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FORMULATION AND EVALUATION OF POLYHERBAL GEL FOR ANTI-INFLAMMATORY ACTIVITY

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Abstract:

Herbal formulations have growing demand in the world market. In the present study, three medicinal plants *Jasmium grandiflorum*, *Cynodon dactylon (L) pers*, and *Andrographis paniculata* having significant anti-inflammatory was selected for formulation as ploy herbal gels. The gels were prepared using the dried ethanolic extract of *Jasminum grandiflorum, Andrographis panculata* and *Cynodon dactylon (L) pers*. Formulation batches i.e F1 and F2 were prepared using gelling agent like carbapol-934 in various concentration. Polyherbal gel formulations were evaluated for its pH, appearance and homogenecity, viscosity, spreadability and skin irritation studies. Assessment of Anti- inflammatory activity was done by carrageenan induced rat paw edema and formalin induced rat paw edema. Polyherbal gels were found to possess anti-inflammatory effect in acute and chronic models.

Polyherbal gel also showed synergistic effect as compared to individual's gels which can be useful for the treatment of local inflammation.

Keywords:*Jasminum grandiflorum Linn*(JGL), *Andrographis paniculata Linn*(APL), and *Cynodon dactylon* (L) pers(CDL), polyherbal gel, anti-inflammatory activity.

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Introduction:

Herbal medicine is the study of pharmacognosy and the use of medicinal plants, which are the basis of traditional medicine. The major use of herbal medicine is for health promotion.

World Health Organisation (WHO) has defined herbal are finished and labeled medicinal product that contain active ingredients, aerial and underground parts of the plants or other plant material or combination. Herbal medicines are still the mainstay of about 75-80% of the world population. Health medicines consist of plant or its part to treat injuries, disease or illnesses and are used to prevent and treat diseases. Herbal medicines are the oldest from of health care known to mankind.

The aim must be to link every proprietary product and every formulation with a definite concept of the plant on which it is based, not only as regards its actions, but also the medicinal plant has a specific image, and knowledge is required of the plant drug and its uses. The literature survey revealed that various plants scattered throughout the plant kingdom exhibit anti-inflammatory activity. Few well known examples are *Acacia nilotica, Withania somnifera, Glycyrrihza glabra, Boswelia serrata, Phyllanthus amarus, Eclipta Alba* etc. which contain flavonoids and are reported for their anti-inflammatory activity. The plant selected for presented work are Andrographis paniculata, Jasminum grandiflorum, Cynodon dactylon which contain high percentage of glycosides, flavonoids and glycosides and other components responsible for anti-inflammatory activity.[3] Thus, an attempt was made to study anti-inflammatory activity of individual combination of extract in a single dosage form which may show synergetic anti-inflammatory activity.

Gel formulations are used to deliver the drugs topically because of easy application, increase contact time and minimum side effects as compare to other topical preparation and oral administration.[1]

Traditionally, andrographis has been used for liver complaints and fever, and as an anti-inflammatory and immune modulator. In clinical trials, andrographis extract has been studied for use as an immuno-stimulant in upper respiratory tract infections and HIV infection.[4]

The plant jasminum grandiflorum leaves useful in fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, ottorhoea, otalgia, strangury, dysmenorrhoea, ulcers, wound and corns. The plant cynodon dactylon has been long used in the traditional medicines to treat various ailments such as anascra, cancer, convulsions, cough, cramps, diarrhea, dropsy, dysentery, epilepsy, headache, hemorrhage, hypertension, hysteria, measles, rubella, snakebite, sores, stones, tumours, urogenital disorders, warts and wounds.[2]

The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction, prompted us to investigate and evaluate anti- inflammatory potential of Cynodon dactylon along with Andrographis paniculata and Jasminum grandiflorum by incorporating into polyherbal topical gel and assessing its anti- inflammatory activity. An attempt will be made to find out synergistic activity by combination of the extracts.

Experimental:

Collection of plant material:The leaves were collected from the home garden of Koradi village, Kamptee dist., Nagpur, India. During the month of December 2020. The aerial part of Cynodon dactylon

was collected from medicinal plant garden of Kamla Nehru College of Pharmacy, Butibori Nagpur and authenticated by R.T.M. Nagpur University, Nagpur.

Authentication of plants:-

The plant was identified and authenticated by Dr. N.M. Dongarwar, Associate professor at Botany, Department of Rashtrasant Tukdoji Maharaj Nagpur University.

Figure 1: Andrographis paniculata Nees

Figure 2: Cynodon dactylon (L), pers

Figure 3: Jasminum grandiflorum Linn

Preparation of extract: The collected fresh leaves of Andrographis paniculata, jasminum grandiflorum and aerial part of Cynodon dactylon were dried in hot air oven at 400^0C to avoid degradation of phytoconstituents. After drying, the plant materials were coarsely powdered and kept in well closed container. About 185gm, 100gm and 125gm powder of Andrographis paniculata, jasminum grandiflorum and aerial part of Cynodon dactylon respectively were defatted with Pet. Ether (60-800) in soxhlet apparatus. After defatting, it was further extracted with methanol. The collected extracts were concentrated by distillation to recover the solvent. Concentrated extracts were kept in desiccators till further used.

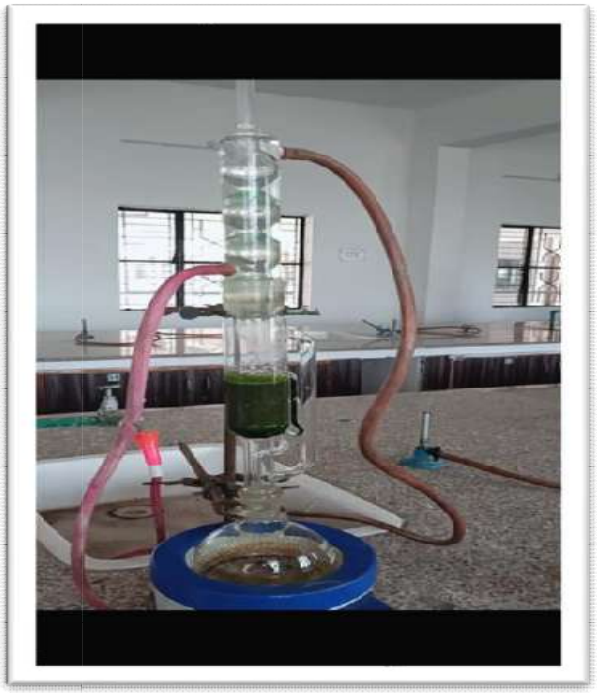

Figure 4: Soxhlet extraction

Preparation of polyherbal gel:

Herbal gel was prepared using carbopol-934 as a gelling agent in 1% w/w concentration with deionized water using mechanical stirrer, add Triethanlamine in it. Prepared gel base by mixing of methyl paraben, prolyl paraben and propylene glycol 400. Then the herbal extract of APL, JGL and CYL was added to the gel and mixing of extract in gel base. Prepared gel was filled in collapsible tubes and stored at a cool and dry place. Gels of individual plant extracts as well as polyherbal gels were prepared. The same procedure was used for preparation of Diclofenac sodium gel as a standard.[5]

Table 1: Composition of different formulation of polyherbal gel

Ingredients (quantity in %)	F1	F2	F3
EEAP	1	1	2
EEJG	1	2	1
EECY	2	1	1
Methylparaben	2	2	2
Propylparaben	2	2	2
Propylen glycol 400	1	1	1
Carbapol-934	1	1	1
Triethanolamine	q.s	q.s	q.s
Distilled water	q.s	q.s	q.s

Evaluation of polyherbal gel:

pH:

pH of individual and polyherbal gel formulation was determined by using a pH meter.

Appearance and Homogeneity:

The developed individual and polyherbal gels were evaluated for physical appearance and homogeneity by visual observation.

Viscosity:

The viscosity of individual and polyherbal gels was measured by Brookfield viscometer (Model RVTDV II) at 100 rpm using spindle no. 6.

Spreadability:

The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20cm x 20cm) after one min. The standard weight applied on the upper plate was 125gm.

Spreadability was calculated using the following formula:- S= M × L/ T Where,

S= Spreadability,

M= weight in the pan (tied to upper slide),

L= Length moved by the slide,

T= Time (in sec.)

Skin irritation studies:

The wistar rats of either sex weighing 150-200gm were used for skin irritation studies. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gels containing extracts were used on test animal. Gel base was applied on the back of animal taken as control. The animals were treated daily upto seven days and finally the treated skin was examined visually for erythema and edema.[6]

Extrudability:

The gel formulations were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 0.5gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good,>70% extrudability: fair).

Stability study:

The stability of gels was assessed using the ICH guidelines

Primary Dermal Irritation Index (PDII):

Dermal irritation is the production of reversible damage to the skin following the application of a test substance for up to 4 hours. Primary dermal irritation index (PDII) is a method for classifying topical formulations into various categories based on acute toxic reactions observed upon single application of a formulation on skin. Based on the PDII score, the formulation can be graded as irritating or non-irritating.[7]

Primary Dermal Irritation Index (PDII) = PDII observed on 12 + 24 + 48 + 72 hrs.

***In vitro* diffusion study**:

The diffusion studies of the prepared gels were carried out in franz diffusion cell for studying the dissolution release of gels through a cellophane membrane. Gel sample (1gm) was taken in cellophane membrane and the diffusion studies carried out at 37±1º c using distilled water as dissolution medium. Five millilitres of each sample was withdrawn periodically at 1,2,3,4,5,6,7,8,9,10,11,12 and 24 h and each sample was replaced with equal volume of dissolution medium. The samples were analysed for the drug content by using distilled water as blank.[8]

Swelling index:

To determine the swelling index of prepared topical gel, 1 gm of gel was taken on petri dish and then placed separately in a 50 ml beaker containing 10 ml distilled water. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows

Swelling Index[9,10] (SW) % = [(Wt – Wo) / Wo] × 100

Where, (SW) % = Equilibrium percent swelling, Wt = Weight of swollen gel after time t, Wo = Original weight of gel at zero time.

Grittiness:

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence obviously the gel preparation fulfils the requirement of freedom from particular matter and form grittiness as desired for any topical preparation.[11]

Drug content:

Drug concentration in polyherbal gel was measured by UV spectrophotometer. Drug content was measured by dissolving specific quantity of polyherbal gel in solvent with the help of Sonication. Absorbance was measured after suitable dilution at 242 nm in UV/ VIS spectrophotometer.

Pharmacological Studies:

Chronic toxicity studies:

Half a gram of the herbal gel, as the test substance, was applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a suitable semi- occlusive dressing for 4 hours and was then removed. At the end of the exposure period, i.e. 4 hours, residual test substance was removed without altering the existing response or the integrity of the epidermis. Observations were recorded an hour after the removal of the patch. Control animals (rat) were prepared in the same manner and 0.5 gm of the gel base, i.e. gel formulated using all the ingredients except the herbal mixture, was applied to the control animals and observations were made similar to the test animals (rat). Both the control and the test animals were observed every day for any occurrence of skin irritation or toxic reactions such as edema or erythema. The skinirritation was scored between 0 and 4 where 0 means no skin erythema and eschar formation and 1, 2, 3 and 4 stood for very slight, well defined, moderate and severe erythma to eschar formation, respectively. It also scored from 0–4, where 0 stood for no edema and 4 stood for severe edema. .

Assessment of Anti-inflammatory activity:

Both in-vivo and in-vitro methods are available for the evaluation of anti-inflammatory agents but among the in-vivo methods the carrageenan induced paw edema method is widely used for acute anti-inflammatory study. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, Chondrous crispus. The edema, which develops in rats paw after carrageenan injection, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin, the edema maintained between the 1st and 2nd phase is attributed to the release of kinin like substances and the 2nd phase is attributed to the release of prostaglandins like compound.[12,13]

Animals:

The Wister rats weighing between 150-200gm were procured from Animal house of Kamla Nehru College of Pharmacy, Nagpur, and maintained under constant conditions (temperature 25± 2^0C, Humidity 40-60%, 12 h light/ 12 h dark cycle). During maintenance the animals received a diet of food pellet supplied from animal house and water ad libitum.

Carrageenan induced rat paw edema:

Pedal inflammation in animal was produced according to the method described by Winter et al (1962). Rats were divided in 11 groups of six rats in each. Group I- was applied with gel base and served as control. Group II- standard (Diclofenac sodium Gel 0.5%) and served as reference. Group III - IX application of 1gm of 1%, 2% and 4%

gel of Andrographis paniculata, jasminum grandiflorum and Cynodon dactylon respectively, Group X - XI application of 1.0 gm and 0.5 gm of polyherbal gel respectively. The edema was induced by injecting 0.1 ml of carrageenan (1% w/v) in normal saline into the sub planter region of the left hind paw, after 1 hour of drug application. Paw thickness was measured with the help of Digital Vernier caliper at 0, 30, 60, 120, 180, 240 and 300 min after administration of carrageenan.[14,15]

Formalin- induced rat paw edema:

The formalin-induced rat paw edema model was used for acute as well as chronic inflammation on the basis of formalin concentration. For chronic model 2% of formalin in saline was used. Formalin-induced edema is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandin are known to be involved.

The % inhibition of edema was calculated by formula:

% Inhibition = 1- {a-x/b-y} x 100 where,

a= paw thickness of test animal after treatment x= initial paw thickness of test animal

b= paw thickness of control animal after treatment y= initial paw thickness of control animal.

Table 2:Evaluation of prepared polyherbal gel

Parameter	Diclofenac gel	F1	F2	F3
pH	6.3	6.5	6.8	7
SViscosity	4520	4260	4300	4500
Appearance	White	Pale green	Pale green	Pale green
Spreadability	28.33	24.38	24.83	22.35
Homogenecity	Good	Good	Good	Good
Extrudability	13.1	12.9	13.42	13.15
Swelling index	3.5	3.7	4.1	4.2

Result and discussion:

The polyherbal gels were prepared and evaluated for anti-inflammatory activity by using carrageenan-induced rat paw edema and formalin-induced rat paw edema topically. It was evident that carrageenan-induced edema was commonly used as an experimental in vivo model for evaluating the anti-inflammatory potential of plant extracts and was believed to be biphasic. The early phase observed after 1 hr is related to the production of 5-hydroxytryptamin, histamine, bradykinin and cyclooxygenase products and the late phase is due to neutrophil infiltration as well as continuous production of arachidonic acid metabolites.

The later phase is reported to be sensitive to the most of the clinically effective anti-inflammatory agents. Statistical analysis showed that the edema inhibition by formulation containing extracts were significantly differing from control group at all the concentration tested. The results showed that the anti-inflammatory effect of the formulation containing 3% of A.paniculata gel was better than the effect of standard gel formulation. In the individual formulation of various concentration of plant extracts (1%, 2% and 3%), 3% gel of A. paniculata showed significant inhibition (82.57%) of paw edema in rats comparable to standard Diclofenac gel (0.5%) (78.65%) at 300 min after carrageenan injection.

In combination, formulation applied half dose of individual formulation (0.5 gm) showed synergistic effect (77.22%).The highest inhibition was found at 300 minutes

post carrageenan injection, which is supposed to be due to inhibition of late phase mediators, arachidonic acid product and prostaglandins, of acute inflammation induced by carrageenan (Figure 5).

Formalin-induced rat paw edema model was used for acute as well as chronic inflammation on the basis of formalin concentration. For chronic model, 2% of formalin in saline is used. Formalin-induced edema is biphasic. An early neurogenic component is mediated by substance P and bradykinin followed by a tissuemediated response where histamine, 5-HT, prostaglandin are known to be involved. Statistical analysis showed that the edema inhibition by formulation containing extracts were significantly differing from control group at all the concentration tested.

As compared to acute inflammation, in chronic model, it showed no significant result as compared to standard. In the individual formulation of various concentration,4% gel of A. paniculata formulation showed inhibition (29.30%) of paw edema in rats comparable to standard drug diclofenac gel 0.5% (45.88%), after 9day (Figure 6).

The results showed that the anti-inflammatory effect of the combination formulation applied half dose of individual formulation (0.5 gm) showed equivalent to the effect of standard gel formulation. Also the combination formulation showed synergistic effect (40.21%) as compared to individual formulation. From these results, it is evident that an individual and polyherbal gel of A. paniculata, J. grandiflorum and C.dactylon possesses anti-inflammatory effect in both acute and chronic model.

Moreover polyherbal gel showed synergistic effect as compared to individual gels which can be useful for the treatment of local inflammation.

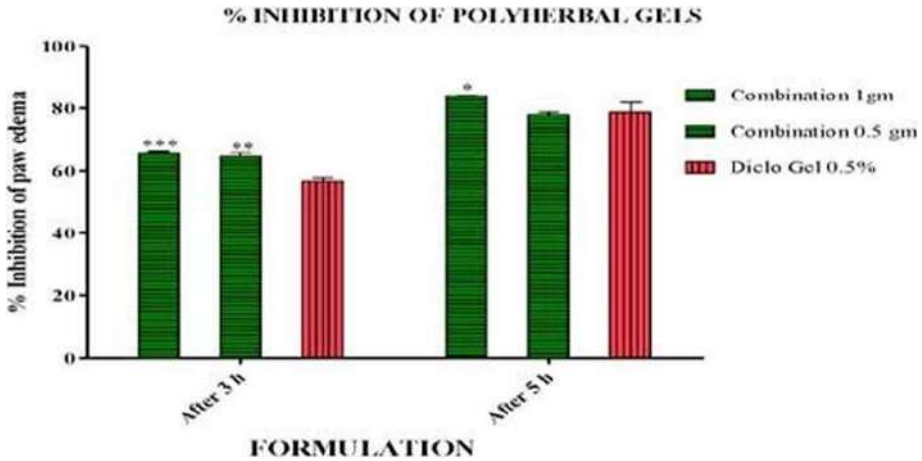

Figure 5: ANTI-INFLAMMATORY EFFECT OF TOPICAL APPLICATION OF POLYHERBAL GELS IN COMBINATION ON THE FIRST PHASE AND LATE PHASE OF CARRAGEENAN-INDUCED PAW

Edema in rat values represent the % mean vs. std value *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.p value calculated by comparing with std by Two-way Anova followed by bonferroni post test.

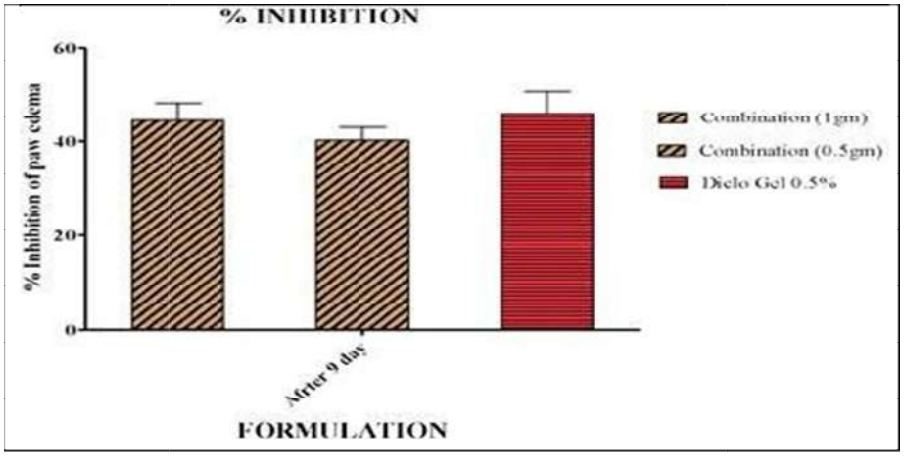

Figure 6: Anti-inflammatory effect of topical application of polyherbal gel

Combination on the late phase of formalin-induced paw edema in rat. Values represent the % mean vs. std value *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. p value calculated by comparing with std by One-way Anova followed by Bonferroni post-test.

Conclusion:

On the basis of the study, the data showed that the polyherbal gels prepared from the dried ethanolic extracts Andrographis paniculata Linn., Jasminum grandiflorum Linn. and Cynodon dactylon (L.) Pers. As phytochemical tests showed the presence of glycosides, carbohydrates, flavonoids, steroids and resin in the methanolic extracts.So many years, The Ayurvedic medicine is very useful for various ailments. The present study deals with referentially information for formulation, identification and for the physical and chemical evaluation of the polyherbal gel. Andrographis paniculata Linn, Jasminum grandiflorum Linn, and Cynodon dactylon linn, pers of the easily available and more economical drugs. Result shows that the polyherbal gel formulation are good in appearance, homogenecity, extrudability, and spreadabilityderivative.(Figure 5 and 6)

List of abbreviation: EEAP-Ethanolic extract of Andrographis paniculata, EEJG-Ethanolic extract of Jasminum grandiflorum, EECY-Ethanolic extract of Cynodon dactylon.

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