FORMULATION AND EVALUATION OF VAGINAL GEL USING SPILLANTHUS CILIATA FOR THE TREATMENT OF MENSTRUAL PAIN

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***Abstract*- Local drug delivery, is the method of treatment that involves the transportation of the therapeutic agent to specific tissue without reaching the remaining part of the body. Vagina is a promising site for local delivery of drugs. Among vaginal formulations, gels are easy to manufacture, comfortable, and have the ability to spread onto the surface of mucus and to achieve an intimate contact with vaginal mucosa. Spillanthus ciliata is commonly known as toothache plant, which has the anti-inflammatory, analgesic and spasmolytic activity. In this study, the formulation and evaluation of vaginal gel using spillanthus ciliata for the treatment of menstrual pain has been carried out. Evaluation including invivo acute dermal toxicity studies has been done. This is a promising formulation because, menstrual pain management other than NSAIDS is most relevant.**

***Key Words*: vaginal route, vaginal gel, primary dysmenorrhea, *spilanthus ciliata*, analgesic, anti-inflammatory, spasmolytic.**

INTRODUCTION

The biological effects of a drug in a patient depend on the pharmacological properties of the drug[1]. These effects arise due to the interactions between the drug and the receptors at the site of action of the drug. The efficacy of this drug-target interaction has been undermined unless the drug is transported to its site of action at such a concentration and rate that causes the minimum side-effects and maximum therapeutic effects. **Local drug delivery**, is the method of treatment that involves the transportation of the therapeutic agent to specific tissue without reaching the remaining part of the body[2]. Therefore, it delivers the medication only to areas of interest within the body. This offers an improved efficacy of treatment and reduces side effects. It differs from the conventional drug delivery system in that, it gets a release in a dosage form while the former functions by the absorption of the drug through the body's semipermeable membrane. If a drug can be preferentially delivered to the desired site of action, thereby sparing the rest of the body, the overall toxicity of the drug will be reduced and the TI will increase[3.]

Vaginal route for drug delivery

The vagina, in addition to being a genital organ with functions related to conception, serves as a potential route for drug administration. Mainly used for local action in the cervico-vaginal region, it has the potential of delivering drugs for systemic effects and uterine targeting.[4]

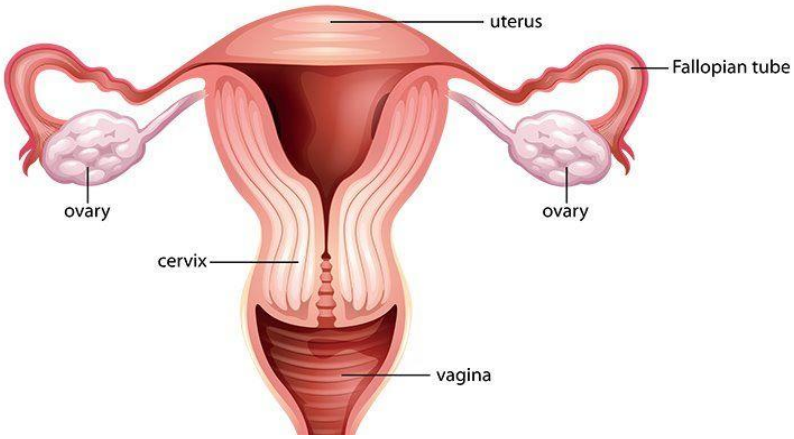

Fig.1 female reproductive system

Vaginal anatomy and physiology with reference to drug delivery

The vagina is a fibro-muscular tube approximately 10 cm in length comprised of three distinct layers namely; An outer adventitial layer, A middle muscularis layer, An innermost mucosal layer.

Vaginal secretions

The vaginal epithelium is usually considered to be a mucosal surface, although it has no goblet cells and lacks the direct release of mucin.Vaginal discharge is a mixture of several components including transudates through the epithelium, cervical mucus, exfoliating epithelial cells, secretions of the Bartholin's and Skene's glands, leukocytes, endometrial and tubal fluids.The cervical mucus contains inorganic and organic salts, mucins, proteins, carbohydrates, urea and fatty acids (lactic and acetic acids). Estrogens and sexual stimulation increase vaginal fluid secretion[4].

Vaginal pH

The vaginal pH of healthy women of reproductive age is acidic (pH 5 4–5); this value is maintained by lactobacilli that convert glycogen from exfoliated epithelial cells into lactic acid.The pH changes with age, stages of menstrual cycle, infections and sexual arousal. Menstrual, cervical and uterine secretions, and semen act as alkalizing agents and increase pH.

Microflora

The vaginal flora is a dynamic and closely interrelated system1. The ecology of the vagina is influenced by factors such as the glycogen content of epithelial cells, glucose, pH, hormonal levels, trauma during sexual intercourse, birth-control method, age, antimicrobial treatment and delivery. Lactobacillus (Döderlein's bacilli) is the most prevalent organism in the vaginal environment together with many other facultative and obligate aerobes and anaerobes[4].

Enzyme Activity

The human genital tract has lower enzymatic activity leading to less degradation of protein and peptide drugs in the vagina than the gastrointestinal tract[6].

Vaginal Gels

Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. The gel is classified as a two-phase system. In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes called as a magma. Single-phase gels consist of organic macromolecules uniformly circulated throughout a liquid in such a way that no apparent boundaries occur between the dispersed macromolecules and the liquid[25].

Among vaginal formulations, gels are easy to manufacture, comfortable, and have the ability to spread onto the surface of mucus and to achieve an intimate contact with vaginal mucosa[18-20]. Moreover, because of their high water content and their rheological properties, they present the further advantage of a hydrating and lubricating action, which is particularly useful in pathological situations characterized by dryness of the vaginal mucosa. The employment of mucoadhesive polymers can improve the time of contact with the mucosa, delaying the loss of the formulation and prolonging the effect[21].

Menstrual Disorders

There are several types of menstrual disorders. Problems can range from heavy, painful periods to no periods at all. There are many variations in menstrual patterns, but in general women should be concerned when periods come fewer than 21 days or more than 3 months apart, or if they last more than 10 days. Such events may indicate ovulation problems or other medical conditions.

Dysmenorrhea (Painful Cramps): Dysmenorrhea is severe, frequent cramping during menstruation. Pain occurs in the lower abdomen but can spread to the lower back and thighs. Dysmenorrhea is usually referred to as primary or secondary:

Primary dysmenorrhea: Cramping pain caused by menstruation. The cramps occur from contractions in the uterus and are usually more severe during heavy bleeding.

Secondary dysmenorrhea: Menstrual-related pain that accompanies another medical or physical condition, such as endometriosis or uterine fibroids.

PRIMARY DYSMENORRHEA	SECONDARY DYSMENORRHEA
Pain which comes from having a period, due to natural chemicals called *Prostaglandins* which are produced in the lining of the uterus. There is no underlying disorder.	The pain occurs as a consequence of an underlying disorder like *Endometriosis, Adenomyosis, Fibroids, or Pelvic Inflammatory Disease* (PID).
Often happens when a girl starts getting her periods, and may improves later in life.	Occurs or starts later in life, than primary dysmenorrhea.
Pain occurs right before menstruation starts, as the *Prostaglandin* level rises and eases out as the period progresses.	Pain often starts earlier in the cycle, than in primary dysmenorrhea, continues through the period and may last even beyond the period.
Can be treated with medicines for pain and other remedies .	Underlying condition needs to be diagnosed and treated.

Fig.2 difference between primary and secondary dysmenorrhea

Herbal medicines and its importance

Herbal medicine is the oldest form of healthcare known to mankind. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Herbal medicines have often retained popularity for historical and cultural ingredients and are used primarily for treating mild and chronic ailments. India has an ancient heritage of traditional medicines; Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian material medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices.

Spillanthus ciliata : The genus Acmella Rich. (Asteraceae) comprises 30 species and 9 additional intraspecific taxa that are mainly distributed in the tropical and subtropical regions around the world [12]. One of the most distinct and recognizable members of the genus is spillanthus ciliata [1]. In particular, this species is famous as a traditional remedy for toothache and for throat, gum infections, that's why it is known in the English nickname, "toothache plant." The whole plant is used as a medicinal remedy in various parts of the world.

Anti-inflammatory activity

Various studies had reported the anti-inflammatory properties of spillanthus ciliata on carrageenan, an inducer of hind paw disorder and a standard phlogistic agent to study anti-inflammatory activity. Reports suggested significant dose-dependent inhibition of paw oedema [6].Mouse treated with methanol extract also presented an anti-inflammatory function which can suppress neutrophilic inflammation in the lungs of the treated animal [23]. The presence of flavonoids was found to be the powerful inhibitors of prostaglandins which were effective at the later stages of acute inflammation [6]. Anti-iflammatory component of spillanthus ciliata, spilathol, was isolated by a bioactivity-guided approach and found to exert an inhibitory effect on NF-κB activation through restrained IκB phosphorylation and degradation, leading to the reduced downstream inflammation mediator expression, including iNOS, COX-2, IL-1, IL-6, and TNF-R. These findings suggest that spilanthol can be a useful inhibitor of inflammatory mediators and is potentially applicable for COX-2 selective nonsteroidal anti-inflammatory drugs (NSAIDs) [27].

Analgesic activity

Researchers have also reported the presence of analgesic properties when coupled with acetic acid and have confirmed to induce abdominal constriction as revealed by a popular methodology, namely tail flick method. The results obtained with these plant extracts proved to be more efficient and effective as to tail flick method thus could be explored as peripherally acting analgesic [24]. In another study by using cold water extract of spillanthus ciliata flowers, possess antinociception activity against persistent pain, possibly by inhibiting prostaglandin synthesis, interrupting nociception transmission, and exerting antihistamine activity [25]. Sedatives have analgesic activity [26], and this mode of action may also contribute to its sensitivity to pain. Interruption of nociception transmission along C-fibers may also play a vital role in inducing analgesia. Another study of antitoothache plant H. longipes revealed that it was used as local anesthetic and analgesic in Mexican indigenous medicine and the results showed that its stem acetone extract and spilanthol from root displayed dose-dependent antinociceptive effect in mice as assessed by Writhing and capsaicin tests [28].

Anti-spasmodic activity

A study demonstrated, for the first time spillanthus ciliata has effective spasmolytic property on isolated rat ileum by inhibiting Ca2+ influx into intestinal smooth muscle. Thus, AFE has great potential as a nutraceutical product/herbal medicine for its overall antispasmodic action in gastrointestinal disorders such as diarrhoea [33]. Moreover, Acmella oleracea extract is an active component in body and beauty care cosmetics as a fast-acting muscle relaxant that may be essential in accelerating the repair of functional wrinkles as well as stimulate, reorganize and strengthen the collagen network and has thus been utilized for anti-aging purposes in the form of anti-wrinkle cream formulations [35] .In addition, A. oleracea shows vasorelaxant potential in rat thoracic aorta. The results showed that the tested extracts exhibited vasorelaxant activity via partial endothelium-induced NO and PGI_2 in dose dependent manner [34].

The menstrual pain is primarily due to the inflammatory mediators and following contraction of uterus. By taking into account of analgesic, anti-inflammatory and antispasmodic properties of spillanthus plant a vaginal gel is formulated for menstrual pain.

Materials and methods

Sample preparation: For the extraction, whole plant were washed and cut into small pieces. Soaked in petroleum ether for dissolving waxes and gums. After evaporation of petroleum ether, grinded well. Crude extract subjected for phytochemical screening.

Fig.3 extraction of spillanthus

PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

The hydroalcoholic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract.

1. Test for Alkaloids

Dragendroff's test: Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

2. Test for Flavonids :

To 1 ml of aqueous extract, add 1 ml of 10% lead acetate. Formation of yellow precipitate indicated the presence of flavonoid.

3. Test for Steroids :

2 ml of organic extract was dissolved in 2 ml of chloroform and was treated with sulfuric acid and acetic acid. The appearance of green confirmed the presence of steroids

4. Test for Terpenoids

Salkowski test was used to detect Terpenoids. Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of Terpenoids.

5. Test for Phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% hydrochloric acid, and the mixture was dried. Deposition of red precipitate was taken as evidence for the presence of phlobatannins.

6. Test for Glycosides

About 2 ml of organic extract was mixed with 2 ml of chloroform to which 2 ml of acetic acid was added carefully. A color change from violet to blue to green indicated the presence of a steroidal nucleus that is aglycone portion of glycoside.

7. Test for Carbohydrates To 3 ml of aqueous extract, 1 ml of iodine solution was added. A purple coloration at the interface indicated the presence of carbohydrates.

8. Test for Saponins 5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube. The mixture was warmed for few minutes.The formation of stable foam, honeycomb in shape was taken as the evidence for the presence of saponins.

FORMULATION OF GEL USING S. CILIATA

The required quantity of extract and polymer (Carbopol 940P and HPMCK4M) was weighed, then it was sprinkled slowly on surface of purified water for 2 h. After that it was continuously stirred by mechanical stirrer, till the polymer was soaked in the water. Finally solution was kept for overnight for complete hydration of polymer. With continuous stirring, triethanolamine was added to neutralize the gel and to maintain the pH of the gel. Now the appropriate quantity of dimethyl sulphoxide (DMSO) was added to the gel, which behaved as the penetration enhancer, followed by addition of required quantity of ethanol to make the soft gel. Care should be taken to avoid incorporation of air into gel. In this way, four formulations (F1–F4) of gel were prepared by different concentrations of extract. Finally, the preparations were packed in wide mouth plastic jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place[32].

Table No.1: Formula for vaginal gel using *S.ciliata*

Ingredients	**F1**	**F2**	**F3**	**F4**
Extract (mg)	200	500	1000	2000
Carbopol (mg)	100	100	100	100
DMSO (ml)	0.2	0.2	0.2	0.2
HPMC (mg)	400	400	400	400
Triethanolamine (ml)	0.9	0.9	0.9	0.9
Methyl paraben (mg)	15	15	15	15
Alcohol (ml)	5	5	5	5
Water (up to ml)	100	100	100	100

EVALUATION OF GELS

pH measurements: pH measurements of the gel were carried out using a digital pH meter.1gm of gel was dissolved in 100 ml of distilled water and it was placed for 2 hr. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Viscosity: The viscosity of the formulation was determined by using the Digital Brookfield Viscometer using spindle no. 6 at 10 rpm and temperature of 25±1°C. A sufficient quantity of gel was filled in appropriate wide mouth container in such way that it should sufficiently allow to dipped the spindle and allowed to settle over 30 min before the measurements[33].

Spreadability: The protocol involved taking 2 g of formulation and placing it on a ground slide and sandwiching it by an analogous glide slide, having a hook attached. A heavy mass was applied to the slides to remove the entrapped air so as to form a uniform film between the slides. The excess gel content was scrapped off from the edges. Following it, the top slide was made to drag 50 g intensity [48]. The time needed by the top slide to cover a distance of 6 cm was determined from the formula:

Washability: The washability of formulations was examined by applying the gel on the skin and then evaluating the ease and the extent of washing it with distilled water and manually observing the effect [48].

Spreadability = MXL/T

Swelling Index: The swelling index of the prepared gel was determined by taking 2 g of gel in a beaker containing 10 mL of distilled water. After 1 hr, the swelled formulation was removed from the beaker and was put on a petridish. The content was re-weighed and the swelling index was estimated from the formula.

Swelling index (Si) =Wt-Wo/Wo X100

Gelation temperature: Gelation temperature (GT) was measured by heating the formulation in a 15-mL borosilicate glass test tube. Into each test tube, 2 mL of formulation solution were placed and heated with gentle stirring until the formulation solution gets gelled. Gel formation was considered as the point where there will be no flow when the test tubes were tilted >90°C [34,43].

***INVITRO* STUDY BY USING CRC**

Invitro study of the S.ciliata extract was done by the concentration response curve (CRC) or dose response curve by using chicken ileum.

Procurement of Chick ileum: Chick ileum was procured from the local market in ramanattukara.

Table: 2 Preparation of physiological salt solution (PSS)

Compound	Tyrode solution (g/L)
NaCl	8
KCl	0.2
$CaCl_2$	0.2
$MgCl_2$	0.1
$MgSO_4$	0.1
$NaHCO_3$	1.0
KH_2PO_4	0.05
Glucose	0.5

All values are in **g/l**. Weighed accurate quantity of the ingredients and dissolved in one liter distilled water. The physiological solution prepared should be clear, and if turbid it is advised to prepare fresh solution before the start of the experiment [35].

Procedure: Fresh entire gastrointestinal tract of healthy cock was obtained from a local market. The caecum was lifted forwards and the ileocaecal junction was identified. A few centimetres of the ileal portion was cut and removed and immediately placed it in the watch glass containing physiological salt solution. The mesentery and adhering tissues were removed with gentle care. Utmost care was taken to avoid any damage to the gut muscle. The ileum was cut into small segments of 2- 3 cm long. To one piece of ileum the thread was tied to top and bottom ends without closing the ileum, and mounted the tissue in the organ bath containing PSS maintained at 32-35˚C and bubbled with air. The magnification from 5-7 folds and bath volume of about 25 ml was maintained, and the tissue was allowed to equilibrate for 30 min before adding Acetylcholine to the organ bath. The Acetylcholine induces the contraction in the ileal smooth muscles which were recorded on Kymograph by using frontal writing lever. Contact time of 30 sec, and 5 min time cycle was kept for proper recording of the responses. The CRC was recorded till ceiling effect to Acetylcholine was obtained. Height of response was measured. The same way different concentrations of spillathus extract was also tested for its spasmolytic activity [35].

***IN VIVO* PHARMACOLOGICAL EVALUATIONS**

STUDY-1: Acute dermal toxicity study of formulated vaginal gel[36]

The study was done as per OECD guideline (NO. 402). Wister albino rats was used. Due to insufficient information on a test chemical, a dose-range finding study using 1 animal at a starting dose **of 200mg/kg body weight** was carried out. Animals are observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours. A period after 48 hours, special attention given for the appearance of any signs of dermal toxicity. There was no signs of toxicity found, so continued dosing 1000, 2000 mg/body weight on further two animals. The observation was continued up to day 14. Mortality and morbidity of the animals was recorded during the experimental observation period. On day 14, surviving rats were rehabituated safely[37,38].

STUDY/METHOD	NO.OF ANIMALS
Acute dermal toxicity study (200,1000,2000 mg/kg Body weight, Dermal route)	3

STUDY-2: Study of analgesic activity by eddy's hot plate method

The hot plate test method was employed to assess the analgesic activity. The experimental animals were divided into control, standard and test groups with six mice in each group. The animals of test groups received test samples at the doses of 250 and 500 mg/kg body weight, positive control group was administered ketoprofen 2% gel. In this test, the animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 ºC. The test samples and the standard drug were administered 30 minutes before the beginning of the experiment. Reaction time was recorded when animals licked their fore or hind paws, or jumped. A cut-off period of 20 seconds was observed to avoid the damage of the paw. The analgesic activity response was recorded from the time between placement and licking of fore or hind paws or jumping movements of the animals [39, 42].

SI.NO	STUDY/METHOD	NO. OF ANIMALS
1	Group-1 Standard (Ketoprofen 2.5% gel Dermal)	6
2	Group-2 control	6
3	Group-3 Treatment group	6

Stability studies: Stability of pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specification. In real-time stability testing, a product is stored at recommended storage conditions and monitored until it fails product specifications. In accelerated stability testing, a product is stored at elevated stress conditions (e.g., high temperatures and/or humidity).

The stability study was performed as ICH guidelines and the formulated gel was filled in plastic container and stored at different temperatures and humidity conditions 40±2ºc/75±5% RH for a three month periods. Samples were withdrawn at periodically (30 day intervals) and evaluated for colour, odour, viscosity, washability, pH and Spreadability

RESULTS AND DISCUSSION

Table no.3 Preliminary phytochemical analysis

Alkaloid	Dragendorff's Test	+
	Mayer's Test	+
Carbohydates	Molish Test	+
Saponin	Froth Test	+
Tannin	Ferric chloride Test	+
Steroids	Salkowski's Test	_
Carotenoids	chloroform test	+
glycoside	Salkowski's test	+

FORMULATION OF GEL USING S. CILIATA

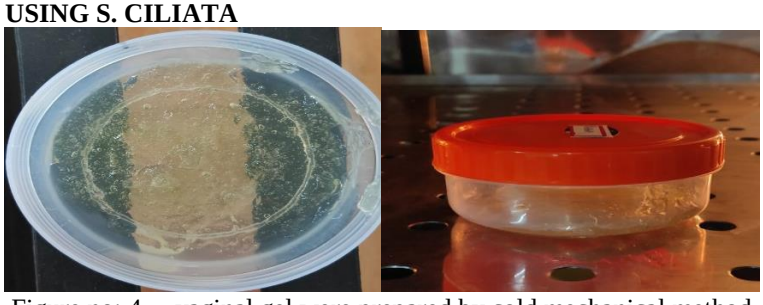

Figure no: 4 vaginal gel were prepared by cold mechanical method

EVALUATION OF GELS

Physical appearance of gel formulation

Colour: colourless **Odour:** characteristic **Taste:** pungent

Measurement of pH: pH measurements of the gel were carried out using a digital pH meter.1gm of gel was dissolved in 100 ml of distilled water and it was placed for 2 hr. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Table No. 4 : pH measurement of gel formulations

Formulation code	pH
F_1	5.5
F_2	5.3
F_3	5.3
F_4	5.4

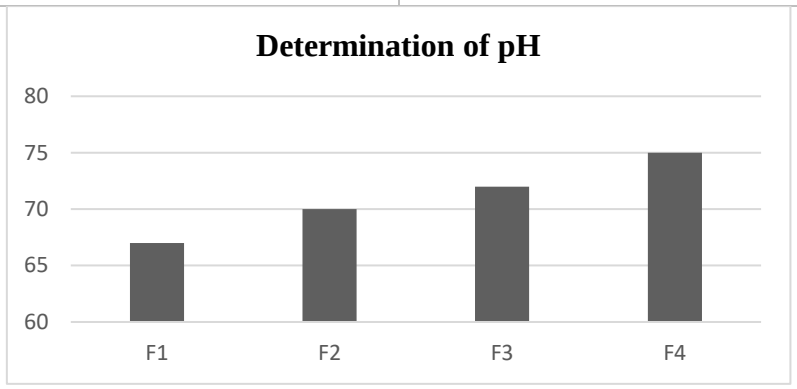

Figure no: 5 Determination of pH of Gel formulations

The pH of various gel formulations (F1 – F4) were determined by using pH meter and pH of all formulations are found to be in the range of 5.3-5.5 that is, as per the pH of vagina.

Viscosity

Viscosity of gel formulations were determined using Digital Brookfield viscometer spindle no.6 at 12 rpm and results are shown in Table no: 5

Formulation code	Viscosity (cps)
F_1	67±0.12
F_2	70±0.45
F_3	72±0.45
F_4	75±0.45

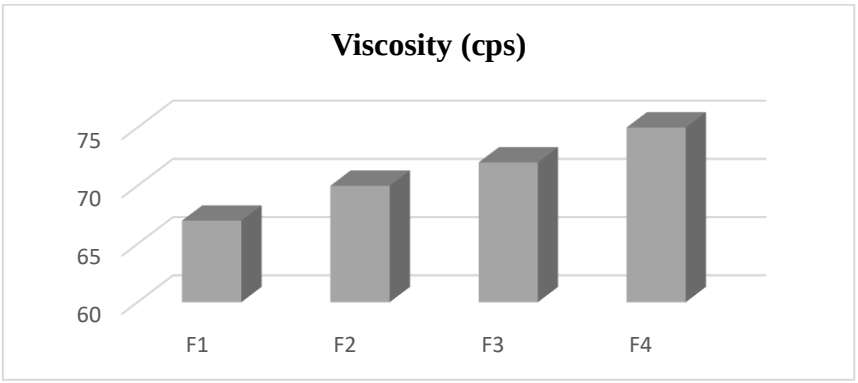

Fig No. 6 : Determination of viscosity of gel formulations

An ideal insitu gel should be liquefied at room temperature so as to allow reproducible administration into the site of application where it undergoes insitu transition to form a strong gel. The prepared gel shows Newtonian flow as their viscosity is in 67- 75 cps range at formulation conditions. However the formulation shows increased viscosity in SVF and in body temperature. From this it was clear that the gel will undergo gelation at physiological conditions.

Spreadability

Table No.6 Measurement of Spreadability

Formulation code	Spreadability (g.cm/sec)
F_1	13.5
F_2	14
F_3	14.2
F_4	15

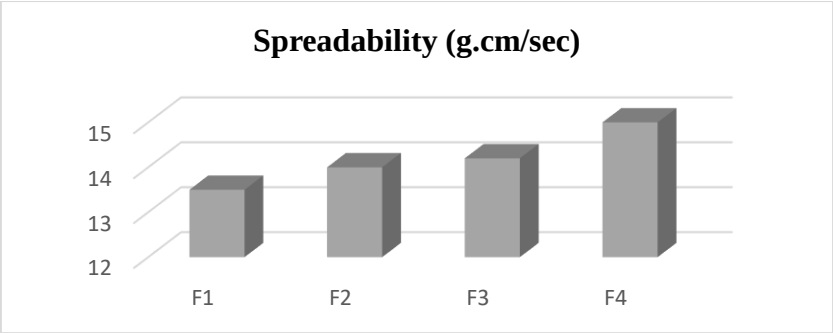

Figure no:7 Determination of Spreadability of gel formulations

The spreadability plays an important role in patient compliance and helps in uniform distribution of formulation into the vagina. A good gel will take less time to spread and thus have good spreadability. The spreadability was found in the range of 13.5- 1 g.cm/sec.

Washability

The washability of formulations was examined by applying the gel on the skin and then evaluating the ease and the extent of washing it with distilled water and manually observing the effect

Table no. 7

Formulation code	Washability
F_1	Good
F_2	Good
F_3	Good
F_4	Good

Swelling index

The swelling index of the prepared gel was determined by taking 2 g of gel in a beaker containing 10 mL of distilled water. After 1 hr, the swelled formulation was removed from the beaker and was put on a petridish.

Table No. 8 Measurement of swelling index

Formulation code	Swelling index (%)
F_1	123
F_2	122
F_3	124
F_4	124

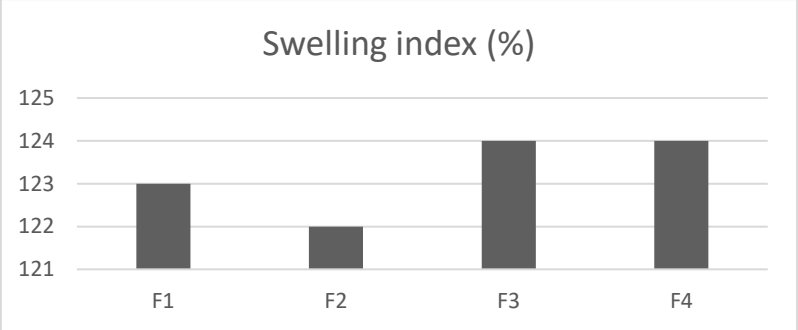

Fig No. 9 Determination of swelling index of gel formulations

The Swelling index of all formulations was examined. The highest value was found in F_3, F_4 and least value was found in F_1. Here as the result shows, there is no significant difference in swelling index of 4 formulations. An inverse relationship exists between the drug release rate and matrix swelling rate. This implies that HPMC swelling is one of the factors affecting drug release. The swelling behaviour of HPMC is therefore useful in predicting drug release.

Gelation temperature (°C) The temperature at which a sharp increase in viscosity is observed is referred to as the gelation temperature

Table No. 9 : Measurement of Gelation temperature (°C)

Formulation code	Gelation temperature (°C)
F_1	30
F_2	34
F_3	31
F_4	35

For vaginal formulations, gelation temperature in the range of 30 °C to 35 °C is suitable since gelation temperature lower than 30 °C may pose difficulties in handling and administration of formulation whereas gelation temperature higher than 35 °C may not allow gelation of
formulation at body temperature resulting in its leakage. After application to vaginal cavity,
formulation may be diluted with vaginal fluid. This dilution may affect the sol to gel transition and gel strength. Therefore, the effect of dilution on gelling ability and gelation temperature of formulations was studied. There was significant increase in the gelation temperature after dilution. The formulations, with HPMC as mucoadhesive agent, retained their gelation temperature within the range of 30-35 °C even after dilution.

***INVITRO* STUDY BY USING CRC**

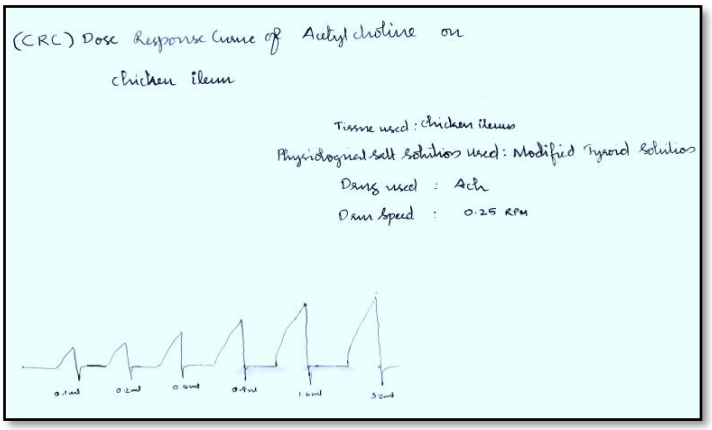

Figure no: 10 Effect of ACh on chicken ileum

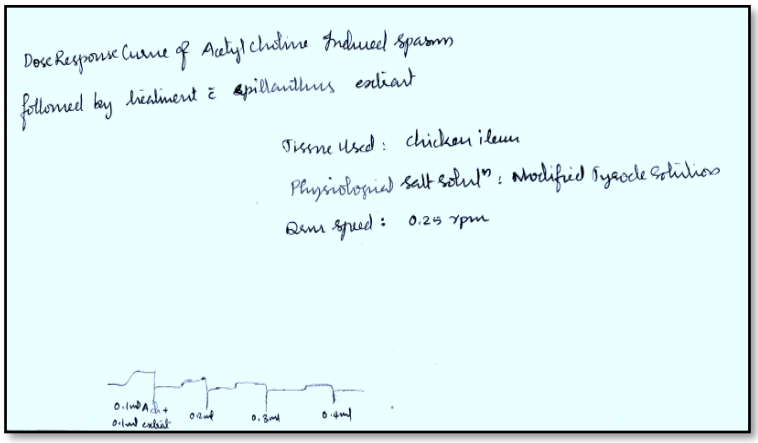

Figure no:11 ACh induced spasm followed by the treatment with *spillanthus* extract

Effect of Ach on chicken ileum reflected an increase in spasmodic activity (response) with an increase in the dose. Ach induced spasm followed by the treatment with spillanthus extract showed prominent antispasmodic activity as shown in the graph.

Table no: 10 dose and height of response in each sample

ACh		**Extract + ACh**	
Dose	Height	Dose	Height
0.1	0.9	0.1+ 0.1	0.5
0.2	1.1	0.2+0.2	0.4
0.4	1.3	0.3+0.3	0.3
0.8	1.5	0.4+ 0.4	0.2

***IN VIVO* PHARMACOLOGICAL EVALUATIONS**

STUDY-1: Acute dermal toxicity study of formulated gel

The study was done as per OECD guideline (NO. 402). Wistar albino rats was used.

Table no: 11 observations after 14 days of acute dermal study

SL.NO	PARAMETER	OBSERVATION
1	Erythema	Nil
2	Oedema	Nil
3	Irritation	Nil
4	Tremors	Nil
5	Convulsions	Nil
6	Salivation	Nil
8	Diarrhoea	Nil
9	Death	Nil

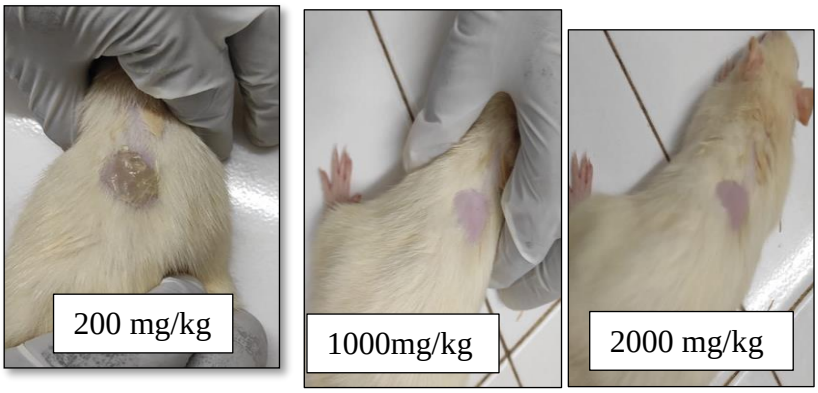

Figure no: 12 showing results of acute dermal toxicity studies

STUDY-2: Study of analgesic activity of gel by eddy's hot plate method

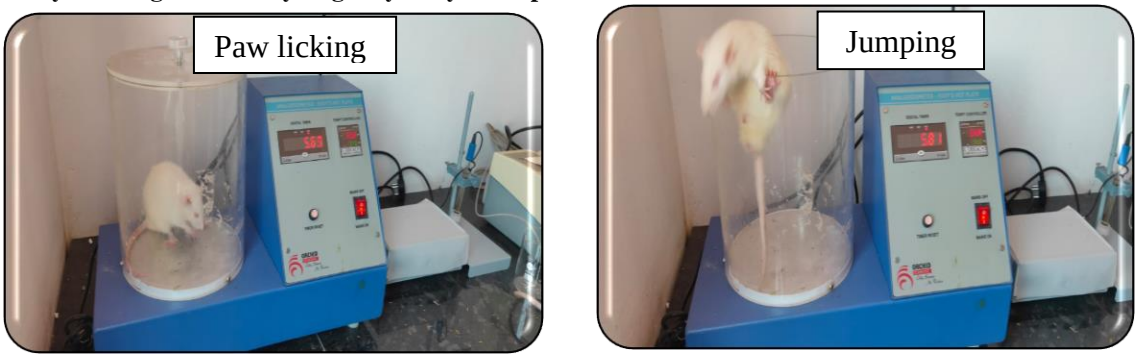

Figure no: 13 observations of analgesic activity by eddy's hot plate method

Table no: 11 response in eddy's hot plate method

Sl.no	Animals	Weight of animal (mg)	Reaction time in seconds	
			Paw licking	jumping
1	Control	C1 = 162	2.36	3.08
		C2 = 172	2.45	3.11
		C3 = 170	2.61	3.01
		C4 = 168	2.58	3.56
		C5 = 167	2.01	3.44
		C6 = 174	2.76	3.21

2	Standard	S1 = 170	8.73	9.88
		S2 = 172	9.11	10.56
		S3 = 169	9.34	10.25
		S4 = 171	8.36	9.01
		S5 = 161	9.49	10.98
		S6 = 170	10.01	10.98
3	Test	T1 = 164	7.91	11.81
		T2 = 172	6.43	7.59
		T3 = 168	7.35	8.45
		T4 = 167	7.66	8.32
		T5 = 171	7.95	8.98
		T6 = 165	6.29	7.94

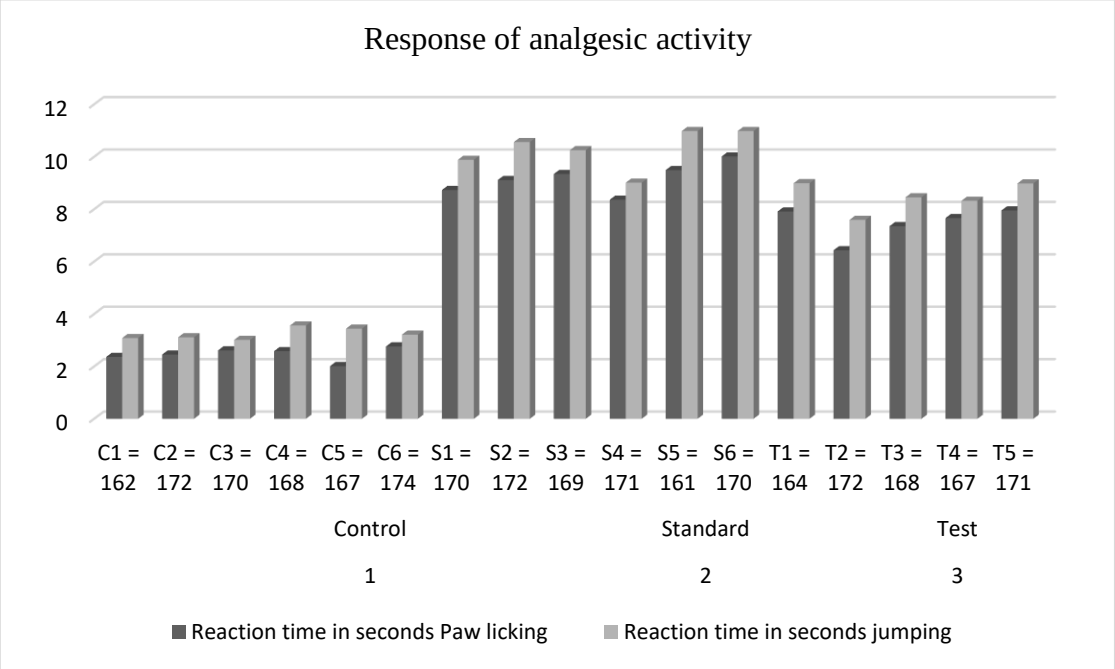

Figure no: 14 Response of analgesic activity by eddy's hot plate method

STABILITY STUDIES

STUDY-1: Best gel formulation F4 was subjected in short term stability studies or accelerated stability studies as per ICH guidelines.

Table No.12 Stability studies of gel formulation (F4)

Duration in (days)	**At 40±2°C/75±5%RH**						
	Evaluation parameters						
	colour	**odour**	**consistency**	**Washability**	**pH**	**Spreadability(g.cm/sec)**	**viscosity**
0	Colourless	Characteristic	thick and transparent	Good	5.5	15	75±0.45
30	Colourless	Characteristic	Thick and transparent	Good	5.5	15	70±0.45
60	Colourless	Characteristic	Thick and transparent	Good	5.6	15	70±0.45
90	Colourless	Characteristic	Thick and transparent	Good	5.6	15	70±0.45

SUMMARY AND CONCLUSION

Local drug delivery, is the method of treatment that involves the transportation of the therapeutic agent to specific tissue without reaching the remaining part of the body. Therefore, it delivers the medication only to areas of interest within the body. The vagina, in addition to being a genital organ with functions related to conception, serves as a potential route for drug administration. Mainly used for local action in the cervico-vaginal region, it has the potential of delivering drugs for systemic effects and uterine targeting. **Primary Dysmenorrhea** or menstrual cramps have a large impact on women's quality of life. Frequent use of NSAIDS and oral

contraceptives are not recommendable, since it has many side effects. So this is an open field for research that finding an alternative way of tackling menstrual cramps other than oral drug delivery. The vagina, as a site for drug delivery, offers certain unique features that can be exploited in order to achieve desirable therapeutic effects. The drug here is a plant, conventionally used as for tooth ache as it has analgesic, anti-inflammatory and spasmolytic properties. A vaginal gel using this plant is a promising development for the treatment of menstrual pain.

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