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FORMULATION AND EVALUATION OF POLYHERBAL GEL FOR ANTI - INFLAMMATORY ACTIVITY

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ABSTRACT: In the present study, three medicinal plants *Cynodon dactylon* (L.) Pers*, Cassia tora* Linn. and *Cassia alata* Linn having significant anti-inflammatory potential were selected to be formulated as polyherbal gels. The gels were prepared using the dried methanolic extract of *Cassia tora* Linn*, Cassia alata* Linn and *Cynodon dactylon* (L.) Pers. Polyherbal gel formulations were evaluated for its pH, appearance and homogeneity, 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. Individual and polyherbal gel of *Cassia alata* Linn*,Cassia tora* Linn. and *Cynodon dactylon* (L.) Pers were found to possess anti-inflammatory effect in acute and chronic models. Polyherbal gel also showed synergistic effect as compared to individual gels which can be useful for the treatment of local inflammation.

INTRODUCTION: Traditional medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for health care. A large number of Indian medicinal plants are attributed with various pharmacological activities as they contain diversified classes of photochemical. The opoids or non-steroidal anti-inflammatory drugs, widely used to reduce the inflammation of various types, suffer from severe side effects like redness, itching etc. As a result, a search for other alternatives seems to be necessary which would be more beneficial. 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, Glycyrrhiza glabra, Boswelia serrata, Phyllanthus amarus, Eclipta alba* etc. which contain flavonoids and are reported for their anti-inflammatory activity. The plants selected for present work are *Cynodon dactylon, Cassia tora* and *Cassia alata* which contain high percentage of flavonoids and other component responsible for anti-inflammatory activity. Thus, an attempt was made to study the anti-inflammatory activity of individual as well as combination of extracts in a single dosage form which may show synergistic anti-inflammatory activity [1].

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

The plant *Cassia tora* and *Cassia alata* Linn has been found to be used traditionally for its various therapeutic properties like, anticancer activity [2], oral anti-inflammatory activity [3, 4], antibacterial activity [5], antioxidant activity [6], skin disorder and wound-healing activity [7]. The plant *Cynodon dactylon* has been found to be used traditionally for various therapeutic properties like, antiviral activity [8], antidiabetic activity [9], antifungal activity [10], antibacterial activity [11], antioxidant activity [12], antiulcer activity [13], skin disorder and wound-healing activity [14, 15].

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 *Cassia tora* and *Cassia alata* 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 [16].

EXPERIMENTAL:

Collection of Plant Material: The leaves of *Cassia tora* and *Cassia alata* were collected from Hingna, MIDC area, of Nagpur. The aerial part of *Cynodon dactylon* was collected from medicinal plant garden of J. L. Chaturvedi college of Pharmacy, Nagpur and identified by Department of Botany, R.T.M. Nagpur University, Nagpur. (Voucher specimens 9549, 9550 and 9551).

Preparation of Extract: The collected fresh leaves of *Cassia alata, Cassia tora* and aerial part of *Cynodon dactylon* were dried in hot air oven at 40^0 C to avoid degradation of phytoconstituents. After drying, the plant materials were coarsely powdered with Willy mill and kept in well closed container. About 185 gm, 100 gm and 125 gm powder of *Cassia alata, Cassia tora* and *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

Preparation of Polyherbal Gel [17-22]: The gel was prepared using the dried methanolic extract of *Cassia tora* Linn., *Cassia alata* Linn. and *Cynodon dactylon* (L.) Pers and using Carbopol-940 (1%) as a gelling agent. 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 (**Table 1**).

TABLE 1: COMPOSITION OF POLYHERBAL GEL

Formulation	Carbapol-940 (%)	Extract (%)	Propylene glycol (%)	Ethanol (%)	Methyl paraben (%)	Propyl paraben (%)	EDTA (%)
C.A., C.T., C.D. Gel 1%	1	1	4	3	0.2	0.02	0.03
C.A., C.T., C.D. Gel 2%	1	2	4	3	0.2	0.02	0.03
C.A., C.T., C.D. Gel 4%	1	4	4	3	0.2	0.02	0.03
Combination	1	6	4	3	0.2	0.02	0.03

Evaluation:

1. **pH:** pH of individual and polyherbal gel formulation was determined by using a pH meter (**Table 2**).
2. **Appearance and Homogeneity:** The developed individual and polyherbal gels were evaluated for physical appearance and homogeneity by visual observation (Table 2).
3. **Viscosity:** The viscosity of individual and polyherbal gels was measured by Brookfield viscometer (Model RVTDV II) at 100 rpm using spindle no. 6 (Table 2).
4. **Spreadability:** The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 gm (Table 2).
5. **Skin irritation studies:** The wistar rats of either sex weighing 150-200 gm 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 aluminum 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. 0.5 gm 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).

TABLE 2: EVALUATION PARAMETERS OF PREPARED POLYHERBAL GELS

Formulation	pH	Appearance	Homogeneity	Spreading diameter after 1 min (mm)	Viscosity (cp)
Diclofenac gel	6.15	White	Good	55	4500
C.A. 1%,	6.35	Light green	Good	48	4700
C.T. 1%	6.30	Light green	Good	42	4700
C.D 1%	6.31	Light green	Good	44	4800
C.A. 2%	6.45	Light green	Good	42	4700
C.T. 2%	6.50	Light green	Good	40	4700
C.D. 2%	6.49	Light green	Good	42	4800
C.A. 4%	6.45	Light green	Good	40	4700
C.T. 4%	6.46	Light green	Good	39	4800
C.D. 4%	6.48	Light green	Good	41	4800
Combination	6.98	Light green	Good	38	4900

7. **Stability study:** The stability of gels was assessed using the ICH guidelines

8. **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.

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

9. **Pharmacological Studies:**

a. **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 skin irritation 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. Primary Dermal Irritation Index (PDII) = PDII observed on 12+24+48+72 hrs 4.

b. **Assessment of Anti-inflammatory activity** [23, 24]**:** 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.

- **Animals:** The Wister rats weighing between 150-200 gm were procured from Animal house of J. L. Chaturvedi College of Pharmacy, R.T.M.

Nagpur University, Nagpur, and maintained under constant conditions (temperature 25± 2C, 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*. These experiments were approved by the Institutional Animal Ethics Committee, R.T.M. Nagpur University, Nagpur (approval no. 648/02/6/CPCSEA).

- **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 1 gm of 1%, 2%, 4% gel of *Cassia alata*, *Cassia tora* 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 (**Fig. 1 and Fig. 2**).

- **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. (Fig 3 and Fig 4)

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.

RESULTS 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 4% of *C. alata* gel was better than the effect of standard gel formulation. In the individual formulation of various concentration of plant extracts (1%, 2% and 4%), 4% gel of *C. alata* 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 1**).

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 tissue

mediated 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 *C. alata* formulation showed inhibition (29.30%) of paw edema in rats comparable to standard drug diclofenac gel 0.5% (45.88%), after 9 day (**Figure 2**).

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 *C. alata*, *C. tora* 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 3 and 4**).

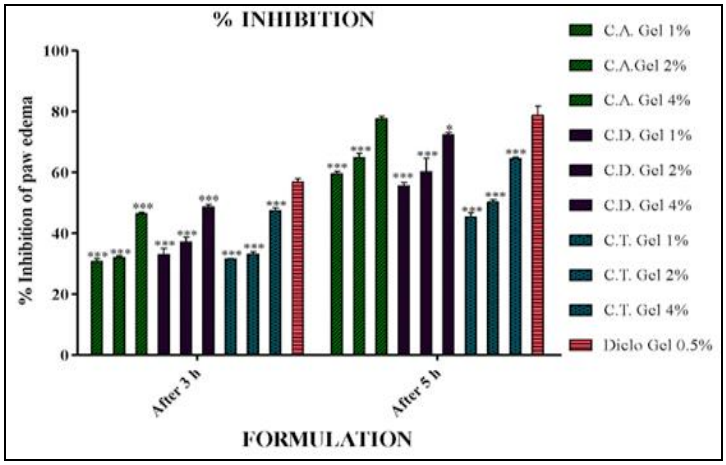

FIGURE 1: ANTIINFLAMMATORY EFFECT OF TOPICAL APPLICATION OF GEL OF *C. ALATA, C. TORA* AND *C. DACTYLON* ON THE FIRST PHASE AND LATE PHASE OF CARRAGEENAN - INDUCED PAW EDEMA IN RAT. values represent the % mean vs. std value. *** p < 0.001, ** p < 0.01, * p < 0.05. p value calculated by comparing with std by two-way anova followed by Bonferroni post test.

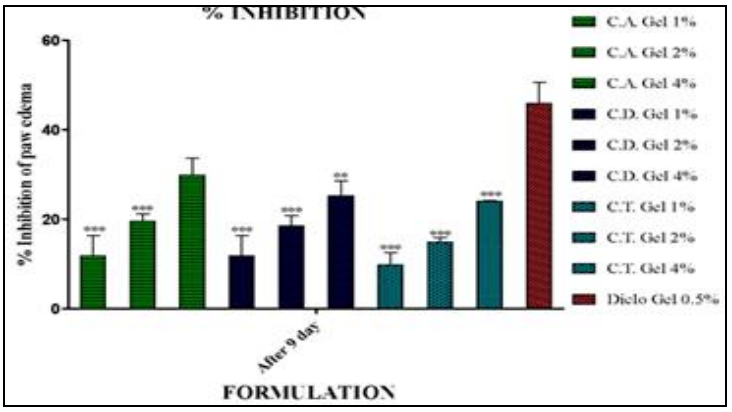

FIGURE 2: ANTIINFLAMMATORY EFFECT OF TOPICAL APPLICATION OF GEL FORMULATIONS ON THE LATE PHASE OF FORMALIN–INDUCED PAW EDEMA IN RAT. Values represent the % mean vs. std value. *** p < 0.001, ** p < 0.01, * p < 0.05.p value calculated by comparing with std by One-way Anova followed by Bonferroni post test.

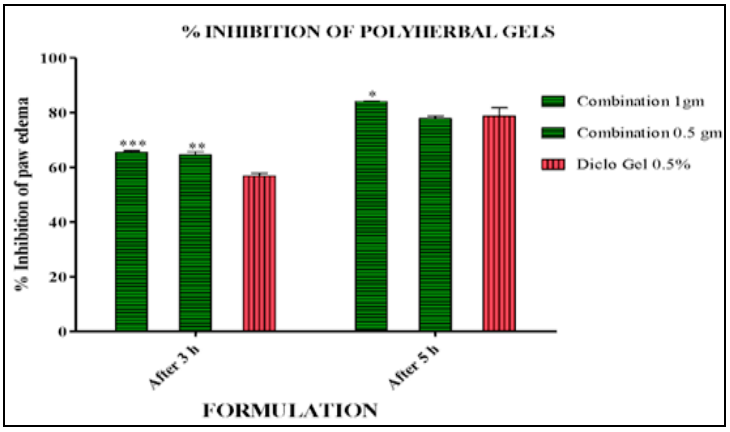

FIGURE 3: ANTIINFLAMMATORY EFFECT OF TOPICAL APPLICATION OF POLYHERBAL GELS IN COMBINATION ON THE FIRST PHASE AND LATE PHASE OF CARRAGEENAN-INDUCED PAW EDEMA IN RAT. Values represent the % mean vs. std value *** p < 0.001, ** p < 0.01, * p < 0.05.p value calculated by comparing with std by Two-way Anova followed by bonferroni post test.

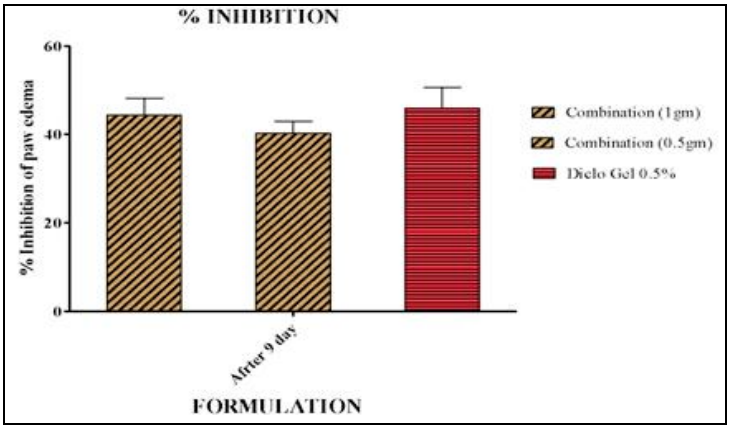

FIGURE 4: ANTI INFLAMMATORY EFFECT OF TOPICAL APPLICATION OF POLYHERBAL GELS COMBINATION ON THE LATE PHASE OF FORMALIN-INDUCED PAW EDEMA IN RAT. Values represent the % mean vs. std value *** p < 0.001, ** p < 0.01, * p < 0.05. p value calculated by comparing with std by One-way Anova followed by Bonferroni post test.

CONCLUSION: On the basis of the study, the data showed that the polyherbal gels prepared from the dried methanolic extracts *Cassia tora* Linn*., Cassia alata* Linn. and *Cynodon dactylon* (L.) Pers.f gave the significant anti-inflammatory activity when compared with standard Diclofenac gel. As phytochemical tests showed the presence of glycosides, carbohydrates, flavonoids, steroids and resin in the methanolic extracts they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exert its activity. The polyherbal gels showed synergistic effect as compared to individual gels which can be useful for the treatment of local inflammation.

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