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Drug Treated Anti - Inflammatory Effect On Pharmacotherapy Activity: Evidence From Various Treatments Using Poly Herbal Gel

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ABSTRACT- Medicinal plants are widely used in the treatment of skin diseases. Topical application of gels at pathological sites offer faster release of a drug to site of action. The poly herbal gel of Curcuma longa, Curcuma caesia and amada showed anti-inflammatory activity in paw edema. Among the three distinct phases of inflammation, the last phase is considered to be the most clinically effective phase to access the anti-inflammatory drugs. It was concluded that the response of PHGs treated group animals in the first phase (1 h) after the post treatment was found to be less effective as compared to standard drug treated animals. It might be possibly due to inadequate release of histamine and serotonin.The anti-inflammatory effect was found to be consistent in the second phase (1–2 h) in all treatment group with maximum effect by standard drug treated animals that possibly indicated the release of phase two mediator. However the PHGs treated group animals at all dose level were found to be effective in phase three (2–3 h), corresponding with the significant release for phase 3 mediators.

INTRODUCTION

Herbal Medicine

Herbal medicine therapies have long been a vital source of first-rate healthcare all over the world. Plants have provided a plentiful source of useful and safe medicinal therapies since ancient times. Despite this, conventional pharmaceuticals are still used by about 80% of the world's population. Herbal medicines are finished, classified medicinal preparations that contain living components, aerial or underground a portion of vegetation or other plant materials, or a combination of them, whether or not in the crude country or as plant arrangements. Plant compounds mixed with chemically defined energetic chemicals, as well as chemically characterized distant aspects of plant life, aren't deemed herbal medication therapies [1].

Herbal medication treatments are still a big business in the US pharmaceutical industry, worth billions of dollars. Approximately 1500 botanicals are offered as dietary supplements; formulations are not subjected to rigorous toxicity testing by the Food and Drug Administration (FDA) to ensure their safety and efficacy. The Indian natural medication business is worth over $1 billion, with plant-based crude drug exports worth around $100 million. Herbal medicine's present market potential in Europe and the United States is estimated to be between $ 80 and $250 billion [2-3].

The ayurvedic notion was recognised and evolved in India between 2500 and 500 BC. The exact meaning of Ayurveda is "science of lives," since it is an ancient Indian system of health treatment that focused on man and his ailment. It is stated that people with good health have a metabolically well-balanced metabolism. The practise of Ayurveda therapies was organised into eight parts and three hundred and eighty chapters, with 314 flowers designated as pharmacological treatments in India [4].

Ayurveda was the name given to the medicinal knowledge of the Indian subcontinent four thousand years

ago. In India, Ayurveda continues to be an essential tool for medical and pharmacological therapy. Plant alkaloids are the most common active ingredients in Ayurvedic medicines.

Many Ayurvedic medications now include pharmacologically active ingredients, and their utility in drug therapy is being determined. Only a small percentage of plants are used in traditional medicines, as stated in the introduction. The Indian subcontinent is home to a large number of medicinal plants that are used in traditional medical treatments [5].

Although almost 15000 medicinal flowers have been reported in India [6,] traditional tribes use just 7,000-7,500 plant life to treat certain illnesses [7-9]. For extraordinary ailments, therapeutic plant life is indexed in many indigenous structures such as Siddha (600), Ayurveda (700), Amchi (600), Unani (700), and Allopathy (30) plant species [10]. According to various estimates, 17,000 species of medicinal plants have been identified, with about 3,000 species being used in the medical field [11]. Chemical concepts derived from herbal assets have become a lot easier to come by, and they've helped a lot with the development of new medicinal flora capsules [12-13]. Saponins, tannins, alkaloids, alkyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids, and phorbol esters all contribute to the valuable medicinal properties of various plants [14].

Among them some are act as synergistic and beautify the bioactivity of other compounds. Artemisinin produced via Artemisia annua plant may be very powerful in opposition to Plasmodium falciparum, P. Vivax and also drug resistant parasite. The most important active components of Artemisia annua are sesquiterpenoid lactone endoperonides named artemisinin and artemisinic acid. For more than century quinine, an alkaloid acquired from the bark of diverse species of cinchona timber has been used in the treatment of Malaria and interestingly changed into one of the first sellers used for the treatment of amoebic dysentery.

Reserpine remoted from uncooked plant extract of Rauwolfia serpentina is used as tranquilizer and in control of high blood pressure. From 2000 years the powdered root of Rauwolfia serpentina has been utilized in remedy of intellectual infection in India. Although synthetic drugs are regularly used in treatment of positive ailment however a great interest and self assurance on plant remedy turned into found [15].

Indian Vedas describe the massive use of natural products and aqueous extract of various plant parts for curing distinctive illnesses. Maximum 30 % of root a part of medicinal plant is utilized in distinctive practices in assessment to other plant elements [16].

Inflammation and anti-inflammatory interest

Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic. Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes [17]. Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers.

Types of Inflammation

There are mainly two types of inflammation which are as follows:

Acute inflammation

It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes.

Chronic infection

It is related to infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis [18].

Inflammation is a commonplace scientific situations and rheumatoid arthiritis (RA) is a persistent debilitating autoimmune disease that affects approximately 1% of the population in evolved nations [19-20]. The classic symptoms of inflammation are neighborhood redness, swelling, pain, warmness and loss of characteristic [21].

Inflammation is a stereotyped reaction, inherent to vascularized tissues, which has the goal of reestablishing tissues homeostasis. The inflammatory process has cell and humoral additives, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic structures (complement, kinins and coagulation), respectively. These components paintings synergistically and concurrently, inflicting vascular changes and leukocyte recruitment to the lesion [22].

Leucocytes (to begin with neutrophils), start to phagocytose micro organism and mobile particles, performing a number one clearance of the lesion. The top of neutrophil recruitment is accompanied with the aid of the appearance of macrophages into the tissue, which phagocytose the final cell and bacterial residues, consisting of apoptotic neutrophils [23]. At the same time, lymphocytes can be activated in the lymph nodes via antigen-imparting cells (e.g., dendritic cells) from the tissue, initiating the manufacturing of antibodies by B cells and the migration of T helper lymphocytes to the inflamed web site. Following the path, stromal and parenchymal cells multiply and reconstitute the tissue, at the same time as most of the remaining macrophages and lymphocytes go away through the lymphatics.

Inflammation is critical for the survival of the host, however is observed by its classical cardinal symptoms rubor, calor, tumor and dolor (redness, warmth, tumor and pain), which can be the main motive of patient discomfort, specially after surgical methods. This impels health professionals to prescribe anti inflammatory drugs, a practice that must be constrained to the shortest duration viable following the patient'slesion or surgical intervention.

The motive for this is the mechanism of motion of NSAIDS, which is the inhibition of the enzyme cyclooxygenase (COX) which takes part inside the synthesis of seasoned- inflammatory lipid mediators called prostanglandins and thromboxanes. Ironically, the identical mediators that result in the preliminary section and symptoms of inflammation are folks who will take part and stimulate the expression of different enzymes that synthesize mediators liable for the decision of irritation, or in other phrases, its cease. For instance, prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) induce the expression of the enzyme 15-lipoxygenase (15-LOX) in its lively shape in leucocytes, which catalyzes a step in the manufacturing cascade of a potent pro-resolving mediator named lipoxin A4 [24].

Lipoxin A4 is a member of a group of lipid mediators of decision that includes resolvins, protectins and the aspirin-brought about analogs of those training. It does now not have immunosuppressive residences, in assessment, it turns on precise cellular mechanisms, consisting of the stimulation of non-phologistic recruitment of monocytes (with out elaborating pro-inflammatory mediators), activation of macrophage phagocytosis of microorganisms and apoptotic cells, boom in phagocyte exit thru the lymphatics, expression of antimicrobial molecules and inhibition of in addition neutrophil and eosinophil infiltration [25].

Another exciting decision pathway, whose discovery created quite a few controversy in this area of studies, is the motion of prostaglandins at the resolution section of inflammation [26]. It turned into tested that COX is expressed also at that point, correlating with the manufacturing of PGD2, prostaglandin 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2 - the non-enzymatic degradation made of PGD2) and currently, prostaglandin F2a (PGF2a). While statistics confirming PGF2a decision residences is restricted, PGD2 and 15d-PGJ2 have properly set up anti inflammatory and pro- resolving results on inflammation fashions, such as Promotion of leukocyte apoptosis, macrophage clearance from infected sites, and control of cytokines and chemokines that adjust leukocyte trafficking . These results are mediated by means of activation of DP1 receptor via PGD2 and inhibition of nuclear aspect γB (NFγB) activation via peroxisome proliferator-activating receptor γ (PPAR γ) via 15d-PGJ2 [27].

Considering all this statistics, the extended or behind schedule use of anti- inflammatory tablets, by blocking the manufacturing of prostaglandins and the further synthesis of pro-resolving mediators, ought to cause a postpone in tissue recovery or even establishment of a continual lesion. Some measures can be taken to avoid harming the patients, which includes decreasing the prescription and use of NSAIDS to the smallest duration essential for symptom remedy, for instance, edema and ache. In addition, the selection of a medicinal drug with little anti-inflammatory hobby but nonetheless suitable analgesic effect, including acetaminophen (Paracetamol), dypirone and diclofenac, or even codeine-NSAIDs combined drugs, ought to additionally be taken into consideration.

Special interest need to also accept to determine precisely the degree of the inflammatory method encountered inside the affected person earlier than the administration of any form of anti inflammatory drug. Knowing the degree of irritation, the choice of applying pills with anti inflammatory interest or simplest analgesic pastime is less difficult. Proceeding this way, it is much more likely that the pharmacological intervention will not interfere with the natural path of inflammation and resolution, consequently, growing the performance in patient treatment and recuperation.

Treatment of anti-inflammatory

Non-steroidal anti-inflammatory tablets (NSAIDs)

Non-steroidal anti-inflammatory tablets (NSAIDs) have been the corner stone of ache management in sufferers with osteoarthritis and other painful situations. In the USA an envisioned five% of all visits to a doctor are associated with prescriptions of non- steroidal anti inflammatory tablets and they are most of the most usually used drugs [28-29]. In 2004, rofecoxib, advertised as a cyclo-oxygenase-2 (COX- 2) selective inhibitor, turned into withdrawn from the market after the outcomes of a randomised placebo managed trial confirmed an accelerated chance of cardiovascular occasions associated with the drug. This locating becomes confirmed in other trials and a cumulative meta-analysis [30-31]. Since then debate has surrounded the cardiovascular safety of cyclo-oxygenase-2 selective inhibitors, observed by comparable concerns approximately conventional non-steroidal anti inflammatory drugs [32]. More lately, america Food and Drug Administration determined against the approval of etoricoxib because of its insufficient hazard-benefit profile [33].

Mechanism of movement of NSAIDs

Traditionally, the analgesic motion of non-steroidal anti-inflammatory drugs (NSAIDs) has been defined on the basis of their inhibition of the enzymes that synthesis prostaglandins.

However, it's miles clear that NSAIDs exert their analgesic impact now not best through peripheral inhibition of prostaglandin synthesis however also thru a ramification of other peripheral and vital mechanisms. It is referred to now that there are 2 structurally wonderful varieties of the cyclo-oxygenase enzyme (COX-1 and COX-2). COX-1 is a constitutive member of normal cells and COX-2 is precipitated in inflammatory cells. Inhibition of COX-2 activity represent the maximum in all likelihood mechanism of action for NSAID-mediated analgesia, whilst the ratio of inhibition of COX-1 to COX-2 by means of NSAIDs ought to decide the probability of detrimental outcomes. In addition, a few NSAIDs inhibit the lipoxygenase pathway, which may additionally itself bring about the manufacturing of algogenic

metabolites. Interference with G-protein-mediated signal transduction by way of NSAIDs can also shape the basis of an analgesic mechanism unrelated to inhibition ofprostaglandin synthesis [34].

Immune Selective Anti-Inflammatory Derivatives (ImSAIDs)

Leukocyte (white blood cell) activation and transmigration are the earliest and most essential occasions that arise in the course of irritation. Leukocytes, which participate in all inflammatory processes, are essential in host defense but immoderate and inappropriate activation can bring about worsening of pathology. Granulocytes, particularly, launch a spectrum of materials which amplify the inflammatory cascade as illustrated in pancreatitis, sepsis, allergies, ischemic reperfusion harm, trauma, hepatitis, and so forth. It is properly widely wide-spread that modulating the excessive activation and migration of leukocytes is a new goal for pharmaceutical improvement [35-36]. In precis, the modern anti inflammatory repository is deficient for a spread of inflammatory and critical care warning signs which advise to be happy by means of the ImSAIDs.

ImSAIDs constitute a completely new technology of biologically derived immuno- modulating anti inflammatory molecules. ImSAIDs are not steroids or NSAIDs and act through a mobile mode of movement that inhibits infection triggered with the aid of the innate immune reaction. ImSAIDs have interaction with co-stimulatory cellular floor receptors to inhibit immoderate activation and infiltration of leukocytes without compromising the immune machine. This upstream technique reduces the release of seasoned-inflammatory cytokines, digestive enzymes, and oxidative burst merchandise thereby inhibiting inflammatory amplification and keeping collateral tissue [37].

REVIEW OF LITERATURE

Sathiyabalan *et al.*, (2017) the anti-inflammatory effect of an ethanol extract of the entire plant of *Petiveria alliacea* was tested. The presence of alkaloid, catechin, coumarin, flavonoids, tannin, saponins, steroid, phenol, glycoside, and terpenoids was discovered in an ethanol extract of the entire plant. After 3 hours of therapy with carrageenen-induced paw edoema, maximum inhibition (76.35 percent) was observed at a dosage of 400 mg/kg of *P. alliacea* whole plant, whereas indomethacin provided 77.34 percent inhibition [38]

Kamau *et al.*, (2016) investigated the anti-inflammatory effects of methanolic extracts of Kigelia africana and Acacia hockii in mice. The leaf extract of K. Africana reduced the diameter of inflamed hind paws in mice by 0.21 percent to
4.98 percent, whereas the stem bark extract of A. hockii reduced the diameter of inflamed hind paws by 0.6 percent to 5.38 percent. The diclofenac decreased the diameter of inflamed hind paws by between 1.11% and 4.9 percent. Saponins, flavonoids, alkaloids, terpenoids, phenolics, and cardiac glycosides were found inthe qualitative phytochemical screening. [39]

Shaikh *et al.*, (2016) the anti-inflammatory activity of selected medicinal plants utilised in Indian traditional medicine was investigated in vivo and in vitro. The COX-1 and 2 inhibitory and antioxidant properties of progressively extracted plant materials such as *Cissus quadrangularis, Plumbago zeylanica, Terminalia bellarica,* and *Terminalia chebula* in water, ethanol, and hexane were examined in vitro. Using carrageenan and the Phorbol Myristate Acetate (PMA) generated mice edoema animal model, the in vivo anti-inflammatory efficacy of selected samples displaying potential COX-2 inhibition was tested. In comparison to COX-1, most plants were shown to decrease COX-2 activity, according to the findings [40]

Nilanjana *et al.*, (2013) studied at the ethanolic extract of *Curcuma longa* rhizomes and observed the presence of flavonoids and amino acids, while the aqueous extract was determined to contain alkaloid

further to flavonoids, amino acids. [41]

Rita *et al.*, (2012) research showed antioxidant, wound healing and anti inflammatory pastime of ethanolic extract of *Curcuma longa* Linn rhizomes. The ethanolic extract prepared through maceration technique turned into subjected to display screen for antioxidant interest the use of DPPH radical scavenging technique and wound recovery pastime using incision, excision, histopathological and dead area wound model and the study was supported with assessment of granuloma tissue to estimate hydroxyproline content material and histopathological evaluation[42].

Bordoloi *et al.*, (2012) studied the antiulcer activity of the ethanolic extract of the rhizome of *C. caesia* on experimental animal models. Four groups of albino rats weighing 150-200 g were taken for the study (*n* = 5). Group A: Control (3%gum acacia 5 ml/kg/day orally for 7 days). Group B: Experimental control (Aspirin 400 mg/kg orally as single dose on 7th day). Group C: Test (*C. caesia* extract 500 mg/kg/day orally for 7 days plus Aspirin 400 mg/kg orally on 7th day) and Group D: Standard (Ranitidine150 mg/kg orally for 7 days and Aspirin 400 mg/kg orally on 7th day). The stomachs of the sacrificed rats were removed. The ulcer index, pepsin activity, free and total acidity and volume of gastric juice in group III and IV showed significant decrease in comparison to group II whereas there was increase in gastric mucus secretion[43].

Rajamma *et al.*, (2012) investigated antioxidant and antibacterial activities of oleoresins isolated from *Curcuma caesia*. Oleoresins were extracted from rhizomes of nine starchy *C. caesia*, using dichloromethane and evaluated for antioxidant and antibacterial activity. Oleoresins from all the species exhibited high DPPH radical scavenging activity and ferric reducing power, which had good correlation with phenolic content The oleoresins inhibited both gram+ve (*Staphylococcus aureus* and *Bacillus subtilis*) and gram–ve (*Escherichia coli*) bacteria. Maximum sensitivity wasobserved in the case of *B. subtilis* [44].

Satija *et al.*, (2011) compared the analgesic and antipyretic activity of different extracts obtained from *C. caesia* and *C. amada* rhizomes. Analgesic and antipyretic activities of the plant extracts was evaluated using chemical model of acute pain and brewer's yeast induced hyperthermia in rats. The writhing and pyrexia were observed at the doses of 250 and 500 mg/kg body weight of rats. Both the plants exerted analgesic and antipyretic activity [45].

Paliwal *et al.*, (2011) investigated the bronchodilating activity of extracts of *C. caesia*. Bronchodilator activity of the extract was studied on the histamine aerosol induced Bronchospasm and preconvulsion dyspnoea in guinea pigs. Treatment with methanolic CC extract 500 mg/kg showed significant protection against histamine induced bronchospasm. In this study CC extract significantly prolonged the latent period of convulsions followed by exposure to histamine aerosol at the dose of 500 mg/kg and showed maximum protection of 34.84% at 4th h as compared to chlorpheniramine maleate (standard) 2 mg/kg, p.o. which indicating its H1 receptor antagonistic activity and supports the anti-asthmatic properties of the plant[46].

Gill *et al.*, (2011) studies two most popular species of genus Curcuma, *C. amada* and *C. caesia* were proved for their anthelmintic activity. In this study, four extracts viz. Petroleum ether, Dichloromethane, ethanol and aqueous extract of rhizomes of
C. amada and *C. caesia* were investigated for anthelmintic activity at three different concentrations. Three concentrations (50 mg/ml, 100 mg/ml and 150 mg/ml) of each extract were studied which included the determination of paralysis time and time of death of earthworms. All the extracts of both the plants exhibited dose dependant activity [47].

Karmarkar *et al.*, (2011) studied the methanolic CC extract rhizome for some *in vitro* antioxidant activity. Effect of methanol extract of *Curcuma caesia* rhizome (MECC) on ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) were evaluated *in vitro* methods like 1, 1-diphenyl-2-picrylhydrazil radical, hydroxyl radical, superoxide anion, nitric oxide, hydrogen peroxide, peroxynitrite and hypochlorus acid. Lipid peroxidation, total phenolic content was also measured by standard assay method [48].

Amit *et al.*, (2011) worked on antifertility impact of curcumin. In study at control and curcumin dealt with albino rats had been discovered for ovulation by means of vaginal smear technique. Ovarian weights have

been measured in control and curcumin handled rats for antigonadotrophic effect. In the manipulate institution there was a ordinary oestrus cycle and ovaries, uteri additionally were regular. In curcumin dealt with organization it become visible that there had been absence of cornified epithelium in vaginal smear in all the rats which persist even after few days of withdrawn of the drug. And also there had been no capabilities of ovulation and ovaries confirmed cystic changes in histopathological examination [49].

Krishnaraj *et al.*, (2010) determined phenol content and antioxidant activity of *C. caesia* in comparison with *Curcuma amada*. The total phenol contents of the methanolic rhizome extracts of *C. amada* and *C. caesia* were 37.64 mg and 44.33 mg Tannic acid equivalents/g dry material, respectively. The reducing power and superoxide, ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] and DPPH radical scavenging activities of *C. caesia* were higher than *C. amada*. These results supported that the non-conventional *C. caesia* could be an economically[50].

Arulmozhi *et al.*, (2006) evaluated anti asthmatic property of *Curcuma caesia*. The hydroalcoholic extract of *Curcuma caesia* (CC extract) was tested for its relaxant effect in guinea pig trachea and also in the presence of various receptor antagonists and enzyme inhibitors. Furthermore, the possible role of hydroalcoholic extract in calcium channel modulation was investigated in depolarized rabbit aorta. The CC extract concentration dependently relaxed the carbachol (1 μM) induced pre contractions and the presence of an antagonist, such as propranalol, glibenclamide, 2′, 5′-dideoxyadenosine, a-chymotrypsin, L-NNA and methylene blue, did not affect the log concentration relaxing response curves of cumulative CC extract to carbachol (1 μM)-induced pre contraction[51].

Chirangini *et al.*, (2004) studied on different rhizome extracts of some members of the medicinal *Zingiberales* are widely used in dietary intake as well as in the traditional system of medicine. Curcumin, the chrome orange-yellow colouring compound present in turmeric rhizomes, has long been known to possess antioxidant property. Chirangini evaluated Crude methanol extracts of the rhizomes of 11 species, including *C. caesia* for their antioxidant properties using sulphur free radical reactivity with curcumin as a reference indicator, *C. caesia* gave good degreeof radio protection [52].

Nita, (2003) studied on curcumin and determined some proof of anti-inflammatory interest of curcumin. In their research they found some of exceptional molecules involved in infection which are inhibited via curcumin including phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis issue (TNF), and interleukin-
12 (IL-12). Curcumin has been proven to be safe in six human trials and has confirmed anti inflammatory interest [53].

AIM AND OBJECTIVE

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. 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.

Curcuma is a genus of about 100 accepted species in the family Zingiberaceae that contains such species as turmeric and Siam tulip. Few known species of *Curcuma* with reported pharmacological activity are *Curcuma longa, Curcuma caesia, Curcuma amada*. Among them, *C. longa* is the one species extensively studied and has ancient traditional medicinal uses. Thus, an attempt was made to study the anti-inflammatory activity of polyherbal formulation in a single dosage.

PLAN OF WORK

1. Literature survey
2. Collection and authentication
3. Extraction of plant material

4. Phytochemical screening of extracts
5. Quantitative of bioactive constituents
6. Formulation of polyherbal topical gel
7. Evaluation of formulation by following parameters:-

a. Organoleptic properties
b. Viscosity
c. Washability
d. Extrudability
e. Spreadability
f. Measurement of pH
g. Drug content

8. *In-vivo* anti-inflammatory activity of prepared gel
9. Result and discussion
10. Summary and conclusion

PLANT PROFILE

Curcuma longa

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native in southeast India, and needs temperatures between 20 °C and 30 °C (68 °F and 86 °F) and a considerable amount of annual rainfall to thrive. Plants are gathered annually for their rhizomes, and propagated from some of those rhizomes in the following season. When not used fresh, the rhizomes are boiled for about 30–45 minutes and then dried in hot ovens, after which they are ground into a deep orange-yellow powder commonly used as a spice in Indian cuisine and even curries, for dyeing, and to impart color to mustard condiments.

Classfication

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Curcuma

Figure 5.1: *Curcuma longa* Rhizomes

Chemical Composition of Turmeric

The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice curcumin, giving the yellow colour to turmeric, was first isolated by Vogel in 1842.

Turmeric contains protein (63%), fat (5.1%), minerals (3.5%), carbohydrates (6.94%) moisture (13.1%) and essential oil (0.8%). The steam distillate of turmeric i.e., essential oils contains, phelladrene (1.00%), sabinene (0.6%), cineol (1.00%), borneol (0.5%), zingiberene (25%) and sesquiterpenes (50%) and curcumin (3.4%) which is responsible for yellow colour[54].

Biological Activity of Turmeric extracts and its components

Extensive research within the last half a century has proven that most of these activities, once associated with turmeric, are due to curcumin. Even though curcumin is the major component, it contains many powerful antioxidants and anti inflammatory compounds. Large number of applications of turmeric extractswas observed [55].

Curcumin

Curcumin, a major yellow pigment found in the rhizome of *cucuma longa*. It is a potent antioxidant, liver detoxifier and protector, gallstone prevention and cholesterol lowering compound. It suppresses the damage to liver cells caused by hepatitis C and stimulates glutathione by the liver. It is a potent cancer prevention and cancer treatment compound. It stimulates apoptosis of cancer cells. It assists in stopping all stages of cancer formation i.e., initiation, promotion and progression. Curcumin in preliminary studies suggests that it is likely to inhibit prostate, breast, skin, colon, stomach, and liver cancers and is suitable for use in conjunction with chemotherapy [56].

Curcuminoids and its Biological activity

In the last few decades, efforts have been made to isolate curcuminoids from different sources, including Curcuma longa, Curcuma zedoaria, and Curcuma aromatica. Several research groups have investigated and compared their antioxidant, cardioprotective, neuroprotective, antidiabetic, antitumor and chemopreventive activities, employing them either individually or as mixtures. The curcuminoids have been shown to be scavengers of free radicals and reactive oxygen species (ROS), such as hydroxyl radicals, superoxide radicals, singlet oxygen, peroxyl radicals and peroxynitrite, whose production is implicated in the induction of oxidative stress [57].

Parts used

Rhizomes, Leave, Root & Stem

Uses

The active compound curcumin is believed to have a wide range of biological effects including anti-inflammatory, antioxidant, antitumour, antibacterial, and antiviral activities, which indicate potential in clinical medicine. In Chinese medicine, it is used for treatment of various infections and as an antiseptic. Turmeric is mostly used in savory dishes, but is used in some sweet dishes, such as the cake *Sfouf*. In India, turmeric plant leaf is used to prepare special sweet dishes, *patoleo*, by layering rice flour and coconut-jaggery mixture on the leaf, and then closing and steaming it in a special copper steamer It is commonly used in Tamil Nadu as a home remedy when someone is suffering from fever. Turmeric paste is often used in Tamil Nadu as an antiseptic in open wounds, while *chun-holud* (turmeric with slaked lime) is used to stop bleeding as home remedies. It is also used as a detanning agent [58].

Curcuma Caesia

Curcuma caesia, black turmeric or black zedoary is a perennial herb with bluish- black rhizome, native to North-East and Central India. Black turmeric is also sparsely found in the Papi Hills of East Godavari, West Godavari, and the Khammam districts of Andhra Pradesh. The rhizome of black turmeric has a high economic importance owing to its putative medicinal properties. In west Bengal, the rhizome of the plant is used in Kali Puja, and hence the plant is called Kali haldi. The cultivation and harvest practices are similar to that of common turmeric which is used in recipes. In the fields, the rhizomes are washed thoroughly and are placedin a wide mouthed cauldron.

Common Names

Curcuma Kuchoor, Kali Haldi, Krishna kedar, Yaingang Amuba, Kala-haldi, Nalla Pasupu, kariarishina,

naru kachora, Aihang

Classfication

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Curcuma

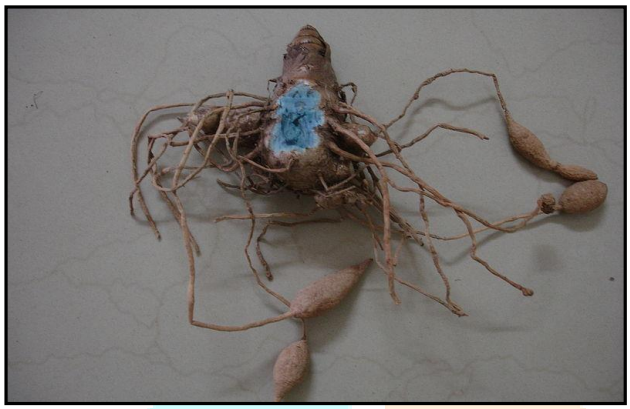

Figure 5. Curcuma caesia Rhizomes

Uses

The rhizomes are used as a rub efficient to rub the body after taking a Turkish bath. It is used in the fresh state-turmeric. The rhizomes of the herb are often used by the Baiga, Sahariya, Agariya, Gond, Korku, and for the treatment of pneumonia, cough, and cold in children, and for fever and asthma in adults [59]. The powder of rhizomes is used by tribal women as a face-pack during their engagement and marriage period. Fresh rhizomes are crushed and applied as a paste on forehead for relief from migraine or applied on the body for sprains and bruises. Apply fresh rhizome paste on snake and scorpion bites. The rhizomes are claimed to have a property of acting against leukoderma, epilepsy, cancer and HIV/AIDS. Apply rhizome paste on the hydrosol using betel leaves. Powdered tuber is orally administered with water in stomachache and bloating.

Curcuma amada

Common Names

Amiyaa haldi, Mango ginger, maamidi, Temu mangga, Ambiya haladi, amragandha,

Classfication

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Curcuma

Figure 5.3: *Curcuma amada* Rhizomes

Curcuma amada (mango ginger) is a plant of the ginger family Zingiberaceae and is closely related to turmeric. The rhizomes are very similar to ginger but have a raw mango taste. They are used in making pickles in south India. The taxonomy of the species is a subject of some confusion as some authorities have considered the name
C. mangga as identical while others describe it as a distinct species with *C. mangga* being found in southern India while *C. amada* is of East Indian origin. Curcuma mangga extracts have shown cytotoxic activities on KB, A549, Ca Ski, HT-29 and MRC-5 cancer cell lines. Mango ginger (*Curcuma amada*) *is* a spice of high usage in pickles, sauce, culinary formulations and traditional/folk systems of medicine for therapeutic actions in Asian countries.

Uses

Curcuma amada used as various type of ayurvetic preparation like Kandu, Vrana, Kasa, Svasa, Hikka, Jvara, Abhighataja Sotha, Karnasula, Sannipata. Appetiser, carminative, digestive,demulcent. Stomachic, appetizer, expectorant, antipyretic, anti-inflammatory. Specific in rheumatism and inflammation of liver, rheumatism, in contusions and sprains. The rhizomes are used externally in the form of paste as an application for bruises and skin diseases and combined with other medicines it is useful in improving quality of blood. Mango ginger (*Curcuma amada*) is a rhizomatous aromatic herb rhizome that are used as flavouring for pickles and other dishes and also valued for their medicinal properties. The fresh as well as dried rhizomes are used for flavouring curries. The fresh cut rhizomes have the flavour and the colour of mango, hence the name mango ginger [60].

EXPERIMENTAL WORK

This chapter deals with the material and methods used for phytochemical extraction, their preliminary chemical screening, formulation, development and evaluation of polyherbal gel and their anti-inflammatory activity of rhizomes of *Curcuma longa, Curcuma caesia, Curcuma amada.*

Materials and Equipments

Table 6.1: Materials used for study

Sr. No.	Chemicals	Supplier
1.	Potassium Mercuric Iodide	Thomas Baker, Mumbai
2.	Iodine	Loba chemie Pvt. Ltd., Mumbai
3.	Potassium Iodide	Loba chemie Pvt. Ltd., Mumbai
4.	Potassium Bismuth Iodide	S. D. Fine Chem. Ltd., Mumbai
5.	Picric acid	Thomas Baker, Mumbai
6.	Sodium nitropruside	Loba chemie Pvt. Ltd., Mumbai
7.	Sodium hydroxide	Loba chemie Pvt. Ltd., Mumbai
8.	Pyridine	S. D. Fine Chem. Ltd., Mumbai
9.	Ferric chloride	Thomas Baker, Mumbai
10.	Gelatin	S. D. Fine Chem. Ltd., Mumbai
11.	Lead acetate	Loba chemie Pvt. Ltd., Mumbai
12.	Nitric acid	S. D. Fine Chem. Ltd., Mumbai
13.	Copper acetate	S. D. Fine Chem. Ltd., Mumbai
14.	Sodium Chloride	S. D. Fine Chem. Ltd., Mumbai

15.	Methanol	Qualigens Fine Chemicals, Mumbai
16.	Ethanol	Qualigens Fine Chemicals, Mumbai
17.	Chloroform	Qualigens Fine Chemicals, Mumbai
18.	Folin-Ciocalteu reagent	Loba chemie Pvt. Ltd., Mumbai
19.	Fehling's solution	Central drug house ltd new Delhi

Table 6.2: Instruments used for study

Sr. No.	Instruments	Supplier
1.	UV -Visible Spectrophotometer	Labindia 3000+
2.	Micro Centrifuge	REMI laboratory, Mumbai
3.	pH Meter	Accumax India, New Delhi
4.	Electronic Balance	Contech Instruments Ltd. , Mumbai
5.	Hot Air Oven	Oracle Equipments, New Delhi
6.	Vortex Apparatus	Ambros Lab Equipments, Ambala
7.	Rotary Vaccum Evaporator	Microtech Scientific Instruments, NewDelhi
8.	Sonicator	Athena Technology, Thane

Selection of plants

The plants have been selected on its availability and folk use of the plant.

Collection of plant material

Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh & healthy plant materials, free from diseases of rhizomes of *Curcuma longa*, *Curcuma caesia*, *Curcuma amada* were collected from ruler area of Bhopal (M.P.) in the month of January, 2022.

Drying

The rhizomes of plants sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. Drying of fresh plant parts were carried out in sun but under the shade.

Storage

Dried rhizomes of *Curcuma longa, Curcuma caesia, Curcuma amada* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Percentage loss

The weight of fresh sample and dried powder was determined and percentage lossdue to drying and loss of water was calculated.

The percentage loss was calculated by using following formula:

$$\%\ \textbf{Loss of drying} = \frac{Loss\ in\ weight\ of\ the\ sample}{Weight\ of\ the\ sample} \times 100$$

Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure will be adopted for the preparation of extract from the shade dried material.

Defatting of plant material

36.51 gram of *Curcuma longa,* 54.21 gram of *Curcuma caesia* and 40.74 gram of *Curcuma amada* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether

(60-80°C) in a maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted rhizomes of *Curcuma longa, Curcuma caesia, Curcuma amada* were exhaustively extracted with hydroalcoholic solvent (ethanol: aqueous: 70:30v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts [61-62].

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extract obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence ofalkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence ofcarbohydrates.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the

presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. **Detection of proteins**

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. **Detection of diterpenes**

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [63].

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin- Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [64]

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10- 50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [64].

Formulation development of polyherbal gel

Method of preparation

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of rhizomes of *Curcuma longa*, *Curcuma caesia*, *Curcuma amada* were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then Carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed [65].

Carbopol 940 – Gelling polymer
Triethanolamine- Gelling agent, pH adjusting agent, neutralizer
Methyl Paraben - Preservative
Distilled Water, Glycerin and Polyethylene Glycol-solvents

Table 6.3: Formulation of polyherbal gel

Ingredients	PHG1	PHG 2	PHG3	PHG4	PHG5	PHG6
Curcuma longa extract	1gm	1gm	1gm	1gm	1gm	1gm
Curcuma caesia extract	1gm	1gm	1gm	1gm	1gm	1gm
Curcuma amada extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.25mg	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation of polyherbal gel [66-69]

A. Appearance and consistency:

The physical appearance was visually checked for the texture of polyherbal gel formulations and observations may be like stated in Table 7.11.

B. Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations may be like stated in table 7.12.

C. Extrudability determination of formulations

The polyherbal gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

D. Determination of SpreadabilityPrinciple:

An important criterion for polyherbal gel is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value.

A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability.

Method:

Two glass slides of standard dimensions (6×2) were selected. The polyherbal gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams
of weight was placed up on the upper slide so that the polyherbal gel formulation between the two slides was traced uniformly to form a thin layer.
The weight was removed and the excess of the polyherbal gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each polyherbal gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)l= length of glass
slide (6cms).
t = time taken is seconds.

E. Determination of pH

The pH of the polyherbal gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

F. Drug content (Phenol content)

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

G. *In-vivo* pharmacological activityAnimals:

Animals Healthy Wistar albino rats weighing between 180 and 200 g were used for the present study. The study protocol was approved by the Institutional Animal Ethics Committee as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India. The

animals were acclimatized to the standard laboratory conditions at 25 ± 2 °C, relative humidity of 44–56%, and light and dark cycle of 12:12 h and fed with standard diet and water ad libitum during the study.

Acute dermal toxicity

The acute dermal toxicity test of polyherbal extract was determined according to the OECD guidelines no. 402[70]. Adult Wistar rats of either sex were used. Nine animals were divided in two groups, each group comprising of three animals. Approximately 24 h before the test, 10% hairs of the body was depilated from the dorsal area of the test animals by suitable depilatory preparation. Group I animals were considered as control, Group II animals received Poly herbal gel of (*Curcuma longa, Curcuma caesia, Curcuma amada* (PHG) (5%) topically at 2000 mg/kg body weight. All animals were monitored for 14 days for changes in fur, eyes, behavior and toxic dermal reactions.

Grouping of animals and treatment scheduled

The rats were divided into 5 groups (n = 6) and were subjected to the following treatment scheduled: Group-I: Normal control (left untreated). Group-II: Negative control (Gel base applied topically) Group-III: Standard (5% w/w diclofenac gel was applied topically) Group-IV: PHG (2%) applied topically Group-V: PHG (5%)applied topically.

Table 6.4: Grouping of animals.

Treatment group	Dose (mg/kg)
Negative Control	Gel Base
Diclofenac gel	5% w/w
Poly herbal gel of *Curcuma longa, Curcuma caesia, Curcuma amada* (PHG)	2% w/w
Poly herbal gel of *Curcuma longa, Curcuma caesia, Curcuma amada* (PHG)	5% w/w

Carrageenan induced paw edema in rats

The anti-inflammatory activities of the test drug under study were evaluated by using the Carrageenan-induced edema model. The 50 mg of each gel base, standard diclogel and PHG 2% & 5% was applied to the plantar surface of the left hind paw of negative control, standard and test drug treated group respectively by gently rubbing 50 times with the index finger. Three hours after the dose, 0.1 ml of 1% Carrageenan solution in normal saline was injected via sub-plantar route in the left hind paw of each animal. The right hind paw however received 0.1 ml of saline. Paw edema was measured every 60 min up to 4 h after the injection of Carrageenan. Digital Vernier callipers (Digi calliper) were used to measure the difference in footpad thickness between the right and left foot. Mean values of treated groups and control group were compared and analyzed using statistical methods [71].

Measurement of paw thickness

The volume of the paw up to the tibiotarsal joint were measured by using digital Vernier caliper, after one, two, three and four hours of the induction of inflammation. The percentage increase in the thickness of the left paw in comparison to the right one of each rat, is an indication of the inflammation produced and was calculated by the following equation:

$$\%\text{increase in paw thickess} = \frac{(\text{Thickness of right paw} - \text{Thickeness of left paw})}{\text{Thickness of right paw}} \times 100$$

$$\%\text{ reduction in paw edema} = \frac{(\%\text{ thickness of Normal control} - \%\text{ thickeness treated group})}{\%\text{ thickness of Normal control}} \times 100$$

Statistical analysis

Statistical calculations were carried out using GraphPad Prism 6, version 6.01. All values are represented as mean ± SEM,(n = 6) animals in each group. Data were analysed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test where $P < 0.1$, $P < 0.01$, $P < 0.001$, $P < 0.0001$ were considered significant.

RESULTS AND DISCUSSION

Results of percentage loss

Table 7.1: Showing the results of percentage loss of *Curcuma longa*

S. No.	Description	Weight in (gms.)	% Loss
1.	Weight of plant material (Rhizomes) in wet, fresh condition	76	51.3%
2.	Weight of plant material (Rhizomes) after drying at room temperature	37	
3.	Loss in weight on drying	76-37=39	

Table 7.2: Showing the results of percentage loss of *Curcuma caesia*

S. No.	Description	Weight in (gms.)	% Loss
1.	Weight of plant material (Rhizomes) in wet, fresh condition	80	47.5%
2.	Weight of plant material (Rhizomes) after drying at room temperature	42	
3.	Loss in weight on drying	80-42=38	

Table 7.3: Showing the results of percentage loss of *Curcuma amada*

S. No.	Description	Weight in (gms.)	% Loss
1.	Weight of plant material (Rhizomes) in wet, fresh condition	101	45.5%
2.	Weight of plant material (Rhizomes) after drying at room temperature	75	
3.	Loss in weight on drying	101-55=46	

Table No. 7.1-7.3 showed the percentage loss of *Curcuma longa*, *Curcuma caesia* and *Curcuma amada* were found 51.3%, 47.5% and 45.5% respectively.

Results of percentage yield

Table 7.4: Results of percentage yield of extract of *Curcuma longa, Curcumacaesia, Curcuma amada*

Hydroalcoholic extracts	Percentage yield (w/w)
Curcuma longa	3.41%
Curcuma caesia	6.35%
Curcuma amada	5.27%

Table No. 7.4 showed the percentage yield of *Curcuma longa*, *Curcuma caesia* and

Curcuma amada were found 3.41%, 6.35% and 5.27% respectively.

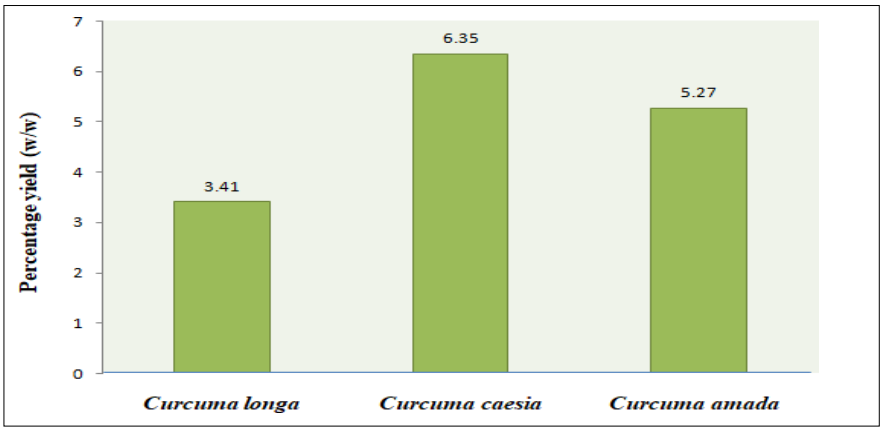

Figure 7.1: Comparative graph of percentage yield (w/w)

Results of phytochemical screening of extract

Preliminary phytochemical screening actually helps in isolating and characterizing the bioactive constituents of plant extracts. A small portion of the dried extracts of *Curcuma longa*, *Curcuma caesia* and *Curcuma amada* underwent phytochemical screening using Kokate (1994) methods to chemical test for alkaloids, glycosides, flavonoids, saponins, phenolics, proteins, and diterpenes separately for extracts of allsamples.

Table 7.5: Result of Phytochemical screening of extract of *Curcuma longa*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Wagner's Test: Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids Lead acetate Test: Alkaline Reagent Test:	 +Ve -Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 -Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

From the table no. 7.5, it could see that, flavonoids, saponins, carbohydrate and phenol were present in hydroalcoholic extract of *Curcuma longa*. The phytochemical screening of *Curcuma longa* revealed negative results for proteins, alkaloids, glycosides and diterpenes.

Table 7.6: Result of Phytochemical screening of extract of *Curcuma caesia*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Wagner's Test: Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids Lead acetate Test: Alkaline Reagent Test:	 +Ve -Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

The phytochemical tests revealed various bioactive secondary metabolites which might be responsible for their medicinal attributes. The hydroalcoholic extract of *Curcuma caesia* had revealed the presence of saponins, proteins, carbohydrate,flavonoids, glycosides and phenol.

Table 7.7: Result of Phytochemical screening of extract of *Curcuma amada*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Wagner's Test: Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids Lead acetate Test: Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 -Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 -Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

Phytochemical screening extract of *Curcuma amada* showed the present ofglycosides, flavonoids, phenol and carbohydrate.

Results of estimation of total phenol and flavonoids content

Total phenol content estimation (TPC)

Table 7.8: Preparation of calibration curve of gallic acid

S. No.	Concentration (µg/ml)	Absorbance* (Mean±S.D)
0	0	0
1	10	0.128±0.001
2	20	0.243±0.002
3	30	0.347±0.003
4	40	0.449±0.002
5	50	0.552±0.002

N=3 Average of three determination

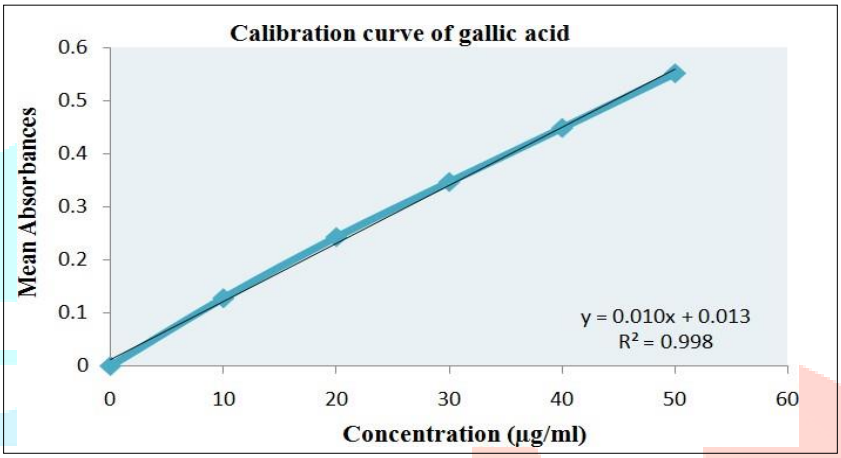

Figure 7.2: Graph of calibration curve of gallic acid

Total flavonoids content estimation (TFC)

Table 7.9: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
0	0	0
1	10	0.167±0.001
2	20	0.311±0.002
3	30	0.465±0.002
4	40	0.599±0.001
5	50	0.741±0.001

N=3 Average of three determination

Figure 7.3: Graph of calibration curve of Quercetin

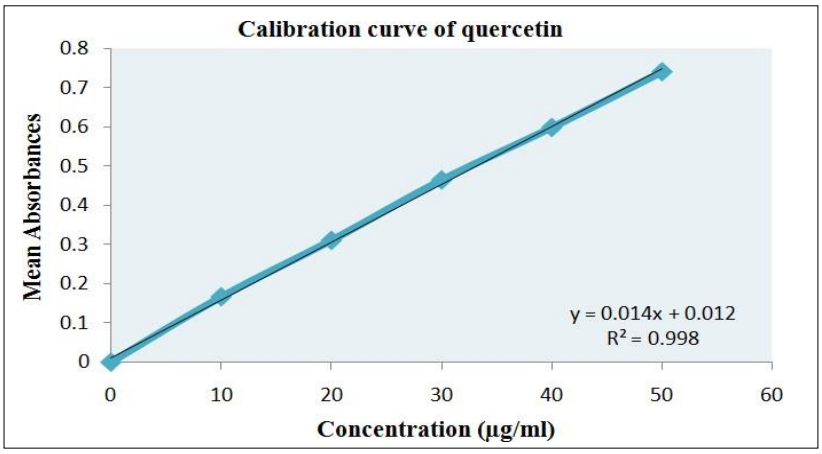

Table 7.10: Estimation of total phenolic and flavonoids content of *Curcumalonga, Curcuma caesia, Curcuma amada*

S. No.	roalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	*Curcuma longa*	0.342	0.124
2.	*Curcuma caesia*	0.541	0.425
3.	*Curcuma amada*	0.474	0.357

Results of formulation development of polyherbal gel

Evaluation of polyherbal gel

Table 7.11: Results of physical appearance

Formulation	Colour	Clogging	Homogeneity	Texture
PHG1	Brown	Absent	Good	Smooth
PHG2	Brown	Absent	Good	Smooth
PHG3	Brown	Absent	Good	Smooth
PHG4	Brown	Absent	Good	Smooth
PHG5	Brown	Absent	Good	Smooth
PHG6	Brown	Absent	Good	Smooth

Results: In the above formulations of gels, it has been noted that all of them hasclear colour, no clogging, good homogeneity and smooth texture.

Results of washability and extrudability

Table 7.12: Results of washability and extrudability

Formulation	Washability	Extrudability
PHG1	Good	Average
PHG2	Good	Average
PHG3	Good	Average
PHG4	Good	Average
PHG5	Good	Average
PHG6	Good	Average

Results: In the above formulations of gels, they have good washability as well asextrudability.

Results of spreadability

Table 7.13: Results of spreadability

Formulation	Spreadability (gcm/sec)
PHG1	18±0.24
PHG2	15±0.57
PHG3	17±0.96

PHG4	20±0.84
PHG5	12±0.51
PHG6	24±0.36

***Mean±S.D., Average of three determinations**

Results: In all above formulations of gel the spreadibility of PHG5 is good.

Determination of pH

Table 7.14: Determination of pH

Formulation	pH
PHG1	7.6±0.2
PHG2	7.0±0.5
PHG3	7.1±0.1
PHG4	6.9±0.4
PHG5	7.2±0.3
PHG6	6.8±0.5

***Mean±S.D., Average of three determinations**

Results: The above formulation of topical gels has different pH value for differentformulation.

Results of Viscosity

Table 7.15: Results of Viscosity

Formulation	Viscosity (cps)
PHG1	2213±12
PHG2	2035±15
PHG3	1985±13
PHG4	2344±18
PHG5	2078±15
PHG6	2496±12

***Mean±S.D., Average of three determinations**

Results: In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHG5 has good viscosity.

Results of phenol content

Table 7.16: Results of phenol content

Formulation	Phenol content (mg/100mg)
PHG1	0.242±0.001
PHG2	0.624±0.002
PHG3	0.521±0.005
PHG4	0.470±0.003
PHG5	**0.878**±0.004
PHG6	0.657±0.002

***Mean±S.D., Average of three determinations**

Results: In the above formulation of different gels the percentage of drug content was found that PHG5 has maximum percentage of drug content.

Acute dermal toxicity

For acute dermal toxicity as per OECD 402 guidelines, the limit test at 2000 mg/kg was performed instead of the main test. The limit test are primarily used for the in vivo studies when we have the primary information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. The present study deals with the natural/ non-synthetic compounds hence the information about the toxicity of the test material has been obtained from literatures for similar tested compounds or similar tested mixtures or products. The toxicity of the test drug under consideration had been assured by thorough literature search. The poly herbal gel of *Curcuma longa*, *Curcuma caesia*, *Curcuma amada* PHG (5%) was safe up to the dose of 2000 mg/kg and no any changes were observed in fur, eyes, and behavior of treated animals. The treated animals also did not show any signs of skin irritation or adverse reaction. The animals were found to be normal and active with no mortality. These formed the basis for considering the formulation safe for dermatological applications.

Anti-inflammatory effect of poly herbal gel of *Curcuma longa, Curcuma caesia, Curcuma amada*

The inflammation induced by the Carrageenan lasts approximately up to 5 h usually consisted of three phases, the first phase is from 0 to 1.5 h, second phase is from 1.5 to 2.5 h and third phase lasts from about 2.5–5 h. The first phase corresponded with the release of histamine and serotonin while bradykinin and prostaglandin for second and third phase respectively.

Table 7.17: Anti-inflammatory activity of Poly herbal gel of (*Curcuma longa, Curcuma caesia, Curcuma amada*) on Carrageenan-induced paws edema

Treatment groups	Paw Thickness (mm)				% Inhibition of Edema at 4 h
	60 min	120 min	180 min	240 min	
Negative Control	6.25 ± 0.147	6.46 ± 0.216	6.64 ± 0.243	6.86 ± 0.188	0
Diclofenac gel 5% w/w	5.56 ± 0.070	4.96 ± 0.030	4.50 ± 0.012	4.13 ± 0.024	34.62
PHG 2% w/w	6.15 ± 0.123	5.74 ± 0.094	5.20 ± 0.057	4.41 ± 0.167	39.46
PHG 5 % w/w	6.043 ± 0.067	6.17 ± 0.064	4.96 ± 0.208	4.57 ± 0.095	35.01

Data are represented as Mean paw thickness of rats (mm) ± SEM, n = 6.

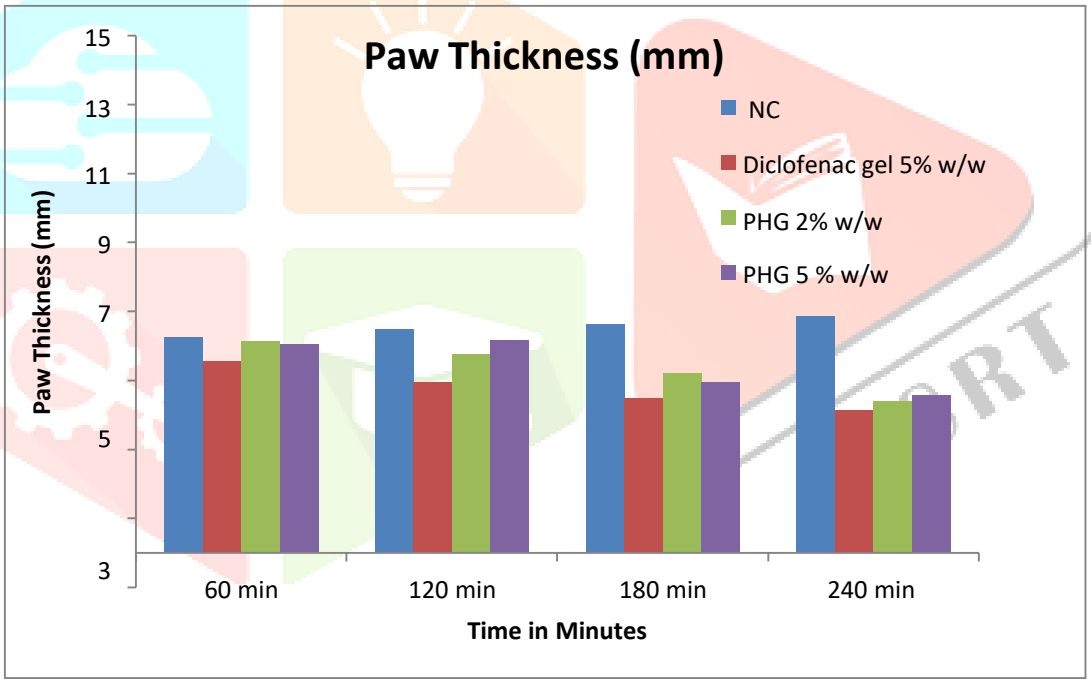

Figure 7.4: Graph of paw thickness

The percentage increased in paw thickness of the Standard diclofenac gel and PHG (2% and 5%) treated groups at 60, 120, 180 and 240 min. after the induction of inflammation. It has been observed that the standard drug treated animals showed maximal anti-inflammatory effect within 120 min after the Carrageenan injection as it represent no percentage increase in paw thickness.

However the poly herbal gel of *Curcuma longa, Curcuma caesia, Curcuma amada* (5%) PHG (2% and 5%) showed significant and maximal anti-inflammatory effect after 180 min of the Carrageenan injection as compared to normal and negative control animals ($P < 0.0001$) as depicted in Fig 7.4. Afterwards beyond 240 min the inflammation again increased and then diminished within 5 h.

SUMMARY AND CONCLUSION

Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio. There are numerous medicinal plants which are widely used in the treatment of skin diseases and also known to possess antimicrobial activity. Topical application of

gels at pathological sites offer great advantages in a faster release of a drug directly to site of action as compared to cream and ointment. Medicinal plants are used all over the world to treat various diseases due to its variety of phytochemical constituents. Ideally, topical therapy is the first line treatment for many skin diseases. Among the various topical formulations, gels have been considered as a potential vehicle due to its non-sticky nature, stable and greater aesthetic value.

Table No. 7.1-7.3 showed the percentage loss of *Curcuma longa, Curcuma caesia* and *Curcuma amada* were found to be 51.3%, 47.5% and 45.5% respectively. Table No. 7.4 showed the percentage yield of *Curcuma longa, Curcuma caesia* and *Curcuma amada* were found to be 3.41%, 6.35% and 5.27% respectively.

From the table no. 7.5, it could see that, flavonoids, saponins, carbohydrate and phenol were present in hydroalcoholic extract of *Curcuma longa*. The phytochemical screening of *Curcuma longa* revealed negative results for proteins, alkaloids, glycosides and diterpenes. The phytochemical tests revealed various bioactive secondary metabolites which might be responsible for their medicinal attributes. The hydroalcoholic extract of *Curcuma caesia* had revealed the presence of saponins, proteins, carbohydrate, flavonoids, glycosides and phenol. Phytochemical screening extract of *Curcuma amada* showed the present of glycosides, flavonoids, phenol and carbohydrate.

In the above formulations of gels, it has been noted that all of them has clear colour, no clogging, good homogeneity and smooth texture. In the above formulations of gels, they have good washability as well as extrudability. In all above formulations of gel the spreadibility of PHG5 is good. The above formulation of topical gels has different pH value for different formulation.

In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHG5 has good viscosity. In the above formulation of different gels the percentage of drug content was found that PHG5 has maximum percentage of drug content.

The topical application of the poly herbal gel of *Curcuma longa, Curcuma caesia* and *Curcuma amada* (5%) showed significant anti-inflammatory activity in Carrageenan induced paw edema as compared to Treated and negative control group animals. The inflammation induced by the Carrageenan lasts approximately up to 5 h after injection. It usually consists of three consecutive phases of approximately 1–
1.5 h each. Usually histamine and serotonin are believed to be involved in first phase while bradykinin and prostaglandins (PG) are involved in second and third phase respectively. Among the three distinct phases of inflammation, the last phase is considered to be the most clinically effective phase to access the anti-inflammatory drugs. It was concluded that the response of PHGs treated group animals in the first phase (1 h) after the post treatment was found to be less effective as compared to standard drug treated animals. It might be possibly due to inadequate release of histamine and serotonin. The anti-inflammatory effect was found to be consistent in the second phase (1–2 h) in all treatment group with maximum effect by standard drug treated animals that possibly indicated the release of phase two mediator. However the PHGs treated group animals at all dose level were found to be effective in phase three (2–3 h), corresponding with the significant release for phase 3 mediators.

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