RESEARCH PAPER

An Experimental study to Evaluate Acute Dermal Toxicity and Vaginal sensitivity of novel Panchavalkala Vaginal Suppository

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ABSTRACT

Introduction : Vaginitis , an inflammatory condition of vaginal mucosa resulting from disruption of normal vaginal flora , being one of the most common pathological condition worldwide . It is characterized by vaginal discharge, pruritus, erythema and edema affecting nearly 90% of women at least once in their lifetime. 1In India, the prevalence of abnormal vaginal discharge is reported to be around 30% .

Conventional treatment using combined antifungal and antimicrobial agents are frequently associated with antimicrobial resistance, local and systemic side effect and disturb the vaginal microbiome.This study introduces a noval herbal vaginal suppository based on Panchavalkala -the barks of five plants -traditionally employed in Ayurveda for the management of Kaphaja yonivyapad or Leaucorrhoea .[3,4]

Methodology : Acute dermal toxicity and Vaginal sensitivity were evaluated through OECD guideline -complaint to acute dermal toxicity (limit test 2000 mg/kg body weight) and a 14 day vaginal sensitivity study (200 mg suppository) using nulliparous Female Wister rats . Parameters assessed included mortality, clinical signs of toxicity, local irritation , body weight changes and detailed histopathological examination of the vagina , uterus and ovaries .

Results : No mortality was observed in any animal across both dermal toxicity and vaginal sensitivity study. There were no signs of systemic toxicity, behavioral alteration, dermal irritation (erythema or edema) or vaginal mucosal inflammation . Histopathological evaluation revealed normal tissue architecture with no evidence of induced inflammation, e Keywords : Herbal formulation , dermal toxicity , vaginal sensitivity study , histopathology , Panchavalkala , vaginal suppository , vaginitispithelial dysplasia or pathological change . Minor physiological variation observed were consistent with normal estrous cycle fluctuation.

Conclusion : This preclinical investigation established the acute dermal and vaginal mucosal safety of Panchavalkal vaginal suppository, validating its potential as a safe , broad spectrum , resistance sparing herbal topical formulation for the management of vaginitis w s r Kaphaja yonivyapad . The results provide a strong scientific foundation for further clinical studies, exemplifying the successful integration of Ayurveda with modern toxicological standards..

Keywords: Herbal formulation , dermal toxicity , vaginal sensitivity study , histopathology , Panchavalkala , vaginal suppository , vaginitis

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INTRODUCTION

Vaginitis is defined as inflammation of the vaginal mucosa as a result of dysfunction of the vaginal flora and is the most common reason for consulting a Gynecologist. [1]

Vaginitis is characterized by inflammation-related complaints accompanied by erythema, pruritus, vaginal discharge and edema. The condition affects women at various life stages and can be caused by various factors, including infections, hormonal changes and irritants. Common types of vaginitis include bacterial vaginosis, yeast infections, and trichomoniasis. The vaginal epithelium is a hormone-responsive, nonkeratinized, stratified squamous epithelium. The prevalence of vaginal discharge in India is estimated to be 30%. [2]

Vaginitis can be observed in 90% of women at least once in their lives, and the association of two or more types of pathogens is observed in approximately 30% of the cases. [1]

Although mixed vaginitis can be treated with treatment approaches that combine antifungal and antibacterial agents,

this approach brings with it disadvantages in terms of increased resistance development and/or systemic and/or local side effects . [5]

This can be correlated to the features of *Kaphaja yoni vyapat*, where there will

be *Kaphavrudhi* all over the body. Management of this condition is by using *Ruksha, Katu, Ushna* dravyas. *Sthanika Chikitsa* such as *Yoni Prakshalana* (douching), *Yoni*

Pichu (tamponing per vagina), *Yoni Varti* (local use of pessaries) and *Yoni*

Dhoopana (fumigation) have been mentioned and these modalities are found effective in treating Vaginitis wsr to *Kaphaja Yoni Vyapat.* [3,4,6] Though there are many modalities there is a need of easy and economic herbal vaginal formulation in the form of suppository although it is likely to exhibit sustained and significant antimicrobial action. Hence this is a need based attempt

The animal study was carried out with the institutional animal ethical committee (Ref: BMK/IAEC/Res-27/2025-10). In view of ascertaining the dermal toxic characteristics of *Panchavalkala vaginal suppository*, acute dermal toxicity study and Vaginal sensitivity study was conducted as per OECD guidelines. [14]

Acute Dermal Toxicity Study

Phase I- Animal Selection, Microenvironmental Standardization and Preparation

Cohort Demographics- Nulliparous, non-pregnant adult female wistar rats were selected to ensure hormonal uniformity.

Environmental Stabilization- All animals were dynamically stabilized and acclimatized to the laboratory testing environment for a mandatory period of five days prior to initiation of dosing.

Thermoregulatory and moisture control- The animal house ambient room temperature was strictly maintained at 22 degree Celsius.

Preparation of the cutaneous field- Before 24 hours of the application of the test formulation (Panchavalkala suppository) the fur from dorsal area of the trunk was carefully removed via depilation. The cleared field corresponded to approximately 10% of the animals total body surface area.

Phase II- Dosage and Application Protocol

Dose calibration- The target test formulation dose was calculated and measured out precisely at a limit threshold of 2000 mg/kg body weight of individual rodent.

Topical Application- The formulation was uniformly applied directly over the prepared, exposed dorsal skin field.

Occlusion Technique- To maintain continuous, uninterrupted contact between the formulation and the skin and to effectively prevent animal from orally ingesting the substance through grooming, the site was securely bound using porous gauze dressing and stabilized it with non irritating surgical adhesive tape.

Exposure Window- The occluded patch assembly was held in contact with cutaneous surface for a fixed exposure period of 24 hrs.

Post Exposure Cleansing- Upon completion of 24 hour exposure window, the dressings were carefully removed. The residual test substance was gently washed away using lukewarm water and the site was carefully wiped with dry sterile gauze without causing abrasion to the skin.

Phase III- Systemic Observation and Screening Timeline

Immediate Post-Dosing Monitoring: Cage side monitoring was initiated immediately following application.

Acute Phase Monitoring: Periodic observations were carried out during first 24 hours, with critical, high frequency surveillance sustained during first 4 hours post application

Phenotypic Toxicity Screening- The wistar rat was systematically evaluated for localised cutaneous reactions and behavioural signs of systemic toxicity.

Results:

Table No.1- Mortality Data

Sl no.	Group	Total no. of animals	Dose	Percent mortality (upto 15 days)
1)	Sighting study	1 female	2000 mg/kg	0
2)	Limit test	4 female	2000 mg/kg	0

Table No.2 - Signs of Toxicity

	Group	Days

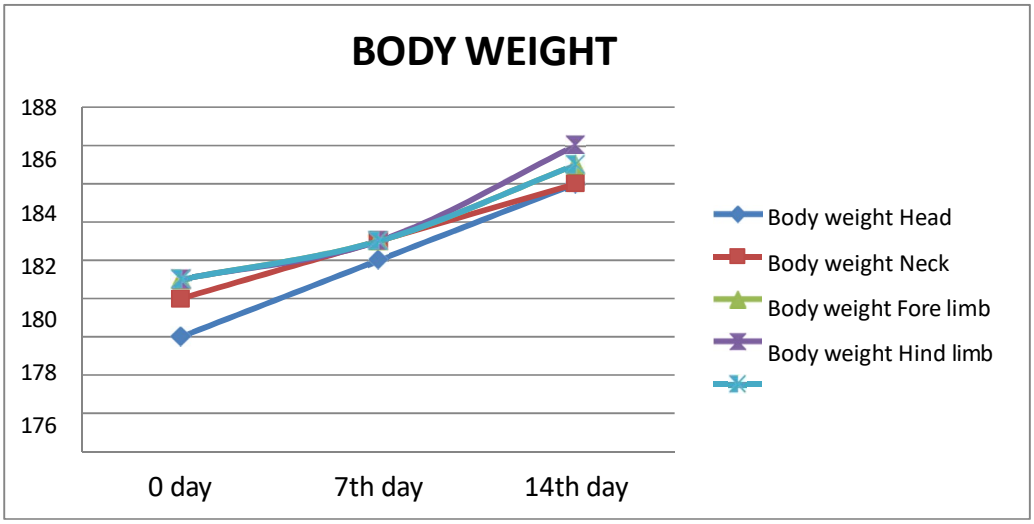
Sl no		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Sightin g	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
2	Limit	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
'0' – No Mortality																

Table No.3 - Skin reactions

Sl no.	Dose	Animals	Observation s	Initiation of exposure(hrs)				Effects noted after initiation of exposure (days)		
				1/2	1	2	4	1	7	14
1	2000 mg/ kg	Sighting test (n=1)	Erythema	0	0	0	0	0	0	0
			Oedema	0	0	0	0	0	0	0
2		Limit test (n=4)	Erythema	0	0	0	0	0	0	0
			Oedema	0	0	0	0	0	0	0
'0' – No reactions										

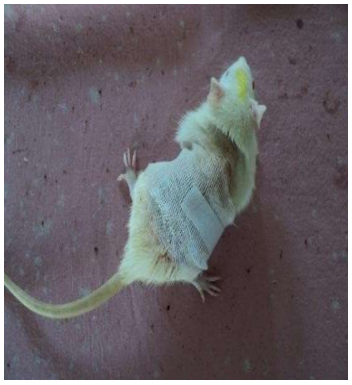
Table No.4 - Body Weight

Sl no.	BODY WEIGHT		
	Day 0	Day 7	Day 14
1	176 gm	180 gm	184gm
2	178gm	181 gm	184 gm
3	179 gm	180 gm	185 gm
4	179 gm	181 gm	186 gm
5	179 gm	181 gm	185 gm



Acute Dermal Toxicity Study Sighting and Limit Study Group

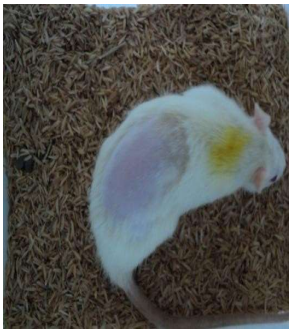
Day-0 **Day -1** **Day-7** **Day-14**



Panchavalkala suppository applied and porous gauze dressing

Day 7 post application skin reaction

Day 14 post application



Procedure for Vaginal sensitivity test

Pre- Experimental Phase

Standard Adaptation- The wistar rats are housed in the laboratory for a 7 days before the study.

Controlled Environment- Temperature of experiment room was strictly maintained at 22 degree Celsius and relative humidity between 30% and 70%.

Sensitivity testing and Baseline phase

A preliminary sensitivity test was conducted to confirm that our suppository formulation is non-irritating and safe for mucosal contact before moving to experimental phase.

Panchavalkal Suppository Insertion and Standardization-

Precise delivery- A standardized suppository of 200 mg was carefully inserted into the vaginal vault of three rats.

Uniformity- The physical dimensions and dosage of the formulation were strictly controlled across all subjects to ensure reproducibility.

Post Insertion observation:

The animals are monitored daily for 2 weeks to evaluate both localized mucosal responses and systemic effects

Clinical Evaluation- Daily visual and behavioral monitoring was performed to check for signs of localized inflammation, abnormal vaginal discharge or systemic distress.

Biometric Tracking- Periodic body weigth measurements are recorded to screen for systemic toxicity or metabolic suppression affecting growth.

Termination and tissue harvesting

Humane Euthanasia: On 15th day, the study was terminated. The rats are humanely sacrificed using an anesthesia (ether) overdose in strict compliance with institutional animal ethics protocols.

Anatomical Dissection: The female reproductive tract was carefully dissected to harvest targeted organs, specifically vagina, uterus and ovaries.

Histopathological and Laboratory Investigation

Tissue fixation: The harvested organs were immediately immersed and fixed in 10% Formalin to preserve cells and prevent autolysis.

Laboratory processing: Fixed tissue samples from experimental groups A, B, and C were dispatched to the histopathology laboratory for:

Haematoxylin and Eosin (H & E) Staining: This is done to microscopically evaluate cellular morphology, structural damage, leukocyte infiltration or signs of localized inflammatory reactions.

Structural Integrity Assessment: To closely analyse and compare the health, thickness and continuity of vaginal epithelial lining and uterine walls across the groups.

Results:

Table No.5 - Body Weight ,Mortality data and Signs of Localized Inflammation

Sl no.	**BODY WEIGHT**			**Group**	**Dose**	**Percent mortality and signs of localized inflammation (upto 15 days)**
	Day 0	Day 7	Day 14			
1	186 gm	190 gm	204gm	Animal A	200 mg / kg	Nil
2	188gm	201 gm	214 gm	Animal B	200mg / kg	Nil
3	189 gm	200 gm	215 gm	Animal C	200mg /kg	Nil

Table No. 6 – Changes in behavior

Sl no	Group	Days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Animal A	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
2	Animal B	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
3	Animal C	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Changes in Fur- Falling of fur, Discoloration, Piloerection
Changes in Eyes-Ptosis, Exopthalamus, Lacrimation, Redness, Pupil constricted, Pupil dilated
Salivation – Viscid, Watery
Respiration- Depression, Stimulation, Failure
Behavioral pattern- Restlessness, Grooming Lying flat on belly, lying flat on side, Lying flat on back, Sleeping
CNS- Defecation, Urination, Squatting, Ataxic gait, Timidity, Writhing, Tremors, Paresis of hind limbs, Paresis of forepaws, Twitches, Convulsions (Clonic, Tonic)

Day -0 Vaginal Sensitivity Study - Application of Suppository at rat vaginal mucosa

Results
Histopathology report
Specimens of Vagina labelled as A B C

Microscopy	A	B	C
Vaginal epithelial thickness	Normal	Decreased	Decreased
Keratinization	Normal	Normal	Normal

Vascularity	Mild	Moderate	Mild
Epithelial dysplasia	Nil	Nil	Nil
Intra epithelial neoplasm	Nil	Nil	Nil
Inflammation	Mild	Nil	Nil

Specimens of Ovaries labelled as A B and C

Microscopy	A	B	C
Preantral follicles/ section of Ovary	17	12	14
Antral follicles / section of ovary	8	10	10
Atretic follicles/ Section of Ovary	3	6	3
Cystic follicles /Section of Ovary	0	1	1
Corpus luteum / Section of Ovary	4	10	7

Specimens of Uterus labelled as A B and C

Microscopy	A	B	C
Endometrial hyperplasia	-	+	-
Endometrial congestion	+	++	+
Endometrial Inflammation	-	-	-
Myometrial hyperplasia	-	-	-
Myometrial congestion	++	+	++
Myometrial Inflammation	-	-	-

Acute Dermal Toxicity study

Sighting study:

The local application of panchavalkal suppository of a single dose of 2000 mg/kg body weight to one female rat resulted in zero mortality during initial 24 hour monitoring period. Immediate observations within first 30 min, 4 hours upto 24 hours post dosing revealed no clinical anomalies on the skin.

Limit test study:

A single dermal application of 2000mg/kg body weight to four additional female rats yielded zero mortality over 14 days of observation. This demonstrates an exceptionally wide systemic margin of safety.

Systemic observations- No adverse changes were recorded in fur conditions like any hair loss or pigmentation, ocular health such as lacrimation, salivation, respiratory rate, behavioural patterns or CNS patterns like tremors etc.

Localised Skin Reactions-

Dermal Grading- Skin irritation was evaluated quantitatively at 30 min, 1 hour, 2 hours, 4 hours 7 days and 14 days of exposure where no irritation was noted.

Erythema and Oedema scores- Both the slighting test animal and limit test animals consistently recorded score of 0 for erythema and oedema formation at every single observation. No redness, no mass or swelling was seen. This confirms that the excipient base and active ingredients within the *Panchavalkala* suppository are non- irritating to skin tissue.

Vaginal Sensitivity Study

Mortality and Behavioural Changes-

Survival Rates- Continuous intra-vaginal administration of a 200 mg formulation dose to three groups (A, B and C) over 14 days resulted in zero mortality.

Behavioral Diagnostics:

Daily observations for autonomic or behavioural changes—specifically tracking fur condition, eye adjustments, salivation status, respiratory depth, grooming activities, and motor-CNS reflex changes—returned nil entries across categories for every animal from Day 0 through Day 14

Localized Vaginal Inflammation-

Mucosal Response- Visual inspections conducted daily for 14 days showed no signs of localised mucosal irritation, swelling or redness around vaginal area.

Discharge profile- No abnormal vaginal discharge was detected in any of the subjects throughout multiday observation timeline.

Histopathological Evaluation-

Vaginal Histopathology-

Epithelial Thickness: Animal A exhibited completely normal vaginal thickness while group B and C demonstrated a decreased epithelial thickness. vaginal epithelial thickness fluctuates substantially depending on the phase of the estrous cycle (estrus features thick, highly cornified epithelium, whereas diestrus features a thin, non- cornified layout).

Keratinization and Growth: It was structurally normal across all the evaluated groups. There was complete absence of epithelial dysplasia or intra epithelial neoplasm in all tissue samples.

Vascularity and tissue defense: It was found mild in group A and C and was moderate in group B. Microscopic cellular inflammation was categorised as mild in animal A and was completely absent in group B and C. Hence formulation does not trigger chemical-induced vaginitis..

Uterine Histopathology

Endometrial Changes: Microscopic review showed endometrial hyperplasia was absent in Animals A and C, but present (+) in Animal B. Endometrial congestion was

recorded as mild (+) in Animals A and C, and moderate (++) in Animal B. No instances of cellular endometrial inflammation were found in any specimen. The isolated appearance of mild hyperplasia in a single animal, in the total absence of inflammation, strongly correlates with normal endogenous estrogen fluctuations (proestrus/estrus phase) during the rat's 4-to-5-day reproductive cycle rather than any pathognomonic toxic effect of the drug.

Myometrial Changes: Myometrial congestion was categorized as moderate (++) in Animals A and C, and mild (+) in Animal B. Both myometrial hyperplasia and myometrial inflammation were completely absent across all groups. In uterine pathology, widespread congestion *unaccompanied* by inflammatory cells (neutrophils or lymphocytes) is an indicator of active hyperemia, a normal physiological event driven by cyclical ovarian hormone surges that increase pelvic blood flow, rather than a toxic or infectious injury.

Discussion

The present investigation successfully established the acute dermal and vaginal mucosal safety profile for the novel Panchavalkala suppository, a standardized herbal formation rooted in classical ayurvedic principle for managment of *Kaphaja yonivyapad* (corresponding to vaginitis). [3,4]

Through acute dermal toxicity testing (limit dose 2000 mg/kg) and a 14 days vaginal sensitivity study (200mg suppository) in nulliparous female Wister rats , the formulation demonstrated an exemplary safety margin with no mortality , systemic toxicity , behavioural alteration or no local irritation was observed in either dermal or mucosal tissues.

Histopathological evaluation of the vagina, uterus and ovaries revealed no evidence of induced inflammation ,epithelial dysplasia or pathological changes and observed physiological variation in epithelial thickness, mild congestion and isolated endometrial hyperplasia were consistent with normal estrous cycle fluctuation rather than toxicological effects.

Conclusion

This study represents the first systematic evaluation of Panchavalkala in vaginal suppository form, bridging ancient ayurveda wisdom with contemporary regulatory standards of safety assessment. [14] The absence of irritant potential on both dermal and vaginal mucosa underscore its promise as a patient friendly, economic and locally acting herbal formulation that may minimize the drawbacks associated with conventional antifungal -antibacterial treatment, such as resistance development and systemic side effects .In conclusion, the results clearly indicate that Panchavalkala vaginal suppository emerges as a toxicologically safe and pharmacologically sound. Its successful development could exemplify the integrative approach in addressing Vaginitis.

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