JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Evaluation of *Dooshivishari Agada* in Mitigating MSG-Induced Growth Hormone Suppression in Wistar Rats: A Randomized Experimental Animal Study Protocol

Dr. Satbir Kumar Chawla

Ph.D. Scholar, P.G Department of Agada Tantra, Institute for Ayurved Studies & Research, Kurukshetra, Haryana, India

ABSTRACT

Background: Monosodium glutamate (MSG) is widely used experimentally to study effects on the hypothalamic–pituitary endocrine axis. Exposure during early life has been linked to impaired growth hormone (GH) secretion, altered growth hormone-releasing hormone (GHRH) signalling, reduced insulin-like growth factor-I (IGF-I), and stunted growth. Several experimental studies have documented these disturbances in GH secretion and hypothalamic–pituitary function following MSG exposure. *Dooshivishari Agada* (DVA), a classical Ayurvedic herbo-mineral formulation traditionally indicated for *Dooshivisha*, has shown antioxidant and protective effects in experimental models, including MSG-associated reproductive toxicity. Its effect on MSG-associated GH suppression, however, remains unexplored.

Objective: To evaluate the effect of *Dooshivishari Agada* on MSG-induced suppression of serum GH and associated growth and safety parameters in Wistar rats.

Methods: This will be a prospective, randomized, controlled experimental animal study. Eighteen healthy Wistar rats will be allocated into three groups of six animals each: control, MSG-exposed, and MSG-exposed followed by DVA treatment. The control group will receive a standard diet and water. The MSG group will receive MSG at 0.20 g/kg/day by oral gavage for 14 days, followed by observation until Day 30. The intervention group will receive MSG at the same dose for 14 days, followed by DVA at 216 mg/200 g body weight/day by oral gavage from Day 15 to Day 30. Blood samples will be collected as per the approved animal protocol to assess serum GH, complete blood count, and liver- and renal-function parameters. Body-weight changes and other predefined growth parameters will also be recorded. Intergroup differences will be analyzed using an appropriate statistical model, with assumptions verified before inferential testing.

Ethical considerations: The study will begin only after approval from the competent Institutional Animal Ethics Committee (IAEC) and will follow applicable institutional and national regulations governing the use of laboratory animals. Animal welfare, humane handling, anaesthesia, and humane endpoints will be built into the protocol.

Expected significance: This study is designed to generate preliminary experimental evidence on the possible protective effect of DVA against MSG-associated endocrine dysfunction, laying the groundwork for further mechanistic and translational research.

Keywords: *Dooshivishari Agada; monosodium glutamate; growth hormone; Wistar rat; Ayurveda; Dooshivisha; experimental toxicology; neuroendocrine toxicity.*

1. INTRODUCTION

Monosodium glutamate (MSG) is a widely used flavour enhancer that has also been studied extensively in experimental toxicology. Research has shown that MSG exposure, particularly during critical periods of development, can affect hypothalamic structures involved in endocrine regulation. Because hypothalamic GHRH signalling drives pulsatile GH secretion, disruption at this level can affect the broader GH–IGF-I axis.

MSG exposure alters rhythmic GH secretion in male rats, including a measurable reduction in GH secretory activity[1]. Neonatal MSG exposure has also been shown to produce a prolonged impairment of GHRH-dependent GH secretion and growth, along with changes in pituitary somatotroph morphology and GH content[2–4].

The research synopsis on which this protocol is based identifies suppression of GH secretion, reduced IGF-I, and impaired growth as key downstream consequences of MSG exposure, driven in part by oxidative stress, excitotoxicity, and neuronal injury.

Ayurveda describes the concept of *Dooshivisha* — latent, residual, or cumulative toxicity that manifests only under specific triggering conditions[5,10]. *Dooshivishari Agada* is a classical formulation indicated for managing this condition, composed of twelve ingredients: *Pippali*, *Dhyamaka*, *Jatamansi*, *Lodhra*, *Ela*, *Suvarchika*, *Kutannata*, *Tagara*, *Kushtha*, *Yashtimadhu*, *Chandana*, and *Gairika*[5,9].

Earlier experimental work on *Dooshivishari Agada* (DVA) has reported antioxidant, antimicrobial, and protective activity[7,8]. DVA has also been shown to ameliorate MSG-induced reproductive toxicity in female Wistar rats[6]. Despite this established protective potential and the well-documented link between MSG exposure and GH dysfunction, no study to date has examined whether DVA can specifically counter MSG-induced GH suppression — a gap this protocol is designed to address.

2. RESEARCH QUESTION

Can *Dooshivishari Agada* mitigate growth hormone suppression caused by MSG exposure in Wistar rats?

3. HYPOTHESIS

Null hypothesis: *Dooshivishari Agada* does not significantly influence MSG-induced suppression of growth hormone in Wistar rats.

Alternative hypothesis: *Dooshivishari Agada* significantly improves serum GH levels and associated growth parameters in MSG-exposed Wistar rats.

4. OBJECTIVES

Primary objective: To evaluate the effect of *Dooshivishari Agada* on serum GH levels in MSG-exposed Wistar rats.

Secondary objectives:

- To evaluate changes in body weight and predefined growth-related parameters following MSG exposure and DVA treatment.
- To assess selected haematological parameters following treatment.
- To assess selected liver- and kidney-function parameters as safety outcomes.
- To compare outcomes in the DVA-treated group against the untreated MSG-exposed group.
- To generate preliminary evidence on the possible role of DVA in mitigating MSG-associated endocrine toxicity.

5. STUDY DESIGN

This will be a prospective, randomized, controlled experimental animal study.

Experimental unit: The individual rat will be considered the experimental unit.

Study duration: The experimental period will extend from Day 1 to Day 30, preceded by a 7-day acclimatization period.

Experimental groups

Group	Intervention	n
Group I – Control	Standard diet and water	6
Group II – MSG	MSG 0.20 g/kg/day orally for 14 days, followed by observation	6
Group III – MSG + DVA	MSG 0.20 g/kg/day orally for 14 days, followed by DVA 216 mg/200 g/day orally from Day 15–30	6

The three-group structure and doses are derived from the study synopsis.

6. EXPERIMENTAL ANIMALS

Healthy Wistar rats will be procured from an authorized laboratory-animal facility. In line with current reporting standards for animal research[12], the final manuscript will report the species and strain, sex, age at allocation, baseline body weight, source, health status, identification method, housing conditions, acclimatization period, and inclusion and exclusion criteria, rather than simply stating “Wistar rats.” The study synopsis specifies healthy Wistar rats and a 7-day acclimatization period under standard laboratory conditions.

7. INCLUSION CRITERIA

Animals will be eligible if they are healthy Wistar rats of the predefined sex and age range, fall within the predefined acceptable body-weight range, show normal activity and feeding behaviour during acclimatization, and show no clinically evident disease or injury.

8. EXCLUSION CRITERIA

Animals may be excluded before randomization if they show clinically evident illness, significant injury or abnormal behaviour, fail to acclimatize, or fall outside the predefined body-weight range. Any animal excluded after randomization, together with the reason for exclusion, will be documented and reported.

9. HOUSING AND HUSBANDRY

Animals will be maintained under standard laboratory conditions with appropriate environmental control, ventilation, and sanitation. Certified laboratory diet and drinking water will be provided according to institutional animal-house procedures, with water available ad libitum unless otherwise specified in the approved protocol. Animals will undergo a 7-day acclimatization period before treatment begins. Housing density, cage dimensions, bedding material, temperature, relative humidity, and light–dark cycle will be recorded in the final manuscript, since these factors can influence animal physiology and reproducibility.

10. RANDOMIZATION AND ALLOCATION

Following acclimatization and eligibility assessment, animals will be randomly allocated to the three experimental groups using a computer-generated randomization sequence, generated before treatment allocation and retained as part of the study records. Where feasible, animals will be identified using coded numbers rather than treatment labels.

11. BLINDING

Personnel performing biochemical assays will remain blinded to group allocation wherever practicable, and the investigator responsible for statistical analysis will preferably receive coded group identifiers until the primary analysis is complete. Any unavoidable unblinding will be documented.

12. TEST SUBSTANCES

12.1 Monosodium Glutamate

MSG will be administered at 0.20 g/kg/day by oral gavage for 14 consecutive days, as specified in the study synopsis. The final manuscript will report the manufacturer, batch number, purity/grade, and preparation details.

12.2 *Dooshivishari Agada*

DVA will be administered at 216 mg/200 g body weight/day by oral gavage from Day 15 through Day 30. DVA is a twelve-ingredient classical Ayurvedic herbo-mineral formulation, as described above, with its ingredient profile documented in the study synopsis.

For publication, the manuscript will additionally document the authenticated source of raw materials, the formulation and manufacturer, batch number, method of preparation, quality-control and standardization data, storage conditions, vehicle, timing of preparation relative to administration, dose calculation, and administration volume, since a reproducible pharmacological effect cannot be claimed without adequately documenting the identity and quality of the formulation.

13. EXPERIMENTAL PROCEDURE

Phase I – Acclimatization

Animals will undergo 7 days of acclimatization before randomization and treatment.

Phase II – MSG Exposure

Animals allocated to Groups II and III will receive MSG at 0.20 g/kg/day by oral gavage for 14 days. Group I will receive the corresponding control treatment according to the approved protocol.

Phase III – DVA Intervention

From Day 15 to Day 30, Group III will receive DVA at 216 mg/200 g body weight/day by oral gavage. Group II will remain untreated following MSG exposure and will be observed until the end of the experimental period, while Group I will continue to receive standard care. Body weight will be measured at predefined intervals, and administered doses adjusted according to current body weight where specified in the approved protocol.

14. OUTCOME MEASURES

14.1 Primary Outcome

The primary outcome is serum growth hormone concentration. The primary comparison will be the difference in GH between the MSG-exposed group and the MSG + DVA group. Because GH secretion is pulsatile, a single serum measurement may not fully capture its secretory dynamics; the timing of blood collection will therefore be standardized across groups and reported precisely.

14.2 Secondary Outcomes

Growth-related parameters: body weight, percentage change in body weight, growth trajectory, and other predefined growth measurements included in the approved protocol.

Haematological parameters: haemoglobin, total leukocyte count, and other CBC parameters specified in the laboratory protocol.

Liver-function parameters (to be prespecified): ALT, AST, ALP, total bilirubin, and albumin/total protein where applicable.

Renal-function parameters (to be prespecified): serum creatinine and blood urea nitrogen/urea.

These outcomes reflect the CBC, LFT, RFT, serum GH, and weight-related measures specified in the study synopsis.

15. BLOOD COLLECTION

The study synopsis proposes collecting approximately 2 mL of blood on Days 1, 15, and 31 under light anaesthesia. This blood-volume schedule will be reviewed and approved by the IAEC and the animal-care veterinarian, taking into account animal body weight, the effects of repeated sampling, and the chosen sampling site. The final manuscript will specify the sampling site, anaesthetic agent and dose, duration of anaesthesia, sample-processing procedure, anticoagulant used for CBC, serum separation method, storage temperature, interval between collection and analysis, and the assay kits, instruments, manufacturers, and catalogue details used.

16. LABORATORY ANALYSIS

Serum GH will be measured using a validated, commercially available assay suitable for rat samples. The manuscript will report the assay manufacturer, assay principle, analytical range, intra- and inter-assay coefficients of variation, sample dilution where applicable, and the number of technical replicates. CBC and liver- and renal-function parameters will be analyzed using validated laboratory methods.

17. HUMANE ENDPOINTS AND ANIMAL WELFARE

Animals will be monitored regularly for abnormal posture, reduced locomotion, abnormal feeding or drinking, marked weight loss, respiratory difficulty, persistent pain or distress, self-injury, severe behavioural abnormality, or any other predefined sign of suffering. Humane endpoints will be established

before the experiment begins. Any animal reaching a predefined humane endpoint will receive appropriate veterinary intervention and, where required, humane euthanasia according to the IAEC-approved procedure, consistent with established guidance on harm–benefit assessment in animal research[11].

18. SAMPLE SIZE

The study will include 18 rats, six per group, as specified in the synopsis. For publication, a formal sample-size justification will be provided based on the primary outcome (serum GH), the expected between-group difference, the expected standard deviation, the significance level, statistical power, and anticipated attrition. If a formal power calculation was not performed prospectively, this will be stated transparently rather than presenting an unsupported retrospective calculation. Because six animals per group is a relatively small sample, the study will be presented as preliminary/experimental unless a power calculation demonstrates adequate power for the primary endpoint.

19. STATISTICAL ANALYSIS

Data will first be assessed for completeness, outliers, and distributional assumptions. Continuous variables will be presented as mean ± standard deviation or median with interquartile range, as appropriate. For comparisons across the three independent groups, one-way ANOVA followed by an appropriate post-hoc multiple-comparison test will be used where the assumptions of normality and homogeneity of variance are satisfied; otherwise, a non-parametric alternative such as the Kruskal–Wallis test with suitable post-hoc comparisons will be applied. Repeated body-weight measurements will be analyzed using a repeated-measures approach or mixed-effects model rather than multiple independent tests at each time point. Statistical significance will be set at $p < 0.05$, and effect estimates with 95% confidence intervals will be reported wherever possible[12]. The statistical analysis plan will be finalized before unblinding.

20. ETHICAL CONSIDERATIONS

The experiment will not begin until approval is obtained from the competent Institutional Animal Ethics Committee, as required by the study synopsis. The study will follow applicable institutional and national regulations governing laboratory-animal experimentation, incorporating the principles of the 3Rs — Replacement, Reduction, and Refinement. The final publication will include the name of the approving ethics committee, the approval/reference number, the date of approval, the applicable regulatory framework, and the humane-endpoint and euthanasia procedures.

21. DATA MANAGEMENT AND QUALITY ASSURANCE

All animals will be assigned unique identification numbers. A predefined study record will document baseline characteristics, randomization, daily treatment, body weight, clinical observations, adverse events, sample collection, laboratory results, exclusions, humane endpoints, and euthanasia. Original laboratory data will be retained for verification, and any protocol deviation will be documented and reported.

22. EXPECTED OUTCOMES

This study is designed to determine whether DVA improves serum GH in MSG-exposed Wistar rats. It is hypothesized that the MSG group will show lower GH and altered growth-related parameters compared with controls, while DVA treatment may attenuate these changes. Effects on CBC, liver, and renal parameters will also be evaluated as safety outcomes. These findings may provide preliminary evidence for further investigation of DVA in experimental endocrine toxicology, consistent with the synopsis's proposal that

DVA may restore MSG-suppressed GH levels while supporting liver, kidney, and haemoglobin-related parameters.

23. STRENGTHS OF THE PROPOSED STUDY

This protocol addresses a clearly identified research gap using a randomized experimental design that includes an untreated MSG-exposed comparison group. It evaluates both efficacy and safety outcomes, applying modern experimental endpoints to a classical Ayurvedic formulation. In doing so, it offers an opportunity to examine the relationship between the Ayurvedic concept of *Dooshivisha* and experimentally induced toxicological effects. The completed study can be reported using current reporting principles for animal research, which set out the Essential 10 as the minimum reporting requirements for animal research manuscripts[12].

24. PROPOSED STUDY FLOW

Animal procurement → 7-day acclimatization → eligibility assessment → randomization → baseline assessment

- Group I: standard care
- Group II: MSG 0.20 g/kg/day × 14 days → observation
- Group III: MSG 0.20 g/kg/day × 14 days → DVA 216 mg/200 g/day × 16 days

↓ Body-weight and clinical monitoring → blood sampling → GH/CBC/LFT/RFT analysis → statistical analysis → interpretation

25. DISSEMINATION PLAN

The completed study will be reported according to current animal-research reporting recommendations. The final manuscript will clearly distinguish prespecified outcomes from exploratory analyses and will report all animals initially allocated, exclusions, protocol deviations, and adverse events, in keeping with the emphasis on transparent reporting that allows readers and reviewers to assess reliability and reproducibility[12].

26. REFERENCES

1. Terry LC, Epelbaum J, Martin JB. Monosodium glutamate: acute and chronic effects on rhythmic growth hormone and prolactin secretion, and somatostatin in the undisturbed male rat. Brain Res. 1981;217(1):129–142.

2. Maiter D, Underwood LE, Martin JB, Koenig JI. Neonatal treatment with monosodium glutamate: effects of prolonged growth hormone-releasing hormone deficiency on pulsatile GH secretion and growth in female rats. Endocrinology. 1991;128(2):1100–1106.

3. Kovacs M, Kineman RD, Schally AV, Flerko B, Frohman LA. Increase in mRNA concentrations of pituitary receptors for growth hormone-releasing hormone and growth hormone secretagogues after neonatal monosodium glutamate treatment. J Neuroendocrinol. 2000;12(4):335–341.

4. Blake CA, Norman RL, Sawyer HR. Effects of neonatal administration of monosodium glutamate on somatotrophs and growth hormone secretion in prepubertal male and female rats. Endocrinology. 1984;115(3):996–1003.

5. Paradkar HSS. Ashtanga Hridaya of Acharya Vagbhata, Uttara Tantra. Varanasi: Chaukhamba Surabharati Prakashan; 2010.

6. Savalagimath JR, Rani J. Effect of Dooshivishari Agada over MSG-induced reproductive toxicity with special reference to ovary and follicle count. AYU. 2017;38(1–2):14–20.

7. Chalakh S, Urkude M, Wanjari A. Physicochemical and phytochemicals investigation of Dooshivishari Agada by HPLC. Int J Ayurvedic Med. 2023;13(4):245–252.

8. Patil SF, Shahapurkar VV, Khanal P. Pre-clinical appraisal of Dooshivishari Agada for antimicrobial, antifungal, and antioxidant activity: in vitro trial. J Ayurveda Integr Med. 2022;13(3):107–112.

9. Kunte AM, Navre KR. Ashtanga Hridaya with Sarvanga Sundara Commentary of Arunadatta and Ayurveda Rasayana Commentary of Hemadri. Varanasi: Chaukhamba Publications; 1994.

10. Acharya YT, editor. Sushruta Samhita with Nibandha Sangraha Commentary. Varanasi: Chaukhamba Orientalia; 2002.

11. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. Laboratory Animals. 2018;52(2):135–141.

12. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol. 2020;18:e3000410.