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Evaluation of *Tripatra Parpati* for Acute Toxicity and Antidiarrheal Activity in Wistar Albino Rats

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Abstract

Background: Diarrhoea is a common gastrointestinal disorder and the leading cause of morbidity and mortality, particularly among children under five. Conventional treatments have (large) limitations with adverse effects. *Ayurveda* offers various alternative drugs that are effective for preventing diarrheal episodes. *Rasa Parpati* is traditionally employed for gastrointestinal disorders, but its antidiarrheal efficacy, particularly with *Bilwa* (*Aegle marmelos*), remains unverified. **Aim:** This study aims to analyse *Tripatra Parpati* and evaluate its acute toxicity and antidiarrheal activity in *Wistar* albino rats. **Methods:** The antidiarrheal potential was assessed using a castor oil-induced diarrhoea model, comparing *Tripatra Parpati* with *Rasa Parpati* and *Bilwa*. Parameters evaluated included diarrhoea onset and defecation percentage inhibition. An acute oral toxicity study was conducted following OECD guidelines 425. HPTLC, FTIR, XRD and SEM-EDAX were employed to assess the physico-chemical structures and components. **Results:** *Tripatra Parpati* was found safe at 2000 mg/kg and significantly reduced diarrhoea severity and frequency compared to control groups, with statistically significant differences. **Conclusion:** These findings support *Tripatra Parpati* as a safe and effective antidiarrheal formulation, particularly beneficial in resource-limited settings.

Major Findings: *Tripatra Parpati* displayed stable physicochemical features and remained safe even at higher doses of 2000mg/kg during toxicity testing. The formulation showed notable antidiarrheal action, achieving around 61% inhibition in castor oil–induced diarrhoea, indicating its therapeutic potential with no significant adverse effects.

Keywords: Acute Toxicity, *A. marmelos,* Antidiarrheal Activity, Castor Oil-induced Diarrhoea, *Rasa Parpati, Tripatra Parpati*

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1. Introduction

Diarrhoea remains a significant health concern, particularly in children under five, contributing to one in nine deaths worldwide. 7.3% of Indian children experienced diarrhoea between 2019 and 2021[1]. Leading to malnutrition, dehydration, and high mortality rates[2-3]. While conventional treatments like antibiotics and bile acid sequestrants are effective, they can disrupt gut microbiota and contribute to antimicrobial resistance[4-6]. Herbal medicines, such as *A. marmelos* (L.) *Correa* (*Bilwa*) has traditionally been used for chronic diarrhoea and dysentery, though their role in infectious diarrhoea requires further scientific validation[7,8].

Ayurveda, an ancient Indian medical science, emphasises holistic well-being by maintaining the balance of *doshas, dhatus, agni,* and *malas* for disease prevention and treatment [9,10]. It has evolved into specialised branches, including Rasa Shastra, which focuses on herbo-mineral formulations known for their quick action, low dosage, and enhanced bioavailability. Among these, *Parpati Kalpana,* introduced by Acharya Nagarjuna, is widely used in gastrointestinal disorders[11-13].

This study aims to develop *Tripatra Parpati*, a novel herbo-mineral formulation combining *Rasaparpati*

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with *Bilwa*, and evaluate its physicochemical properties, acute toxicity, and antidiarrheal activity in Wistar albino rats. By integrating *Ayurvedic* principles with modern pharmacological analysis, this research seeks to provide a safe, effective, and sustainable alternative for diarrhoea management[14-17].

2. Materials and Methods

2.1 Data Collection

The study includes multi-centric outsourcing for the analysis of a drug. *Ashodhit Parad* (mercury) and *Ashodhit Gandhak* (sulphur) were sourced from *Dattatraya Ayurved Rasashala* and authenticated by the Department of Rasa Shastra and Bhaishajya Kalpana Mahatma Gandhi Ayurved College Hospital and Research centre, Salod (H), Wardha. The unripe *Bilwa* fruit, collected from the Herbal Garden, was verified by the Foundation for Revitalisation of Local Health Traditions (Bangalore) and the Department of Dravyaguna (MGACH and RC), Salod (H), Wardha. HPTLC analysis was performed at the Central Research Laboratory, DMIHER, Sawangi Meghe, Wardha, FTIR and XRD analysis was done at Jamnalal Bajaj Science College, Wardha and SEM-EDAX under the Metallurgy Dept. Visvesvaraya National Institute of Technology, Nagpur, Maharashtra, as per the standards outlined in the *Ayurvedic* Pharmacopoeia of India.

2.2 Preparation and Extraction of *Tripatra Parpati* Formulation

API grade castor oil (Merck) was used as the diarrhoea-inducing agent. These substances, mercury and sulphur, were then purified using classical procedures mentioned in the texts of *Ayurved Prakash* and *Rasa Ratna Samuccaya*, respectively; the resulting *Kajjali* was obtained by rubbing equal amounts of purified Mercury and Sulphur in a *Khalva yantra* until the Mercury particles vanished, leaving a fine jet-black powder.

Then dried fruit pulp of *bilwa* was powdered in a grinder before using it in the preparation of *parpati* and its standardisation. The obtained *Kajjali* was heated in ghee-smeared *loha darvi* with a gentle flame directly on the *Angarakosthi*, at a temperature ranging from 110 to 120 °C. *Tripatra Parpati* was formulated by blending the *Kajjali* with the *Bilwa* fruit powder in precise proportions, monitoring *Parpati paka lakshan*. It was then poured onto a banana leaf surface to create a thin flake of *Tripatra Parpati*, following standardisation according to *Ayurvedic* classical protocols.

2.3 Ethical Statement

The acute oral toxicity evaluation of *Tripatra Parpati* was conducted in accordance with OECD Guideline 425[18]. The antidiarrheal study, including all animal experimentation, was performed at the Central Preclinical Laboratory, Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha (Registration No. DMIMS/CPCSEA-IAEC/22-23/09). All experimental procedures strictly adhered to the ethical guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ensuring the humane treatment of animals throughout the study. Following the acute toxicity assessment, *Tripatra Parpati* was further investigated for its antidiarrheal potential using a castor oil-induced diarrhoea model.

2.4 Evaluation of Acute Toxicity

The single-dose acute oral toxicity study was conducted using healthy, young, nulliparous, and non-pregnant Wistar albino rats of both sexes. The study included six rats weighing 140-450 g, with an equal distribution of three males and three females, in which they were housed under standard husbandry conditions, maintaining a controlled temperature of 22°C ± 3°C, relative humidity of 45–55 %, and a 12-hour light/dark cycle provided with a standard pellet diet and had unrestricted access to fresh water. The animals were fasted overnight, with access to water, before dosing. The rats underwent an acclimatisation period of seven days before receiving the trial drug at a dose of 2000 mg/kg body weight. Following the administration of the test substance, food was withheld for an additional 3 to 4 hours. The animals were continuously monitored for 48 hours to assess the onset of any clinical or toxicological symptoms. Additionally, mortality, if any, was recorded over a two-week observation period. On the 15th day, after euthanasia, morphological and histopathological examinations of the liver, kidneys, caecum, colon, and rectum were conducted to evaluate any potential pathological alterations.

2.5 Preparation of Dose

The test drug suspensions were prepared by dispersing 5 ml of finely powdered drug in an appropriate medium to achieve a dosage of 2000 mg/kg body weight. To ensure uniformity and stability, the suspensions were combined with a 10% solution of carboxymethyl cellulose (CMC), a nontoxic binding agent[15].

2.6 Dosage Calculation

To determine the appropriate dosage of *Tripatra Parpati* for rats, the surface area-based conversion formula proposed by Paget and Barnes[19] was utilised. However, in accordance with updated FDA guidelines[20], the calculated dose was determined to be 40.5 mg/kg body weight. To calculate the actual dose administered to each rat based on the dose of 2000 mg/kg, the dose rate (2000 mg/kg) was multiplied by the pre-test weight of each rat (in kg). Fresh test doses were prepared on the day of experimentation based on the individual weight of each animal. The selected dose of 2000 mg/kg was administered orally using an intubation cannula attached to a 2 ml plastic syringe. Additionally, the dose volume did not exceed 1 mL per 100 g of body weight to ensure proper administration.

2.7 Induction of Diarrhoea in Rats

For the first seven days, one control group, one disease-induced group, two standard groups, and one trial drug group were administered the medications listed in Table 1. On the seventh day, the animals were fasted overnight and fed with only tap water. The next day, the control medication, the disease-induced group, the standard drug groups, and the experimental drug group were provided. 1 hour before Castrol oil administration, 2ml/kg was administered orally, placed in cages lined with adsorbent sheets, and watched for 4 hours for diarrhoea and stool consistency. The control group's result was interpreted as 100%. The activity of each group was represented as a percentage inhibition (%) of diarrhoea[14]. The percentage inhibition of defecation was calculated as [(A-B)/A] ×100, where A denotes the mean number of defecations caused by castor oil and B shows the mean number of defecations caused by *Tripatra Parpati*.

Animals were grouped into five groups
Group 1: Normal Control (NC)
Group 2: Castor-oil-induced Diarrhea (DI)
Group 3: *Rasa Parpati* (RP)
Group 4: *Bilwa* (BL)
Group 5: *Tripatra Parpati* (TP)

2.8 Statistical Analysis

Research was carried out and evaluated utilising statistical data and descriptive and inferential statistics, including multiple comparisons, one-way ANOVA, and the student's paired t-test. The analysis was conducted using SPSS 27.0 software and the Tukey test, with a significance level of $p<0.05$.

3. Results and Discussion

The present study investigated the pharmaceutical development, analytical characterisation, acute toxicity, and antidiarrheal potential of *Tripatra Parpati*, an *Ayurvedic* mercurial formulation. The findings highlight its stable formulation, well-defined physicochemical properties, and therapeutic efficacy, particularly in managing diarrhoea.

Diarrhoea is primarily caused by irritation of the enteric mucosal lining or cholinergic toxidrome, both of which promote excessive intestinal motility. Cholinergic toxidrome, induced by increased muscarinic receptor activity, is triggered experimentally by neostigmine, a cholinesterase inhibitor that elevates acetylcholine levels, thereby enhancing gut motility. In contrast, castor oil induces diarrhoea through mucosal

Table 1. Grouping of animals (Anti-diarrheal study)

Group	Group Code	Drug	No of Animal	Dose	Study Duration
Group I	Normal Control (NC)	Distilled water/NS	6	10ml/kg	1day
Group II	Disease Induced (DI)	Castor oil	6	2ml/kg	1day
Group III	*Rasa Parpati* (RP)	RP+castor oil	6	40.5mg/200 g + 2ml/kg	1day
Group IV	Unripe *Bilwa churna* (BL)	BL+castor oil	6	2mg/200 g + 2ml/kg	1day
Group V	*Tripatra Parpati* (TP)	TP+castor oil	6	40.5mg/200 g + 2ml/kg	1day

irritation, leading to inflammation and prostaglandin release, which stimulates the enteric nervous system and further enhances motility. Additionally, inflammation of the mucosal lining disrupts water and electrolyte absorption, exacerbating fluid loss and diarrhea[21-25].

The analytical characterisation of *Tripatra Parpati* confirmed its high purity, stability, and potential bioavailability. Organoleptic and physicochemical assessments highlighted its smooth, crispy texture, blackish colour with a silvery sheen, low moisture content (0.64%), and absence of microbial contamination, all of which contribute to extended shelf life and safety as shown in Table 2.

In acute toxicity, pre-and post-group treatment weight analysis suggested a statistically significant difference, p=0.028, due to diarrheal induction in rats, which causes the weight loss (Tables 3 and 4). The probable mechanism behind weight loss are hyper motility and disparity between intestinal absorption and secretion causes excessive loss of water and electrolytes via feces[34]. Herbo-mineral mixture significantly increased the anti-secretory impact compared to standard drugs. This could be related to increased water reabsorbing capacity and smooth muscle relaxation[34].

HPTLC analysis revealed a diverse range of bioactive compounds, suggesting a complex chemical profile[28]. FTIR analysis confirmed functional groups associated with antioxidant activity, indicating potential pharmacological benefits[29,30]. XRD analysis identified mercury sulphide (HgS) as the dominant phase, reinforcing formulation stability[31,32]. SEM-EDAX analysis demonstrated heterogeneous particle sizes, influencing solubility and bioavailability, with elemental analysis confirming the presence of mercury (43.46 wt. %) and sulphur (41.92 wt. %) in bioavailable sulphide form[33]. These findings collectively validate the

Table 2. Analytical study of *Tripatra Parpati*

Sr. No.	Test Parameters	Test Results
1	Organoleptic characteristics through sensory organs (*Shadba, Sparsha, Rupa, Rasa* and *Gandha*)	*Shadba* - Crispy flake, *Sparsha*- Smooth, crispy, brittle at edges, *Rupa*- Blackish with silvery shiny, *Rasa*- Tasteless, *Gandha*- slightly sweetish/aromatic with sulphurous.
2	Loss on Drying at 105°C[26]	0.64%
3	Total Ash[27]	1.18%
4	Water Soluble Ash	0.5%
5	Acid-insoluble ash	0.05%
6	Total Microbial Contamination	Absent
7	HPTLC (High-Performance Thin Layer Chromatography)[28]	At 254 nm, *Tripatra Parpati* showed a lower concentration of Substance 1 compared to the standard, with multiple unknown compounds and a dominant peak at Rf 0.65. At 301 nm, the analysis revealed several unknown substances with prominent peaks at Rf 0.55 and 0.61, underscoring the formulation's complex chemical profile.
8	FTIR (Fourier Transform Infrared Spectroscopy)[29,30]	*Tripatra Parpati* reveals a complex chemical structure with diverse functional groups, including alcohols, alkanes, alkynes, carbonyls, ethers, and halo compounds. These findings indicate the presence of both organic and inorganic constituents, suggesting esterification reactions and potential carbonate formation during its preparation.
9	XRD (X-ray Diffraction Method)[31,32]	*Tripatra Parpati* confirms mercury sulphide (HgS, Metacinnabarite) as the major crystalline phase, exhibiting a cubic structure with a lattice constant of 5.846 Å and a density of 7.73 g/cm^3. Dominant peaks at specific 2θ angles indicate well-defined crystallographic planes, while minor peaks suggest the presence of additional/secondary phases, aligning with traditional mercurial usage in *Ayurvedic Rasa Shastra*.
10	SEM-EDAX (Scanning Electron Microscopy with Energy Dispersive X-ray Analysis)[33]	The analysis reveals a heterogeneous microstructure in *Tripatra Parpati*, with a wide range of particle sizes, intensities, and surface roughness. The varied particle orientations and morphologies indicate a complex composition typical of traditional *Ayurvedic* formulations.

Table 3. Comparison of the weight of rats in the acute toxicity group at pre- and post-treatment Student's paired t-test

	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	t-value
Pre t/t	310.50	6	78.55	32.07	1.83±1.47	3.05 P=0.028, **S**
Post t/t	308.66	6	79.35	32.39		

Table 4. Comparison of the onset of diarrhoea at 1 hour, 2 hours, 3 hours and 4 hours in six groups

Group	1 hour	2 hour	3 hour	4 hour
Normal Control	0±0	0±0	0±0	0±0
Disease Induced	0±0	7±0.89	5.16±0.75	1.33±1.21
Rasa Parpati	0±0	4.83±0.75	3.50±0.54	1±0.89
Bilwa	0±0	4.33±0.81	3.50±0.54	0.83±0.75
Tripatra Parpati	0±0	4.66±0.81	2.83±0.40	0.83±0.75
F-value	-	72.53	80	2.13
p-value	-	0.0001, S	0.0001, S	0.10, NS

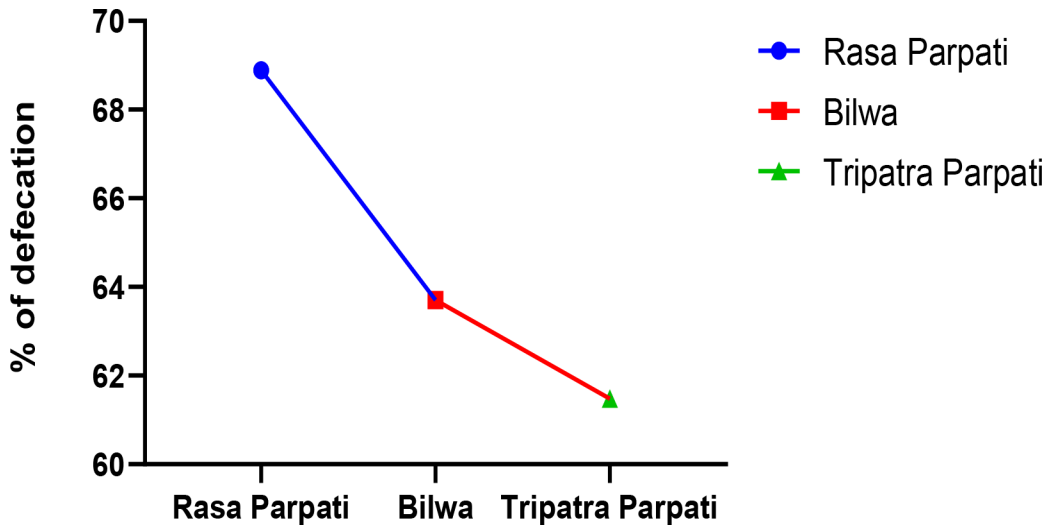


Figure 1. Observations on the percentage of defecation in *Rasa Parpati*, *Bilwa* and *Tripatra Parpati.*

pharmaceutical integrity and safety of *Tripatra Parpati*, while also identifying areas for improvement, such as moisture content and extractive values, to optimise stability and therapeutic efficacy. The percentage of defecation in *Rasa Parpati, Bilwa* and *Tripatra Parpati* is shown in Figure 1.

The acute toxicity evaluation confirmed that *Tripatra Parpati* is well-tolerated, with no severe adverse effects observed even at 2000 mg/kg. Although a statistically significant reduction in mean body weight ($p = 0.028$) was recorded, this did not indicate systemic toxicity. The histopathological evaluation of critical organs in rats that received a single oral dose of 2000 mg/kg indicated primarily standard structure. The liver revealed unremarkable hepatocytes and localised necrosis, indicating minimal hepatic stress. Renal tissues exhibited intact parenchyma and tubules, with only slight inflammatory infiltration, indicating mild nephropathy. Examination of intestinal sections (colon, rectum, and caecum) revealed intact glandular structures, minor lymphocytic infiltration, and found haemorrhage or necrosis in some colon sections. The mere existence of Goblet cells indicated that mucosal function had been maintained. These findings collectively indicate that the formulation is relatively safe at high doses, with no significant toxicological

concerns. Affirming its short-term safety, though long-term studies are required to ensure sustained safety and tolerability.

The antidiarrheal study demonstrated that *Tripatra Parpati* significantly reduces diarrhoea, achieving a 61% inhibition rate in castor oil-induced diarrhoea. Compared to *Rasa Parpati* and *Bilwa, Tripatra Parpati* showed moderate efficacy, with peak inhibition observed between the 2nd and 3rd hours post-treatment.

Although all treatment groups exhibited weight reduction, this was attributed to disease-induced stress rather than drug-related adverse effects. The disease-induced group displayed increased bowel activity, confirming significant gastrointestinal distress. *Rasa Parpati* and *Bilwa* led to a notable increase in faecal output, with *Rasa Parpati* demonstrating the strongest effect, while *Tripatra Parpati* exhibited moderate efficacy. However, all treatments significantly reduced wet faecal output, underscoring their antidiarrheal potential.

All the treated rats were normal, survived, and showed no change in behaviour with no significant difference in morphological and physiological parameters. So, the tested dose was nontoxic and effective in diarrhoea inhibition.

4. Conclusion

The study findings establish *Tripatra Parpati* as a promising antidiarrheal agent with a well-defined safety profile and stable physicochemical properties. Its mechanism likely involves regulating intestinal motility and fluid secretion, aligning with *Ayurvedic* therapeutic principles. While short-term safety is confirmed, further studies, including chronic toxicity assessments and clinical trials, are essential to fully establish its long-term safety and therapeutic efficacy.

Optimising formulation parameters, such as moisture content and extractive values, may further enhance its pharmaceutical and therapeutic potential, making it a strong candidate for clinical applications in gastrointestinal disorders.

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Author Contribution

Yogi Tajane conceptualized the study, conducted the investigation, performed formal analysis, managed data curation, and writes and reviews the manuscript. Bharat Rathi guided the study design, validated the methodology, and critically reviewed the manuscript. Anita Wanjari conceptualized the study, supervised the analytical procedures, validated the data, and contributed to writing and reviewing the article. Bhagyashree Jibkate assisted in the investigation and data curation. Makrand Sonare validated and performed formal analysis of the in-vivo studies. Mujahid Khan contributed to writing and editing the original draft. All authors have read and agreed to the published version of the manuscript.