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FORMULATION AND EVALUATION OF A HERBAL ANTI INFLAMMATORY GEL CONTAINING *TAMARIDUS INDICA* LEAVES EXTRACT

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ABSTRACT

Tamarindus indica L., (Tamarind), family, Leguminosae, is tropical plant used in the indigenous system of medicine for the treatment of various ailments**.** The warm leaf decoction has been used to reduce the swelling in the joints by dipping the legs or hands in it and is more convenient to design a transdermal formulation, which deliver medication to systemic circulation effectively. The study was aimed to develop a herbal topical gel containing *Tamarindus indica* leaf extract using carbopol-940 as gelling agents and to investigate the anti-inflammatory activity of suitable gel formulation. Gels were prepared using carbopol-940 at different concentration (1%, 1.5% and 2%). Prepared formulations were evaluated for various physicochemical properties like appearance, pH, spreadability and viscosity. Based on *in vitro* permeation study, the best gel formulation was chosen and it was subjected to *in vitro* anti-inflammatory activity studies and skin irritation studies and kept for stability studies for a period of three months. It was inferred from the result that gel formulation was good in appearance and homogeneity. The value of spreadability indicated that these gels were easily spreadable by small amount of shear. The preparation was stable under normal storage conditions and complies with skin irritation test. The *in-vitro* anti inflammatory studies showed that the potency of the extract was retained while formulating the gel and have a significant activity when compared to the standard drug (Diclofenac) and its gel. Hence the newly formulated gel (TF2) can be considered as an anti-inflammatory gel. future in-vivo studies should be perform to confirm the results obtained and utilize the formulation for health care needs.

Key Words: *Tamarindus indica*, Anti-inflammatory activity, carbopol-940.

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INTRODUCTION

Preparations from plant origin become important in modern medicine. According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Traditionally, the use of plant preparations as Constituent or due to blend of constituents. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The studies mainly focused towards Transdermal drug administration, which deliver certain medication to systemic circulation in a more convenient and effective way than with conventional dosage form. TDDS can minimize first-pass metabolism associated with gastro-intestinal administration of drugs and maintain constant drug level in blood. *Tamarindus*

indica L., (Tamarind), family, Leguminosae, is tropical plant used in the indigenous system of medicine for the treatment of various ailments traditionally, the warm leaf decoction has been used to reduce the swelling in the joints by dipping the legs or hands in it. Certain studies showed that the aqueous extract of *Tamarindus indica L*. leaves contains some pharmacologically active substances, was moderately toxic and possessed significant anti-inflammatory and antinociceptive acivities (1). Here an attempt was made to utilize this property of the plant and to develop a new, effective and safe anti-inflammatory gel which can reduce swelling and other associated symptoms.

MATERIALS AND METHODS

Preparation of the plant extract

The freshly collected fresh mature leaves of *Tamarindus indica L* were pulverized and shade dried at room temperature to constant weight for (10) days. The leaf powder was subjected to soxhlet extraction using distilled water. The extract obtained was concentrated, evaporated and the residue was kept in desicator.

Preparation of topical gels (2)

The gel was prepared using the aqueous extract of *Tamaridus indica*. The gel was prepared using Carbapol-940 (1%, 1.5%&2%), propylene glycol 400, ethanol, methyl paraben, propyl paraben, EDTA, tri-ethanolamine and distilled water in a quantity sufficient to prepare 100 g of gel in case of blank gel.

Water required for these formulations was divided in to two parts. In one part the exact amount of extract was dissolved and to this calculated quantity of propylene glycol 400 and ethanol was added and in other part, carbapol-940 was dissolved and to this solution methyl paraben, propyl paraben and EDTA was added. Both of these solutions were mixed in a beaker and tri-ethanolamine was added to the mixture drop wise to obtain the gel consistency.

Table-1 Composition of gel formulation

Formulation Code	Drug (%)	Carbapol 940 (%)	Ethanol (%)	Propylene glycol (%)	Methyl Paraben (%)	Propyl Paraben (%)	EDTA (%)	Water (%)
TF1	1	1	3	4	0.2	0.02	0.03	Upto100g
TF2	1	1.5	3	4	0.2	0.02	0.03	Upto 100g
TF3	1	2	3	4	0.2	0.02	0.03	Upto 100g

Evaluation of Herbal Gel (3)

Appearance and homogeneity-All developed gels were tested for physical appearance and homogeneity by visual observation

Viscosity-Viscosity of gel was measured by using Brookfield viscometer using spindle no.64 at 30 rpm

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. 500 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).

Spreadability (4) -Spreadability of the formulation was determined by using an apparatus designed and developed in the pharmaceutics laboratory. Two rectangular glass plates of standard dimension were selected. 500mg of sample was placed on one of the glass plate. Second plate was placed over the other one to sandwich sample between plates. A 20gm weight was placed on the top of upper plate to provide a uniform thin film of the sample between the plates. Weight was removed; excess of the ointment sample was scrapped off from the edges. The top plate was then subjected to pull by using string to which 20gm weight was applied. The time required by the upper plate to travel a distance of 6 cm and separate from the lower plate was noted. Spreadability was calculated using formula

$$S = M. L / T$$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

Drug content (5)-A 100mg of the gel was dissolved in 100 ml of phosphate buffer pH 6.8 .The volumetric flask containing gel solution was shaken for 2hr on a mechanical shaker to allow the drug to dissolve

completely. The solution was filtered and the drug content was determined spectrophotometrically at 275 nm using phosphate buffer (pH 6.8) as blank.

In Vitro **Release (6)-**The in vitro release experiments were carried out by using Franz-diffusion cells apparatus from different formulations. An exact amount of formulations (1.0 g) was spread out on membrane positioned between the donor and receptor chambers with an available diffusion area. The receptor compartment was filled with phosphate buffer pH 6.8 and continuously stirred with a small magnetic bar at a speed of 50 rpm during the experiments to ensure homogeneity and maintained at 37.2±0.5^{0}C. The samples were withdrawn at various time intervals and replaced with the same volume of PBS. Sink conditions were met in all cases. The samples were analyzed spectrophotometrically at 275 nm.

Skin irritation test-The albino mice of either sex weighing 20-22gms were used for this test. The intact skin was used. The hair was removed from the mice 3 days before the experiment. The animals were divided into two batches and each batch was again divided into two groups. The gel containing drug was used on test animal. A piece of cotton wool soaked in saturated drug solution was placed on the back of albino mice taken as control. The animals were treated daily up to seven days and finally the treated skin was examined visually for erythema and edema.

In-Vitro **Anti- Inflammatory studies (7-9)**

Protein Denaturation-5ml 0.2% w/v bovine serum albumin in Tris HCl buffer saline and different concentrations of extracts in methanol were taken in test tubes and heated at 72°C for 5 minutes, cooled for 10 minutes. The absorbance of these solutions was determined at 660nm. The experiment repeated with standard (diclofenac)also. The IC 50 was calculated and compared with standard**.**

Proteinase Inhibitory Action-The reaction mixtures (2ml) contained 0.06 mg trypsin, 1ml 25mM Tris HCl buffer (pH7.4) and 1ml aqueous solution of plant extracts of different concentration (100,200,300,400,500µg/ml). The mixtures were incubated at 37ºC for 5min. Then 1ml of 0.8%(w/v) casein was added. The mixtures were incubated for an additional 20 minutes. Then 2ml of 70 %(w/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280nm against buffer as blank. IC 50 was calculated and compared with standard (diclofenac).The same procedure is repeated for selected gel formulation TF2 and standard diclofenac marketed gel. Here specified amount of gel which was equivalent to 1mg/ml of extract were taken and dissolved in distilled water and used for anti inflammatory studies**.**

Stability study-The stability study was performed as per ICH guidelines. The formulated gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, *viz*. 25±2 ºC / 60±5% RH, 30±2 ºC / 65±5% RH, 40±2 ºC / 75±5% RH for a period of three months and studied for viscosity, drug content and physical appearance.

RESULTS AND DISCUSSION

The dried powder of leaves was extracted with water. Herba gel of aqueous extract T.indica was prepared using carbapol 940 as gelling agent at different concentration. Composition of herbal gel is shown in table-1. The physico chemical properties of gel formulation are shown in table-2. From the result it is concluded that all gel formulation showed god appearance and homogeneity. The physical appearance of gel was green in nature. All preparations were easily spreadable, with acceptable bio adhesion and fair mechanical properties. The pH values ranged from 6.52 to7.26, which are considered acceptable to avoid the risk of irritation after skin application. Viscosity is an important physical property of topical formulations, which affects the rate of drug release; in general, an increase of the viscosity vehicles would cause a more rigid structure with a consequent decrease of the rate of drug release. Viscosity increased from 3245.31 cPs to 4208.35cPs as polymer concentration increased. Increased consistency was ascribed to enhanced polymeric entanglements, thereby increasing the resistance to deformation.

Table-2 Physico chemical properties of gel formulation

Formulation	Homogeneity	pH	Extrudability	Drug content (%)	Spreadability (gm, cm/sec)	Viscosity (cps)
TF1	**	6.81	**	94.6	31.87	3245.31
TF2	***	6.52	***	95.9	21.34	3789.28
TF3	**	7.26	**	97.2	17.61	4208.35

Note- Excellent*, Good**, Satisfactory ***

In vitro **dissolution** profile of T. indica gels containing different concentration of carbopol and marketed formulation (dicloenac gel MFG) are shown in table-3 and fig-1. Release profiles of various gel formulations across the cellulose membrane deplicated that drug release decrease with increase in concentration of the gelling agent. The drug release values were also found lower for the formulation in which polymer concentration was kept high (Table 3). Viscosity is negatively related to the release of active substance from formulations and its penetration through the diffusion barriers. The decrease in the release could be attributed to increased micro viscosity of the gel by increasing polymer concentration. Thus, both high concentration of polymer and high viscosity compete each other in decreasing the release of active substance from the formulation. Formulation TF2 has shown a better release pattern compared with other formulation. Thus considering all the result of evaluation studies TF2 was selected as best formulation and was taken for further studies.

Table-3 Cumulative % Drug release from gel formulations at different time intervals

Time (min)	% Drug diffused			
	TF1	**TF2**	**TF3**	**MFG (Diclofenac gel)**
0	0	0	0	0
15	13.09	39.76	24.64	35.59
30	27.97	44.16	37.26	47.38
45	35.83	50.95	57.27	51.83
60	56.07	68.93	66.78	63.93
75	71.59	75.21	74.52	73.92
90	85.95	89.04	84.76	82.38
120	90.10	96.76	88.93	94.04

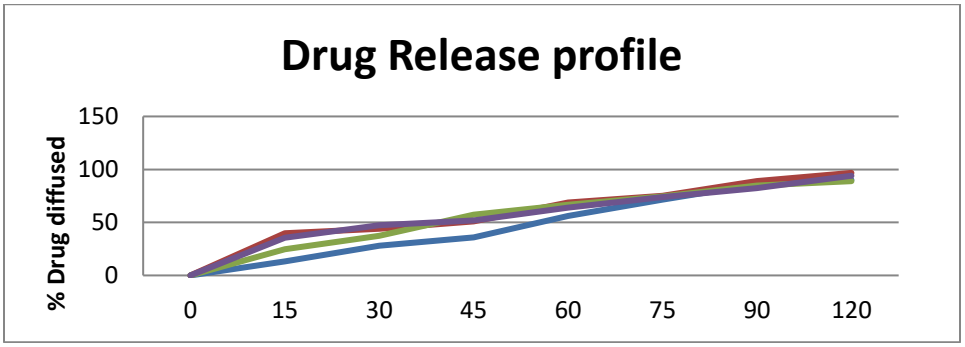

Fig-1 ***In-vitro*** **drug release**

The results of skin irritancy studies are shown that the prepared gels do not produce any skin irritation and are well tolerated by mice.

Anti inflammatory studies of both extract and gel shows that the potency of the extract was retained when it was formulated as gel and has got a significant activity compared to standard drug and its gel (Table-4).

Table-4 *In vitro* anti inflammatory studies

Content	Protein denaturation	Protein inhibition
Standard diclofenac	50	61
Methanol extract	72	81
Gel Standard marketed preparation	52	58
Gel extract	76	88

The result of stability studies showed there were no significant changes in the viscosity, drug content and physical appearance of the gel, after storing at different temperature conditions for three months. These results indicate that drug remain stable after stability studies.

CONCLUSION

The aqueous extract of Tamarindus indica L.contain some pharmacologically active constituents (cardiac glycosides, flavonoids, steroidal nucleus, tannins and terpenoids) and possessed analgesic and anti-inflammatory activities purported to be mediated via peripheral (probably through inhibition of prostaglandin synthesis) and central mechanisms. This supports the use of the plant in ethnomedical and folkloric practices in alleviating pain. The study was aimed to develop a herbal topical gel containing *Tamarindus indica* leaf extract using carbopol-940 as gelling agents and to investigate the anti-inflammatory activity of suitable gel formulation.. Based on *in vitro* permeation study, the best gel formulation was chosen and it was subjected to *in vitro* anti-inflammatory activity studies and skin irritation studies and kept for stability studies for a period of three months. The *in-vitro* anti inflammatory studies showed that the potency of the extract was retained while formulating the gel and have a significant activity when compared to the standard drug (Diclofenac) and its gel. Hence the newly formulated gel (TF2) can be considered as an anti-inflammatory gel. Future *in-vivo* studies should be perform to confirm the results obtained and utilize the formulation for health care needs.

REFERENCE:

1. Akor S.T, Wampana B & Sodipo O.A. Antinociceptive and Anti-Inflammatory Activities of the Aqueous Leaf Extract of Tamarindus indica L. in Albino Rats. *Journal of Plant Studies*,2015;.4(2):44-56
2. Bele AA, Jadhav VM and Kadam VJ, Formulatin\on and evaluatin of herbal drug, *Drug invent today*,2010;2(7):369-372.
3. Arvind Negi, Nimisha Sharma1, Mamta F. Singh. Formulation and Evaluation of an Herbal Anti-Inflammatory Gel Containing *Eupatorium* Leaves Extract, *Journal of Pharmacognosy and Phytochemistry* 2012;1(4):113-117.
4. Tanwar Y. S. , Amit Kumar Jain. Formulation And Evaluation Of Topical Diclofenac Sodium Gel Using Different Gelling Agent. *AJPRHC* 4(1):1-6
5. Sera UV, Ramana MV. Invitroskin absorption and drug release – a comparison of four commercial hydrophilic gel preparations for topical use. *The Indian Pharmacist*. 2006, 73: 356-360.
6. Kumar Tarun Guleri, Kaur Loveleen Preet. Formulation And Evaluation Of Topical Gel Of Aceclofenac *Journal of Drug Delivery & Therapeutics*; 2013, 3(6), 51-53
7. Dr.KandelwalK.R. *Practical Pharmacognosy*.12 th edition Pune NiraliPrakashan 2004;p.10-29
8. Leelaprakash,G and MohanDass,S. (2011). In-vitro anti-inflammatory activity of methanol extract of Enicostemmaaxillare. *International Journal of Drug Development & Research*.3 (3),p.189-196
9. Anil kumar,M.(2010). Ethnomedicinal plants as anti inflammatory and analgesic agents. Ethnomedicine: *A Source of Complementary Therapeutics*. 6 (1), p.267-293.