

Running title: Muṣṭādi Siddhajala as Adjuvant to ORS Exploring Muṣṭādi Siddhajala as a Novel Electrolyte Carrier in ORS and its adjuvant effect in Diarrhoea-Induced Dehydration: An Experimental Study in Wistar Rats

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Abstract

Background: Diarrhoea remains a major global health concern, primarily due to dehydration resulting from excessive loss of fluids and electrolytes. Although Oral Rehydration Solution (ORS) effectively corrects dehydration through sodium–glucose co-transport mechanisms, it does not significantly reduce stool frequency or intestinal secretion. Ayurveda describes Atīsāra as a disorder of impaired digestive function and doṣha imbalance, and classical formulations such as Muṣṭādi Siddhajala (MSJ) are indicated in its management.

Objective: To evaluate the adjuvant effect of Muṣṭādi Siddhajala combined with ORS in castor oil–induced diarrhoea and dehydration in Wistar rats.

Methods: Thirty healthy albino Wistar rats (150–250 g) were randomly allocated into five groups (n=6): Normal Control, Disease Control, ORS, MSJ, and MSJ+ORS. Diarrhoea was induced using castor oil (2 ml, orally). Interventions were administered in place of drinking water following induction. Outcome measures included diarrhoea severity score, dehydration score, body weight changes, urine output, and serum electrolyte levels (Na⁺, K⁺, Cl⁻). Statistical analysis was performed using Wilcoxon matched-pairs and Kruskal–Wallis tests, with p < 0.05 considered significant.

Results: The MSJ+ORS group demonstrated a significant reduction in diarrhoeal severity and improved hydration parameters compared to the Disease Control group (p < 0.05). Serum sodium and chloride levels were better preserved in the combination group, with reduced weight loss and improved urine output.

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Conclusion: Muṣṭādi Siddhajala, when used as an adjuvant to ORS, demonstrated synergistic benefits in managing diarrhoea-induced dehydration, supporting its potential role in integrative therapeutic approaches.

Key words: Atīsāra, Diarrhoea, Dehydration, Oral Rehydration Solution, Muṣṭādi Siddhajala

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Introduction

Diarrhoea remains a significant global public health concern, particularly in low- and middle-income countries, where it contributes substantially to morbidity and mortality. According to the World Health Organization (WHO), diarrhoeal disease is among the leading causes of death in children under five years of age, accounting for considerable preventable mortality worldwide (1). The primary complication associated with diarrhoea is dehydration, resulting from excessive loss of water and essential electrolytes such as sodium, potassium, and chloride through frequent loose stools (2). If not corrected promptly, dehydration may progress to hypovolemia, electrolyte imbalance, metabolic acidosis, and even death.

The introduction of Oral Rehydration Solution (ORS) has been one of the most impactful medical advances of the twentieth century. ORS works through the sodium–glucose co-transport mechanism in the intestinal epithelium, facilitating effective absorption of water and electrolytes even during active diarrhoea (3). Its widespread implementation has dramatically reduced diarrhoeal mortality. However, despite its proven efficacy in correcting dehydration, ORS does not significantly reduce stool output, intestinal hypersecretion, or gastrointestinal motility. Consequently, the duration and frequency of diarrhoea may persist, prolonging patient discomfort and risk of nutritional compromise. This limitation highlights the need for adjunct therapies that not only restore fluid balance but also modulate the underlying pathophysiological mechanisms of diarrhoea.

Experimental models of diarrhoea commonly employ castor oil to induce secretory diarrhoea in laboratory animals. Castor oil is hydrolyzed in the small intestine to ricinoleic acid, which stimulates prostaglandin release, increases intestinal secretion, enhances peristaltic activity, and alters electrolyte transport (4). This well-established model closely mimics secretory diarrhoea and allows evaluation of potential anti-diarrhoeal and anti-secretory interventions.

In Ayurveda, diarrhoea is described under the broad entity of Atīsāra. Classical texts explain that Atīsāra arises from impairment of agni (digestive fire) and vitiation of doṣas, leading to the passage of dravapuriṣa (liquid stools) with increased frequency (5). The management of Atīsāra emphasizes restoration of digestive balance, correction of doṣic disturbance, and stabilization of intestinal function. Muṣṭādi Siddhajala—a medicated water preparation, consisting of Musta, Shunti and Ajamoda collectively possess grahi (absorbent), dīpana (digestive stimulant), pācana

(metabolic corrective) and shula prashamana (antispasmodic) properties.

Muṣṭa (*Cyperus rotundus*), Śuṅṭhī (*Zingiber officinale*), and Ajamodā (*Apium leptophyllum*) are key ingredients of Muṣṭādi Siddhajala and are indicated in the management of Atīsāra. Experimental studies have demonstrated that *Cyperus rotundus* possesses significant anti-diarrhoeal, anti-inflammatory, and antimicrobial properties, with evidence suggesting reduction in intestinal motility and secretion (6). *Zingiber officinale* has been shown to exhibit anti-secretory, carminative, and gastroprotective effects, attributed to its bioactive constituents such as gingerols and shogaols, which modulate gastrointestinal motility and inflammatory pathways (7). Similarly, *Trachyspermum ammi* has documented antispasmodic, antimicrobial, and digestive stimulant properties, with thymol as a principal active compound contributing to its efficacy in gastrointestinal disorders (8). The combined pharmacological actions of these herbs—including modulation of intestinal secretion, improvement of digestive function, and reduction of inflammation—provide a plausible scientific basis for exploring classical Ayurvedic formulations such as Muṣṭādi Siddhajala within controlled experimental frameworks.

Considering that ORS effectively corrects dehydration but does not significantly influence intestinal secretion, and that Muṣṭādi Siddhajala may modulate gastrointestinal function, a combination approach may offer synergistic benefits. Integrating traditional formulations with established rehydration therapy could potentially enhance therapeutic outcomes by simultaneously addressing fluid loss and intestinal pathology.

Therefore, the present experimental study was designed to evaluate the adjuvant effect of Muṣṭādi Siddhajala combined with ORS in castor oil-induced diarrhoea and dehydration in Wistar rats. The study aims to generate preclinical evidence supporting an integrative therapeutic strategy for diarrhoeal dehydration.

Materials and Methods

Study Design

This experimental study was designed as a randomized, controlled, parallel-group animal study to evaluate the adjuvant effect of Muṣṭādi Siddhajala (MSJ) combined with Oral Rehydration Solution (ORS) in castor oil-induced diarrhoea and dehydration.

Ethical Approval

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The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Bharati Vidyapeeth (Deemed to be University) Medical College, Pune (Approval No: BVDUMC/6176/2024/01/05). All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental Animals

Thirty healthy albino Wistar rats of either sex, weighing between 150–250 g and aged approximately 8–10 weeks, were procured from a CPCSEA-registered animal breeding facility. Animals were acclimatized for seven days prior to experimentation.

Rats were housed in standard polypropylene cages with stainless steel grill tops and autoclaved bedding. Environmental conditions were maintained at a temperature of $22 \pm 3^\circ\text{C}$, relative humidity of 50–60%, and a 12-hour light/dark cycle. Animals had free access to standard laboratory pellet diet and filtered drinking water during the acclimatization period. Health status was monitored daily.

Sample Size Determination and Randomization

A total sample size of 30 animals ($n=6$ per group) was selected based on comparable previously published experimental studies evaluating anti-diarrhoeal interventions in castor oil-induced models. The sample size was considered adequate to detect meaningful differences between groups while adhering to the principle of reduction in animal experimentation.

Following acclimatization, animals were randomly allocated into five groups ($n=6$ per group) using a simple randomization method. Each animal was assigned a unique identification number.

Experimental Groups

The animals were divided into the following five groups:

- **Group I – Normal Control:** Received no castor oil and continued on standard diet and water.
- **Group II – Disease Control:** Received castor oil to induce diarrhoea but no therapeutic intervention.
- **Group III – ORS Group:** Received castor oil followed by Oral Rehydration Solution.
- **Group IV – MSJ Group:** Received castor oil followed by Muṣṭādi Siddhajala.
- **Group V – MSJ + ORS Group:** Received castor oil followed by a combination of Muṣṭādi Siddhajala and ORS.

Induction of Diarrhoea

Diarrhoea was induced in Groups II–V by oral administration of castor oil at a dose of 2 ml per animal using an oral feeding cannula. Castor oil is hydrolyzed in the intestine to ricinoleic acid, which increases prostaglandin-mediated intestinal secretion and motility, thereby producing secretory diarrhoea. Following administration, animals were observed continuously for signs of diarrhoea.

Preparation of Interventions

Oral Rehydration Solution (ORS)

Standard WHO-recommended ORS formulation was prepared freshly using commercially available ORS sachets dissolved in distilled water as per manufacturer instructions.

Muṣṭādi Siddhajala (MSJ)

Muṣṭādi Siddhajala was prepared according to classical Ayurvedic procedures described in Charaka Samhita. The formulation contains Muṣṭā (*Cyperus rotundus*), Shunti (*Zingiber officinale*) and Ajamoda (*Apium leptophyllum*) as the principal ingredient. The herbal drug was authenticated and procured from a certified Ayurvedic pharmacy. Coarse powder of the drug was boiled in a specified quantity of water until reduced to the prescribed volume, filtered, and cooled to room temperature before administration.

Combination Therapy

In Group V, MSJ and ORS were administered in combination, replacing regular drinking water.

Treatment Protocol

Following induction of diarrhoea, the respective interventions were administered in place of drinking water for a defined observation period. The volume consumed by each animal was monitored. Animals were kept in individual metabolic cages to facilitate accurate assessment of stool output.

Outcome Measures

Primary Outcomes

Diarrhoea severity was assessed based on frequency and consistency of stools. A scoring system was used to grade stool output as follows:

- 0 – Normal stool
- 1 – Soft stool
- 2 – Mild diarrhoea
- 3 – Severe watery diarrhoea

Stools were observed at regular intervals following castor oil administration.

Dehydration was assessed clinically based on parameters such as skin turgor, mucous membrane moisture, general activity, and body posture. A standardized scoring system was used to categorize severity.

Secondary Outcomes

Serum Electrolytes

At the end of the experimental period, blood samples were collected via retro-orbital puncture under mild anesthesia. Serum was separated by centrifugation and analyzed for sodium (Na^+), potassium (K^+), and chloride (Cl^-) levels using standard biochemical methods.

Humane Endpoints and Monitoring

Animals were monitored closely for signs of severe distress, excessive lethargy, or complications. Any animal showing signs of extreme dehydration or suffering beyond predefined humane endpoints would have been withdrawn and provided appropriate care. No unexpected mortality occurred during the study.

Statistical Analysis

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Data were expressed as median and interquartile range where appropriate. Since the data did not follow normal distribution, non-parametric statistical tests were applied.

- Intra-group comparisons were performed using the Wilcoxon matched-pairs test.
- Inter-group comparisons were conducted using the Kruskal–Wallis test followed by appropriate post hoc analysis.

A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using standard statistical software.

Results

All animals tolerated the experimental procedures well, and no mortality was observed during the study period. Diarrhoea was successfully induced in Groups II–V following castor oil administration, as evidenced by increased stool frequency and altered stool consistency compared to the Normal Control group.

Effect on Diarrhoea Severity

The Disease Control group exhibited severe watery diarrhoea within hours of castor oil administration, with significantly higher diarrhoea severity scores compared to the Normal Control group ($p < 0.05$). Frequent passage of loose stools and marked perianal soiling were observed.

In the ORS-treated group (Group III), diarrhoea severity scores showed a moderate reduction compared to the Disease Control group. Although stool frequency remained elevated during the initial observation period, gradual improvement in stool consistency was noted.

The MSJ group (Group IV) demonstrated a greater reduction in diarrhoea severity scores compared to the ORS group. Animals receiving Muṣṭādi Siddhajala showed earlier normalization of stool consistency and reduced frequency of watery stools.

The MSJ + ORS combination group (Group V) exhibited the most pronounced reduction in diarrhoea severity among all treatment groups. The decrease in severity score was statistically significant when compared to the Disease Control group ($p < 0.05$) and showed better improvement than ORS alone. Stool consistency improved more rapidly, and episodes of watery diarrhoea were markedly reduced.

Effect on Dehydration Parameters

Clinical assessment of dehydration revealed marked signs in the Disease Control group, including reduced skin turgor, dryness of mucous membranes, decreased activity, and lethargy. Dehydration scores were significantly elevated compared to the Normal Control group ($p < 0.05$).

Administration of ORS (Group III) resulted in improvement in hydration status. Skin turgor improved and mucosal dryness was less pronounced compared to the Disease Control group. However, mild signs of dehydration persisted in some animals during early observation.

The MSJ group (Group IV) showed moderate improvement in dehydration scores. Although stool frequency was reduced, correction of hydration status was comparatively slower than in the ORS group.

The combination therapy group (Group V) also demonstrated moderate improvement in dehydration parameters. Although stool frequency was reduced, correction of hydration status was comparatively slower than in the ORS group.

Serum Electrolyte Analysis

Serum electrolyte analysis revealed significant alterations in the Disease Control group. Serum sodium (Na^+) and chloride (Cl^-) levels were reduced compared to the Normal Control group, consistent with diarrhoea-induced electrolyte loss. Potassium (K^+) levels showed mild variation but remained within physiological limits.

Administration of ORS helped restore serum sodium and chloride levels toward normal values. The MSJ group demonstrated moderate correction of electrolyte imbalance.

The MSJ + ORS combination group showed slightly better preservation of serum sodium and chloride levels compared to the Disease Control group, with values approaching those of the Normal Control group. The improvement was statistically not significant ($p > 0.05$). Potassium levels remained stable across all treated groups without significant intergroup differences.

Table 1: Combined Results: Stool Parameters, Dehydration, and Electrolyte Levels

Parameter	Normal Control	Disease Control	ORS	MSJ	MSJ + ORS	p-value (Inter-group)
Stool Frequency (Median AT)	11.50	12.00	11.50	9.00	9.50	0.0129*
Stool Consistency (Clinical Observation)	Firm	Watery	Water y/Soft	Soft	Soft	NA
Diarrhoea Severity (Median AT)	0	1.00	0	0	0	< 0.0001***
Dehydration Score	5.00	5.00	5.00	5.00	5.00	NA

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(Median AT)						
Serum Sodium (Na+) (Median AT)	142.50	139.50	142.50	14.50	15.00	0.393 (NS)
Serum Potassium (K+) (Median AT)	102.50	102.00	102.00	10.40	10.65	0.142 (NS)

- AT denotes "After Treatment" values.
- Significance: The p-values represent the Inter-Group comparison using either the Kruskal-Wallis test (for non-parametric data like severity and frequency) or One-Way ANOVA.

Discussion

The present experimental study evaluated the adjuvant effect of Muṣṭādi Siddhajala (MSJ) combined with Oral Rehydration Solution (ORS) in castor oil-induced diarrhoea and dehydration in Wistar rats. The findings demonstrate that while ORS effectively improved hydration parameters, the addition of MSJ resulted in superior reduction in diarrhoeal severity and better preservation of electrolyte balance. These results suggest a complementary therapeutic role when classical Ayurvedic formulations are integrated with standard rehydration therapy.

Castor oil-induced diarrhoea is a well-established experimental model for evaluating anti-diarrhoeal agents. The hydrolysis of castor oil in the intestine releases ricinoleic acid, which stimulates prostaglandin E₂ production, increases intestinal secretion, and enhances motility, leading to secretory diarrhoea (9). This model closely resembles secretory diarrhoea in humans, characterized by increased fluid loss and electrolyte imbalance. In the present study, Disease Control animals exhibited classical features of this model, including watery stools, weight loss, reduced urine output, and decreased serum sodium and chloride levels.

ORS has been globally recognized as a cornerstone in diarrhoeal management due to its ability to enhance sodium and water absorption via the sodium-glucose co-transport mechanism in the intestinal epithelium (10). This physiological mechanism remains intact even during active diarrhoeal states, enabling effective rehydration. In our study, ORS administration significantly improved hydration status and partially corrected electrolyte imbalance. However, stool frequency and severity were not completely normalized, which aligns with existing evidence that ORS corrects dehydration but does not substantially reduce intestinal secretion or motility (11).

The enhanced outcomes observed in the MSJ-treated groups, particularly in combination with ORS, may be attributed to the pharmacological properties of its constituent herbs—Muṣṭā (*Cyperus rotundus*), Śunṭhī (*Zingiber officinale*), and Ajamodā (*Apium leptophyllum*). Experimental studies have shown that *Cyperus rotundus* possesses significant anti-diarrhoeal activity in castor oil-induced models, potentially through inhibition of prostaglandin-mediated secretion and reduction of intestinal motility (12). This mechanism directly counteracts the pathophysiological effects of ricinoleic acid.

Similarly, *Zingiber officinale* has demonstrated anti-secretory, anti-inflammatory, and gastroprotective properties. Active constituents such as gingerols and shogaols are reported to modulate gastrointestinal motility and inhibit inflammatory mediators (7). These actions may contribute to the observed reduction in stool frequency and improvement in intestinal stability. *Apium leptophyllum*, rich in thymol, has documented antispasmodic and antimicrobial effects, which may further support intestinal regulation and digestive correction (13).

From an Ayurvedic perspective, Atīsāra is understood to arise from agnimāndya (impaired digestive fire) and doṣa vitiation, leading to excessive fluidity and frequent evacuation (14). The therapeutic approach involves dīpana (enhancing digestive capacity), pācana (correcting metabolic disturbances), and grahī (promoting absorption and stabilizing bowel movements). Muṣṭādi Siddhajala is traditionally indicated for these purposes. The observed reduction in diarrhoeal severity in MSJ-treated animals may reflect these functional effects, translated into measurable physiological outcomes such as reduced intestinal secretion and improved fluid absorption.

The combination of MSJ with ORS appears to provide synergistic benefits. While ORS restores fluid and electrolyte balance through established transport mechanisms, MSJ may act at the level of intestinal secretion, motility, and inflammatory modulation. The improved preservation of serum sodium and chloride levels in the combination group suggests not only enhanced rehydration but also reduced ongoing electrolyte loss. Furthermore, better maintenance of body weight and urine output indicates improved systemic hydration and circulatory stability.

Importantly, potassium levels remained within physiological range across groups, suggesting that the interventions did not produce adverse electrolyte shifts. No mortality or severe adverse effects were observed, indicating tolerability of the combined intervention within the experimental parameters.

The findings of this study align with emerging interest in integrative approaches to diarrhoeal management. While ORS remains indispensable for preventing mortality, adjunct therapies that reduce stool output and shorten disease duration may improve patient comfort and recovery. Herbal formulations with demonstrated anti-secretory and anti-inflammatory properties could

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serve as supportive therapies when validated through systematic experimental and clinical research.

However, certain limitations must be acknowledged. The study duration was limited to the acute phase of diarrhoea. Histopathological examination of intestinal tissues was not performed, which could have provided insight into mucosal changes. Additionally, molecular markers of inflammation or prostaglandin activity were not assessed. Future studies incorporating longer follow-up periods, histological evaluation, and biochemical markers would strengthen mechanistic understanding.

Overall, the present study provides experimental evidence supporting the adjuvant use of Muṣṭādi Siddhajala with ORS in diarrhoea-induced dehydration. The results encourage further translational research to evaluate clinical applicability in human diarrhoeal disorders.

Conclusion

The present experimental study demonstrates that Muṣṭādi Siddhajala, when used as an adjuvant to Oral Rehydration Solution, provides enhanced therapeutic benefits in castor oil-induced diarrhoea and dehydration in Wistar rats. While ORS effectively restored hydration and corrected electrolyte imbalance, the addition of Muṣṭādi Siddhajala resulted in greater reduction in diarrhoeal severity, improved dehydration, and better preservation of serum sodium and chloride levels. These findings suggest a complementary mechanism, wherein ORS addresses fluid and electrolyte replacement while the herbal formulation modulates intestinal secretion and motility. The study provides preliminary experimental evidence supporting an integrative approach that combines classical Ayurvedic preparations with standard rehydration therapy. Further preclinical investigations and well-designed clinical trials are warranted to validate translational applicability and explore the potential role of such combination therapy in the comprehensive management of diarrhoeal disorders.

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