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Research Article

PHARMACOGNOSTIC STANDARDIZATION AND EXPERIMENTAL EVALUATION OF THE FERTILITY-SUPPORTIVE ACTIVITY OF (*MUSTA*) *CYPERUS ROTUNDUS LINN*. IN A STRESS-INDUCED REPRODUCTIVE DYSFUNCTION MODEL

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ABSTRACT

Materials and Methods

The study was carried out in two major components: pharmacognostic and experimental evaluation. Fresh rhizomes of *Cyperus rotundus* were collected, dried, powdered, and subjected to organoleptic, microscopic, physicochemical, fluorescence, phytochemical, HPTLC, and HPLC analyses. The experimental study was conducted in pre-treatment and post-treatment phases using a stress-induced reproductive dysfunction model. Animals were allocated to control, negative control, standard, *Musta churna* high-dose, *Musta churna* low-dose, *Musta kashaya* high-dose, and *Musta kashaya* low-dose groups. The duration of the diestrus phase, uterine weight, number of pups, and ovarian histopathological changes were assessed. Data were expressed as mean ± SEM and analyzed using appropriate statistical tests, including one-way ANOVA and post-hoc analysis.

Results

In the pre-treatment phase, *Musta churna* high dose showed the shortest diestrus duration among the trial groups (8.17 ± 0.75 days) and the highest mean number of pups (10.00 ± 1.00). In the post-treatment phase, *Musta churna* high dose showed improvement in diestrus duration (10.83 ± 0.75 days) and the highest number of pups (11.00 ± 1.00). *Musta churna* high dose and *Musta kashaya* high dose showed the highest uterine weight in the post-treatment phase (442.67 ± 6.43 mg). The overall intergroup differences for the major parameters were statistically significant ($P < 0.001$).

INTRODUCTION

Infertility is an important reproductive health concern affecting individuals and couples worldwide. The woman in whom there is hindrance of any kind to the normal process of conception is *Vandhya*[1]. Normal reproductive function depends on the coordinated functioning of the hypothalamic-pituitary-ovarian axis, ovaries, uterus, and other physiological systems.

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Various factors may interfere with reproductive function and contribute to disturbances in cyclicity, ovulation, implantation, and fertility.[7] Stress is considered one of the factors capable of influencing reproductive physiology. Prolonged stress may alter normal reproductive hormonal activity and may be associated with disturbances in the reproductive cycle. Therefore, experimental models involving stress-induced reproductive dysfunction may be useful for evaluating substances with potential reproductive protective or fertility-supportive effects.

Many drugs have been documented in Ayurvedic classics which are safe and effective in yonidosha. Musta is one such known drug which is mentioned in Sushruta Samhita[2] and Asthanga Hridaya[3] as

Yonidoshahara. In Bhaishajyaratnavali, *Musta* is one of the ingredients in a *Yoga* by the name *Kumarakalpa druma* which is used as *Vandhyatvahara* [4]. *Musta* is main ingredient in the *Mustadiyapana basti* which is used as *Virya vardhaka* [5]. Ayurveda describes several concepts and therapeutic approaches related to conception and maintenance of pregnancy. Musta (*Cyperus rotundus* Linn.), belonging to the family Cyperaceae, is an important medicinal plant described in Ayurvedic literature. It is traditionally attributed with *Tikta, Kashaya* and *Katu rasa, Sheeta virya*, and *Ruksha guna. Musta* is also described as possessing *Yonidoshahara karma* and is included in certain traditional formulations used in reproductive disorders.[8]

The present study was undertaken to scientifically evaluate *Musta* through pharmacognostic and phytochemical investigations and to assess its experimental effects in a stress-induced reproductive dysfunction model. Both Musta churna and *Musta kashaya* were evaluated at two dose levels during pre-treatment and post-treatment phases. In case of Pcos one of the factor, is increased androgen level and previous work has proved that *Musta* acts as antiandrogen [10]

Aim and Objectives

Aim

To evaluate the *Garbhasthapana Karma* and fertility-supportive potential of *Musta* (*Cyperus rotundus* Linn.) through pharmacognostic, phytochemical, and experimental evaluation [9].

Objectives

1. To authenticate and evaluate the rhizome of *Cyperus rotundus* through pharmacognostic studies.
2. To determine the physicochemical characteristics of Musta powder.
3. To conduct preliminary phytochemical screening of aqueous and alcoholic extracts.
4. To evaluate the chromatographic profile using HPTLC and HPLC with rutin as the reference standard.
5. To assess the effect of *Musta churna* and *Musta kashaya* on stress-induced changes in reproductive parameters.
6. To evaluate the duration of the diestrus phase, uterine weight, number of pups, and ovarian histopathological changes.
7. To compare the effects of pre-treatment and post-treatment administration.

Materials and Methods

Collection and Processing of the Drug

Fresh rhizomes of Musta were collected for the study. The wet weight of the collected rhizomes was 2 kg. After drying, the weight was reduced to 1.2 kg. The dried drug was processed into powder for pharmacognostic and experimental evaluation.

Parameter	Observation
Fresh rhizome collected	2 kg
Dried rhizome obtained	1.2 kg

Organoleptic Evaluation

The macroscopic and organoleptic characteristics of *Musta* rhizome were evaluated based on external appearance, shape, colour, taste, odour, and fracture.

Table 2. Organoleptic characteristics of Musta

Parameter	Observation
External surface	Rough with striations
Shape	Ovoid
Colour	Dark brown externally and creamish internally
Taste	Bitter
Odour	Pleasant
Fracture	Short

The powdered drug was smooth in texture, brownish in colour, characteristic in odour, and Kashaya in taste.

Preparation of Extracts

Aqueous and alcoholic extracts were prepared from 200 g of coarse powder of *Cyperus rotundus*.

Table 3: Characteristics of aqueous and alcoholic extracts

Parameter	Aqueous extract	Alcoholic extract
Coarse powder taken	200 g	200 g
Extract obtained	4.0 g	7.0 g
Colour	Light brown	Dark brown
Consistency	Powder	Powder
Odour	Characteristic	Characteristic
Taste	Slightly bitter	Bitter

Powder Microscopy

Powder microscopy of *Cyperus rotundus* rhizome was performed to identify characteristic diagnostic features.

The powder showed:

- Abundant simple and compound starch grains
- Tannin-containing structures
- Pitted xylem vessels
- Oval oil globules
- Parenchymatous cells
- Groups of fibres

These microscopic characteristics supported the identification of the crude drug.

Fluorescence Analysis

The crude drug powder was treated with different chemical reagents and observed under daylight, UV 254 nm, and UV 366 nm.

Table 4: Fluorescence characteristics of *C. rotundus* powder

Treatment	Daylight	UV 254 nm	UV 366 nm
Powder as such	Brown	Dark green	Black
Powder + ethanol	Light brown	Brown	Black
Powder + NaOH	Brownish black	Greenish brown	Black
Powder + dilute HCl	Brownish yellow	Dark yellowish	Yellowish black

Physicochemical Evaluation

The powdered rhizome was evaluated for selected physicochemical parameters.

Table 5: Physicochemical characteristics of *Cyperus rotundus*

Parameter	Observed value
Foreign matter	0.81%
Loss on drying	12.65%
Total ash	3.72%
Acid-insoluble ash	2.50%
Water-soluble extractive	4%
Alcohol-soluble extractive	1%
Ph	5.38
Volatile oil	0.4%

Preliminary Phytochemical Screening

Aqueous and alcoholic extracts were subjected to preliminary qualitative phytochemical screening.

The analysis demonstrated the presence of:

- Alkaloids
- Flavonoids
- Saponins
- Glycosides
- Tannins and phenolic compounds
- Carbohydrates
- Resin
- Gums

Proteins were demonstrated in the aqueous extract, whereas steroids were absent in both extracts.

Inorganic Constituents

Qualitative analysis demonstrated the presence of iron, sodium, calcium, magnesium, potassium,

chloride, sulphate, and phosphate. Carbonate and nitrate were not detected.

3.9 HPTLC Analysis[6].

HPTLC analysis was carried out using rutin as the reference standard.

The standard rutin demonstrated a maximum Rf value of 0.20. The aqueous extract showed a major peak with an Rf value of 0.14, whereas the alcoholic extract showed a peak at an Rf value of 0.17.

Table 6: HPTLC summary

Parameter	Aqueous extract	Alcoholic extract
Number of peaks	10	6
Maximum Rf value	0.14	0.17
Area reported	349.2	621.7

The chromatographic profiles indicated the presence of multiple constituents in both extracts.

HPLC Analysis

HPLC analysis was performed using rutin as the reference standard.

Table 7: HPLC profile

Sample	Retention time	Area
Standard rutin	11.238 min	2,995,053
Aqueous extract	11.144 min	10,854
Alcoholic extract	11.215 min	21,603

The retention times of the aqueous and alcoholic extracts were close to that of the reference standard rutin.

Experimental Study

The experimental study was carried out in two phases:

1. **Stage I – Pre-treatment phase**
2. **Stage II – Post-treatment phase**

The animals were divided into the following groups:

- Control
- Negative control
- Standard
- *Musta churna* high dose
- *Musta churna* low dose
- *Musta kashaya* high dose
- *Musta kashaya* low dose

The major outcome parameters were:

1. Duration of diestrus phase.
2. Uterine weight.
3. Number of pups.
4. Ovarian histopathological changes.

Results

Effect on Diestrus Phase

Pre-treatment phase

The control group showed a mean diestrus duration of 13.00 ± 0.89 days. The negative control group showed 7.67 ± 0.52 days. Among the treatment groups, *Musta churna* high dose demonstrated the shortest diestrus duration.

Table 8: Diestrus phase in the pre-treatment groups

Group	Diestrus duration (days), Mean ± SEM
Control	13.00 ± 0.89
Negative control	7.67 ± 0.52
Standard	9.83 ± 0.75
Musta churna high dose	8.17 ± 0.75
Musta churna low dose	10.50 ± 0.55
Musta kashaya high dose	10.67 ± 0.82
Musta kashaya low dose	11.17 ± 0.98
Overall P value	<0.001

Musta churna high dose demonstrated the lowest diestrus duration among the trial groups, suggesting a comparatively better effect on normalization of estrous cyclicity.

Uterine Weight in the Pre-treatment Phase

Table 9: Uterine weight in pre-treatment groups

Group	Uterine weight (mg), Mean ± SEM
Control	366.67 ± 11.55
Negative control	400.00 ± 5.00
Standard	432.67 ± 6.43
Musta churna high dose	390.67 ± 10.07
Musta churna low dose	370.00 ± 10.00
Musta kashaya high dose	426.00 ± 5.29
Musta kashaya low dose	382.67 ± 6.43
Overall P value	<0.001

Among the trial groups, *Musta kashaya* high dose showed the highest uterine weight.

Number of Pups in the Pre-treatment Phase

Table 10: Number of pups in pre-treatment groups

Group	Number of pups, Mean ± SEM
Control	5.00 ± 2.00
Negative control	4.67 ± 1.53
Standard	6.67 ± 0.58
Musta churna high dose	10.00 ± 1.00
Musta churna low dose	8.67 ± 1.15
Musta kashaya high dose	5.00 ± 1.00
Musta kashaya low dose	4.33 ± 1.15
Overall P value	<0.001

The highest mean number of pups was observed in the *Musta churna* high-dose group.

Post-treatment Results

Diestrus Phase

Table 11: Diestrus phase in post-treatment groups

Group	Diestrus duration (days), Mean ± SEM
Control	18.83 ± 1.33
Negative control	7.67 ± 0.52
Standard	14.67 ± 0.82
Musta churna high dose	10.83 ± 0.75
Musta churna low dose	12.67 ± 1.37
Musta kashaya high dose	14.00 ± 1.10
Musta kashaya low dose	15.50 ± 0.55
Overall P value	<0.001

The control group showed the greatest prolongation of the diestrus phase. *Musta churna* high dose demonstrated the greatest improvement among the trial groups.

Uterine Weight

Table 12: Uterine weight in post-treatment groups

Group	Uterine weight (mg), Mean ± SEM
Control	320.00 ± 10.00
Negative control	400.00 ± 5.00
Standard	432.67 ± 6.43
Musta churna high dose	442.67 ± 6.43
Musta churna low dose	398.00 ± 7.21
Musta kashaya high dose	442.67 ± 6.43
Musta kashaya low dose	397.33 ± 6.43
Overall P value	<0.001

Both *Musta churna* high dose and *Musta kashaya* high dose showed the highest mean uterine weight.

Number of Pups

Table 13: Number of pups in post-treatment groups

Group	Number of pups, Mean ± SEM
Control	4.33 ± 0.58
Negative control	5.67 ± 0.58
Standard	6.67 ± 0.58
Musta churna high dose	11.00 ± 1.00
Musta churna low dose	9.00 ± 1.00
Musta kashaya high dose	7.33 ± 1.15
Musta kashaya low dose	6.67 ± 1.15
Overall P value	<0.001

The highest number of pups was observed in the *Musta churna* high-dose group.

Comparison Between Pre-treatment and Post-treatment Phases

The post-treatment control group showed greater prolongation of the diestrus phase compared with the pre-treatment control group. *Musta churna* high dose demonstrated comparatively favourable findings in both phases.

Uterine weight increased from 390.67 ± 10.07 mg during pre-treatment to 442.67 ± 6.43 mg during post-treatment in the *Musta churna* high-dose group. A similar value was observed with *Musta kashaya* high dose during post-treatment.

The number of pups in the *Musta churna* high-dose group increased from 10.00 ± 1.00 during pre-treatment to 11.00 ± 1.00 during post-treatment.

Histopathological Findings

Histopathological examination of ovarian tissue demonstrated differences in follicular development and corpus luteum formation among the groups.

In the pre-treatment phase, the *Musta churna* high-dose group showed corpus luteum, primary follicles, and Graafian follicles containing antrum and oocyte.

In the post-treatment phase, the *Musta churna* high-dose group showed secondary follicles, primary follicles, and corpus luteum. These observations suggested comparatively favourable ovarian activity.

The *Musta kashaya*-treated groups also demonstrated various stages of follicular development, including primary follicles, secondary follicles, Graafian follicles, and corpus luteum.

Discussion

The present study was designed to evaluate the pharmacognostic characteristics and experimental fertility-supportive potential of Musta (*Cyperus rotundus* Linn.) in a stress-induced reproductive dysfunction model.

The pharmacognostic findings supported the identity of the crude drug. The characteristic macroscopic features included a rough and striated surface, ovoid shape, dark-brown external colour, creamish internal appearance, bitter taste, and pleasant odour. Powder microscopy demonstrated characteristic starch grains, oil globules, pitted xylem vessels, parenchymatous cells, fibres, and tannin-containing structures.

The physicochemical analysis provided basic quality parameters for the drug. Preliminary phytochemical screening revealed the presence of several groups of phytoconstituents, including alkaloids, flavonoids, saponins, tannins, phenolic compounds, glycosides, carbohydrates, gums, and resin.

HPTLC and HPLC analysis were performed using rutin as the reference standard. The HPLC retention times of the aqueous and alcoholic extracts were close to that of the rutin standard, suggesting chromatographic correspondence under the experimental conditions.

The experimental component of the study demonstrated that stress was associated with prolongation of the diestrus phase. This alteration was particularly evident in the control groups. Treatment with Musta produced comparatively favourable effects on several reproductive parameters.

Among the treatment groups, *Musta churna* high dose showed the best overall effect on the diestrus phase. In the pre-treatment phase, the mean diestrus duration was 8.17 ± 0.75 days, while in the post-treatment phase it was 10.83 ± 0.75 days. These values were lower than those observed in the corresponding control groups.

The effect of *Musta* on reproductive outcome was further reflected by the number of pups. *Musta churna* high dose showed the highest mean number of pups in both experimental phases, with values of 10.00 ± 1.00 and 11.00 ± 1.00 in the pre-treatment and post-treatment phases, respectively.

Musta kashaya high dose showed a notable effect on uterine weight. In the pre-treatment phase, the mean uterine weight was 426.00 ± 5.29 mg. In the post-treatment phase, the mean uterine weight increased to 442.67 ± 6.43 mg, which was equal to that observed with *Musta churna* high dose.

Histopathological examination provided supportive morphological observations. The presence of primary and secondary follicles, Graafian follicles, and corpus luteum in treated groups indicated active ovarian structures. The *Musta churna* high-dose group demonstrated comparatively favourable follicular development and corpus luteum formation.

From an Ayurvedic perspective, the observed activity of Musta may be interpreted in relation to its traditionally described *Yonidoshahara* action. *Musta* possesses *Tikta, Kashaya*, and *Katu rasa* along with *Sheeta virya* and *Ruksha guna*. These properties have traditionally been considered relevant in conditions involving *Dosha* imbalance affecting the reproductive system.

The experimental findings indicate that *Musta churna* and *Musta kashaya* may have differential effects on reproductive parameters. *Musta churna* at the higher dose showed comparatively better effects on estrous cyclicity and number of pups, whereas *Musta kashaya* at the higher dose showed a favourable effect on uterine weight.

However, the present findings are based on an experimental animal model. The exact biochemical, endocrine, antioxidant, or other mechanisms responsible for these observations were not established in the present study and require further investigation.

Conclusion

The present study provided pharmacognostic, physicochemical, phytochemical, and chromatographic characterization of *Musta* (*Cyperus rotundus* Linn.).

The pharmacognostic evaluation demonstrated characteristic macroscopic and microscopic features of the rhizome. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, phenolic compounds, carbohydrates, resin, and gums. HPTLC and HPLC analyses demonstrated chromatographic profiles with peaks corresponding to the reference standard rutin.

In the experimental study, *Musta churna* high dose demonstrated the most consistent improvement in the duration of the diestrus phase and number of pups in both pre-treatment and post-treatment phases. The highest mean number of pups was observed in the *Musta churna* high-dose group, with values of 10.00 ± 1.00 during pre-treatment and 11.00 ± 1.00 during post-treatment.

Musta kashaya high dose demonstrated a favourable effect on uterine weight. During the post-treatment phase, both *Musta churna* high dose and *Musta kashaya* high dose showed the highest mean uterine weight of 442.67 ± 6.43 mg.

Histopathological observations demonstrated follicular development and corpus luteum formation in the treated groups, with comparatively favourable findings in the *Musta churna* high-dose group.

Overall, the findings suggest that *Cyperus rotundus* may have fertility-supportive activity in the stress-induced reproductive dysfunction model used in this study. Among the evaluated interventions, Musta churna at the higher dose showed the best overall effect on estrous cyclicity and reproductive outcome, while *Musta kashaya* at the higher dose demonstrated a favourable effect on uterine weight.

Further studies using larger sample sizes, detailed hormonal investigations, mechanistic studies, toxicological evaluation, and well-designed clinical

trials are required before extrapolating these findings to human infertility management.

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