8.1 Introduction

Majority of drugs existing in the market constitute the natural products and even the semi-synthetic or synthetic drugs have originated from the natural sources. The therapeutic potential of medicinal plants used in several traditional systems has been established through scientific studies from across the globe. The interest of the scientific community in correlating the phytoconstituents of a plant and their botanical properties with its pharmacological activity has increased. The plant extracts and isolated phytoconstituents have expressively contributed to the new drug discoveries. Finding viable, robust, and druggable lead candidates is a challenging task in drug discovery and development of pharmaceuticals for use in humans. As it involves the transformation of screening hits to the drug candidate, it demands for both experience and expertise. The new drug development is a very expensive, time-consuming, and complex task. Usually, it takes about 12 years from the discovery of new lead to its appearance in the clinic as a therapeutic agent. The diminution in the new drug approvals and escalating development cost are major challenges in the new drug discovery. Although the arrival of combinatorial chemistry has rationalized the drug discovery process, it does not increase the success rate. Primarily, drug discovery focuses on the identification of new chemical entities possessing potential characteristics of druggability. Natural products have formed the basis of useful therapeutic agents for centuries. Plants have continued to serve mankind with discoveries of new remedies. Several phytoconstituents like flavonoids, triterpenoids, alkaloids, steroids, and phenols have been documented to possess interesting anti-inflammatory properties. Many phytoconstituents exhibited potent activities at micromolar concentrations against well-established biomarkers of inflammation. The active components obtained from natural products used as traditional medicines appear to be the main sources of drug discovery in modern medicines. Despite the advances in the allopathy field, plants are continuing to be the source of potential therapeutic agents in the modern and traditional system of medicine. Therefore, the isolation of pure compounds from the natural sources, and characterization of pharmacologically active compounds have been continued.

8.1.1 Systematic Approach in Use of Different Animal Models for Evaluations

The role of pharmacology in modern medicine is to search new therapeutic drugs by using appropriate models and elucidate the mechanism for therapeutic targeting by other novel

molecules. The experimental models based on pharmacological principles should provide physiologically and clinically relevant model system to predict the intended therapeutic indication. Pharmacological model can be considered relevant when the effects obtained in the preclinical model are linked with the results in the clinical setting. Vogel et al. [2] described various *in-vivo* and *in-vitro* methods for the pre-clinical assessment of anti-inflammatory drugs. Before the execution of actual assay, it should be planned appropriately concerning the sample size, statistical methods, route of administration, and use of positive control. Although many phytoconstituents are studied for anti-inflammatory activity, studies involving delineation of mechanisms of action, pharmacokinetics, and safety of phytoconstituents are still desirable. Inappropriate planning and execution of the drug screening programs seem to be the main reason behind the declined success of phytoconstituents based drug discovery programs. While selecting animal models for the screening of anti-inflammatory activity, consideration should be given to correlate mechanisms behind well-established animal models. [1]

8.1.2 Animal Models and Mechanisms for Screening of Anti-Inflammatory Activity

Acute Inflammation

1. Histamine/5-HT-Induced Paw Edema
2. Bradykinin-Induced Paw Edema
3. Carrageenan-Induced Paw Edema
4. Dextran-Induced Paw Edema
5. Lipopolysaccharide (LPS)-Induced Paw Edema
6. Arachidonic Acid-Induced Ear Edema
7. Croton oil/TPA-Induced Ear Edema
8. Oxazolone-Induced Ear Edema
9. Acetic Acid/Compound 48/80-Induced Vascular Permeability
10. Pleurisy Model

Sub-Acute Inflammation

1. Granuloma Pouch Model

Chronic Inflammation

1. Cotton Pellet-Induced Granuloma
2. Formalin-Induced Paw Edema
3. Complete Freund’s Adjuvant (CFA)-Induced arthritis

8.1.3 Carrageenan-Induced Paw Edema

Carrageenan-induced paw edema model is widely used to assess the anti-inflammatory activity of several natural and synthetic compounds. It is the distinctive model of the acute inflammation having greater reproducibility. Carrageenan is a non-antigenic phlogistic agent with the devoid of any visible systemic effects. Sulphated sugars present in carrageenan are liable for the activation of complement system and the inflammatory mediators. Stimulation of phospholipase A2 by carrageenan initiates the early phase of inflammation, whereas the cytotoxic effects progress the inflammation. Carrageenan dilates postcapillary venules that result in exudation of inflammatory fluid and cells. This process involves the release of several proinflammatory mediators. These events represent the early exudative inflammatory phase and its inhibition terminate the inflammatory process. Carrageenan model is typically linked with the activation of the cyclooxygenase pathway. Glucocorticoids and prostaglandin antagonist exhibit anti-inflammatory activity in this preclinical model. The edema developed by carrageenan is represented as biphasic curve. The first phase of carrageenan-induced inflammation is partly assigned to the injection trauma and released of acute phase mediators especially the serotonin and histamine. Prostaglandins are the main players for the occurrence of second phase of carrageenan-induced inflammation, which occurs around 3 hr after carrageenan injection. [3-9]

Advantages:

- Widely used and well-established working model of the inflammation.
- Inflammation-induced by carrageenan is acute, non-immune, and reproducible.
- Involvement of multiple mechanisms allow this model as a preliminary test for the screening of anti-inflammatory drugs.
- Biphasic response after subplantar carrageenan injection enable this model to predict the probable biological targets of test drug in the inflammation.
- This model is sensitive to cyclooxygenase inhibitors and suitable for the assessment of NSAIDs that act by the cyclooxygenase inhibition which is involved in prostaglandin synthesis.

Limitations:

- To eradicate the effects of stress, animals should be acclimatized at least one week before the commencement of an experiment.
- The investigator should be trained to record the stable and reproducible paw volumes using sophisticated equipment like plethysmometer.
- Rise in paw edema is based on the concentration of injected carrageenan.
- Typically, the maximum edema response produced by carrageenan is too difficult to inhibit. Therefore, the carrageenan type and preparation of its solution needs careful attention.

8.2 Materials and methods

8.2.1 Skin irritation study

This test was performed on Wistar/Sprague-Dawley rats. The animals given standard animal feed and had free access to water ad libitium. Animals were divided into four groups, each batch containing six animals. (Table 8.1) Dorsal hairs at the back of the rats were removed one day prior to the commencement of the study and kept individually in cages to avoid contact with the other rats. Two groups of each were used for control and standard irritant. Another two groups were used as a test. The 50 mg of the gel formulation was applied over one square centimeter area of whole and abraded skin of different animals. Aqueous solution of 0.8% formalin was used as standard irritant. The animals were observed for seven days for any signs of edema and erythema.

Table 8.1 Groups of animals for skin irritation study

	Groups	No. of animals
Group I	Normal control	6
Group II	Standard irritant	6
Group III	Polyherbal Gel	6
Group IV	Polyherbal Spray	6
	Total No. of animals	24

8.2.2 Evaluation of anti-inflammatory activity

Carrageenan-induced paws edema model [Protocol No.-MSU/IAEC/2021-22/2117**]**

Wistar/Sprague-Dawley rats of either sex were housed in cages placed in an animal room with a constant temperature of 22 °C and a fixed 12-hour light-dark cycle. All animals will be handled and housed according to the CPCSEA guidelines, Department of Animal Husbandry and Dairying, Ministry of Fisheries Animal Husbandry and Dairying Government of India (DAHDMoFAH&D). The rats were given standard rat chow and water ad libitum. After acclimatization rats were randomly allocated to groups which were as follows (Table 8.2)

Table 8.2 Groups of animals for Evaluation of anti-inflammatory activity

Groups		**Administered samples**	**No. of animals**
I	Normal Control	Purified water	6
II	Test I (Gel)	Developed Polyherbal Gel	6
III	Test II (Spray)	Developed Polyherbal Spray	6
IV	Test III (Diclofenac Gel)	Diclofenac Gel (1%)	6
V	Test IV (Diclofenac Spray)	Diclofenac Spray (4%)	6
Total No. of Animals			30

Male or female Sprague-Dawley or Wistar rats were used. The animals were starved overnight. To ensure uniform hydration, the rats received 5 ml of water by stomach tube. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan (in saline) into the plantar side of the left hind paw. The route of administration was topical for Polyherbal Gel and Polyherbal Spray. The dose of the prepared formulations was 0.5 gm (Gel) and 0.5 ml (Spray). The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically (Figure 8.1) immediately after injection, again 3 and 6 h, and eventually 24 h after challenge.[2]

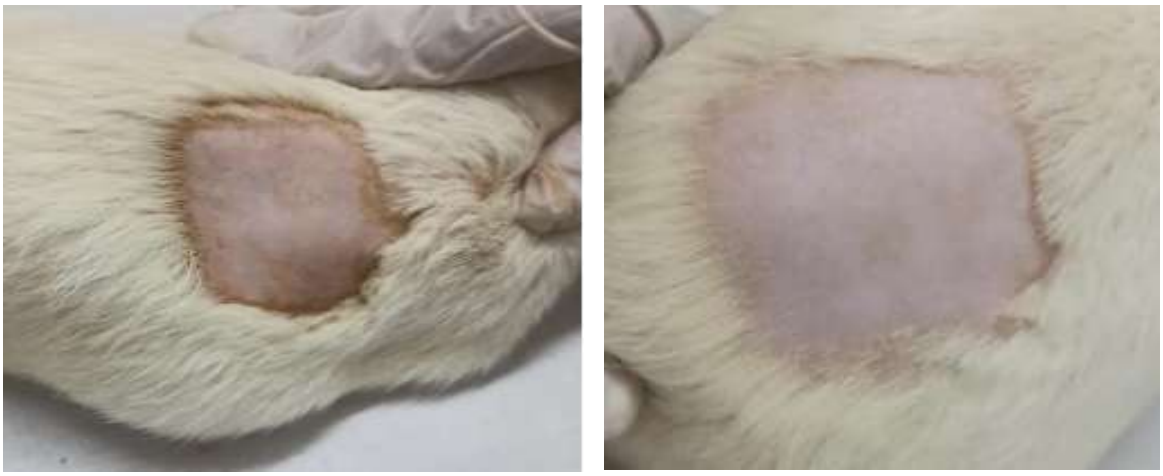
Figure 8.1 Photograph of Digital plethysmometer

8.3 Results and discussion

8.3.1 Skin irritation test

The prepared polyherbal gel and polyherbal spray were evaluated for its skin irritant effect, where no erythema or edema was observed for all the formulations, even after 10 days of study, Figure 8.2 (a and b) indicating that the prepared polyherbal formulations were found to be safe. Group II animals (Standard Irritant) were observed with well-defined Erythema/oedema (Score 2).

Erythema/oedema	Score
None	0
Slight	1
Well defined	2
Moderate	3
Scar formation	4



(a) (b)

Figure 8.2 (a) Photograph of skin of rat after application of Polyherbal Gel

(b) Photograph of skin of rat after application of Polyherbal Spray

8.3.2 Evaluation of anti-inflammatory activity

Table 8.3 Effect of developed formulations and diclofenac formulations on carrageenan-induced paws edema in rats.

	Inhibition of edema (%)					
Groups (n=6)	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
Carrageenan	-	-	-	-	-	-
Carrageenan+ Diclofenac Gel	60.0	63.0	62.0	65.0	61.0	59.0
Carrageenan +Diclofenac Spray	55.0	56.0	58.0	57.0	54.0	53.0
Carrageenan+ Polyherbal Gel	65.0	67.0	68.0	69.0	68.0	67.0
Carrageenan + Polyherbal Spray	51.0	52.0	53.0	52.0	54.0	50.0

8.3.3 Conclusion of *in-vivo* studies

The developed polyherbal gel and polyherbal spray were evaluated for their skin irritation study. Over a 07-day study period, no erythema or edema was observed for any of the formulations. These results indicate that the prepared polyherbal formulations are safe for use. The pharmacological screening was carried out by using the carrageenan-induced edema model to evaluate the possible anti-inflammatory activity of the two developed formulations. As shown in Table 8.3, the inhibition of edema was 67.3% for Polyherbal Gel, highly effective to that produced by the standard Diclofenac Gel 61.66%, indicating an anti-inflammatory effect. The inhibition of edema of Diclofenac Spray was 55.5% and Herbal Spray was 52%. From this study it can also be concluded that Carbopol 934 successfully used as gel base and Ethanol used as solvent for Spray. It can be concluded that both the developed formulations Gel and Spray have an anti-inflammatory effect better than respective anti-inflammatory formulations of diclofenac available in the market. However, developed Gel has better activity than developed Spray formulation.

8.4 References

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