

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

407

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ABSTRACT

Background: Gynoveda® BEEHJ Ayurvedic Tablets are an Ayurvedic formulation intended for use in the management of male infertility. As formulations used in fertility management may require repeated and prolonged administration, systematic evaluation of their safety following repeated exposure is important. Repeated-dose toxicological assessment provides information regarding potential cumulative effects and target-organ toxicity.^[1] **Objective:** To assess the systemic and target-organ toxicity of Gynoveda® BEEHJ Ayurvedic Tablets following repeated oral administration at therapeutic-equivalent, intermediate, and high dose levels in male and female Wistar rats. **Methods:** Forty healthy Wistar rats, comprising 20 males and 20 females, were randomly allocated

into four groups: normal control, low-dose (225 mg/kg/day), medium-dose (450 mg/kg/day), and high-dose (1125 mg/kg/day) groups. The doses were derived from the marketed human dose using body-surface-area-based allometric scaling.^[2] The formulation was administered orally by gavage for 28 consecutive days. Animals were monitored for mortality, clinical and behavioural abnormalities, body weight, and food and water consumption. At the end of the treatment period, haematological and serum biochemical investigations were performed, followed by necropsy and histopathological examination of major organs, consistent with the endpoints recommended for repeated-dose oral toxicity assessment.^[1] **Results:** No mortality or treatment-related clinical signs of toxicity were observed in any treatment group during the 28-day study period. Animals demonstrated normal body-weight progression and maintained normal food and water consumption. No treatment-related abnormalities were observed in

clinical or behavioural parameters. Haematological and serum biochemical parameters showed no treatment-related toxicological pattern. Histopathological examination of major organs revealed no treatment-related lesions. A mild elevation of serum phosphorus was noted in the high-dose satellite animals; no other significant biochemical abnormality was reported. The study established a No Observed Adverse Effect Level (NOAEL) of 1125 mg/kg/day for both male and female rats. **Conclusion:** Repeated oral administration of Gynoveda® BEEHJ Ayurvedic Tablets for 28 days did not produce evidence of systemic or target-organ toxicity in Wistar rats at doses up to 1125 mg/kg/day. The findings support a favourable sub-acute safety profile of the formulation under the experimental conditions of the present study.

INTRODUCTION

Male infertility is a multifactorial condition associated with abnormalities in sperm concentration, motility, morphology, reproductive function, and other physiological factors. Management may require prolonged therapeutic interventions, making the safety of formulations intended for repeated use an important consideration.

Ayurvedic formulations have traditionally been used in reproductive health and may contain multiple botanical and other ingredients administered in combination. Although traditional use provides a basis for therapeutic application, systematic preclinical toxicological evaluation remains important for establishing the safety profile of formulations intended for repeated or prolonged consumption.^[10,17]

Repeated-dose toxicity studies are particularly relevant for identifying potential cumulative effects, target-organ toxicity, alterations in physiological functions, and the reversibility of treatment-related changes.^[1,3]

The Organisation for Economic Co-operation and Development (OECD) Test Guideline 407 provides a standardized framework for evaluating repeated-dose oral toxicity in rodents over a 28-day period. The guideline incorporates clinical observations, body-weight monitoring, food consumption, haematological and clinical biochemical investigations, gross pathological examination, and histopathological evaluation of major organs.^[1]

Clinical observations are important components of animal toxicity studies because changes in general appearance, behaviour, neurological status, autonomic activity, and other clinical

signs may provide early evidence of systemic toxicity.^[3,4]

Gynoveda® BEEHJ Ayurvedic Tablets are formulated for use in the management of male infertility. The marketed dosage is two tablets after breakfast and two tablets after dinner, corresponding to a total daily dose of four tablets.

The therapeutic-equivalent rat dose was calculated using body-surface-area-based allometric scaling. Such interspecies dose conversion is commonly used during preclinical drug development to estimate an appropriate experimental dose from a human therapeutic dose.^[2]

Considering the intended repeated use of the formulation, the present study was undertaken to evaluate its sub-acute oral toxicity in male and female Wistar rats following 28 days of repeated administration according to OECD TG 407.^[1]

1. METHODOLOGY

1.1 Ethical Approval

The protocol was approved by the Institutional Animal Ethics Committee on 11 April 2026 and the study was conducted in accordance with applicable requirements for laboratory animal experimentation.^[18]

1.2 Test Substance

The test substance was **Gynoveda® BEEHJ Ayurvedic Tablets**.

The marketed human dosage is four tablets per day, administered as two tablets after breakfast and two tablets after dinner. Each tablet weighed approximately 550 mg, resulting in a total daily human dose of 2200 mg.

1.3 Dose Selection and Justification

The rat-equivalent dose was calculated using the body-surface-area-based Km correction method:

$$\text{Rat dose (mg/kg)} = \text{Human dose (mg/kg)} \times \text{Km (human)} / \text{Km (rat)}$$

This approach is consistent with established methods for extrapolating doses between laboratory animals and humans during preclinical investigations.^[2]

For a 60-kg human, the Km factor was 37, while the corresponding factor for a 200-g rat was 6.2. The human dose was calculated as:

$$2200 \text{ mg/day} \div 60 \text{ kg} = 36.67 \text{ mg/kg/day}$$

The corresponding rat dose was:

$$36.67 \times 6.2 = 227.33 \text{ mg/kg/day}$$

This value was approximated to **225 mg/kg/day** as the therapeutic-equivalent dose. The three experimental dose levels were therefore:

- **Low dose (1×): 225 mg/kg/day**
- **Medium dose (2×): 450 mg/kg/day**
- **High dose (5×): 1125 mg/kg/day**

1.4 Experimental Animals

A total of 40 healthy Wistar rats, comprising 20 males and 20 females, were used. Animals were 6–7 weeks of age and weighed approximately 150–200 g at procurement.

Animals were acclimatized before initiation of treatment and maintained under controlled environmental conditions. Standard laboratory animal care procedures were followed.^[18]

1.5 Grouping of Animals

The animals were randomly assigned to four groups, with 10 animals per group comprising five males and five females.

Group	Treatment	Dose
C	Normal control	—
T1	Low dose	225 mg/kg/day
T2	Medium dose	450 mg/kg/day
T3	High dose	1125 mg/kg/day

1.6 Preparation and Administration of Test Substance

The BEEHJ tablets were crushed into a fine powder and triturated with sufficient distilled water to prepare the dosing suspension. The formulation was administered orally by gavage once daily for 28 consecutive days.

The repeated oral administration protocol was designed in accordance with the principles of OECD TG 407.^[1]

1.7 Clinical Observations and General Toxicity Assessment

Animals were observed daily throughout the treatment period for clinical or behavioural signs suggestive of toxicity. Parameters included general appearance, skin, fur, eyes, mucous membranes, gait, posture, response to handling, autonomic activity, and neurological

abnormalities.

Clinical observations are an important component of repeated-dose toxicity assessment because overt systemic toxicity may first become evident through changes in behaviour, appearance, neurological function, or autonomic activity.^[3,4]

Body weight and food and water consumption were also monitored during the study period.

1.8 Haematological and Serum Biochemical Investigations

At the end of the 28-day treatment period, blood samples were collected for haematological and serum biochemical assessment. Haematological parameters included haemoglobin, white blood cell count, haematocrit, red blood cell count, platelet count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, neutrophils, lymphocytes, eosinophils, and monocytes.

Serum biochemical parameters included total and direct bilirubin, SGOT, SGPT, alkaline phosphatase, total protein, albumin, blood urea nitrogen, calcium, phosphorus, creatinine, total cholesterol, triglycerides, random blood glucose, sodium, potassium, and chloride.

These parameters are routinely incorporated into repeated-dose systemic toxicity assessment to identify potential effects on haematopoietic, hepatic, renal, metabolic, and electrolyte homeostasis.^[1,23]

1.9 Necropsy and Histopathological Examination

Following completion of the treatment period, selected animals underwent necropsy. Major organs, including the brain, heart, lungs, liver, kidneys, spleen, adrenal glands, testes, ovaries, and uterus, were collected and preserved for histopathological examination.

Histopathology is an important endpoint in repeated-dose toxicity studies because microscopic examination may identify organ injury that is not apparent from clinical or biochemical findings.^[1,21,23]

1.10 Satellite Recovery Group

Following completion of the 28-day treatment period, four animals from the high-dose group, comprising two males and two females, were maintained as a satellite recovery group for an additional 14 days without treatment.

Recovery groups may provide additional information regarding persistence, delayed occurrence, or reversibility of treatment-related findings.^[1]

1.11 Statistical Analysis

The data were analysed using GraphPad Prism version 11.0. Main-study data were analysed using ordinary one-way analysis of variance (ANOVA) followed by Dunnett's test. Satellite-group data were analysed using a paired-sample t-test. Statistical significance was interpreted as $p < 0.05$.

2. RESULTS

2.1 Clinical Signs, Mortality, Body Weight, Food and Water Consumption

No mortality occurred in any of the control or treatment groups throughout the 28-day treatment period. Daily clinical observations did not reveal treatment-related abnormalities in either male or female rats. Animals maintained normal skin, fur, eyes, mucous membranes, gait, posture, and response to handling. No abnormal autonomic activity, lacrimation, piloerection, altered pupil size, abnormal respiratory pattern, clonic or tonic seizures, stereotypic behaviour, or unusual excretory changes were observed.

Normal body-weight progression was observed throughout the treatment period. Food consumption and water intake remained comparable with the normal control group. The absence of treatment-related clinical signs, mortality, or adverse effects on body weight and food consumption is consistent with the parameters commonly evaluated in repeated-dose oral toxicity studies.^[1,2,3]

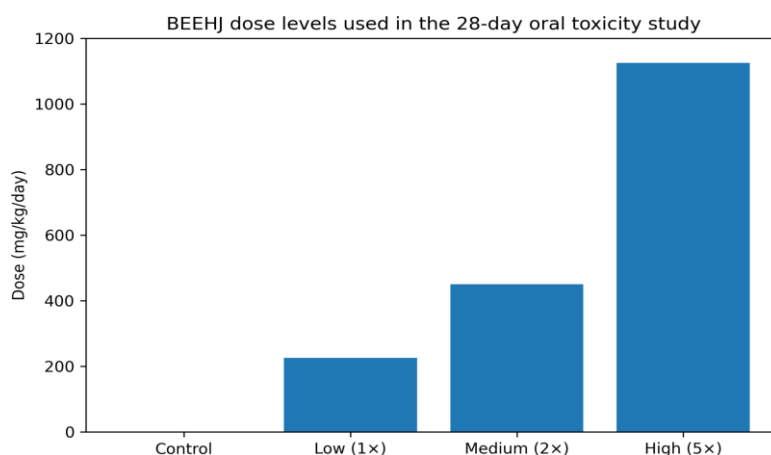
2.2 Haematological Findings

Haematological parameters were assessed separately in male and female rats.

In male animals, haemoglobin, WBC count, haematocrit, RBC count, platelet count, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils, and monocytes did not demonstrate statistically significant treatment-related differences compared with the normal control group.

Similarly, no statistically significant treatment-related alterations were observed in the corresponding haematological parameters of female rats.

No treatment-related pattern of haematological toxicity was therefore identified.

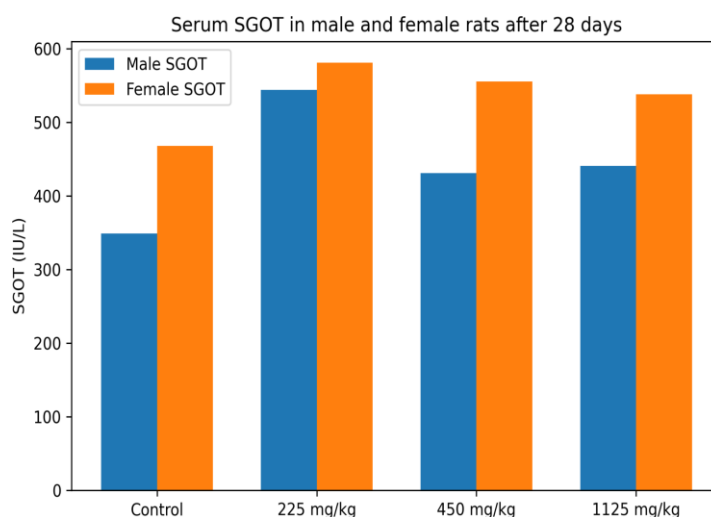


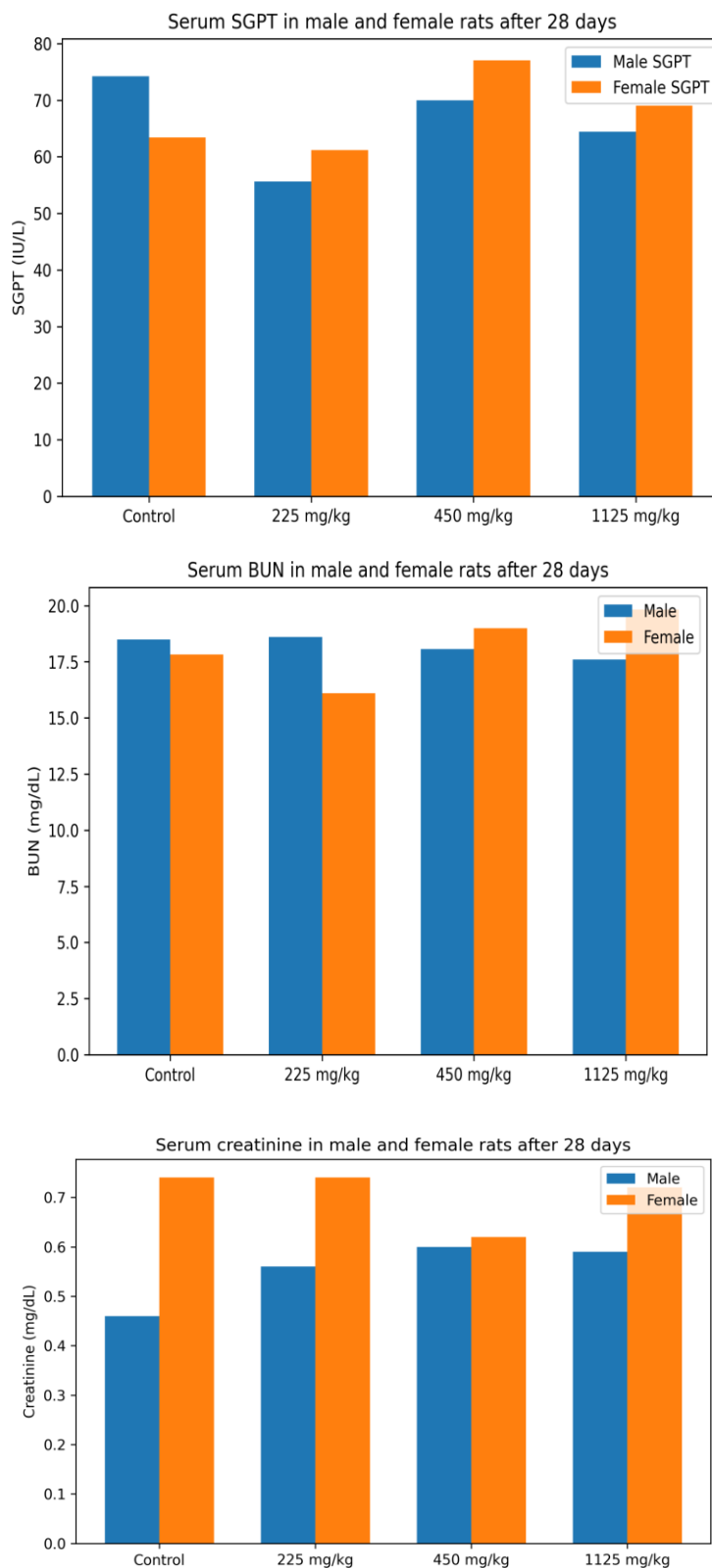
2.3 Serum Biochemical Findings

Serum biochemical assessment did not reveal a consistent treatment-related alteration in hepatic or renal function.

Total bilirubin, direct bilirubin, SGOT, SGPT, alkaline phosphatase, total protein, albumin, BUN, calcium, creatinine, cholesterol, random blood glucose, and serum electrolytes remained without a treatment-related toxicological pattern across the dose groups.

Evaluation of serum biochemical markers is an established component of repeated-dose toxicity testing because alterations in hepatic enzymes and renal function markers may provide evidence of target-organ toxicity.^[1,23,24] A mild elevation in serum phosphorus was reported in the high-dose satellite animals. The study report noted that no significant difference was observed in the other haematological and serum biochemical parameters.





2.4 Gross Necropsy and Histopathological Findings

Gross examination of the major organs did not reveal treatment-related abnormalities.

Histopathological examination of the brain, heart, lungs, liver, kidneys, spleen, adrenal

glands, testes, ovaries, and uterus demonstrated no lesions suggestive of drug-induced toxicity in animals receiving 225, 450, or 1125 mg/kg/day for 28 days.

The absence of treatment-related histopathological changes across major visceral and reproductive organs provides additional evidence that repeated administration did not produce detectable target-organ injury under the conditions of the study.^[1,21,22]

2.5 Recovery/Satellite Group Findings

The high-dose satellite group was followed for an additional 14 days after cessation of treatment. No delayed or persistent toxicological effects were identified. A mild elevation in serum phosphorus was observed in the satellite group, while the remaining evaluated biochemical parameters were reported without significant abnormalities. Histopathological examination did not demonstrate treatment-related lesions.

3. DISCUSSION

The present study evaluated the sub-acute oral safety of Gynoveda® BEEHJ Ayurvedic Tablets following repeated administration in Wistar rats for 28 days. The study incorporated the major endpoints recommended for repeated-dose oral toxicity evaluation under OECD TG 407.^[1]

The absence of mortality and treatment-related clinical signs across all treatment groups indicates that repeated oral administration of BEEHJ was well tolerated under the conditions of the study. Animals maintained normal behaviour, posture, gait, skin and fur condition, mucosal appearance, and autonomic activity. Body-weight progression is an important general indicator of systemic toxicity in repeated-dose studies.^[1,21] In the present investigation, animals demonstrated normal weight gain throughout the treatment period, with no adverse effect on food or water intake. Haematological evaluation did not demonstrate treatment-related changes in erythrocytic, leukocytic, or other evaluated blood parameters. These findings suggest that repeated administration did not produce detectable adverse effects on the haematopoietic parameters evaluated.

The serum biochemical findings provide additional evidence regarding the hepatic and renal safety of the formulation. Liver-associated parameters, including bilirubin, SGOT, SGPT, alkaline phosphatase, total protein, and albumin, did not show a consistent treatment-related pattern. Similarly, renal-associated parameters including BUN and creatinine did not

demonstrate changes indicative of progressive renal dysfunction.

Histopathological examination is an important endpoint in repeated-dose toxicity assessment because it can reveal structural injury that may not be apparent from clinical observations or biochemical investigations.^[1,21,22] In the present study, no treatment-related lesions were identified in the examined visceral or reproductive organs.

The overall findings are consistent with the broader toxicological literature on Ayurvedic and polyherbal formulations, in which systematic acute and repeated-dose studies have been used to establish tolerability and identify potential target-organ toxicity.^[7,9] The safety evaluation of herbal medicines requires particular attention because multi-component formulations may contain numerous pharmacologically active constituents and may also be affected by issues relating to contaminants, adulteration, standardization, and quality control.^[10-17,25] Therefore, controlled preclinical toxicological studies provide important complementary evidence to traditional use and clinical experience.

An important finding of the present study is that the highest administered dose of **1125 mg/kg/day**, corresponding to five times the therapeutic-equivalent dose, did not produce treatment-related adverse effects in either male or female rats. On the basis of the study observations, the investigators established the **NOAEL as 1125 mg/kg/day** for both sexes under the conditions of the study.

However, the findings should be interpreted within the scope of the experimental model and study duration. A 28-day repeated-dose study provides evidence regarding sub-acute toxicity but does not by itself establish safety for all possible durations of human use.

Longer-duration toxicological studies and appropriately designed human clinical safety data would provide complementary evidence for prolonged administration.^[1,6]

4. CONCLUSION

The present study demonstrates that repeated oral administration of Gynoveda® BEEHJ Ayurvedic Tablets at doses of 225, 450, and 1125 mg/kg/day for 28 consecutive days was well tolerated in male and female Wistar rats.

No mortality, treatment-related clinical signs, behavioural abnormalities, adverse effects on body-weight progression, or alterations in food and water consumption were observed.

Haematological and serum biochemical investigations did not demonstrate a treatment-related toxicological pattern. Gross examination and histopathological evaluation of major visceral and reproductive organs revealed no treatment-related pathological abnormalities. The high-dose recovery group did not demonstrate delayed or persistent treatment-related toxicity. Based on the overall findings, the **No Observed Adverse Effect Level (NOAEL)** was established at **1125 mg/kg/day in both male and female rats**, representing five times the therapeutic-equivalent dose under the study conditions.

Overall, Gynoveda® BEEHJ Ayurvedic Tablets demonstrated a favourable sub-acute oral safety profile in Wistar rats when administered repeatedly for 28 days.

5. CONFLICT OF INTEREST

Two authors are employees of Gynoveda Femtech Pvt. Ltd., the sponsor of this study. The authors declare no other competing interests.

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