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Comparative evaluation of Punarnavadi guggul tablet formulation for anti-inflammatory activity

Research Article

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Abstract

Punarnavadi guggul represents a traditional polyherbal formulation utilized in the treatment of dermatological conditions, jaundice, dropsy, edema, hyperuricemia, and rheumatism. The present investigation sought a comparative analysis of the three marketed formulations of *Punarnavadi Guggul* tablet for its in vivo anti-inflammatory properties. The anti-inflammatory effects were assessed using the carrageenan-induced rat paw edema model and the cotton pellet granuloma method. The animal groups treated with laboratory-prepared formulation PF (150 mg/kg) showed significant antiinflammatory activity comparable to the standard drug. Existing literature indicates that guggulsterone E, Z, and deodarone are implicated in the anti-inflammatory effects, whereas gallic acid, ascorbic acid, polyphenols, and flavonoids contribute to its antioxidant properties. The formulated tablet exhibited pre-eminent anti-inflammatory properties when compared to commercial formulations, but all formulations showed noteworthy bioactivity.

Keywords: Anti-inflammatory activity, Carrageenan, Cotton pellet, Polyherbal, *Punarnavadi guggul*.

Introduction

Inflammation is a dynamic sequence as a protective response against various etiological agents, either infectious or noninfectious, by the body (1). The inflammatory process constitutes a pathological reaction of living tissues to injuries, culminating in the localized aggregation of plasma fluid and blood cells. This physiological response serves to eradicate or mitigate the propagation of harmful agents while simultaneously facilitating the removal of necrotic cells and tissues that arise as a consequence. The currently available anti-inflammatory pharmacological agents impose significant challenges within the realm of medical science, exhibiting restricted utility due to their associated adverse effects (2). Punarnavadi Guggul is an Ayurvedic preparation acclaimed for its therapeutic efficacy, particularly in the management of diverse health disorders. This formulation amalgamates the advantageous properties of Guggul (*Commiphora* species) with a selection of other herbal constituents, rendering it effective in addressing conditions such as obesity, inflammation, hypothyroidism, and chronic renal failure. The formulation primarily comprises the desiccated roots of *Boerhaavia diffusa* Linn. (Nyctaginaceae) and the heartwood of Cedrus deodara (Roxb.). Loud. (Pinaceae), The stems of *Tinospora cordifolia* Miers. (Menispermaceae), The fruits of *Terminalia chebula* Retz. (Combretaceae), In conjunction with *Commiphora mukul* Engl. (Burseraceae) to create the Punarnavadi guggul tablet formulation (3,4). In the present investigation, the Laboratory prepared formulations (PF) and commercially available formulations of Punarnavadi guggul of various manufacturing companies, designated as Marketed formulation 1 (PN), Marketed formulation 2 (PM), and Marketed formulation 3 (PP), were subjected to comparative analysis regarding their anti-inflammatory efficacy. A review of the literature has indicated that guggulsterone E and Z, derived from Commiphora mukul (5), along with deodarone from *Cedrus deodara* (6) polyphenols and flavonoids, bioactives of Punarnava, are implicated in the mediation of anti-inflammatory activity (3,7). Consequently, Formulation and comparative evaluation of the Punarnavadi guggul formulation concerning its anti-inflammatory properties has been undertaken. The anti-inflammatory effects of the formulations were comparatively assessed utilizing the carrageenan-induced rat paw edema method and the cotton pellet granuloma method at dosages of 100 and 150 mg.

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Methodology

Physicochemical investigation

The assessment of the physicochemical parameters associated with the pharmaceutical substances constitutes an essential procedure for

identifying adulteration or improper handling of drugs. This evaluation encompasses various foreign organic matter, extractive values (alcohol and water soluble), ash values (Total ash value and acid insoluble ash value), Loss on drying (Table 2).

Ash content: The powdered botanical drugs were analyzed for total ash value and acid-insoluble ash value in accordance with the standardized protocols outlined by the quality control regulations for herbal products as established by the World Health Organization (8).

Determination of extractive values: The extractive values were ascertained utilizing an array of solvents, namely alcoholic and hydroethanolic solvents. Approximately 5g of dried, coarsely ground plant material was introduced into a sealed glass conical vessel. Subsequently, 100 mL of the aforementioned solvents was added. The sealed conical vessel was permitted to remain undisturbed for a period of 24 hours while subjected to continuous agitation. Following this, the mixture was filtered, and 25 mL of the resultant filtrate was transferred to a pre-weighed petri dish. The liquid was then subjected to evaporation until a dry state was achieved in a hot air oven maintained at 105°C, followed by a cooling phase of 30 minutes in a desiccator prior to weighing (8).

Moisture content: A precisely measured 1 g sample was placed into a pre-dried crucible. The samples underwent desiccation in an oven maintained at a temperature range of 100-110°C for a duration of 2 hours, subsequently removed, allowed to cool within a desiccator, and then reweighed. This methodology was persistently applied until equilibrium in weights was attained. The moisture content within the samples was computed utilizing the following equation: % moisture = (ab-ac) * 100/ab, where ab represents the combined weight of the dish and the sample prior to drying (g), and ac denotes the combined weight of the dish and the sample subsequent to drying (g) (8).

Foreign organic matter: A quantity of 100 g of the drug was accurately measured and uniformly distributed across a white tile, ensuring that there was no overlap. The sample was then examined visually or with the aid of a lens with a magnification of 10x or greater. Any foreign organic materials were subsequently isolated. Upon completion of the separation process, the weight of the foreign organic matter was recorded, and the w/w percentage present within the sample was calculated.

Phytochemical Screening

The bioactivity of herbal constituents was determined by the phytoconstituent present in it. Therefore hydroalcoholic extract of plant parts was screened to ascertain the presence of phytoconstituents by using different chemical tests as per standard procedures (8) (Table 3).

Plant Material and Formulation Preparation

The validated raw materials were acquired from Natural Remedies Pvt. Ltd., Bangalore, and utilized for the formulation development. The raw materials underwent comprehensive pharmacognostic, physical, and chemical assessments as per standard protocols. Three batches of the formulation were formulated from these evaluated raw materials, *viz*. PF1, PF2, and PF3, following the methodology delineated in Baishajya Ratnawali (9). The tablets formulated successfully met the conventional physical and chemical standards, as well as the evaluative criteria specified for tablet formulation.

Preparation of Punarnavadi Guggul tablet formulation

The Punarnavadi guggul tablets were prepared by the dry granulation method. Dried raw materials Haritaki, deodar, gudvel, and punarnava were procured from Natural Remedies, Bangaluru. Materials were grounded by a mechanical grinder and sifted through a mesh number 20 to create 300 mg pills. For a 300 mg tablet of the Punarnavadi guggul formulation mentioned in Table 1 (10).

Table 1: Ingredients of Punarnavadi guggul formulation

Ingredients	Quantity
Boerhaavia diffusa	1 part
Cedrus deodara	1 part
Terminalia chebula	1 part
Tinospora cordifolia	1 part
Commiphora mukul	4 parts
Pulverised sugar	1 part
Talc	0.25 part
Crospovidone	0.75 part

Evaluation parameters of granules

Angle of repose

The fixed height funnel method was employed to determine the angle of repose of tablet blends. The funnel (10 mm inner diameter) was designed to allow the mixtures to flow easily onto the platform. The angle of repose was determined by measuring the diameter of the powder cone using the formula: Tan θ = h/r. Where 'h' represents the height and 'r' denotes the radius of the powder cone, respectively (11,12).

Bulk density

To assess the apparent bulk density, a specific amount of tablet mixtures was added to a graduated cylinder, and both the volume and weight of the cylinder were recorded (12).

Bulk Density = Weight of powder / Total Volume of the powder

Tapped density

A graduated cylinder containing a measured tablet blend was allowed to fall from a height of 10cm onto a hard surface every 2 seconds, falling solely under its weight. The tapping continued until there was no noticeable difference in the volume (11).

Tapped density = Powder weight / Powder tapped volume

Carr's index

The compressibility index created by Carr is calculated as (12):

$$CI = (\rho t - \rho a) / \rho t = (Va - Vt) / Vt$$

Where ρt and ρa denote tapped and poured bulk density, and Vt and Va represent tapped and poured bulk volume, respectively.

Hausner's ratio:

Hausner's ratio was calculated using the formula, and the results were expressed as a percentage (11).

$$H = Dt / Db$$

While Dt indicated the density of the powder after it was tapped, Db represented the density of the powder when assessed in bulk.

Assessment of tablets:

The Thickness of the 20 formulated tablets of each batch was calculated by Vernier caliper.

Uniformity in weight

Weight variation was determined using 20 tablets. The tablets were weighed separately, and computed the average weight of the 20 tablets was computed. Then determine the upper and lower limits (12).

Hardness and brittleness

The Electro lab friabilator apparatus and the Pfizer hardness tester were utilized to assess the friability levels and hardness of 20 tablets (11).

Time for disintegration

The disintegration test was conducted using a disintegration apparatus with water as the medium. The container was filled with 900 mL of disintegration medium and maintained at a temperature of 37 ± 0.2°C. Six tablets were placed in each of six tubes, and a plastic disc was positioned over the tablets. The test tubes were allowed to oscillate up and down at a frequency of 29-32 cycles per minute. The time taken for the tablet to disintegrate was determined, and the duration required for all tablets to pass through mesh 8-10 (11,13).

In vitro release investigation

The dissolution studies of polyherbal tablets were evaluated employing the USP dissolution apparatus II with 900 mL of 0.1 M phosphate buffer maintained at 37±0.5 °C and a stirring rate of 100 revolutions per minute. The absorbance at 254 nm was measured with a UV spectrophotometer after various 5 ml samples were taken and replaced with an equal volume of simulated fluid at 1, 2, 4, and 8 hours, respectively. The samples were then filtered using Whatman filter paper before measuring the absorbance (11,13).

Design of an Anti-Inflammatory Study

The Anti-inflammatory study was done by using two animal models: Carrageenan induced rat paw oedema method and cotton pellet granuloma method at 100 and 150 mg doses.

Animals used

Sprague-Dawley rats of either sex (150-180 g) were used for the present investigation. They were maintained under standard environmental conditions and were fed with a standard pellet diet and water *ad libitum*. Twelve hours before the start of the experiment, rats were deprived of food but given free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee (Registration No.92/1999/CPCSEA).

Acute toxicity study

Sprague Dawley rats of either sex (n=3) were randomly selected and kept in their cages for at least 5 days prior to dosing for acclimatization to the laboratory conditions. The animals were fasted overnight with free access to water. The hydroalcoholic extracts of Punarnavadi guggulu tablet were administered orally with an initial dose of 2000 mg/kg body weight. The general behaviour of the animals was observed continuously for the initial 4h and intermittently for the next 6h and then again at 24h and 48h following drug administration. The following parameters were used for observation: stimulation (hyperactivity, irritability, tremor, convulsion, piloerection), depression (sedation, anaesthesia, loss of reflex, analgesia), respiration, diarrhoea, salivation, and motor activity. The mortality was observed for seven days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered a toxic dose. However, if the mortality was observed only in one rat out of three animals, then the same dose was repeated to confirm the toxic effect (OECD, Guideline 425 200) (14).

Carrageenan-induced Rat paw oedema

The rats were divided into ten groups (n=6). An acute inflammatory response was elicited through the administration of 0.1 ml of 1% carrageenan (Himedia RM 1576) in normal saline, to the sub-plantar area of the right hind paw of rats, previously demarcated with ink at the level of the lateral malleolus, and subsequently immersed in a perspex cell up to this designated mark. The volumetric measurement of the paw was conducted at time intervals of 0, 1st, 3rd, and 5th hours post-carrageenan injection, utilizing a plethysmometer (Ugo Bastile). Group I was administered normal saline (3 ml/kg) orally, Group II received diclofenac (10 mg/kg) orally, and Groups III through X were subjected to treatment with formulations PF, PN, PM, and PP at dosages of 100 and 150 mg, respectively. The animals underwent pretreatment with the respective drug one hour prior to the administration of carrageenan (15–17).

Cotton pellet-induced granuloma

Following the removal of their pelage, the rodents were subjected to light ether anesthesia, after which 20 mg of sterile cotton pellets were implanted, one within each rodent's axillary region. A control vehicle, indomethacin (10 mg/kg), along with formulations PF, PN, PM, and PP at dosages of 100 and 150 mg, were administered via the oral route for seven consecutive days, commencing from the date of cotton pellet implantation. On the eighth day, the subjects were sedated, and the cotton was surgically excised and

meticulously cleared of any extraneous tissue. The pellets were subsequently desiccated to a constant weight at a temperature of 60°C. The increase in the desiccated weight of the pellet was utilized as an indicator of granuloma formation (15,16,18).

Statistical analysis

The results were expressed as mean ± S.E.M. The significance statistical analysis was performed by two-way ANOVA, $p \leq 0.001$ implied significance.

Results and Discussion

Table 2: Physicochemical analysis of plant parts

Sample	Foreign organic matter (% w/w)	Total ash (% w/w)	Acid insoluble ash (% w/w)	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)	Loss on drying (% w/w)
Guggul	3.78	4.2	1.0	33.0	59.3	5.70
Hirda	0.35	4.5	4.51	46.38	69.4	6.37
Punarnava	1.6	1.0	0.8	4.38	11.2	8.36
Gudvel	1.18	9.2	1.74	3.14	11.2	7.41
Deodara	0.5	2.0	0.94	19.65	4.20	8.33

(% w/w = Percent weight by weight)

Table 3: Phytochemical Screening

Plant Constituent	Test Reagent	Guggul	Hirda	Gudvel	Punarnava	Deodara
Steroids	Salkowaski reaction	+	+	+	-	+
Triterpenoids	Liebermann-Burchard test	-	-	-	+	+
Alkaloids	Dragendorff's reagent	-	-	+	+	-
	Mayer's reagent	-	-	+	+	-
	Hager's reagent	-	-	+	+	-
	Wagner's Reagent	-	-	+	+	-
Tannins	Ferric chlorideTest	-	+	+	-	-
	Lead acetate test	-	+	+	-	-
	Potassium dichromate test	-	+	+	-	-
Flavonoids	Shinoda test	-	-	-	-	+
Carbohydrates	Molish's test	+	+	+	+	-
	Fehling's test	+	+	+	+	-
Proteins	Biuret test	-	+	-	-	-
	Xanthoproteic test	-	+	-	-	-
Coumarins	Fluorescence test	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-

Evaluation Parameters of Tablet Blend

Based on the findings from the angle of repose, Carr's Index, and Hausner ratio, the powder mixtures of tablet blend exhibit favorable flow characteristics and effective packing capability (Table 4).

Table 4: Evaluation parameters of tablet blend for prepared formulations

Parameter	PF1	PF2	PF3
Bulk density (g/cm³)	0.88 ± 0.17	0.89 ± 0.21	0.91 ± 0.14
Tapped density (g/cm³)	0.97± 0.23	1.03 ± 0.17	1.01± 0.31
Angle of repose	$30.76^0 \pm 0.08$	$31.25^0 \pm 0.18$	$31.76^0 \pm 0.14$
Compressibility Index (%)	18.36	16.41	13.89
Hausner's Ratio	1.25 ± 0.09	1.18 ± 0.05	1.27 ± 0.04

The value shown in the tables is the mean of three determinations.

Table 5: Evaluation parameters of polyherbal tablets

Parameters	PF	PM	PN	PP
Uniformity of weight (mg)	306.67 ± 2.08	307.05 ± 5.29	308 ± 3.65	310.25 ± 32.86
Thickness (mm)	4.32 ± 0.181	5.12 ± 0.250	4.99 ± 0.314	4.16 ± 0.147
Friability (%)	0.169 ± 0.046	0.324 ± 0.51	0.141 ± 0.35	0.172 ± 0.024
Tablet Hardness (Kp)	3.33 ± 0.29	2.67 ± 0.76	3.17 ± 0.29	2.75 ± 0.69
Disintegration time (min)	11.23 ± 0.65	12.64 ± 0.41	13.65 ± 0.21	14.62 ± 0.82

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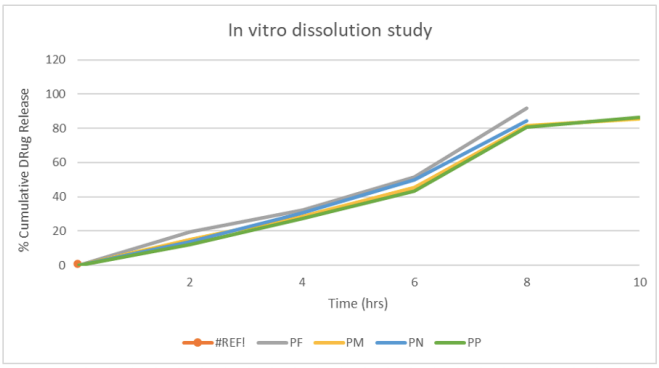
The evaluation parameters for each batch of tablets were within acceptable limits. The herbal tablets exhibit low friability, indicating that they are dense and hard to break. It was found that all the formulations showed a disintegration time ranging from 11.23 to 14.62 minutes, which may be due to the binding property of guggul (Table 5). Based on the results of the drug release profile, the formulation PF reaches its peak release of 91.6% after eight hours.

Figure 1: Dissolution study



Anti-inflammatory activity assessment by Carrageenan-induced rat Paw oedema method

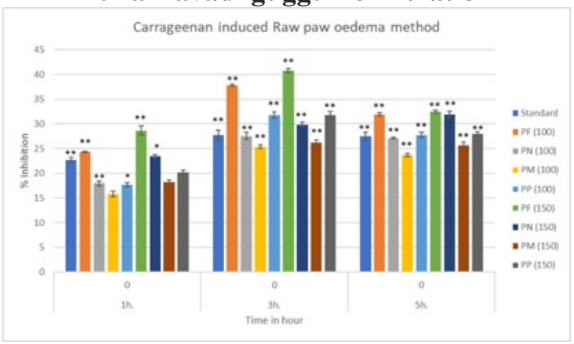
The formulations that were prepared and subsequently marketed exhibit a remarkable ($p<0.001$) inhibition of carrageenan-induced paw oedema in rats, with the results delineated in Table 6. Administration of doses of (100 and 150 mg) of these formulations results in a considerable ($p<0.001$) and dose-dependent attenuation of the swelling provoked by carrageenan at the 1st, 3rd, and 5th hours. The formulations PF, PN, PM, and PP demonstrated a maximum inhibition of paw oedema volume at 31.93 %, 27.13 %, 23.90 %, and 27.74 %, respectively, at a dosage of 100 mg/kg, whereas they exhibited a maximum inhibition of 32.46 %, 31.93 %, 25.66 %, and 28.00 %, respectively, at a dosage of 150 mg/kg. The formulations PF, PN, and PP maintained statistical significance at the 1st, 3rd, and 5th hours, whereas the formulation PM exhibited significance only at the 3rd and 5th hours, across both 100 and 150 mg/kg dosages, while the standard drug diclofenac achieved a maximum inhibition of paw oedema volume at 27.76 % (Figure 2).

Table 6: Anti-inflammatory activity of Punarnavadi guggul formulation on carrageenan-nduced rat paw oedema

Treatment	Dose (mg/kg)	Carrageenan induced paw oedema (mL)			% inhibition		
		1st h.	3rd h.	5th h.	1st h.	3rd h.	5th h.
Control	-	1.402±0.045	1.825±0.039	1.910±0.069	-	-	-
Standard	10	1.083±.0073**	1.330±0.085**	1.385±0.088**	22.71	27.76	27.48
PF	100	1.06±0.026**	1.13±0.029**	1.3±0.01**	24.37	37.8	31.93
PN	100	1.07±0.044**	1.28±0.065**	1.30±0.070**	17.95	27.57	27.13
PM	100	1.180±0.08	1.36±0.111**	1.45±0.115**	15.81	25.38	23.90
PP	100	1.15±0.025*	1.245±0.029**	1.38±0.0559**	17.71	31.78	27.74
PF	150	1.00±0.037**	1.08±0.027**	1.29±0.011**	28.65	40.82	32.46
PN	150	1.15±0.054*	1.32±0.026**	1.39±0.071**	23.42	29.86	31.93
PM	150	1.146±0.035	1.340±0.05**	1.42±0.046**	18.19	26.21	25.66
PP	150	1.118±0.025	1.245±0.030**	1.41±0.044**	20.21	31.78	28.00

*Significant at $p<0.01$, **significance at $p<0.001$, p-value was calculated by comparing with control by ANOVA, values are expressed as ± SEM

Figure 2: Graph showing percentage inhibition of Punarnavadi guggul formulation



Anti-inflammatory activity assessment by Cotton pellet induced granuloma

The formulations at tested doses of 100 and 150 mg/kg produced a significant ($p<0.01$) reduction in granuloma weight when compared to the control group. The formulations PF, PN, PM, PP at 100 mg of dose shows % inhibition of 36.35 %, 32.01 %, 29.37 %, 34.09 % respectively whereas at dose 150 mg it shows % inhibition of 45.01 %, 43.12 %, 33.15 %, 44.25 % respectively and the effects were comparable to that of the standard drug indomethacin 10 mg/kg which shows the % inhibition of 43.69 % (Table 7, Figure 3).

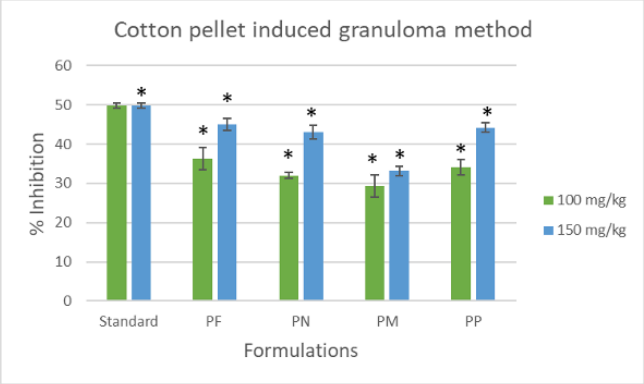
Table 7: Anti-inflammatory activity of Punarnavadi guggul on cotton pellet induced granuloma method.

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	% inhibition
Control	-	88.5±1.258	-
Standard	10	49.83±0.654*	49.83
PF	100	56.33±2.883*	36.35
PM	100	60.16±0.8062*	29.37
PN	100	62.5±2.798*	32.01

PP	100	58.33±1.961*	34.09
PF	150	48.66±1.585*	45.01
PM	150	50.33±1.778*	33.15
PN	150	58.16±1.229*	43.12
PP	150	49.33±1.174*	44.25

* Significant at p<0.01, p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM

Figure 3: Cotton pellet granuloma method for Punarnavadi guggul formulation.



Discussion

In the current study, three distinct batches of the punarnavadi guggul tablet were formulated in a laboratory and subsequently compared with three separate batches of commercially available formulations from different manufacturers. The tablets were formulated utilizing the direct compression technique. Preliminary formulation investigations were conducted on the tablet blends. The findings pertaining to bulk and tap density, angle of repose, Carr's Index, and Hausner ratio indicated that the powder mixtures exhibited favourable flow characteristics and excellent packing capabilities. Moreover, the formulated tablets underwent evaluation based on weight variation, hardness, drug content, and friability, with the results being compared against those of other polyherbal tablets for consistency. All tablet weights across the formulations were determined to be within the parameters established by the USP, which specified a range from 295 to 310 mg. The hardness and friability levels of the tablets produced from each batch of herbal tablets conformed to acceptable standards. The herbal tablets demonstrated a relatively low friability, implying a dense compaction. It was observed that all formulations exhibited disintegration times ranging from 11.23 to 14.62 minutes (Table 5). According to the analysis of the drug release profile, formulation PF attained its peak release of 91.6% after a duration of eight hours (Figure 1). In addition, the formulations were assessed for their anti-inflammatory efficacy. The carrageenan-induced paw oedema and cotton pellet-induced granuloma methodologies were employed as experimental models for the assessment of anti-inflammatory activity. All formulations demonstrated significant anti-inflammatory effects. The formulation-maintained significance at the 1st, 3rd, and 5th hours, exhibiting dose-dependent activity; however, when comparing the prepared formulations with various marketed formulations for anti-inflammatory activity, the highest percentage of inhibition was observed in PF, followed by PP, whereas formulation PM exhibited a lower percentage of inhibition for both acute and chronic activities at dosages of 100 and 150 mg/kg. The discrepancies in the percentage of inhibition exhibited by the different formulations can be attributed to variations in the concentrations of active constituents such as guggulsterone E and Z, punarnavin, deodarone, polyphenols, and flavonoids present, which are integral to the anti-inflammatory activity.

Conclusion

In the current research endeavor, efforts were directed towards the comparative analysis of the Punarnavadi guggul tablet formulation concerning its anti-inflammatory efficacy. Upon conducting the comparison, it was discerned that the formulation designated as PF exhibited the highest percentage of inhibition, followed by formulation PP; formulation PM demonstrated a comparatively lower percentage of inhibition during both acute and chronic phases. The observed discrepancies in the percentage of inhibition among the various formulations may be attributed to the differences in the concentrations of active constituents present, such as guggulsterone E and Z, punarnavin, and deodarone, which are integral to the manifestation of anti-inflammatory effects. The results clearly spell out the importance of using standard raw materials to ensure proper activity.

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