formulation under repeated exposure conditions.

The formulation represents a complex polyherbal and herbo-mineral system comprising ingredients with complementary pharmacodynamic properties, including *Shatavari, Ashwagandha, Kumari, Ashoka, Lodhra, Punarnava, Kutki, Shivlingi, Putranjiva, Jivanti, Hareetaki,* along with mineral components such as

Shilajit, Vanga, Kasis, Tankana, and Hingu. From a systems pharmacology perspective, such combinations are traditionally designed not through single-target agonism or antagonism, but through multi-target physiological modulation, where nourishing *(Shatavari, Ashwagandha, Jivanti)*, uterine regulatory *(Ashoka, Lodhra)*, metabolic and detoxification-supporting *(Punarnava, Kutki, Hareetaki)*, and bioavailability-enhancing components *(Shilajit, Hingu, mineral bhasmas)* collectively act to maintain functional homeostasis across endocrine, reproductive, and metabolic axes. This balanced interaction may explain the absence of systemic toxicity even at supra-therapeutic exposure levels (6) (7).

Against this pharmacological background, the absence of mortality and treatment-related clinical signs suggests that repeated oral administration does not induce overt systemic toxicity or functional impairment of major physiological systems. The lack of behavioural, neurological, or autonomic abnormalities further indicates preservation of central nervous system integrity and general physiological stability throughout the treatment period.

This overall safety trend is supported by the absence of adverse effects on growth parameters. Normal body weight progression, along with stable food and water intake across all groups, indicates that the formulation does not interfere with metabolic efficiency, nutrient assimilation, or gastrointestinal function. These findings collectively reflect good systemic tolerability under repeated exposure conditions.

From a hematological perspective, most parameters remained within physiological limits, suggesting that erythropoietic and immune functions were not disrupted. The observed increase in platelet count, does not appear to be associated with any adverse toxicological effects. This is supported by the absence of changes in liver and kidney function parameters, normal histopathological findings, and lack of systemic toxicity. The effect is therefore likely to be a non-adverse, possibly adaptive physiological response to treatment.

Similarly, serum biochemical parameters indicated preserved organ function. Liver enzymes (SGOT, SGPT, ALP) and renal markers (BUN, creatinine) did not exhibit consistent or dose-dependent alterations, and all values remained within normal biological ranges. Importantly, the absence of corresponding histopathological changes reinforces that these biochemical variations do not reflect functional organ toxicity.

Within lipid metabolism, an increase in triglyceride levels was observed, particularly in female rats during the treatment phase. However, this alteration was reversible, as evidenced by normalization in the satellite group after the recovery period. The absence of any associated hepatic structural changes further supports the interpretation that this represents a transient metabolic modulation rather than a sustained toxic effect.

This interpretation is strengthened by histopathological findings, which serve as the most definitive endpoint in toxicity evaluation. Across all dose groups, including the high-dose group, vital organs such as the liver, kidneys, heart, lungs, brain, spleen, adrenal glands, and reproductive organs exhibited normal architecture without evidence of degeneration, inflammation, necrosis, or other pathological alterations. The preservation of tissue integrity at doses up to five times the human equivalent further confirms a wide safety margin.

The inclusion of a high-dose and satellite recovery arm provides additional confidence in the safety profile of the formulation. The absence of toxicological changes even at supra-therapeutic exposure levels, along with reversibility of minor biochemical variations, suggests that any observed alterations are adaptive rather than harmful in nature. Importantly, the recovery group findings further demonstrate that no delayed or persistent toxicity occurs following cessation of treatment.

Taken together, these findings suggest that the JEEHV formulation maintains systemic and organ-level safety under repeated exposure conditions. From a mechanistic perspective, the multi-component nature of the formulation may contribute to physiological buffering, where adaptogenic, metabolic, and regulatory constituents act in concert to prevent excessive perturbation of individual biological pathways, thereby maintaining homeostatic balance.

These results are consistent with the broader literature on polyherbal formulations, which often demonstrate favorable safety profiles due to their multi-target and modulatory nature. (8)

5. CONCLUSION

The present sub-acute oral toxicity study of Gynoveda® JEEHV Ayurvedic Tablets, conducted in Wistar rats in accordance with OECD Test Guideline 407, demonstrates that the formulation is safe and well tolerated following repeated administration for 28 days. No mortality, clinical signs of toxicity, or treatment-related adverse effects were observed at therapeutic, intermediate, or high dose levels.

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