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ORIGINAL RESEARCH

Analgesic and Anti-Inflammatory Properties of Two Hydrogel Formulations Comprising Polyherbal Extract

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Background: Nature represents a basic source of medicinal scaffolds that can develop into potent drugs used in the treatment of many diseases.

Aim: The present study was planned to evaluate the combined effects of polyherbal methanolic extract of the herbs (fruit of capsicum, bark of cinnamon, rhizome of turmeric and rhizome of ginger) that were individually well known for their analgesic and anti-inflammatory activities. Furthermore, we aimed to develop hydrogel formulation of this polyherbal extract and to characterize and evaluate its analgesic and anti-inflammatory potential.

Materials and Methods: *Zingiber officinale* (R.), *Capsicum annuum* (L.), *Curcuma longa* (L.), and *Cinnamomum verum* (J.) polyherbal extract (GCTC) was prepared by maceration and evaluated for analgesic and anti-inflammatory potential. Then, two different types of hydrogel formulation were prepared. One is pH-based hydrogel in which carbopol-940 was used and the other is temperature-based gel in which methocel-K100 was used as gelling agent. Different concentrations of polyherbal extract (GCTC), at 1%, 3% and 5%, were used in hydrogel formulation. These prepared hydrogel formulations were characterized and evaluated for analgesic and anti-inflammatory potential.

Results: Results show that polyherbal extract and all the developed formulations of polyherbal extract (GCTC), at concentrations of 1%, 3% and 5%, have significant analgesic and anti-inflammatory effects with good appearance, homogeneity, spreadability, extrudability and stability.

Conclusion: It was concluded from this project that polyherbal extract (GCTC) and its hydrogel have significant analgesic and anti-inflammatory potential.

Keywords: inflammation, formulation, carbopol, methocel, carrageenan

Introduction

The complex process of inflammation and pain is interconnected and includes the multiple existence like the increment of permissibility via blood vessels, protein denaturation and its mutation.[1] Topical drug delivery system is one of the most important drug delivery systems in which drug produces localized effect by penetrating through skin's deeper tissues after its application, as it prevents the drug destruction by metabolism, pH variations and enzymatic activities.[2]

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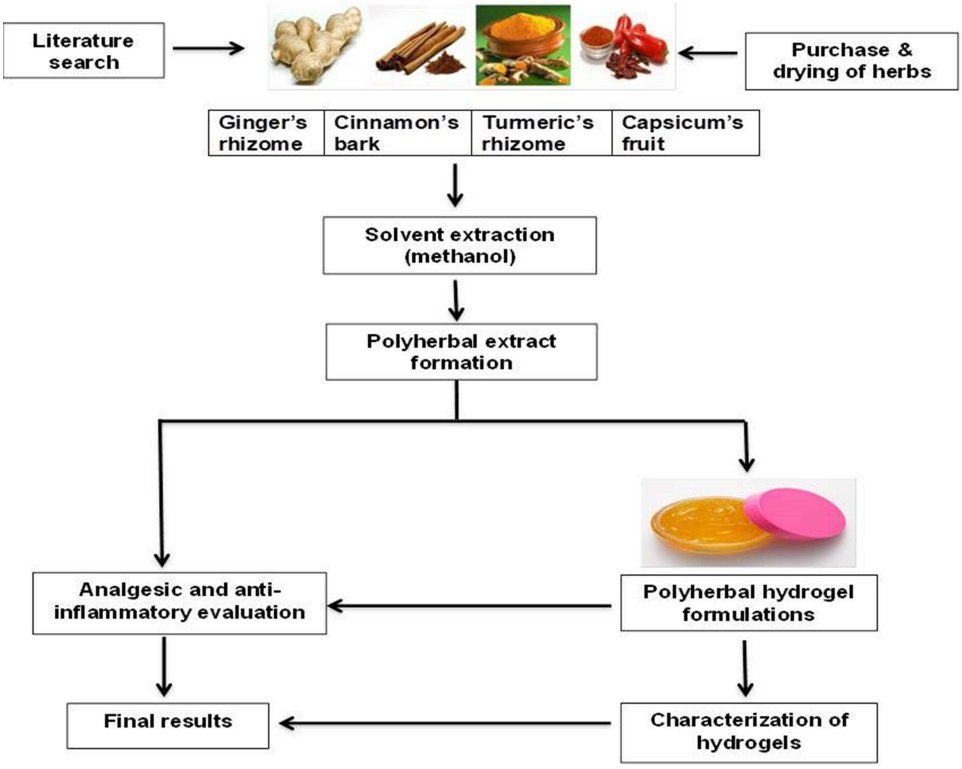
Graphical Abstract



Hydrogel is a type of semi-solid formulation in which natural and synthetic polymers are used as gelling agents. Hydrogels dosage form are widely used in different industries like cosmetics, food and pharmaceuticals because of their biocompatibility potential, hydrophilicity, controlled drug release and smart drug delivery.[3] *Curcuma longa* (L.), commonly called turmeric, belongs to the Zingiberaceae family. It is a rich source of polyphenolic curcuminoids, like curcumin, bis-demethoxy-curcumin and demethoxy-curcumin, that are responsible for both analgesic and anti-inflammatory properties.[4] Other compounds like volatiles (63.6%), including ar-turmerone (45.5%) and α-turmerone (13.4%) as major compounds and sesquiterpene hydrocarbons were the second class (18.0%) with α-zingiberene (5.3%) as predominant. Another major constituent was the monoterpene hydrocarbon α-phellandrene (6.3%).[5] It has anti-inflammatory, antiseptic, analgesic, anti-oxidant, anti-coagulant, and anti-tumor potential and can be used as disinfectant; it is also cardiovascular protective and can cure skin problems.[6]

Capsicum annuum is a member of the Solanaceae family. It contains carbohydrates, vitamins and dietary fibers. The main active pungent ingredient of capsicum is capsaicinoids. There are two prominent types of capsaicinoids: one is dihydro-capsaicin and the other one is capsaicin.[7] Other capsaicinoids include dihydrocapsaicin (41%), nordihydrocapsaicin (7%), norcapsaicin (7%), homocapsaicin (3%) and homodihydrocapsaicin (2%). These capsaicinoids have both analgesic and anti-inflammatory properties. They have potent carminative, anti-coagulant, hepatoprotective, immuno-protective, cardio-protective, anti-carcinogenic, and anti-arthritis potential and are commonly used to cure nerve damage, and uterus pain during and after pregnancy.[8]

Zingiber officinale (R.) (called ginger in English) belongs to the Zingiberaceae family.[9] These terpenes are responsible for their anti-inflammatory and analgesic potential. Ginger has significant anti-oxidant, anti-arthritic, anti-cancer, anti-inflammatory, anti-diabetic, anti-coagulant, anti-fungal and anti-helminthic properties. It is also used to cure nausea, motion sickness, migraine, obesity, emesis, hepatotoxicity and high blood pressure.[10] *Cinnamomum verum* J.S. Presl

relates to the Lauraceae family. Trans-cinnamaldehyde, linalool and eugenol are major constituents that are derived from volatile oil of cinnamon's bark. Their analgesic and anti-inflammatory properties are due to these constituents.[11] Cinnamon has anti-oxidant, anti-diabetic, anti-coagulating, antiseptic, analgesic and anti-arthritic properties. It also has ability to improve immunity and to prohibit the development of blackheads and pimples.[12] Topical semi-solid dosage forms of these herbs are available individually for their analgesic and anti-inflammatory potential.[13–21]

Although all these four herbs are separately well known for their analgesic and anti-inflammatory potential, their combined effect has not been studied and any dosage form has not been prepared. Therefore the current study was planned to find out the combined analgesic and anti-inflammatory potential of polyherbal extract, hydrogel of the polyherbal hydrogel and characterization of hydrogel.

Materials and Methods

Fresh capsicum fruit (*Capsicum annuum* L.), cinnamon bark (*Cinnamomum verum* J.), rhizome of turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* R.), were purchased from local market of Multan Punjab, Pakistan and identified by Dr. Zaffar Ullah Zaffar (Associate Professor), Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan. Identification number of capsicum is kew-2698415, cinnamon's is kew-2721292, ginger's is kew-273361 and turmeric is kew-235249; these were kept in the herbarium of the department for further reference. Methanol, Carbopol 940, Propylene glycol,Ethanol, Tri-ethanolamine (Duksan Pure Chemicals Co., Ltd., South Korea), Methocel 100 (Colorcon, Ltd. England), Carrageenan (Foodmate Co. Ltd. Shanghai) and Diclofenac sodium (Novartis Pharma, Pakistan, Ltd.) were purchased.

Preparation of Extracts

Cinnamon bark 600.0 g, 800.0 g ginger rhizome, 1000.0 g turmeric rhizome and 600.0 g of capsicum fruit were washed and cleaned properly, then cut into small pieces and dried completely under shade for several days. 500.0 g of each dried sample was macerated separately with 1000 mL of methanol as solvent in a closed bottle with frequent shaking for 7 days. After that, the soaked sample-solvent mixture was filtered and filtrate was evaporated by rotary evaporator R-200 (Buchi, Switzerland) to concentrate the residue at 40–60°C under reduced pressure (4–6 mm of Hg). The semi-solid extract of each plant material was weighed (capsicum extract 50.0 g, cinnamon 40.0, ginger 50.0 g and 25.0 g turmeric extract) and stored in a glass vial below 20°C for further processing. The marc was then re-macerated to get appreciable amount of active principle for 3 days and repeat the same procedure.[22] The results of % yield of methanolic extract of different plant materials are shown in Table 1.

Preparation of Polyherbal Extract (GCTC)

Polyherbal extract was prepared by taking 20.0 g of extract of all plants, that is, ginger, cinnamon, turmeric and capsicum (Latin names deleted), and properly mixing it in vortex mixer to get 80.0 g of polyherbal extract, which was named GCTC.

Table 1 Percentage Yields of Methanolic Extract of Different Herbs (GCTC)

Serial No.	Drug Name	Crude Drug (g)	Solvent (mL)	Extract (g)	Extractive Value (%)
1	Ginger	500	1000	50	5.0
2	Turmeric	500	1000	25	2.5
3	Capsicum	500	1000	50	5.0
4	Cinnamon	500	1000	40	4.0

Experimental Animals

Animals used in the experiment were Wistar albino rats (male and female), weighing 150–250 grams. These animals were purchased from animal house of Faculty of Pharmacy, Bahauddin Zakariya University, Multan. These animals had free access to water and food (standard pellet diet). Experiment was approved by bio-ethical committee of Bahauddin Zakariya University Multan vide letter No BZ-234-PH-8/1.

Preparation of Hydrogels

Preparation of pH-Based Hydrogel

Hydrogel with carbopol 940 was prepared by taking 93.0 g distilled water in a glass beaker and placing it on a magnetic stirrer. 1.0 g of carbopol 940 was slowly added with continuous stirring for about an hour to avoid the formation of clumps. After that, 5.0 g of propylene glycol (PG) containing polyherbal extract GCTC (1.0 g of polyherbal methanolic extract in 1% C(GCTC), 3.0 g in 3% C(GCTC) and 5.0 g of polyherbal methanolic extract in 5% C(GCTC) and 3–4 drops of tri-ethanolamine was added, with continuous stirring till the formation of gel.[23] This tri-ethanolamine neutralizes fatty acids, adjusts pH, and solubilizes oils and other ingredients that are not completely soluble in water.

Preparation of Temperature-Based Hydrogel

Hydrogel with methocel K 100 was prepared by taking 93.0 g distilled water in a glass beaker and placing it in a water bath. 1.0 g of methocel K-100 was slowly added in hot water with continuous stirring to avoid the formation of clumps. After that, 5.0 g of propylene glycol containing polyherbal extract GCTC (1.0 g of polyherbal methanolic extract in 1% M(GCTC), 3.0 g in 3% M(GCTC) and 5.0 g of polyherbal methanolic extract in 5% M(GCTC), was mixed it well till a semi-solid gel was formed.[24] See Table 2.

Characterization of Hydrogel

pH Measurement

pH of all hydrogels was determined by using digital pH meter. 1.0 g gel was dissolved in distilled water and kept for 2 hours. pH of all formulations was noted in triplicate form and result was the average of all triplicate readings.[23] Their values are shown in Table 3.

Table 2 Formulation of Hydrogels with and without Polyherbal Methanolic Extract (GCTC)

Serial No.	Formulation	Ingredients					
		Carbopol 940	Methocel K-100	Polyherbal Extract	Propylene Glycol	Distilled Water	Tri-Ethanolamine
1	Sample IA	1 g	_	_	5 g	94 g	2–3 drops
2	Sample Ia	1 g		1 g	5 g	93 g	2–3 drops
3	Sample Ib	1 g		3 g	5 g	91 g	2–3 drops
4	Sample Ic	1 g		5 g	5 g	89 g	2–3 drops
5	Sample IIA	_	1 g	_	5 g	94 g	_
6	Sample IIa	_	1 g	1 g	5 g	93 g	_
7	Sample IIb	_	1 g	3 g	5 g	91 g	_
8	Sample IIc	_	1 g	5 g	5 g	89 g	_

Notes: Sample IA is pH-based hydrogel without polyherbal extract, sample Ia is pH-based hydrogel containing 1.0 g of polyherbal methanolic extract, sample Ib is pH-based hydrogel containing 3.0 g of polyherbal methanolic extract, sample Ic is pH-based hydrogel containing 5.0 g of polyherbal methanolic extract, sample IIA is pH-based hydrogel without polyherbal extract, sample IIa is temperature-based hydrogel containing 1.0 g of polyherbal methanolic extract, sample IIb is temperature-based hydrogel containing 3.0 g of polyherbal methanolic extract and sample IIc is temperature-based hydrogel containing 5.0 g of polyherbal methanolic extract.

Table 3 pH Measurement of Hydrogels with and without Polyherbal Methanolic Extract (GCTC)

Sr. No.	Formulations	pH
1	Sample IA	7.1
2	Sample Ia	7.3
3	Sample Ib	7.2
4	Sample Ic	7.4
5	Sample IIA	7.2
6	Sample IIa	7.3
7	Sample IIb	7.3
8	Sample IIc	7.5

Physical Appearance

Visual evaluation of freshly prepared formulations was done to check its clarity, color, odor and general appearance by using standard protocols.[25] Results are shown in Table 4.

Homogeneity and Grittiness

Greasiness and homogeneity were observed by taking small amounts of hydrogel between thumb and index finger and presence of any coarse particle was observed. It was also done by applying small amount of gel on back-side of hand and rubbing.[26] Results are shown in Table 4.

Spreadability Test

Spreadability test reveals the extendibility of gel when applied on skin. It was done by taking two glass slides, drawing a circle of 7.5 cm on one of them, placing 1.0 g of prepared gel at the center of the circle and placing the other slide on it. 100.0 g of weight was placed on upper slide for 60 sec and diameter of pressed gel was noted by help of the scale. The same procedure was repeated 3 times for each formulation, the means calculated and then the formula of spreadability was used, which is given below:

$$Spreadability(S) = \frac{M \times L}{T}$$

Table 4 Physical Characterization of Hydrogel Formulations with and without Polyherbal Extract (GCTC)

Sr. No	Formulation	Spreadability %	Visual Screening	Porosity %	Extrudability	Swelling (%)	Homogeneity
1	Sample IA	10.1	Good	72.00	Excellent	2.73	Smooth
2	Sample Ia	10.6	Good	71.50	Excellent	2.50	Smooth
3	Sample Ib	11.0	Good	71.00	Good	1.9	Smooth
4	Sample Ic	12.0	Good	69.00	Good	1.73	Smooth
5	Sample IIA	7.1	Good	68.00	Good	11.9	Smooth
6	Sample IIa	7.16	Good	66.50	Good	11.1	Smooth
7	Sample IIb	7.22	Good	64.97	Good	10.6	Smooth
8	Sample IIc	7.31	Good	62.90	Good	10	Smooth

where M = weight (g) or load placed on slides, L = length (cm) of pressed gel moved on glass slide and T = time in seconds for which weight was tied on slides.[27] Results are shown in Table 4.

Washability

Small amount of formulation was applied on proper clean skin and washed with water to check ease of washability.[28] Results are shown in Table 4.

Rheological Study

The freshly prepared hydrogel formulation was observed for its rheological properties with the help of Brook field, model RV and spindle CP41 (Rheometer) with a controller of temperature by using standard protocol. In this study, viscosity of different hydrogel formulations was observed at different temperatures and shear stresses.[29] Viscosity was inversely proportional to the temperature. Results of rheological studies are shown in Figure 1.

Extrudability

Aluminum collapsible tube was taken and 5.0 grams of prepared gel was added by pressing thoroughly and folding the crimped end to restrain its rolling back. If cap was removed and the seal opened, then pressure was applied till gel extrudes. Then weight of extruded gel was measured and extrudability percentage for each formulation was calculated.[30] Results are shown in Table 4.

Swelling Test

Japanese industrial standard was used to measure the swelling index. According to protocol, 0.2 g pre-dried gel was immersed in 50.0 mL of deionized water at room temperature for about 16 hours. The stainless-steel net of 120 mesh was used to filter hydrogel after the swelling of immersed gel. After that, swelling can be calculated by using the formula given below:

Swelling = C/ B *100

where C = weight of pre-dried gel which is immersed and B = weight of marc after filtration.[31] Results are shown in Table 4.

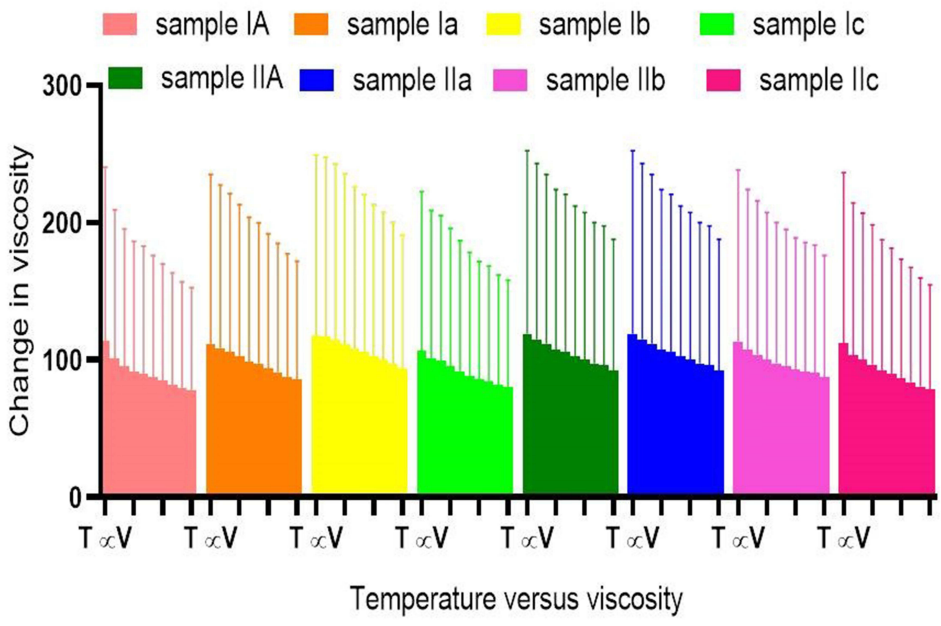


Figure 1 Rheological studies of hydrogel formulations with and without polyherbal extract GCTC.

Table 5 Stability Test of Hydrogel Formulations with and without Polyherbal Extract (GCTC)

Name of Formulation	Storage Condition								
	2–8°C/ 60–70%RH ±5%			32°C/ 60–70%RH ±5%			40°C/ 60–70%RH ±5%		
	15 Days	30 Days	45 Days	15 Days	30 Days	45 Days	15 Days	30 Days	45 Days
Sample IA	0	0	0	0	0	0	0	0	0
Sample Ia	0	0	0	0	0	0	0	0	0
Sample Ib	0	0	0	0	0	0	0	0	0
Sample Ic	0	0	0	0	0	0	0	0	0
Sample IIA	0	0	0	0	0	0	0	0	0
Sample IIa	0	0	0	0	0	0	0	0	0
Sample IIb	0	0	0	0	0	0	0	0	0
Sample IIc	0	0	0	0	0	0	0	0	0

Note: "0" indicates that no change was observed.

Porosity Test

0.2 g of pre-dried hydrogel was soaked in 50.0 mL of ethanol for 24 hours. After that, gel was removed from ethanol and weight of swelled gel was determined after cleaning with blotting paper to dry out extra ethanol. Then the percentage of porosity was calculated using the following formula:

$$Porosity = \frac{W_2 - W_1}{\rho v} \times 100$$

where W1 = refers to the pre-dried gel weight before immersion, W2 = refers to the hydrogel weight after swelling, P = refers to the ethanol density and v = refers to hydrogel volume.[26] Results are shown in Table 4.

Stability Test

Freshly prepared formulations were observed for colour, odor and for general appearance then stored in different conditions of temperature and humidity. Stability of all formulations was examined for 60 days by noticing its color, odor and general appearance. Results are shown in Table 5.[28]

Skin Irritation Test

Six healthy rats were taken and an area of 5.0 cm was shaved on the back of each rat. All gel formulations were applied one by one at room temperature. The rats were observed for about 7 days for any reaction.[23] Results are shown in Table 6.

Analgesic Activity by Tail Flick Method

Analgesic activity of polyherbal extract and hydrogel prepared from polyherbal extract was carried out by using standard protocol. In this method, a water bath was used and temperature was maintained at about 50–52°C, in which 3.0–4.0 cm tail of rat was dipped. Five groups of rats (1, 2, 3, 4, and 5) for analgesic activity of polyherbal extract (GCTC) and eight group of rats (A, B, C, D, E, F, G and H) for polyherbal hydrogel formulations, each group having five rats were used in this experiment. Normal saline was given to group 1. Solution of diclofenac sodium (100 mg/kg) (positive control) in normal saline was given to group 2. Solution of polyherbal extract of 200 mg/kg, 400 mg/kg and 800 mg/kg was given to groups 3, 4, and 5. Group A is control group and diclofenac sodium 1% gel was applied topically on hind paws of group B (positive control). Different formulations of 1%, 3% and 5% of pH base hydrogel were applied to groups C, D and E, respectively. Similarly, temperature-based gels of 1%, 3% and 5% were applied to the F, G and H groups, respectively. Avoiding the cut off time was 30 sec. Doses for analgesic activity of polyherbal extract were administered orally via oral

Table 6 Toxicity Test of Hydrogel Formulations with and without Polyherbal Extract (GCTC)

Formulation	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sample IA	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample Ia	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample Ib	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample Ic	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample IIA	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample IIa	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample IIb	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction
Sample IIc	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction	No reaction

gavage, while polyherbal hydrogel formulation were applied topically. Observations were first taken at zero time, then at 60 min, 90 min, 120 min and 180 for oral polyherbal extract administration and at zero time, 30 min, 60 min, 90 min and 120 min of application for hydrogel formulations.[32]

Analgesic Activity by Hot Plate Method

Hot plate was used in this method and temperature was set at 55–56°C. Animal was placed on this plate and time was recorded with stop-watch till they start licking their paw or start jumping. Five groups of rats (1, 2, 3, 4, and 5) for analgesic activity of polyherbal extract (GCTC) and eight groups of rats (A, B, C, D, E, F, G and H) for polyherbal hydrogel formulations, each group having five rats, were used for this experiment. Normal saline was given to group 1. Solution of diclofenac sodium tablet (100 mg/kg) in normal saline was given to group 2 (positive control). Solutions of polyherbal extract of 200 mg/kg, 400 mg/kg and 800 mg/kg were given to groups 3, 4, and 5, respectively. Group A was control group and diclofenac sodium 1% gel was applied topically on hind paws of group B (positive control). Different formulations of 1%, 3% and 5% of pH base hydrogel were applied to groups C, D and E, respectively. Similar temperature-based gels of 1%, 3% and 5% were applied to the F, G and H groups, respectively. Doses for analgesic activity of polyherbal extract were administered orally via oral gavage, while polyherbal hydrogel formulation was applied topically. Observations were taken first at zero time, then at 60 min, 90 min, 120 min and 180 for oral polyherbal extract administration and at zero time, 30 min, 60 min, 90 min and 120 min of application for hydrogel formulations.[33]

Anti-Inflammatory Activity

Five groups of rats (1, 2, 3, 4, and 5) were used for anti-inflammatory activity of polyherbal extract (GCTC) and eight groups of rats (A, B, C, D, E, F, G and H) were used for polyherbal hydrogel formulations prepared by two different methods. Five rats were present in each group. Group 1 and A were used as control groups and received only normal saline, group 2 was standard control group to which diclofenac sodium 10 mg/kg was given. 200 mg/ kg of extract was given to group 3, 400 mg/kg was given to group 4 and 800 mg/kg was given to group 5, respectively. These doses were given orally with oral gavage, then after one-hour carrageenan was injected through intradermal route in hind right paw

to produce oedema. Diclofenac sodium 1% gel was used in standard group B (positive control). 1%, 3% and 5% pH based hydrogel was applied to groups C, D and E, respectively. Similarly, 1%, 3% and 5% temperature based hydrogel was applied to groups F, G and H, respectively. These gels were applied by rubbing before one hour of carrageenan injection. Paw volume of right hind paw was measured first at zero time via plethysmometer then at next reading taken after 30 min of injection. Next observations were taken at 60 min and at 120 min after injection.[34]

$$\%Inhibition = \frac{Cv - Tv}{Cv} \times 100$$

where Cv = paw volume of control group and Tv = paw volume of test group.

Discussion

Herbal remedies have been used to improve health and to cure a number of ailments all over the world from the beginning of mankind. Today's drugs are prepared from plants on large scale because the use of herbal medicine is more preferred than other chemical-based medicine due to their easy availability, high efficacy and less side effects.[35]

Extraction

Extraction is a technique that involves the separation of active constituents of tissues of plants and animals by using suitable selective solvents and by following standard protocols. It helps to attain desired constituents for their therapeutic effects.[36] Percentage yield by extraction indicates the potential of the solvent to extract different constituents from sample material.[37] In the present research work, percentage yields of methanolic extract of different herbs like ginger, turmeric, capsicum and cinnamon have been shown in Table 1. Due to presence of -OH (hydroxide) group methanol is polar in nature and more likely to dissolve polar substance. This indicates that ginger and capsicum have higher percentages of polar molecules as compared to turmeric and cinnamon.

Characterization of Hydrogels

pH of topical formulations should be near to skin pH to avoid any erythema. In current research work, pH measurement of all hydrogels was carried out and results have shown that all formulations were basic in nature and safe to use. Visual inspection of semi-solid formulation helps to make sure that there is no separation of phases, there is uniformity in color and no foreign substance.[38] Visual inspection of freshly prepared formulations was performed to check their clarity and physical appearance like its color, odor and general appearance. The current hydrogel formulations have yellow color and pleasant characteristic odor with no clumps and have smooth general appearance.

Homogeneity test of hydrogel shows the uniformity in formulation and checks for the presence of any particles or clumps. No clumps or clusters were observed in current hydrogel formulation, therefore, it is concluded that prepared gel by both methods has good homogeneity. Rheological study of hydrogel shows the effect of different temperature and shear stress on velocity.[38] Velocity of hydrogel formulation decreases with increase in temperature. Therefore, viscosity is inversely proportional to the temperature. Results of rheological studies are shown in Figure 1.

Spreadability of hydrogel topical formulation is a very important property that shows the ease of dispersion of gel after its application with small shear stress. If the spreadability is good, so will be its application. Uniformity in application also helps in uniform therapeutic effects.[39] In the present study, hydrogel formulations are very easy to apply with very small shear stress, therefore it has good spreadability, which has been shown in Table 3.

Washability shows the extent to which a gel can be removed or washed after application on skin.[40] In the present study, hydrogel formulation is easy to wash with water after its application but yellow color stain remains at the site of application because of turmeric. Swelling test shows the ability of gel to expand and also shows its elasticity. When water was added to the gel, it permeates the matrix of the gelling agent.[41] The current formulations have good swelling percentage, which has been shown in Table 3. The swelling of carbopol-based gel is better than the methocel-based gel because of greater compatibility of extract in carbopol then methocel.

Extrudability test of gel shows the amount of force which is required to squeeze out. High consistency gel requires more force for extrusion as compared to less viscous gel. For acceptance of gel its extrusion is also

important.[42] Extrudability of current different hydrogel formulations is fine, good, better and very good, as shown in Table 4. The porosity test indicates the pore size in hydrogel, which also shows its penetration through skin. Porosity was affected by cross-linked matrix of gel; when there is more cross-linking, porosity would be decreased and with decrease of cross-linking porosity would be increased.[26] The current study has shown that with an increase in the amount of polyherbal extract (GCTC) there is a decrease in porosity, as shown in Table 3.

The stability test checks the effects of temperature and humidity on the gel's general appearance (color, odor and homogeneity), spreadability and extrudability.[42] Stability of all hydrogel formulations at the mentioned temperature and humidity for 60 days were examined and results are shown in Table 4.

Skin irritation test is used to determine the allergic and irritant potential of the prepared dosages.[23] The prepared topical hydrogel formulations are observed for any irritation, redness or itching through skin irritation test for seven days and its results have been shown in Table 5, which indicated that current hydrogel formulations are safe to use.

Analgesic Activity

Anti-nociceptor prohibits the impression of pain by acting on peripheral or central receptors of pain either by enhancing its threshold or by prohibiting the propagation of its reflexes.[43] In the present research work, analgesic activity of polyherbal extract (GCTC) was analyzed by tail flick and hot plate in dose dependent manner. The potent analgesic effect was shown by delaying the response time of rats. Different concentrations of polyherbal extract (GCTC) ie 200 mg/kg, 400 mg/kg and 800 mg/kg and all hydrogel formulations with mentioned concentrations were tested and results of tests are shown in Figures 2–7. Results have shown that polyherbal extract has anti-nociceptors including capsaicin in capsicum extract (significant peripheral and central analgesic potential by defunctionalizing the nociceptor fibers and inducing counter-irritant effect),[44] curcumin from turmeric (inhibits the impression of pain by acting on central receptors),[45] cinnamaldehyde from cinnamon extract (exhibits analgesic potential both in peripheral and central receptors by suppressing the formation of reflexes)[46] and total polyphenols flavonoids and tannins from ginger extract (exhibits the peripheral and central analgesic activity by inhibiting synthesis of the mediators of reflexes of pain).[47] The combined effect of these active constituents prohibits the impression of pain. As the concentration of polyherbal extract increases, the prohibition of reflexes also increases. Test groups have also shown the analgesic effect and significant activity was shown by test group with higher concentration (800 mg/kg) of polyherbal extract and by sample Ic and sample IIc. Because topical application of dosage form offers the advantage of local, enhanced drug delivery to affected tissues with a reduced incidence of systemic adverse effects and also prevents hepatic metabolism, it has therefore been observed that the analgesic potential of polyherbal hydrogel formulations is greater compared to polyherbal methanolic extract GCTC.

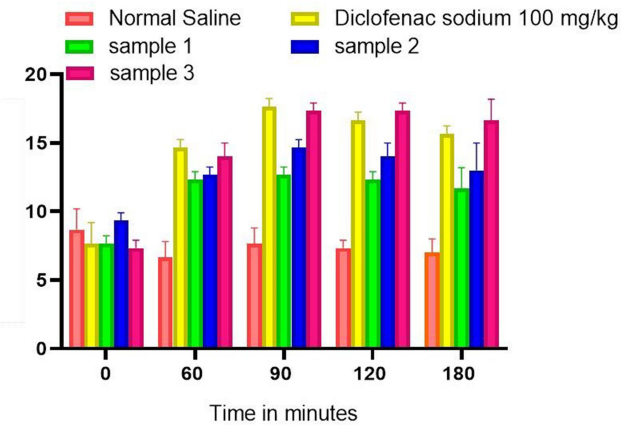


Figure 2 Analgesic activity of polyherbal extracts (GCTC) by tail flick method.

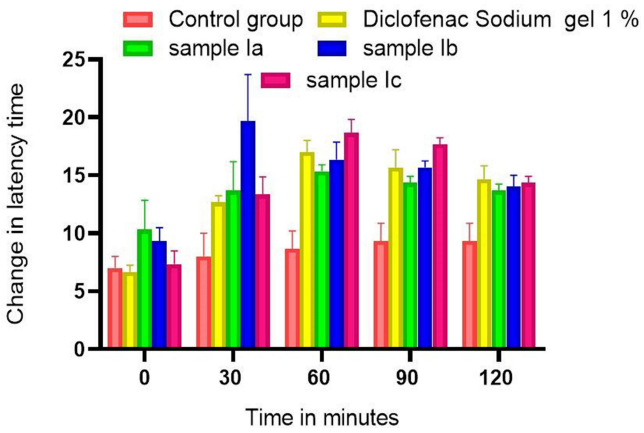


Figure 3 Analgesic activity of polyherbal hydrogel (GCTC) with carbopol 940 by tail flick method.

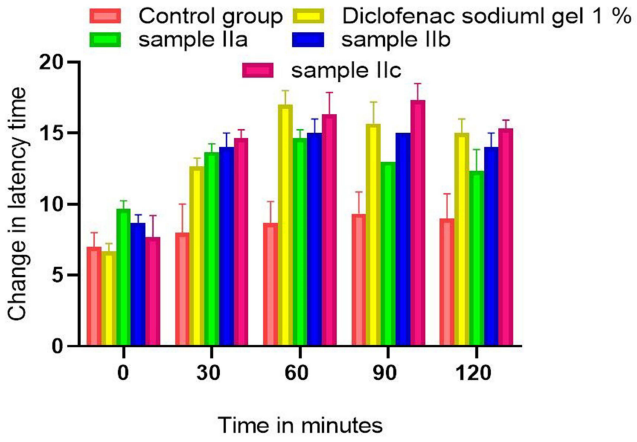


Figure 4 Analgesic activity of polyherbal hydrogel GCTC with methocel by tail flick method.

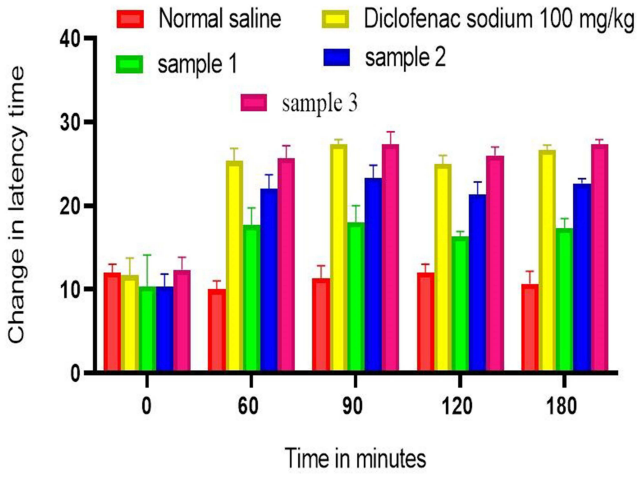


Figure 5 Analgesic activity of polyherbal extract GCTC by hot plate method.

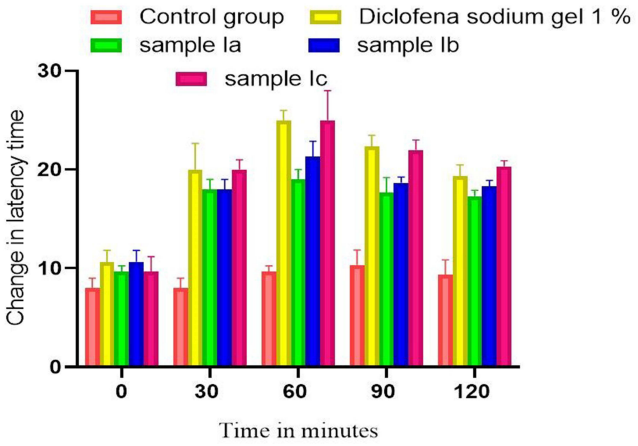


Figure 6 Analgesic activity of polyherbal hydrogel GCTC with carbopol 940 by hot plate method.

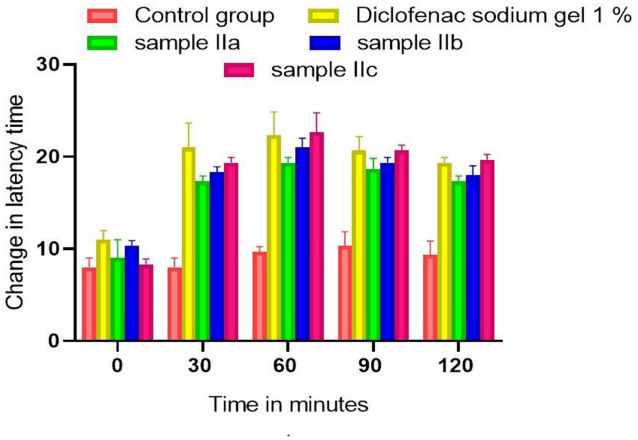


Figure 7 Analgesic activity of polyherbal hydrogel GCTC with methocel by hot plate method.

Anti-Inflammatory Activity

The body has many ways of protecting itself; one of the most vital methods the body uses to protect itself is through the process of inflammation. Elongated inflammation can be prohibited by a search for natural medicines.[48] In the present research work, the anti-inflammatory activity of polyherbal extract (GCTC) was carried by carrageenan-induced paw oedema method. The edema induced by carrageenan is through biphasic response. The first phase mediates the release of histamine, serotonin and kinins while the release of prostaglandin and slow reacting substances occurs in the second phase.[49] Results show that polyherbal extract has anti-inflammatory potential due to combine effect of active constituents of different herbs, including capsicum, cinnamon, ginger and turmeric. Active constituents include total polyphenols flavonoids of ginger (reducing inflammation by inhibiting the histamine, serotonin, kinin and prostaglandin release),[47] curcumin of turmeric (inhibiting inflammatory cytokines by inhibiting cyclooxygenase-2 (COX-2), lipoxygenase and inducible nitric oxide synthase (iNOS) enzymes),[50] type-A proanthocyanidins from cinnamon (inhibits the formation of prostaglandin which releases in second phase of carrageenan inducing edema)[51] and capsaicin from capsicum (in early phase of edema has no significant effect but have predominant effect in second phase by inhibiting neutrophils and inflammatory cytokines).[52] The standard groups (diclofenac sodium 10mg/kg) showed the significant anti-inflammatory effect by inhibiting COX and consequently inhibiting prostaglandin production[46] as compared to control groups. Different concentrations of polyherbal extract (GCTC), ie 200 mg/kg, 400 mg/kg and 800 mg kg, and hydrogel formulations with mentioned concentrations were tested in comparison to standard (diclofenac sodium 1% gel) and

control group and results of test are shown in Figures 8–13. Significant anti-inflammatory effect has been observed in a test group with higher concentration (800 mg/kg) and by sample Ic and sample IIc. It has been observed that polyherbal hydrogel formulations also have more anti-inflammatory activity then polyherbal methanolic extract GCTC, which shows that topical dosage form of these herbs has greater absorbance.

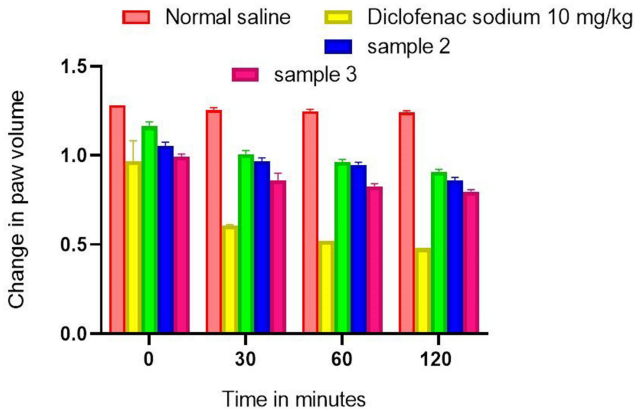


Figure 8 Anti-inflammatory activity of polyherbal extracts GCTC.

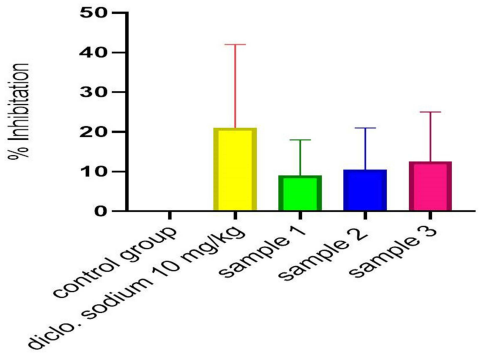


Figure 9 Percentage inhibition of anti-inflammatory activity of polyherbal extract GCTC.

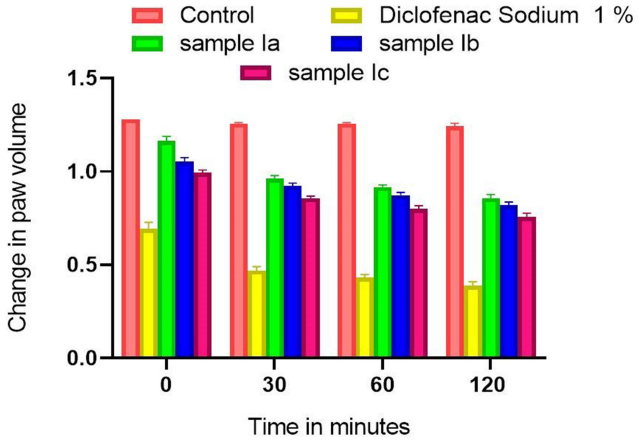


Figure 10 Anti-inflammatory activity of polyherbal hydrogel GCTC with carbopol 940 by carrageenan induces paw edema.

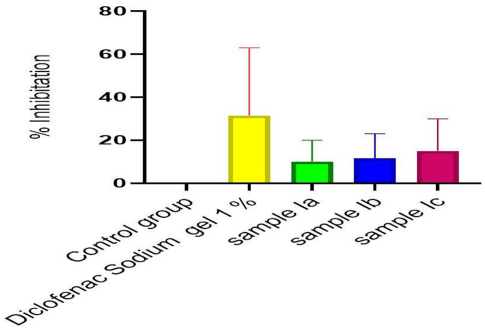


Figure 11 Percentage inhibition of anti-inflammatory activity of polyherbal hydrogel GCTC with carbopol 940 by carrageenan induces paw edema.

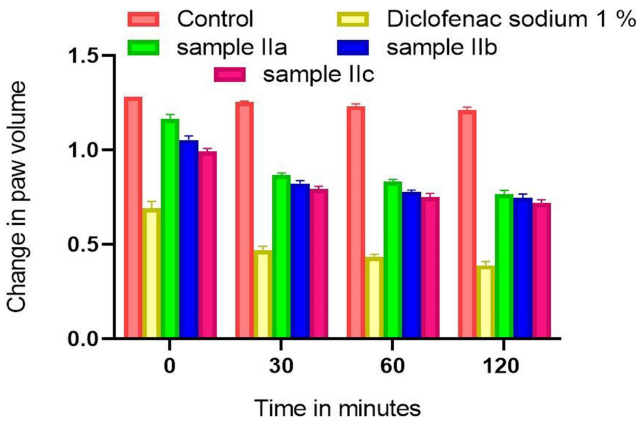


Figure 12 Anti-inflammatory activity of polyherbal hydrogel GCTC with methocel by carrageenan induces paw edema.

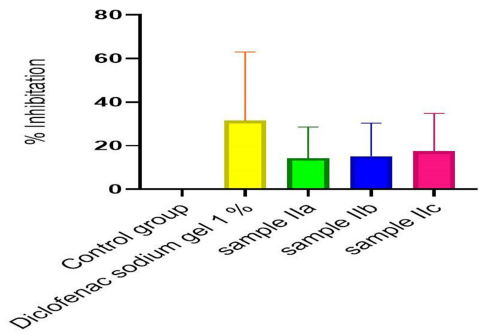


Figure 13 Percentage inhibition of anti-inflammatory activity of polyherbal hydrogel GCTC with methocel by carrageenan induces paw edema.

Conclusion

This study confirms that polyherbal methanolic extract (fruit of capsicum, bark of cinnamon, rhizome of turmeric and rhizome of ginger) had significant anti-inflammatory and analgesic activity. Furthermore, the polyherbal gel formulations of the extracts also revealed higher analgesic and anti-inflammatory potential as compared to the polyherbal methanolic extract.

Abbreviations

GCTC, ginger, cinnamon, turmeric and capsicum; COX, Cyclooxygenase enzyme; sample IA, pH-based hydrogel without polyherbal extract; sample Ia, pH-based hydrogel containing 1.0 g of polyherbal methanolic extract; sample Ib, pH-based hydrogel containing 3.0 g of polyherbal methanolic extract; sample Ic, pH-based hydrogel containing 5.0

g of polyherbal methanolic extract; sample IIA, pH-based hydrogel without polyherbal extract; sample IIa, temperature-based hydrogel containing 1.0 g of polyherbal methanolic extract; sample IIb, temperature-based hydrogel containing 3.0 g of polyherbal methanolic extract; sample IIc, temperature-based hydrogel containing 5.0 g of polyherbal methanolic extract.

Ethical Approval and Consent to Participate

All procedures and techniques used in this study were performed according to National Research Council Guidelines. Laboratory animals were approved by bio-ethical committee of Bahauddin Zakariya University Multan vide letter No BZ-234-PH-8/1 were also used in their accordance.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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