Pharmaceutico-Analytical Standardization and In Vivo Evaluation of Acute Toxicity, Genotoxicity, Anti-Genotoxic Effect and Spermatogenic Action of Musalyadi Churna

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ABSTRACT

Introduction: Infertility is a major reproductive health problem today in which Male infertility may be contributory to 30 to 40 % of infertile couples. *Musalyadi Churna* is most commonly utilized classical Ayurvedic formulation in the management of impotency and infertility which are a major increasingly worldwide concern. It is a combination of herbal drugs like *Musali* (*Chloro-phytum borivilianum*), *Vidarikanda* (*Pueraria tuberosa* DC), *Guduchi* (*Tinospora cordifolia* (Willd Miers ex Hook. f. and Thoms.), *Kapikacchu* (*Mucunna prurita* Hook.), *Gokshura* (*Tribulus Terrestris* Linn.), *Shalmali* (*Bambax malabaricum* DC) and *Amalaki* (*Phyllanthus Emblica* Linn.).

Methods: *Musalyadi Churna* will be prepared as per the reference of classical text *Sharangdhar Samhita* for pharmaceutical study and then its analytical study will be done. In experimental studies To assess the toxicity of *Musalyadi Churna*, its acute oral toxicity on Wistar/SD rats will be performed using OECD 423 guidelines; Genotoxicity and Antigenotoxicity will be performed using Chromosomal aberration assay on 36 Swiss albino mice and Spermatogenesis study will be conducted on 60 Wistar/SD male rats as per OECD guidelines and scientific research articles.

Expected Results: Observations of pharmaceutical and experimental studies will be noted and presented in the form of tables, chart, photographs etc.

Conclusion: As the ingredients of *Musalyadi Churna* are easily available and the method of preparation does not require much time and manpower, therefore if this formulation showed significant efficacy then this will open a new gateway for the management of infertility.

Key Words: *Musalyadi Churna*, Genotoxicity, Spermatogenesis, Infertility

INTRODUCTION

Ayurveda is a life science which given hope to the world for the management of impotency and infertility related diseases. Modern lifestyle has a critical role in developing a wide range of diseases among which diseases related to the reproductive system are one of the major concern. Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples.^{1,2} There are numerous formulations in Ayurveda which are mentioned to possess significant *Vajikarana* properties which are related to both aphrodisiac as well as sper-

matogenic action. Many formulations which are available in the market and frequently utilized in treating impotency and infertility are either from the classical text of Ayurveda or derived from *Vajikarana* drugs mentioned in Ayurveda. *Musalyadi Churna* is one of such formulation which is mostly used by Ayurvedic physicians.³ It is a combination of *Musali*, *Vidarikanda*, *Guduchi* Satva, *Kapikacchu*, *Gokshur* *Shalmali* and *Amalaki*. However, there is a lack of scientific evidence related to Acute Toxicity, Genotoxicity, Anti-Genotoxic effect and Spermatogenic action of *Musalyadi Churna*. Moreover,

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the efficacy level of *Musalyadi Churna* is not known which is needed to understand its valuability in terms of cost-effectiveness and superiority/inferiority in comparison to standard drug. Thus present work will be able to fulfill these gaps of scientific evidence and if significant positive results obtained in this work then it will be a valuable contribution to the management of impotency and infertility.

MATERIAL AND METHODS

Study design –This is the pharmaceutical, analytical and experimental study. The study will be conducted at M.G.A.C.H.R.C., Salod (H), Central Animal House, DMIMS (DU), Wardha and other Lab of National Repute and as recognized or recommended by DMIMS (DU). Ethical approval has been got from Institutional Ethics committee (IEC) and the Institutional animal ethics committee (IAEC).

Present work will be conducted under the following headings;

a) Pharmaceutical study: This study is related to drug preparation in which three different batches of *Musalyadi Churna* will be prepared to establish pharmaceutical standardization. The pharmaceutical study will be done in the following steps (figure 1);

- I) Procurement of Raw materials: All required raw materials will be procured from the field and authentic reliable source.
- II) Authentication of Raw materials: Raw drugs will be verified and authenticated by Department of Dravya Guna of MGAC and RC. Raw drug will be standardized as per A.P.I.

III) Extraction of Guduchi Satva:⁴

गुडूची खण्डशः कृत्वा कुट्टयतिवा सुमरुदयेत्।
वस्त्रेण वधितं तोयं सत्रावयेत्तच्छनैः शनैः।
शुद्धशङ्खनभिं चूर्णमेतैः सम्मिश्रयेद्भषिक्।
- *Yogratnakar Rajyakshma Chikitsa*

The procedure of Extraction of Guduchi Satva:

Figure 1: Flow diagram of the unit procedure of Extraction of

Guduchi Satva.

IV) The classical method of Preparation of Musalyadi Churna (Table 1):³

मुसलीकन्दचूर्णं तु गुडूचीसत्वसंयुतम्।
वानरीगोकषुराभ्यां च शाल्मलीशरकरामलैः ॥
आलोड्य घृतदुग्धाभ्यां भक्षयेत्कामवृद्धये ॥

Sharandhar Samhita Madhyam khanda 6/160

Table 1: Ingredients and quantity of Musalyadi Churna:

Sr. No.	Ingredients	Part Used	Quantity
1	Musali	Rhizome	1 Part
2	Vidarikanda	Rhizome	1 Part
3	Guduchi Satva	Stem bark extract	1 Part
4	Kapikacchu	Seed	1 Part
5	Gokshur	Fruit, Root	1 Part
6	Shalmali (Semal Musali)	Root	1 Part
7	Amala	Fruit	1 Part
8	Khanda Sharkara	-	1 Part

The procedure of preparation of Musalyadi churna (figure 2):

Figure 2: Flow diagram of the unit procedure of preparation of Musalyadi churna:

b) ANALYTICAL STUDY:

For analytical study description (organoleptic parameters) and physicochemical parameters like Acid insoluble ash, Total ash, Water-soluble extractive, pH, HPTLC, Loss on drying at 105°C, GCP, Particle size, Microbial contamination, Pesticide residue will be done.

c) EXPERIMENTAL STUDY:

The experimental study is divided into three parts i.e Acute oral Toxicity, Chromosomal Aberration Assay and Spermatogenesis study of *Musalyadi churna*. These study will be conducted as per OECD guidelines and Scientific research articles.

The animal will be acclimatized for 14 days prior beginning of the study. They will be housed at a relative humidity ($55 \pm 5\%$), 12 h/12 h light-dark cycle, ambient temperature ($22 \pm 1^\circ\text{C}$). Animals will be fed with Amrit brand of food and distilled water ad libitum.

Study design and Experimental procedures:

1] Acute oral Toxicity:

Three female rats will be used for acute oral toxicity study according to OECD (Organization for Economic Coopera-

tion Development) guidelines 423. The study will involve approximately 12 animals. They will be dosed at 2000 mg /Kg body weight. Rats will be administered the test material suspended in suitable vehicle i.e Cows Milk and Cows butter. The animals will be deprived of feed 4 hours before dosing and 2 hours after dosing. Water will be allowed ad libitum. All the animals will be observed for 14 days after dosing. During the first 30 minutes after dosing, animals will be observed individually at least once and periodically during the first 24 hours and once daily thereafter, for a total of 14 days. Then signs of ill health, behavioural changes etc will be recorded for individual animals (table 2).

The body weights will be recorded on test day 1 (pre-administration fasting weight) and days 7 and 14 post-treatment or at death and then after necropsy pathological study will be done.⁵

2] Genotoxicity and Anti-Genotoxicity study in Mice

Table 2: Grouping for Genotoxicity and Anti-Genotoxicity study

The mice will be divided into the following groups:

Groups	Drug	No. of animals
Group I (Normal Control)	-	6
Group II (Vehicle control)	<i>Godugdha, Goghrita</i>	6
Group III (Positive control)	N-Nitroso-N-methylurea (NMU)/ Cyclophosphamide(CYP) Single-dose administration and cell harvesting after 24 hrs.	6
Group IV (Test group)	<i>Musalyadi Churna</i>	6
Group V(Pre-treatment)	Mice will be given first <i>Musalyadi Churna</i> and then NMU/CYP.	6
Group V(Post-treatment)	Mice will be given first NMU/CYP and then <i>Musalyadi Churna</i>	6

For this study, chromosomal aberration assay will be followed. Mice will be dosed with respective treatments and then the animals will be sacrificed by cervical dislocations and bone marrow cells will be harvested. Then Colchicine will be administered intraperitoneally and slides will be prepared. The harvested cells will be incubated at 37°C and then centrifuged at 1000 rpm. A total of 100 well-spread metaphase plates will be scored for chromosomal aberrations at a magnification of 1000 X (100 X 10) for each group. Then different types of chromosomal aberration will be scored. Then statistical significance will be determined.^{6,7}

3] Spermatogenesis Study of *Musalyadi churna* in Male Rats

Table 3: Grouping for Spermatogenesis Study of *Musalyadi churna* in Male Rats

The rats will be divided as follows:

Groups	Drug	Dose	No.	Duration	Route
Group I (Normal Control)	-	-	6 Rats	70 days	-
Group II (Disease control)	Nicotine	0.6 mg/kg	6 Rats	70 days	Oral
Group III (Vehicle Control)	Goghrita, Godugdha	10 ml/Kg b.wt	6 Rats	70 days	Oral

Group IV (Stand- ard drug)	Nicotine+ Clomifen citrate	0.6 mg/ kg b.wt	6 Rats	70 days	Oral
Group V (Test dose I)	Nicotine + <i>Musalyadi</i> <i>Churna</i>	0.6mg/ kg+ (6 gm - human dose)	6 Rats	70 days	Oral
Group VI (Test dose-II)	Nicotine + <i>Musalyadi</i> <i>Churna</i>	0.6mg/ kg+ (9 gm - human dose)	6 Rats	70 days	Oral
Group VII (Test dose-III)	Nicotine + <i>Musalyadi</i> <i>Churna</i>	0.6mg/ kg+ (12 gm - human dose)	6 Rats	70 days	Oral
Group VIII (Only Test dose)	<i>Musalyadi</i> <i>Churna</i>	(6 gm - human dose)	6 Rats	70 days	Oral
Group IX (Only Test dose)	<i>Musalyadi</i> <i>Churna</i>	(9 gm - human dose)	6 Rats	70 days	Oral
Group X (Only Test dose)	<i>Musalyadi</i> <i>Churna</i>	(12 gm - human dose)	6 Rats	70 days	Oral

Rats will be fed with rat feed and water ad libitum. Nicotine 99% will be dissolved in physiological saline and will be given orally by gastric tube for 70 days. The same volume of saline will receive by control Rats (Table 3).

On the 71st day, animals will be allowed to fast for overnight and will be subjected to euthanasia. Tissues will be collected in ice-cold containers for various biochemical estimations like Nicotine, Serum Cholesterol, ALP Tissue, Tissue Acid Phosphatase, ALP Serum, Total Protein and also epididymal Sperm Count and Sperm Morphology also seen.^{8,9}

Animals required

- Species/Common name: Wistar/ SD Rats and Swiss Albino mice
- Age / weight / size: 180-200gms for rats and 20-25 gms for mice
- Gender: Both
- Sample Size: Number to be used 12 rats For Acute Toxicity study approx. 12 female rats will be used. For Spermatogenesis study 60 male rats and for Genotoxicity study 36 mice of both sexes will be used.
- Number of days each animal will be housed: Approx 3 months

- Proposed source of animals: Central Animal House, DMIMS (DU), Wardha
- The rationale for animal usage: Animal usage is necessary for this study to determine the safety, spermatogenic potential and antigenotoxic effect of *Musalyadi churna*.
- Estimated number of animals essential because minimum of 6 animals per group are required for statistical significance.

Dose: The dose for the experimental is calculated as per clinical human dose of *Musalyadi churna* (12000 mg per day) to an animal dose based conversion factor of FDA guidelines. Dose route is oral for all the studies.

Formula= Human dose/60 x body surface area ratio convertibility factor.

DATA ANALYSIS

Unpaired t-test and One way ANOVA will be applied to get the results of the study.

EXPECTED OBSERVATION

Observations will be noted and presented in the form of tables, chart, photographs etc. *Musalyadi churna* is a combination of herbal ingredients hence it is predicted that this formulation will found non-toxic in Acute Oral toxicity test. *Musalyadi churna* will have significant spermatogenic action as well as it possesses genoprotective efficacy and it does not have any genotoxicity.

DISCUSSION

Plants derived medicines have played a major role in health care of ancient and modern cultures.¹⁰ However according to modern science, each drug needs to be scientific, pre-clinically and clinically evaluated for the global acceptance.¹¹ In the medical field, the analytical studies of Ayurvedic formulations help to form the base for standardization.¹² Standardization of Ayurvedic medicines on pharmaceutical and analytical level is the most important aspect before beginning research on pre-clinical and clinical ground. *Musalyadi churna* is a herbal formulation primarily indicated for diseases of the male reproductive system. It will be prepared as per ingredients and proportion mentioned in Table 1 and as per reference of Sharangdhar Samhita. Genotoxicity and Anti-genotoxicity studies will be performed as per the groups mentioned in Table 2. All drugs which have targeted action on the human reproductive system is needed to be tested for their genotoxicity profile as even a small change in the genome may result in significant abnormality in offspring. The anti-genotoxic study provides information on the efficacy of

drugs against preventing the hazardous effect of chemicals on chromosomes. Spermatogenesis study will be performed as per the grouping mentioned in Table 3. Spermatogenesis studies are the key to know whether a drug stimulate sperm production, sperm motility and content of seminal fluid which are considerable factors of fertilization mechanism.

Animal studies give us the guidelines and help us to solve the queries on the extent of the use of medicine in regards to its safety margins. The individual drugs of this formulation have proven their beneficial effect in impotency and infertility.¹³⁻²⁵ Studies related to oligospermia was reported by Varma et al.²⁶ Other toxicity related studies were reported.^{27,28} However there is no established scientific evidence related to the range of combined effect of these ingredients in the form of *Musalyadi Churna*. Moreover, this formulation has target action on the reproductive system and thus its genotoxic profile need to be established and this impediment will be solved by present work.

Strength and limitations

To the best of our knowledge, there is no work done until today on this topic. This study will provide spermatogenesis effect of Musalyadi Churna on Rodents with genotoxicity profile. This study is limited to animal experiment which has few constraints while applying obtained results in human being. This represents that animal experiments have their importance but clinical studies are needed for finalization of the efficacy of any drug.

CONCLUSION

Available formulations on infertility are too much costly and their ADR, as well as lack of genoprotective evidence, is a matter of concern. The present study is an attempt to discover a new cost-effective spermatogenic formulation with establishing its evidence related to genoprotective action with spermatogenesis profile. As the ingredients of *Musalyadi churna* are easily available and the method of preparation does not require much time and manpower, therefore if this formulation showed significant efficacy then this will open a new gateway for the management of infertility.

Availability of data and materials – Nil

Consent for publication – Not applicable

Competing interests - Authors don't have any competing interest.

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