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HERBAL VAGINAL CONTRACEPTIVE GEL FORMULATION: HYDRO-ALCOHOLIC EXTRACT OF *ACACIA LEUCOPHLOEA ROOT*

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Abstract*:* In present study, *Acacia leucophloea* root contains contraceptive preparations exclude demerits of marketed contraceptives. It brings intravaginal mucoadhesive gel formulate with antifertility action, better strength, attuned with the aqueous vaginal atmosphere and miscible by vaginal fluid medium. In this formulation using hydro-alcoholic extract of roots of *Acacia leucophloea* be side with other appropriate pharmaceutically suitable ingredients. Antifertility action of the formulated hydro-alcoholic extract of the roots of *Acacia leucophloea* was carried. Intravaginal gels have the improvement of being non-irritant, water miscible, water washable, simply spreadable, companionable with the aqueous vaginal environmentally and miscible with the vaginal fluid. The process employed desired quantity of hydro-alcoholic extract from the roots of *Acacia leucophloea* in gel conformation in the vagina to prevent conception.

Keywords: - Hydroalcoholic, *Acaica leucophloea*, intra-vaginal.

Introduction: Increasing population is one of the major problems; various countries of the world are suffering from. Various means like condom, intrauterine device, oral contraceptives, vaginal pessaries, gel, foam, cream etc. have been used and proved successful to put a bar over it but still there is a need to develop a formulation to overcome the disadvantages which were associated with these traditional devices. such as intrauterine device have to be implanted lone by the consultant and it gives distress through interaction, method of usage of the condom and vaginal pessaries sometime give an unpleasant and uncomfortable feeling. Beside these, synthetic oral contraceptives which are free from such disadvantage of administration shows various side effect like blood pressure, improving in risk of cancer, weight gain etc.

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Vaginal gel formulation takes advantage over the other formulation because of its ease of application and good efficacy. But synthetic compound in this formulation can reason local touchiness at situate of request and few other side effects.

Herbal preparation had always more popularity over the synthetic because of their good efficacy, less side effects and safe uses etc. Various herbs and plants have been proved with antifertility and contraceptive effects. The folklore uses of the plants and herbs for such activity can be used for the development of herbal contraceptive as there is still a need of such formulation with herbal contents.

In the present study plant *Acacia leucophloea* was selected on the basis of its folklore use and literature survey. *Acacia leucophloea* belonging family Mimonasease. By habit it is a large thorny tree of about 35 m of height. It is distributed in south and Southeast Asia and contagious to drab India, Sri Lanka, Bangladesh, Burma and Thailand. The plant has been used for its various medicinal properties like antimicrobial, antioxidant[4], anthelmintic[5], expectorant[6], gastrointestinal, respiratory disorders[7] and blood disinfectant[8]. It is also using in skin illnesses (leprosy), sore, gum draining, mouth sore, thirsty cough, dysentery, diabetes infection and Snake-bite[9]. Its gum and decoction

of bark and root is used for contraception and menstrual criticisms[10].

Materials and Methods: The plant samples were procure from the Bastar region of Chhattisgarh, India, and were completely identified and authentic by Dr. N. Shiddhamallaya, Botanist, Regional Research Institute (Ayurveda), Central Council for Research in Ayurveda and Siddha, Ashoka pillar, Jayanagar, Bangalore. A voucher case no. is (RRCBI/mus.5-36).The roots were shadow dry and before pulverized. The gunpowder used for extraction for further study. Soxhlet extraction of the ground roots was passed available for 48 hrs with 50% alcohol and the extract therefore found was then dissolved.

Procedure of Gel Preparation: For Formulation F1, F2, F3,F4 1g of hydroalcoholic extract from roots of *Acacia leucophloea* was weighed and dissolved in 15ml of glycerin by help of mild hotness (solution A). Weighed amount of carbapol 934P,0.5 gm, 1 gm, 2 gm, and 5 gm respectively was additional to the 75ml of purified water and enthused until dissolved and then maintain the pH 4.5-5.5 by the 10% NaOH (solution B). Solution A and B were varied methodically and the ultimate weight was finished up to 100g. For Formulation F5, F6, F7,F8 and F9 1g hydro-alcoholic extract from roots of *Acacia leucophloea* was weighed and dissolved in 15ml of glycerin by the help of slight heat after that mixed thoroughly methyl paraben & propyl paraben (solution A). Evaluated amount of HPMC 3gm, 5 gm, 10 gm, 11gmwas additional to the 75ml of purified water and enthused to melt the similar (solution B). Solution A and B were mixed carefully and the final weight was made up to 100g.

Evalution of *Acacia Leucophloea* Gel

pH: The pH of the various gel preparations was evaluated by digital pH meter. The pH quantity was passed available by calibrated digital type pH meter by dropping the glass electrode and the reference electrode absolutely into the gel system so as to cover the electrodes.

Spreadability Study: Spreadability was evaluated by kit suggested by *Mutimer et al* (1956) in which appropriately adapted in the lab and used for the study. It contains of a woody block in which was providing by an elevator at unique end. By this process, spreadability was slow on the base of 'Slip' and 'Drag' features of gels. A pulverized glass slither was secure on this chunk. An extra of gel (about 2 gm) below study was place on this powdered slide. The gel was formerly inserted among this slide and added glass slide taking the measurement of secure crushed slide and providing through the hanger. One Kg weight was situated on the upper of the dual slides for 5 minutes to eject air and to deliver unchanging film of the gel among the slides. Additional of the gel was argued off from the limits. The top plate was then exposed to attraction of 50 gms. Through the assistance of cord devoted to the hook and the time (in seconds) vital by the highest slide to protection a space of 7.5 cm be famous. A smaller break designates improved Spreadability.

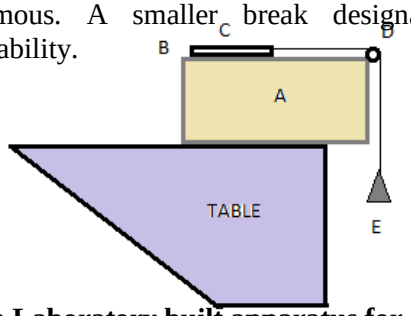

Fig. 1: Laboratory built apparatus for spread ability

Where,

A = Wooden box
B = Lower glass slide
C = Upper glass slide
D = Pully
E = Weighing pan

Spreadability was then considered by the following formulary:

$$S = M \times L / T$$

Where, S = is the spreadability,
M = is the weight in the pan (tied to the upper slide),
L = is the length moved by the glass slide and
T = represents the time taken to separate the slide completely from each other.

Extrudability Study of Gel:

A good gel extrude optimal from the gel with slender hea viness useful. The extrudability of formulations from alu minium collapsible pipes was dogged using universal tub e filling machine.

Aluinium folding pipes full with 10g gels were detained amid two locks. A tube was beaten and extrudibility of th e preparation was resolute in footings of heaviness in gra ms obligatory to extrude a 0.5 cm. band of gel in 10 seco nds.

More amounts extruded healthier was extrudability. The dimension of extrudability of each preparation was in triplicate and the regular standards are obtainable. The extrudability was than planned by the following formula[12]:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm^2)

***In vitro* bio-adhesion study:** The bio-adhesive probable of the gel was considered in contrast with the promoted clotrimazole gel (Candid-V® gel) by an *in vitro* method

reported by Nakamura *et al.*[11], Candid-V® gel was used for the evaluation. Concisely, an agar plate (1%, w/w) was organized in pH 4.5 citrate phosphate buffer. Test sample, 50mg was located at the middle of plate. After 5 min, the agar plate was devoted to a USP disintegration test device (Fig. 2) and stimulated up and down in pH 4.5 citrate phosphate buffer at 37±1°C. The taster on the plate was engrossed into the solution at the lowermost point and was out of the solution at the uppermost point. The dwelling time of the test illustrations on the plate was distinguished visually.

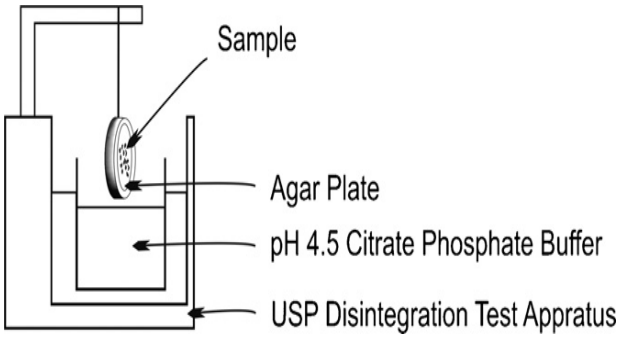

Fig. 2: *In vitro*bioadhesion by USP disintegration test apparatus

Viscosity: Viscosity evaluate flow characteristic of formulations. Alterations in viscosity causes of the product are revealing of modification in constancy or efficiency of the product. Viscosity of Aceclofenac Gel was resolute by Brookfield synchronic viscometer by spindle no 64 at spindle haste 12 rpm at room temperature for 5 min.

Vaginal Irritation Test: The study protocol was approved by the Institutional Animal Ethics Committee. Gels (0.5 g) were useful in to the vagina of the rabbits. After 72 hours, the gel was waged and the subsequent features such as sensitization (hypersensitive response), photosensitization, edema and additional redness were experiential in test animals and in switch by visual examination.

Stability study of optimized formulation: All the certain formulations were exposed to a steadiness challenging for three months as per ICH norms at a temperature of 40ºC ± 2ºC / 75% ± 5% RH. All designated formulations were investigated for the % potency of the formulation.

Evaluation of Anti-Fertility Activity of Vaginal Gels in Rats: The estrus cycle of the female rats was observed. The estrous cycle of a female rat consisting Proestrous, Estrous, Metestrous and Diestrous stages. The animals were considered and the vaginal insults were ready and the female generative sequence was observed initial in the morning, seven existences a week. Few drops of saline were occupied into a dispenser; introduced it into the vagina, enchanting upkeep not to trace the cervix. Saline was barred into the vagina and reserved two or three periods. The fillings of the dispenser were positioned on a microscope slide. The cells (epithelial, cornified, leukocyte) of the slur were experiential by a microscope and the estrous set of the female rat was evaluated. The estrous cycle of the female rat was charted at smallest for two cycles earlier with the animal in the test group.

The vaginal gels were managed intra-vaginally on the twilight among the proestrousphase and the estrous stage; the female rats were reserved for breeding by the male rats since breeding usually happens on the nightly amongst the beings of proestrous and estrous. The females were then remote the subsequent morning. The female rats were then detected for pregnancy and number of disorders after 21 days.

Spermicidal Activity: Spermicidal activity of the organized hydro-alcoholic extract from the roots of *Acacia leucophloea* was also resolute by *in-vitro*, Sander Crammer Control test for influential the spermicidal action.

Semen test of strong male volunteers were used for the study. The samples of motility more than 80% were used for the study. 10 µl of sperm suspension and 10 µl of the hydro-alcoholic extract from the roots of *Acacia leucophloea* (1:1) were located on a glass slide. Then it was diverse consistently and observed below binocular microscope at an exaggeration of 10×45. With the support of stopwatch, the period for the termination of motility of spermatozoa was calculated. The motility of the sperms was experiential at different time pauses.

Spermicidal action of the formulations was conceded out by the *in vitro*, Sander Crammer Control test for decisive the spermicidal activion.

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Results and Discussion: Vaginal has a pH of 4.5-5.5, and vaginal gel is calculated to be in that pH range. The pH of produce can be effect not only the solubility of drugs in the design, but may also move it's possible to

reason skin irritation. Alteration in pH through the self-life of crop may also be revealing of constancy problematic.

The pH of the various gel formularies was gritty by using digital pH meter. 2 gm of gel was enthused in purified water till even suspension forms. The volume was made upto 40 ml and pH dimension was approved out by a standardized digital type pH meter by dropping electrode completely into the gel method so as to shelter the electrodes.

Viscosity is a vital parameter for embodying the gels as it moves the spreadability, extrudability and announcement of drug. The column 4 of Table 1 presented the data of viscosity. The viscosity of gels was enlarged with the rise in carbopol and HPMC gratified which may be owing to the growth in formation of three dimensional cross linking structure of gel, as expected.

The result of vaginal irritation assessment was shown in column 7 of Table 1.All preparations were initiation to be nonirritant to vagina of white rabbits.

Table 1. pH, viscosities, extrudability, spradability and vaginal irritation of *Acacia leucophloea root* Gel formulation

S. No	Formulation	pH	Viscosity (cps)	Extrudability (gm/ cm^2)	spread ability (gm.cm/sec)	Irritation
1.	F1	5.12	82700	15.93	21.88 ± 0.71	-
2.	F2	5.32	84400	16.36	25.85 ± 0.45	-
3.	F3	5.45	92800	15.43	21.43 ± 1.07	-
4.	F4	5.33	93400	17.68	23.91 ± 0.52	-
5.	F5	5.23	56800	15.72	24.51 ± 0.51	-
6.	F6	5.45	66400	16.87	25.13 ± 0.90	-
7.	F7	5.33	72000	16.96	23.65 ± 0.48	-
8.	F8	5.22	73600	16.85	24.53 ± 1.00	-
9.	F9	5.43	78400	15.50	25.13 ± 0.82	-

***In vitro* bioadhesion Study:** The bio-adhesive evaluation of *Acacia leucophloea* gel and profitable preparation (Candid-V® gel) was assessed by *in vitro* process. The preservation times obtainable by *Acacia leucophloea* gel and Candid-V® gel were 475±3.0 and 24±1.5min, correspondingly (n = 3). The retaining period exposed by *Acacia leucophloea* gel was meaning fully advanced as associated to Candid- V® gel. This obviously designates that the *Acacia leucophloea* gel may have advanced house time in vagina as likened to Candid-V® gel. The enlarged bio-adhesivity of *Acacia leucophloea* gel can be credited to the attendance of Carbopol as its bioadhesive possible is uppermost at vaginal pH[13].

Accelerated Stability Studies of Gel: The accelerated steadiness studies were achieved as per ICH guidelines for 12 weeks before starting the accelerated stability study we observed the Precipitation and change in appearance follows in certain of the batches (F3, F4, F5, F6, F8andF9) of polymer based gel comprising *Acacia leucophloea root extract* which might be owing to the unsuitability in the system. Hence, these batches were superfluous and residual batches (F1, F2 and F7) were measured for additional study and %effectiveness of the formulation was experiential.

Table 2: Accelerated stability studies of gels

S.No.	Formulation	% potency of the formulation Periods of study in week						
		1st	2nd	4th	6th	8th	10th	12th
1	F1	99.12	99.00	99.56	99.67	99.32	98.98	99.79
2	F2	100	99.78	99.96	99.90	100	100	99.50
3	F7	98.87	99.53	98.17	98.89	99.61	98.38	98.18

Anti-fertility Activity of Vaginal Gels in Rats: The animals reproduced subsequently management of the vaginal gels. Nobody of the female rats which were directed *Acacia leucophloea* gels brought pups, though completely rats which conventional the *placebo* gels brought pups. Thus, *Acacia leucophloea* vaginal gels presented 100% anti-fertility consequence *in-vivo* in rats.

The spermicidal effect of hydroalcoholic extract of *Acacia leucophloea*was observed 15-120 seconds after mixing. And spermicidal effect of gels was established in the series of 20 seconds to 5 minutes.

Conclusion:-The developed formulation was non-synthetic herbal based anti-fertility compositions having high spermicidal activity for intra-vaginal administration comprising hydro-alcoholic roots extract of *Acacia leucophloea* and pharmaceutically acceptable excipients, in suitably formulated dosage forms for intravaginal administration and a method of contraception in a female subject to prevent pregnancy.

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