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Research Article

Investigation of Anti Inflammatory Activity of Triphala Hydrogels in Carrageenan Induced Paw Edema Model

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A B S T R A C T

Inflammation is a reaction of a living vascularized tissue to an injury, conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes has serious stroke effects. So, number of herbal medicines are recommended for the treatment of inflammation that has no side effects. Hence our study focused to investigate bioactive compounds for anti-inflammatory activity. Folk medicine and ethnopharmacological data can provide a broad range of plants with promising anti-inflammatory activity. Triphala, an ayurvedic formula composed of three different plants. Terminalia chebula Retz., Terminalia bellerica Roxb(Combretaceae) and Phyllanthus emblica L is widely used for various microbial infections. Triphala is now evaluated for its anti-inflammatory action. Triphala is formulated into a hydrogel of different concentrations and is evaluated for in-vitro anti-inflammatory activity by membrane stabilization method at 100, 250 and 500 mg/ml and in-vivo evaluation is done by Carrageenan -induced paw edema method. The results obtained are compared with standard NSAID diclofenac hydrogel. The prepared hydrogel of triphala showed a dose-dependent anti-inflammatory potential in in-vivo and in-vitro methods which provide scientific basis for anti-inflammatory activity of Triphala.

Keywords: Triphala, Carrageenan, Anti inflammatory

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INTRODUCTION

Inflammation is a reaction of living tissues towards injury. [1]The complex biological reaction to harmful agents such as microbes, pathogens, damaged cells that contains vascular responses, activation of leukocytes and various systemic reactions causes inflammation.[2] The key features of inflammation are redness, swelling, warmth and pain. [3]The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It is metabolized to prostaglandins and thromboxane A 2 by the Cyclooxygenase (COX) pathway and to eicosanoids and leukotrienes (LT's) by the 5-lipoxygenase (5-LOX) pathway, which are known to act as chemical mediators in a variety of inflammatory events.[4]Inspite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world's population (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material.The fact is well recognized by the WHO which has recently compiled on inventory of medicinal plants listing 20,000 species. [5]

Triphalab (Sanskrit; tri=three and phala=fruits) consists of dried fruits of the three-plant species *Emblica officinal is* (Family Euphorbiaceae), *Terminalia bellerica* (Family Combretaceae) and *Terminalia chebula* (Family Combretaceae) shown in Fig 1 that are native to the Indian subcontinent and it is a well-recognized and revered polyherbal medicine. [6]Tannins, gallic acid, ellagic acid and chebulinic acid are the major constituents of triphala, which are potent antioxidants that may account for the observed immunomodulatory activity of the formula.[7,8,9] COX and 5-LOX, which are both major enzymes involved in inflammation and carcinogens is are inhibited by

chebulagic acid, a constituent in *Triphala*.[10] *Triphala* is used as a pillar of gastrointestinal treatment in ayurvedic medicine; however, the complexity of the three *rasayanas*, or rejuvenative herbs, in the formulation allows for many applications. Moreover, studies have validated a number of potential uses of *Triphala*, which include free radical scavenging, antioxidant, immunomodulating, antistress, appetite stimulation,wound healing, gastric hyperacidity reduction, dental caries prevention, antipyretic, analgesic, antibacterial, antimutagenic, anticarcinogenic, adaptogenic, hypoglycemic,anticancer,hepatoprotective, chemoprotective, radioprotective and chemo preventive effects.[11]

Topical drug delivery system is widely used for skin diseases like bacterial infection, fungal infection, eczema etc. Direct delivery of drug to the site of action is the main advantage of topical application of drug.[12]

Hydrogel is a water-swollen and cross-linked polymeric network produced by the simple reaction of one or more monomers. Hydrogels have the tendency to absorb considerable amounts of water within their interstices. The demonstration of such phenomena in hydrogels is due to the availability of polar hydrophilic moieties, for example, SO_3H, OH, NH_2, COOH, $CONH_2$, etc., along the polymer network as branched groups. The tendency of water absorption in hydrogels is due to the swelling character, which is monitored by the hydrophilicity of attached groups, swelling media and crosslinked bonding strength. [13,14,15,16]

Figure 1: Triphala Churna

MATERIALS AND METHODOLOGY:

Preliminary phytochemical study:

Collection of Plant Material:

The plant selected is Triphala. It was procured from Himalaya Herbal Health Care in Madhapur. Triphala churna is manufactured by Siddh: ayu. It is packed in a plastic container with a batch no. W214480219. Its manufactured date is 08/2021 and expiry date is 07/2023.

Chemicals:

The standard group used in this study is Diclofenac bought from local market. All chemicals and reagents used in this experiment were procured from Sri Venkateshwara college of Pharmacy.

Equipment's and Instruments:

Plethysmometer, UV spectrophotometer, Centrifuge, Incubator, Digital pH meter, Brookfield Viscometer, Autoclave, Sonicator,Weighing balance.

Glassware:

Test tube, volumetric flask, tripod stand, wide mouth conical flask, filter paper, measuring cylinder, flat bottom flask.

Procurement of Animals:

Healthy adult male Wistar rats (200-250 g) were used in this study. All the animals were obtained from VAB bio sciences. Animals were kept in cages in standard temperature regulated rooms with air-cooling and 12 hours light and dark cycle and had free access to water and standard laboratory diet. They were allowed to acclimatize to the laboratory conditions and trained to acclimatize to restrainer for a period of 1 week before the experiments were conducted. Food was withdrawn 12 hours prior to drug administration until the completion of study. The study was approved by the Institutional Animal Ethics Committee IAEC/SVCP/2022/05 and all the experiments were performed as per the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA) guidelines.

The herbal drug is evaluated for the presence of various phytochemical constituents by the following tests shown in table 1.

Table 1: Preliminary phytochemical tests for triphala

S.No	Phytoconstituents	Name of tests	Procedure	Observation
1.	Alkaloids	Mayer's test	2ml of extract + few drops of HCl + Mayer's reagent	Cream Precipitation
		Wagner's test	2ml of extract + few drops of HCl + Wagner's reagent	Reddish brown colour
2.	Carbohydrates	Molish test	2ml of extract + 2 drops of molish reagent + few drops of conc. H_2SO_4	Violet or reddish colour
3.	Steroids	Salkowski's test	2ml of extract + 2ml of chloroform + 2ml of conc. H_2SO_4	Chloroform layer appears red and acid layer shows greenish-yellow fluorescence
4.	Flavonoids	Lead acetate test	2ml of extract + few drops of lead acetate solution	Yellow precipitation
5.	Terpenoids	Copper acetate test	2ml extract dissolved in water + 3-4 drops of copper acetate solution	Emerald green colour
6.	Tannins	Braymer's test	2ml of extract + 2ml of H_2O + 2-3 drops of 5% $FeCl_3$	Black green or bluish colour
7.	Saponins	Foam test	2ml extract + 4ml distilled H_2O. Mix well and shake vigorously.	Foam formation
8.	Phenols	Ferric chloride test	2-3 ml of extract + few drops of 5% $FeCl_3$ solution	Deep blue-black colour

Preparation of Hydrogels:

Triphala was used for the preparation of hydrogel. The hydrogel was prepared using Carbopol 934, Propylene glycol (PG), Tri-ethanolamine (TEA) and distilled water. The known amount of Carbopol 934 was slowly dispersed into the required quantity of water with continuous stirring to get uniform dispersion and allowed overnight for proper hydration. The accurately weighed quantity of standardized aqueous extract along with other excipients was poured into the fixed amount of hydrated Carbopol 934 dispersion with constant mixing. Same procedure was used to prepare diclofenac hydrogels also.

Evaluation of hydrogels:

1. pH measurement:

The pH values of formulated Hydrogels were determined using a Digital pH meter (Syntronics®, India). The pH meter was initially calibrated with standard buffer tablets of pH 4.0, pH 7.0, and pH 9.0. 1gm of prepared Hydrogel was dissolved in 100ml of distilled water to make a 1% aqueous solution. The pH was measured by dipping the glass electrode into the prepared aqueous solution. The pH is measured in triplicate and the standard deviation was calculated.

2. Spreadability:

The Spreadability is done to denote the extent of the area in which the gel spreads readily on application to the skin. The gels were placed between 2 horizontal plates of 20×20 cm and a standard weight of 200gm was added to the upper plate to determine the Spreadability. The spreading diameter was noted after one minute. The diameter obtained was measured in cm and calculated by using the formula.

$S = M \times L / T$

3. Viscosity:

The viscosity of the prepared Hydrogels was determined by Brookfield viscometer using spindle no 64 at 10 rpm and the temperature was maintained at 25℃. The hydrogel formulations were taken in a beaker and allowed the spindle to rotate. The reading was then recorded.

4. Drug Content Determination:

1gm of formulated Hydrogel was diluted with pH 7.4 phosphate buffer saline and the mixer was filtered through the membrane filter. The absorbance was then determined by scanning the sample using UV Spectrophotometer at 235 nm.

In-Vitro Evaluation for Anti-Inflammatory Activity:

Method: Membrane Stabilization Method

The anti-inflammatory activity of the extracts was determined using human red blood cell (HRBC) – membrane stabilization assay developed by Shine et al. and modified by Sikder et al. Venous human blood was collected from a normal male adult who had not consumed anti-inflammatory or contraceptive medicaments during two weeks before taking the sample.

The blood was mixed with equal amount of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The resulting mixture was centrifuged at 3000rpm for 10min; the supernatant was removed and the packed cells washed 3 times with Isosaline solution (0.9%. pH 7.2). The assay mixture was prepared by mixing 1mL phosphate buffer (pH 7.4), 2mL hyposaline solution (0.36%) and 0.5mL HRBC suspension (10% v/v) with 1mL of each plant extracts of various concentrations (100,250 and 500 µg/mL) and standard drug diclofenac sodium (100, 250, 500µg/mL) respectively. A reaction mixture with distilled water instead of plant sample was used as control and phosphate buffer as blank. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm. The percentage of haemolysis was calculated using the following equation:

Haemolysis (%) = (Optical density of test sample/Optical density of control) * 100

Protection (%) = 100 – [(Optical density of test sample/Optical density of control)] * 100

In-vivo Evaluation for Anti-Inflammatory Activity:

Method:

Carrageenan-Induced Paw Edema Model

Total number of animals was divided into 5 groups of 3 each.The animals were weighed and numbered. Inflammation in the right hind paw of rat was induced by an injection of 0.2mL of 1% (w/v) of carrageenan in saline subcutaneously in the plantar side of the right hind paw of rat. The paw volume was measured using Plethysmometer before the carrageenan injection and then each hour up to 6 times after 12hrs and 24hrs.The rats were randomly divided into five groups (n = 3). The group 1 (control group) received hydrogel without drug (3 ml/kg body weight), while the group 2 received hydrogel with the standard anti-inflammatory drug- the diclofenac (10 mg/kg). The group 3 received hydrogel with Triphala (100mg/kg). The group 4 received hydrogel with Triphala (200mg/kg).The group 5 received hydrogel with Triphala (300mg/kg).The hydrogels are applied topically by gently rubbing on the paw. The animals were pretreated 1hour before the administration of Carrageenan.

RESULTS AND DISCUSSION

The herbal drug selected was evaluated for the prescence of phytochemical constituents. The tests revealed the prescence of carbohydrates, steroids, flavinoids, terpenoids, tannins, saponins and phenols. The results are shown in table 2.

Table 2: Phytochemical screening of Triphala

S. No	Phytoconstituent	Name of test	Aqueous extract
1.	Alkaloids	Mayer's test	-
		Wagner's test	-
2.	Carbohydrates	Molish test	+
3.	Steroids	Salkowski's test	+
4.	Flavonoids	Lead acetate test	+
5.	Terpenoids	Copper acetate test	-
6.	Tannins	Braymer's test	+
7.	Saponins	Foam test	+
8.	Phenols	Ferric chloride test	+

Placebo Preparation of Hydrogels:

Table 3: Placebo preparation of hydrogels

Hydrogels	**HF 1**	**HF 2**	**HF 3**	**HF 4**
Carbopol 934	0.5%	1%	1.5%	2%

Table 4: Evaluation tests for placebo hydrogels

Tests	**HF 1**	**HF 2**	**HF 3**	**HF 4**
Viscosity	562	689	745	985
Spreadability	4.3	4.7	5.4	6.8

Four placebo hydrogels HF 1, HF 2, HF 3 and HF 4 were prepared with concentrations 0.5%, 1%, 1.5% and 2% of Carbopol 934 respectively shown in table 3. HF 3 placebo hydrogel was selected as it has better spreadability, homogeneity and consistency as shown in table 4.

Preparation of Hydrogels:

The hydrogels were prepared by taking 1.5% of Carbopol 934 and 30ml of water. Five formulations were prepared F1, F2, F3, F4 and F5. Hydrogel F1 contains no drug and it serves as a control. Hydrogel F2 contains standard drug-diclofenac and it serves as a standard. Hydrogels F3, F4 and F5 contains Triphala of 100mg, 200mg and 300mg respectively as shown in table 5.

Table 5: Formulation of Hydrogels

Ingredients	**F1(Control)**	**F2(Diclofenac)**	**F3(Triphala)**	**F4(Triphala)**	**F5(Triphala)**
Carbopol 934	0.45 gm	0.45 gm	0.45 gm	0.45 gm	0.45 gm
Water up to (ml)	30	30	30	30	30
Drug	-	0.01 gm	0.1 gm	0.2 gm	0.3 gm
PG	2 ml	2 ml	2 ml	2 ml	2 ml
TEA	3 drops	3 drops	3 drops	3 drops	3 drops

Standard Graph of Diclofenac in Methanol:

Table 6: Absorbance of diclofenac sodium at various concentrations

Concentration(µg/ml)	**Absorbance**
4	0.169
6	0.289
8	0.43
10	0.547
12	0.639
14	0.782

The absorbance of pure Diclofenac was taken at various concentrations of 4, 6, 8, 10, 12, 14µg/ml as shown in table 6. The standard graph of diclofenac in methanol is plotted by taking concentration on X-axis and absorbance on Y-axis as shown in fig 2. The absorbance values for 4, 6, 8, 10, 12 and 14µg/ml concentrations are 0.169, 0.289, 0.43, 0.547, 0.639 and 0.782 respectively. The slope value obtained from the graph is 0.0567x – 0.0293 and R^2 value is 0.994.

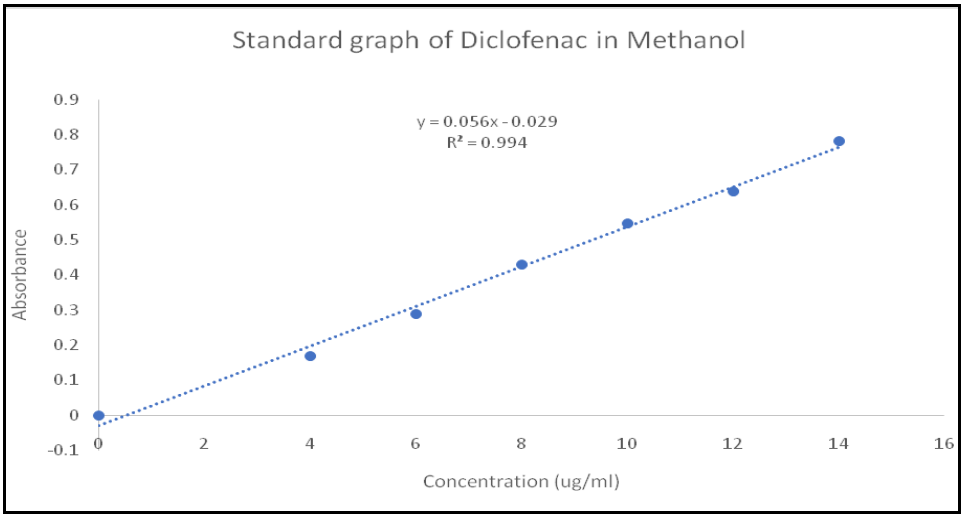

Figure 2: Standard graph of Diclofenac in methanol

Standard Graph of Triphala in Methanol:

Table 7: Absorbance of Triphala at various concentrations

Concentration(µg/ml)	Absorbance
5	0.198
10	0.382
15	0.601
20	0.788
25	0.989

The standard graph of Triphala in methanol is plotted by taking concentration on X-axis and absorbance on Y-axis. The absorbance values for 5,10,15,20 and 25µg/ml concentrations are 0.198, 0.382, 0.601, 0.788 and 0.989 respectively as shown in table 7. The slope value obtained from the fig 3 is 0.0395 xs and R^2 value is 0.9999.

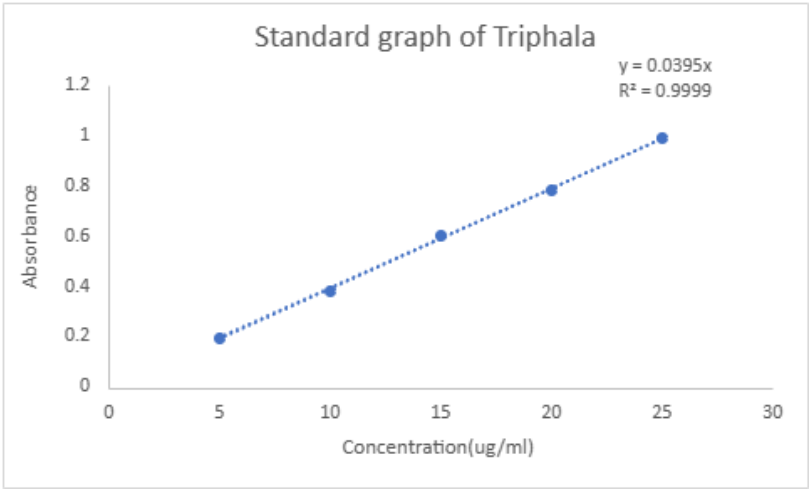

Figure 3: Standard graph of Triphala in methanol

Evaluation Tests of Hydrogels:

Table 8: Evaluation tests of hydrogels

Hydrogels	Ph	Viscosity	Spreadability (g.cm/s)	% Drug content
F1-Control	4.71	743.8	5.4	-
F2-Diclofenac	4.29	862	5.8	93.36%
F3-Triphala (100 mg)	4.85	850	5.6	93.81%
F4-Triphala (200 mg)	3.94	900	5.5	95.25%
F5-Triphala (300 mg)	3.57	925	5.7	94.7%

Hydrogels were prepared using diclofenac sodium and three different concentrations of Triphala. The prepared hydrogels were evaluated based on different tests i.e., pH, viscosity, spreadability and percentage drug content. The results obtained are shown in table 8.

The pH of F1(Control), F2(Diclofenac), F3 (Triphala-100mg), F4 (Triphala-200 mg), F5 (Triphala-300 mg) was found to be 4.71, 4.29, 4.85, 3.94, 3.57 respectively.The viscosity of F1(Control), F2 (Diclofenac), F3 (Triphala-100mg), F4 (Triphala-200mg), F5 (Triphala-300 mg) was found to be 743.8Pa S, 862 Pa S, 850 Pa S, 900 Pa S, 925 Pa S respectively. The spreadability of F1(Control), F2 (Diclofenac), F3 (Triphala-100mg), F4 (Triphala-200mg), F5(Triphala-300mg) was found to be 5.4g.cm/s, 5.8 g.cm/s, 5.6 g.cm/s, 5.5 g.cm/s,5.7 g.cm/s respectively.

The ideal pH of the skin is 4 to 5.5. The formulated hydrogels are found to possess pH near to skin pH. Hence there is no irritation observed on the skin.

The optimum viscosity of hydrogels is 25-4540 Pa S. The formulated hydrogels are found to possess viscosity within the optimum viscosity range.

The optimum spreadability of hydrogels is 5.4-7.2 g.cm/s. The formulated hydrogels are found to possess spreadability within the optimum spreadability range.

The percentage drug content of F2 (Diclofenac), F3(Triphala-100mg), F4(Triphala-200mg), F5(Triphala-300mg) was found to be 93.36%, 93.81%, 95.25%, 94.7% respectively from the standard graphs. The percentage drug content is approximately uniform in the formulated hydrogels.

Hence the prepared hydrogels of Triphala and Diclofenac are found to have ideal characteristics to use.

In-vitro Anti-Inflammatory Assay:

Table 9: Haemolysis percentage and percentage protection of Triphala and Diclofenac

Groups	Drugs	Haemolysis percentage	Percentage protection
1	Triphala-100mg	54.54%	45.46%
2	Triphala-250mg	45.45%	54.55%
3	Triphala-500mg	34.09%	65.91%
4	Diclofenac-100mg	25%	75%
5	Diclofenac-250mg	20.45%	79.55%
6	Diclofenac-500mg	13.63%	86.37%

The in-vitro anti-inflammatory activity was determined by membrane stabilization method.

Figure 5: Haemolysed sample in centrifuge tube

During inflammation, lysis of lysosomal membrane may occur which release their enzyme components that produce a variety of disorders. Since human red blood cell membranes are similar to lysosomal membrane, the inhibition by hypotonicity and heat induced lysis of red blood cell membrane will be taken as a measure of the mechanism of anti-inflammatory activity. Finally the haemolysis of red blood cells take place. Injured red cell membrane produce the cell more susceptible to secondary damage through free radical induced lipid peroxidation. Leakages of lysosomal constituents cause the further tissue inflammation and damage upon extra cellular release. Therefore membrane stabilization of lysosomes are important to control the inflammatory response. This will lead to prevention of leakage of its constituents.

The percent inhibition of heat induced haemolysis of red blood cells at different concentrations of Triphala in the range of 100-500 µg/ml, is shown in table 7.

Triphala was able to inhibit haemolysis in a concentration dependent manner. Inhibition percentages of haemolysis from Triphala were in the range from 34.09% to 54.54% at the concentrations of 100 to 500 µg/ml. The protection percentage of Triphala-100 µg, Triphala-250 µg, Triphala-500 µg was found to be 45.46 %, 54.55 %, 65.91 % respectively and the protection percentage of diclofenac-100 µg, diclofenac-250 µg, diclofenac-500mg was found to be 75%, 79.55%, 86.37% respectively.

The highest protection percentage of Triphala is found at 500 µg /ml i.e., 65.91% and diclofenac at 500 µg /ml i.e., 86.3% is shown in table 9.

Stabilisation of liposomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage by extracellular release. The extract may inhibit these processes. Hence forth contributing to its anti-inflammatory activity. The results are also depicted in the form of bar diagrams fig 6 & 7.

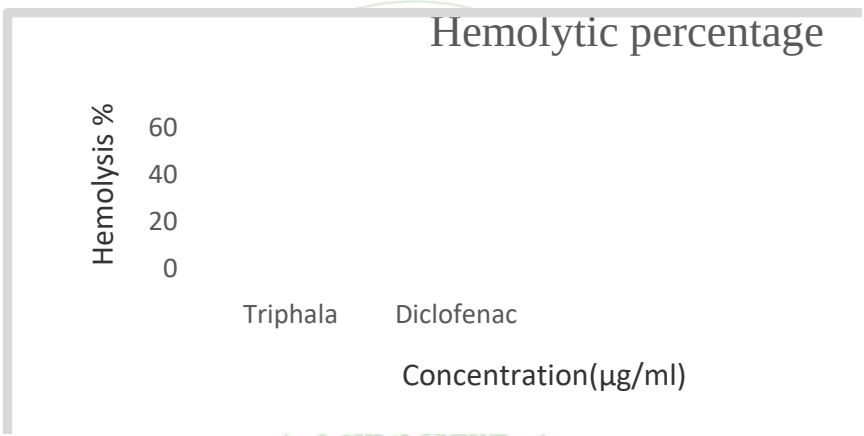

Figure 6: Haemolytic percentage

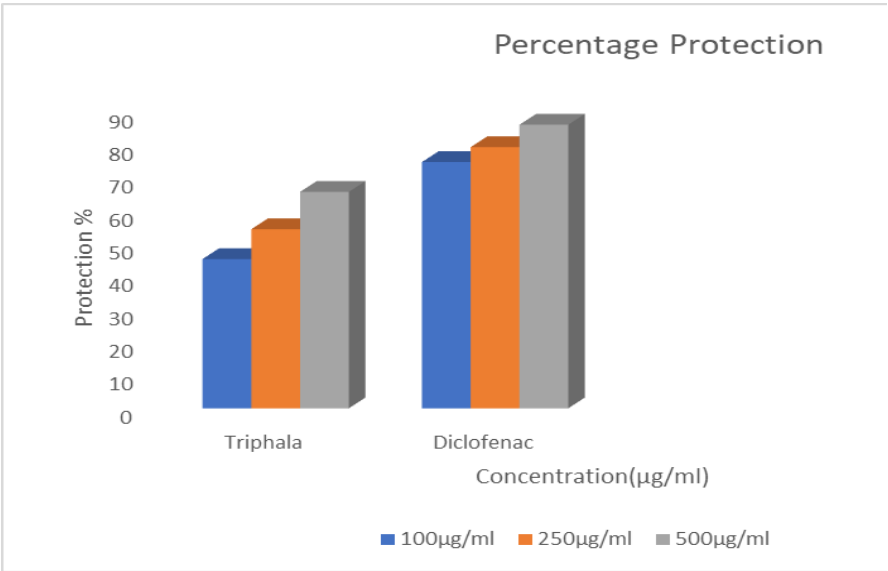

Figure 7: Percentage Protection

In-vivo Anti-Inflammatory Activity

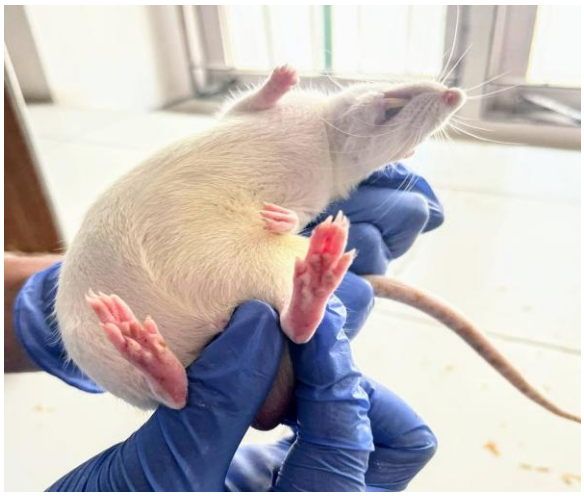

Figure 8: Inflammation in mice

Table 10: Invivo anti-inflammatory activity by carrageenan induced paw edema model a=p <0.0001, b=p<0.001

Groups		Before	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	12 hours	24 hours
Group 1 (control)	M±SEM	0.5±0.063	0.7±0.033	0.6±0.033	0.7±0.0360	0.6±0.033	0.7±0.036	0.4±0.036	0.4±0.047	0.3±0.055
Group 2 (Diclofenac)	M±SEM	0.4±0.036	0.46±0.038	0.43±0.035	0.42±0.035	0.4±0.039	0.36±0.035	0.29±0.036	0.24±0.040	0.24±0.060
Group 3 (Triphala) 100 mg	M±SEM	0.36±0.049	0.52±0.036	0.69±0.036	0.56±0.032[a]	0.54±0.036[b]	0.51±0.042[b]	0.35±0.036	0.32±0.036	0.3±0.036
Group 4 (Triphala) 200mg	M±SEM	0.38±0.030	0.5±0.030	0.65±0.030[c]	0.54±0.030[a]	0.53±0.040[b]	0.49±0.036[b]	0.45±0.036[a]	0.35±0.055	0.33±0.060
Group 5 (Triphala) 300mg	M±SEM	0.37±0.042	0.49±0.033	0.63±0.036[a]	0.54±0.036[a]	0.52±0.036[a]	0.49±0.042[a]	0.43±0.042[a]	0.33±0.049[a]	0.31±0.042

The invivo anti-inflammatory activity of Triphala was evaluated by the carrageenan induced paw edema model. Several inflammatory mediators for example, histamine, serotonin, kinins, PG's, complement and proinflammatory cytokinins, play a major role in paw edema caused by carrageenan. The release of PG's is closely associated with leukocytes migration to the inflamed site. The presence of PG's particularly PGE2, in the inflammatory exudates from the infected foot can be demonstrated at 3 hour and thereafter as shown in fig 8.

Data in table depicts that 100, 200, 300 mg/kg b.w of triphala and 10 mg/kg b.w diclofenac treated animals induced with inflammation using carrageenan significantly ($p<0.05$) inhibited paw edema in a dose dependent manner. Diclofenac (10mg/kg b.w), 100, 200 and 300mg/kg b.w of triphala supressed paw edema of rats at 2ndand 3nd hour onwards by 0.46±0.038mm, 0.56±0.030mm, 0.65±0.030mm and 0.63±0.036mm respectively when compared with control group. The test drug treated group as well as standard drug treated groups have shown significant reduction in the paw edema.

When testdrug treated groups are compared with standard group, Triphala hydrogel (100mg) showed significant effect 3 hours onwards to 5hrs with a mean value 0.56±0.030, 0.54±0.036 and 0.51±0.042. Group four Triphala hydrogel (200mg) showed significant effect 2 hours onwards to 5hrs with a mean value 0.65±0.030and at 3 hours it showed significant effect with a mean value 0.54±0.030, 0.53±0.040 and 0.49±0.036 respectively. Group five Triphala hydrogel (300mg) is compared with group two standard, it showed significant effect 2 hours onwards with a mean value 0.63±0.036, 0.54±0.036 and at 4 hours it showed significant effect with a mean value 0.52±0.036 and 0.49±0.042 respectively. Up to 5 hours the results were significant and with gradual passage of time the significant effect declined. Diclofenac showed significant effect up to 12 hours.

The results obtained show that Triphala has significant anti-inflammatory activity which is comparable to the standard drug Diclofenac.

CONCLUSION

Non-steroidal anti-inflammatory drugs (NSAIDS) are commonly used for the management of inflammatory conditions. However there drugs have several adverse side effects especially gastric ulcers. Therefore, the search for natural sources and phytochemicals with anti-inflammatory activity has greatly increased in recent years. In this present study, the anti-inflammatory potential of Triphala (polyherbal hydrogel) using invitro and invivo inflammation models were evaluated.

Based on the present investigation, it can be concluded that the in-vivo & in-vitro anti-inflammatory activity of Triphala might be attributed to the presence of the various secondary metabolites like tannins, saponins, steroids, alkaloids, reducing sugars, terpenoids and flavonoids.These experimental findings support the traditional use of this plant for the treatment of various ailments especially against pain and inflammatory conditions. However, further investigations are required to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action responsible for the anti-inflammatory activities of Triphala.

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