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Preparation And *In-Vitro* Characterization Of Anti-Rheumatic Polyherbal Capsules

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Abstract:

Rheumatoid arthritis is an inflammatory illness that produces chronic inflammation in the joints as a result of synovial hyperplasia and progresses to the point of causing considerable irreversible bone loss. Although a wide number of drugs are available to help control pain and slow the progression of Rheumatoid arthritis, there is currently no known treatment that may completely cure the condition. Herbal drugs can provide an alternative source of treatment for Rheumatoid arthritis sufferers while simultaneously addressing the limitations associated with current allopathic prescription therapy techniques, according to recent research. Boswellia serrate, Zingiber officinale (Ginger), Tribulus terrestris (Tribulus), Camellia sinensis (Green tea), Withania somnifera (Aswagandha) and Piper longum were used in the current investigation to search for an effective and safer alternative formulation. Polyherbal capsules were prepared and evaluated as part of the investigation. FTIR investigations revealed that the extracts and additives used in the creation of granules did not interact with one another, and four formulations (PCS1, PCS2, PCS3, and PCS4) were created utilising the extracts and the additions lactose, starch, and talc (as well as other ingredients). PCS4 had a larger in vitro drug release than other strains, making it a good candidate for additional studies of antiarthritic efficacy in vivo.

Keywords: Polyherbal, Boswellia, Arthritis, Inflammation, Capsules.

Introduction

Rheumatoid arthritis is an inflammatory disease that causes persistent inflammation owing to synovial hyperplasia and develops to significant irreparable bone damage. Other symptoms include stiffness and lack of physical mobility, as well as systemic aspects such as cardiovascular, pulmonary, physiological, and skeletal diseases (McInnes and Schett, 2011).It is more common in women than in males in all populations. RA often develops (in over 80% of cases) in the Middle Ages. Treatment for RA includes medications and lifestyle adjustments. Nonsteroidal anti-inflammatory medicines (NSAIDs), such as salicylic acid, and steroids (usually cortisone injection) are being used in therapy (Kavanaugh, 2007).Although these medications alleviate pain, they are unable to restore damaged tissues. Although a wide range of medications are provided to manage pain and reduce the course of RA, no treatment is known to entirely cure the illness. Furthermore, stomach ulcers have been found in RA patients who are using NSAIDS on a regular basis, as well as adrenal suppression caused by steroids (Cowan, 2007).

Herbal medicines can provide an alternate source of relief for RA sufferers while also addressing the limitations associated with current allopathic medication therapy approaches. Boswellia serrata (Salai/Salaiguggul) is a medium to large-sized branching tree of the family Burseraceae (Genus Boswellia) that grows in arid mountainous areas of India, Northern Africa, and the Middle East. Oleo gum-resin is extracted from the trunk of the tree and placed in a specially designed bamboo basket to

remove the oil content and solidify the resin. The oleo gum-resins include 30-60% resin, 5-10% essential oils that are soluble in organic solvents, and the remainder is polysaccharides. The resinous component of Boswellia serrata contains monoterpenes, diterpenes, triterpenes, tetracyclic triterpenic acids, and four main pentacyclictriterpenic acids, namely -boswellic acid, acetyl—boswellic acid, 11-keto—boswellic acid, and acetyl-11-keto—boswellic acid Acetyl-11-keto-boswellic acid is the most effective inhibitor of 5-lipoxygenase, an enzyme responsible for inflammation, of these four boswellic acids (Siddique, 2011).

Zingiber officinale rhizomes are used to make ginger. The plant is a member of the Zingiberaceae family. It has been widely utilised as a medicinal herb and spice from ancient times. Zingiber officinale has played crucial roles in the treatment of a wide variety of ailments, including asthma, diabetes, stroke, constipation, and others, due to the presence of phytochemical components and as a useful therapeutic agent (Tanaka, 2013; Kumar et al., 2013).

Ribel-Madsen et al. isolated synovial cells from synovial membrane or synovial fluid to test ginger's anti-inflammatory activity in vitro. TNF- activated the cells. Ginger-treated cells inhibited the production of cytokines IL-1 and IL-6, indicating an anti-inflammatory action, in a manner comparable to betamethasone. According to research, gingerol and gingerdione have strong analgesic and anti-inflammatory properties via blocking PGE2 production (Ribel-Madsen et al., 2012). These unique effects were caused by 6-shogaol, gingerdiols, and proanthocyanidins. The antiarthritic effect of crude extract ginger from Zingiber officinale was tested in rheumatoid arthritis and streptococcal cell wall induced arthritis animal models (Funk et al, 2009; Abdullah Al-Nahain et al., 2014).

Tribulus terrestris L. belongs to the caltrop family (Zygophyllaceae) and has been used as a traditional folk medicine to treat inflammation, high blood pressure, edoema, and urinary infections. T. terrestris includes flavonoids, steroidal saponins, and lignanamides, according to several phytochemical investigations. Furthermore, studies have shown that T. terrestris extracts have vasodilatory, anti-apoptotic, and antioxidant activities, and that they may have impacts on cardiovascular disease, hypertension, diabetes, cancer, and fungal infections (Zheleva-Dimitrova et al., 2012). T. terrestris has also been shown in studies to be useful in treating tracheitis, edoema, and inflammation, as well as protecting joints from inflammatory damage (Mishra et al., 2013). The downregulation of NO synthase 2, COX-2, TNF-, and IL-6 mediated the molecular processes of ETT in OA (Park et al., 2017).

Green tea is one of the most popular beverages eaten globally, and it is made from the Camellia sinensis leaves. GT and its bioactive components have been linked to the prevention and treatment of a number of diseases. This plant contains a variety of bioactive components, including antioxidants, vitamins, and flavonoid-like polyphenols. The presence of the catechinsepicatechin, epigallocatechin, epicatechin-gallate, and epigallocatechin-gallate (ECGC) was thought to heal arthritis (Fechtner et al., 2017). These chemicals have substantial anti-inflammatory and antioxidant effects and can treat a variety of ailments including inflammatory disorders, obesity, diabetes, cardiovascular disease, and cancer (Barbalho et al., 2019)

In the present investigation search for an effective and safer alternative formulation was performed using the above herbs and polyherbal capsules were prepared and investigated.

Materials and methods:

Herbs and Extraction

Crude drugs of Boswellia serrata, Zingiber Officinale, Tribulus terrestris, Camellia sinensis, Withania Somnifera and Piper longum were bought from the local herbal store and duly authenticated by a certified botanist. About 1kg of the dried powder was extracted with N-Hexane, Dichloromethane, Methanol, Ethanol, Water respectively in a Soxhlet apparatus. The extracts were concentrated and traces of the solvents were completely removed under reduced pressure and stored in vacuum desiccators for further use. The percentage yields of the extracts were 5.2, 6.8, 21.5, 28.4, 26.1 and 19.7 respectively.

Chemicals:

All the drugs and chemicals used in the study were procured from Himedia lab, Chennai and Ozone international, Mumbai and are of analytical grade.

Preformulation study

As a part of preformulation the compatability of the extracts with additives was investigated using FTIR. The flow properties of the powders like densities, Carr's index, Husner's Ratio and angle of repose were also investigated as per standard procedures (Aulton, and Taylor, 2017).

Construction of Calibration Curve

Selection of wavelength

In a 100 mL standard volumetric beaker, properly weigh 100mg of Admixture was dissolved in 100 mL of methanol, then fill the beaker to the top with methanol to produce a concentration of 1000 g/mL of extracts. From the aforesaid solution of stock, 10 mL was pipetted out and put into a 100 mL standard volumetric beaker, where the volume was increased to 100 mL with methanol to get a concentration of 100 g/mL by diluting the solution with methanol. In addition, the solution was diluted with methanol to reach a concentration of 50 g/ml. The aforementioned solution was scanned between 200 and 400 nm in wavelength against the reagent blank to obtain the results.The calibration curve of the extracts as per table can be constructed bypreparing two stock solutions (stock solution I and stock solution II)(Amol*et.al*, 2020).

Preparation of stock solution I

100 mg of mixture of Extracts was accurately weighed with the help of weighing balance. This weighed mixture was dissolved in Methanol and make up the volume up to 100mL in a volumetric flask. This is called stock solution I. It contains 1000 µg / ml of admixture.

Preparation of stock solution II

From the stock solution I 10 ml of solution was pipette out and make up the volume up to 100mL. This is called as stock solution II. It contains 100 µg / ml of admixture.

Preparation of aliquots

The aliquots were prepared from stock solution II whose concentration ranging from 2 to 10µg/ml. The absorbance was measured at 251 nm by using UV Spectrophotometer against the reagent blank.

Preparation of Granules

Starch, lactose, and extract mixture were weighed into a clean mortar using the method of doubling the bulk. Tragacanth mucilage (10% w/v) was prepared in another mortar by measuring 10 g of tragacanth and levigatingwith 55mL of water. It was then transferred quantitatively into an amber bottle. Water was added to make up to the 100mL mark (Aulton ME and K. M. Taylor, 2017). The tragacanth mucilage was then added to the powdered mixture until a damp mass was formed. The damp mass was screened through mesh no. 8 and then dried in a hot air oven at 40°C. The dried granules were then sieved with mesh no. 16 and subjected to the analysis of flow properties. The resultant best suitable quantities were tabulated.

Table 1: Quantities for admixture of extracts

Slno.	Extract name	PCS1 (g)	PCS2 (g)	PCS3 (g)	PCS4 (g)
1	Boswellia serrate (n–hexane)	200	50	50	50
2	Zingiber officinale (Dichloromethane)	50	200	50	50
3	Tribulus terrestris (methanolic)	50	50	200	50
4	Camellia sinensis (water:Ethanol – 1:1)	50	50	50	200
5	Withania somnifera (Aqueous)	50	50	50	50
6	Piper longum (Aqueous)	50	50	50	50

Table 2: Optimized formula for preparation of Poly Herbal Granules

Sl.no.	Granules name	Quantity		
		Extracts (g)	Starch (g)	Lactulose (g)
1	PCS1	20	3.2	1.5
2	PCS2	20	1.8	3
3	PCS3	20	1.2	3.5
4	PCS4	20	3.8	1

Preparation of Polyherbal capsules

Granules were weighed and filled into capsules using a capsule filling machine wherein each capsule contained 400mg of polyherbal extracts. The dose was adjusted using talc as the additive. The capsules were then packaged and appropriately labeled pending further tests.

EVALUATION OF PREPARED CAPSULES

Weight variation test: 20 capsules were taken and were weighed individually. The average weight of each capsule was calculated and compared with the individual capsule weight to the average. The capsules pass the IP. test if not more that 2 capsules are outside the percentage limit and if no capsule differs by more than 2 times the percentage limit (Jyothi*et.al*, 2017).

In-vitro **Disintegration test:** The test was carried out on 6 capsules using capsule disintegration tester with distilled water at 37ºC ± 2ºC was used as a disintegration media and time taken for complete disintegration of the 6 capsules in the apparatus was measured(Jyothi*et.al*, 2017).

Drug Content Uniformity

Ten capsules were selected randomly; eachwas emptied and 50mL of Phosphate Buffer pH 6.8added. The mixture was filtered, and Phosphate Buffer pH 6.8was used to top it up to 100 mL. The Drawell's UV spectrometer was used in measuring the absorbance at 251nm after serially diluting the solutions. The recorded absorbance was then inserted into the calibration equation and the percentage content determined for each capsule.

Stability studies

Stability testing was carried out to provide evidence of how the quality of the manufactured capsules may change with time under the influence of environmental factors such as temperature and humidity. They were important and necessary for observing drug's degradation in the process of time. Stability study was carried out in climatic chamber at 25±2°C/60±5% relative humidity for 6 months and 40±2°C/75±5% relative humidity for 6 months.

In-vitro **Dissolution studies (British Pharmacopoeia, 2007):**

In vitro dissolutionstudy was done using USP type II paddle dissolution apparatus. In 900mL of Phosphate Buffer pH 6.8 the release medium wastransferred into each of the six dissolution vessels. The water jacket was heated to 37 ± 2°C to represent the body's temperature and a randomly selected capsule (fitted with a sinker), dropped into the dissolution medium. An aliquot of the sample was withdrawn at regular time intervals (5, 15, 30, 35, 40, 45, and 60minutes), and the same volume of Phosphate Buffer pH 6.8wasused to replace the volume withdrawn. The replacement process was done to maintain sink conditions. The samples were filtered and analyzed with the UV spectrometer at 251 nm. The absorbance obtained was inserted into the calibration equation to obtain the amount of drug released at each time point. A graph of cumulative drug released against time was then plotted to obtain the dissolution profile of the formulatedcapsules in Phosphate Buffer pH 6.8 (Kumadoh et al., 2015).

Results and discussion

FTIR studies:

The individual spectra for Extract, Starch and Lactulose as per their structural formula were observed within the standard ranges. Hence the materials used for the formulation were confirmed to be respective components. The FTIR spectrum of the mixture of Extracts along with Starch and Lactulose

their characteristic peaks. Hence the components are confirmed to be incompatible and may not affect the formulation stability during its shelf life.

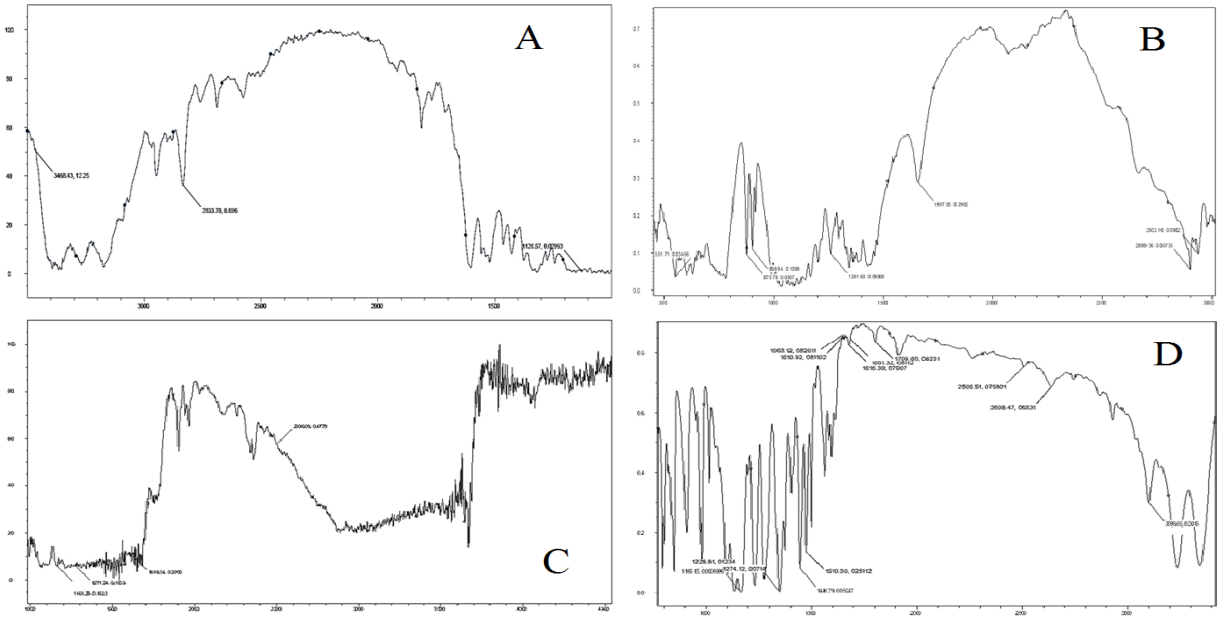

Figure 1: FTIR studies of Polyherbal capsules. A. Extract mixture, B. Pure LActulose, C. Pure Starch, D. Admixture

Calibration curve of the polyherbal capsules

The calibration curves of the polyherbal formulations were drawn and the curves were linear and can be extrapolated for the estimation of the drug content in the capsules. The curves were represented in figure 2.

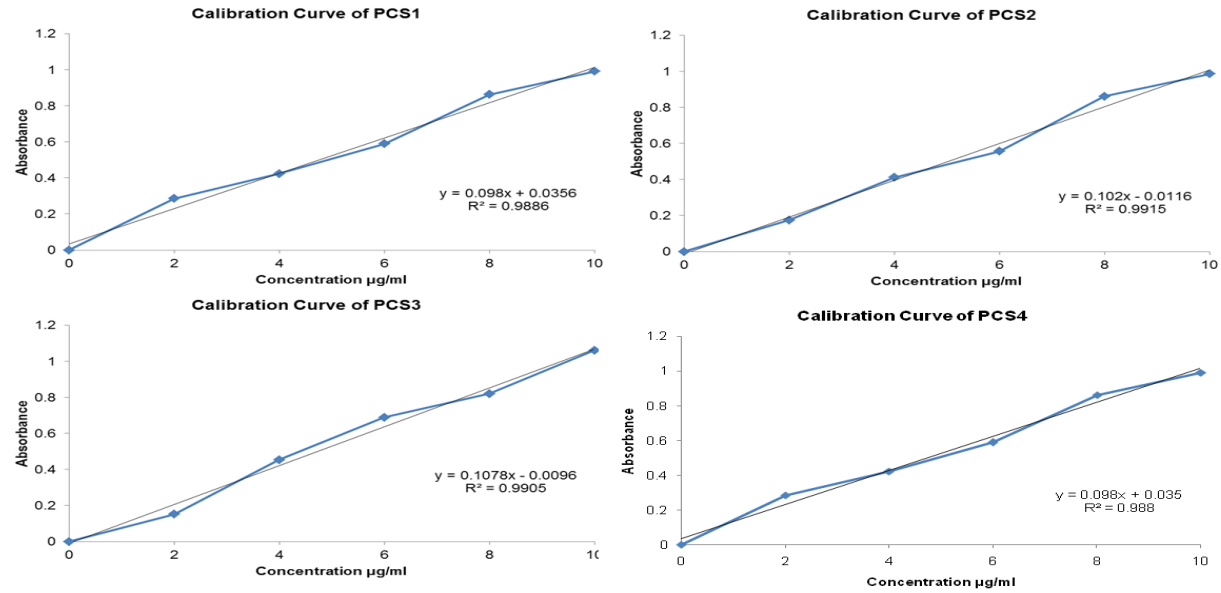

Figure 2: Calibration curves of polyherbal capsules

Flow properties of polyherbal capsules

Physical Evaluation of granules:

The angle of repose of all six formulations was shown within the range of 27 to 30 indicating good flow properties of the granules.Bulk density was found to be between 0.41 – 0.46 g/ml. Tapped density was found to be between 0.50 – 0.54 g/ml. Carr's index was found to be in the range of 8.00 – 17.64. All the granules are well within the specification limit.Hausner's ratio was found to be between 1.08 – 1.31. With this the granules were found to be free flowing material and showed suitability to be compressed as capsules of expected weight.

Table 3: Flow properties of Extract powder mixture

Slno.	Extract mixture	Tapped density	Bulk density	Carr's index	Hausner'sratio	Angle of Repose
1	PCS1	2.85±0.08	2.14±0.06	24.91	1.33	36.51
2	PCS2	2.91±0.07	2.22±0.12	23.71	1.31	30.97
3	PCS3	2.76±0.11	2.15±0.05	22.10	1.28	26.11
4	PCS4	2.96±0.09	2.02±0.08	31.75	1.46	41.06

Table 4: Flow properties of prepared Optimized Poly Herbal Granules

Slno.	Granules name	Tapped density	Bulk density	Carr's index	Hausner'sratio	Angle of Repose
1	PCS1	1.30±0.05	1.20±0.16	7.69±1.27	1.08±0.08	21.62±1.52
2	PCS2	1.23±0.03	1.14±0.08	7.31±0.97	1.07±0.07	22.36±1.81
3	PCS3	1.66±0.08	1.54±0.06	7.22±1.11	1.07±0.03	20.41±1.57
4	PCS4	1.31±0.07	1.21±0.03	7.63±1.52	1.08±0.04	22.98±1.65

Physical Evaluation of polyherbal Capsule:

All the compressed capsules were evaluated for weight variation, content uniformity and disintegration time.

Table 5:Physical evaluation of Polyherbal capsules

S.No	Formulation code	Weight variation	Disintegration time (sec)	Content uniformity

1.	PCS1	2.51	9 min 50 sec	100.01
2.	PCS2	2.48	12 min 15 sec	98.56
3.	PCS3	1.99	8 min 10 sec	99.67
4.	PCS4	2.60	11 min 25 sec	100.12

The capsule parameters observed are given in table. The granules were filled in the capsules using capsule filling machine. The maximum weight variation of the capsules was 2.60%, which falls within the acceptable weight variation range of ±5%, hence the capsules of all batches passed the weight variation test. Disintegration time is an important parameter of capsule. An ideal capsule should disintegrate within 15min. The capsules of all the batches disintegrated within 13 minutes 30 seconds. The drug content in the capsules was also in the limits of ±3%. So ti can be advocated that the extract distribution in each capsule is as per the fixed dose.

***In-vitro* dissolution studies**

The dissolution profiles of polyherbal Capsule prepared with super disintegrant starch were shown good dissolution profiles comparing with the capsules prepared by using starch. It was also observed that increase in the concentration of starch increased the rate of drug release. Out of all the six formulations, PCS4 showed maximum dissolution within 2 hours and the data obtained for this formulation was analyzed.

Table 6: In-Vitro Dissolution studies

Time in min	**PCS1**	**PCS2**	**PCS3**	**PCS4**
5	22.32±1.35	20.02±1.25	20.02±1.22	23.16±2.03
10	27.16±2.51	26.45±1.85	21.06±2.06	24.68±1.36
15	27.16±1.69	26.93±2.47	23.96±2.54	26.67±3.08
30	29.12±3.51	28.92±4.66	26.93±3.61	31.45±3.68
45	30.26±2.85	34.63±4.02	32.23±5.99	37.64±5.07
60	33.41±2.68	37.12±3.58	34.36±4.87	45.72±4.11
90	42.62±3.08	50.60±3.97	58.25±4.52	67.73±4.75
120	62.23±3.66	69.25±4.01	82.12±2.96	96.35±4.62

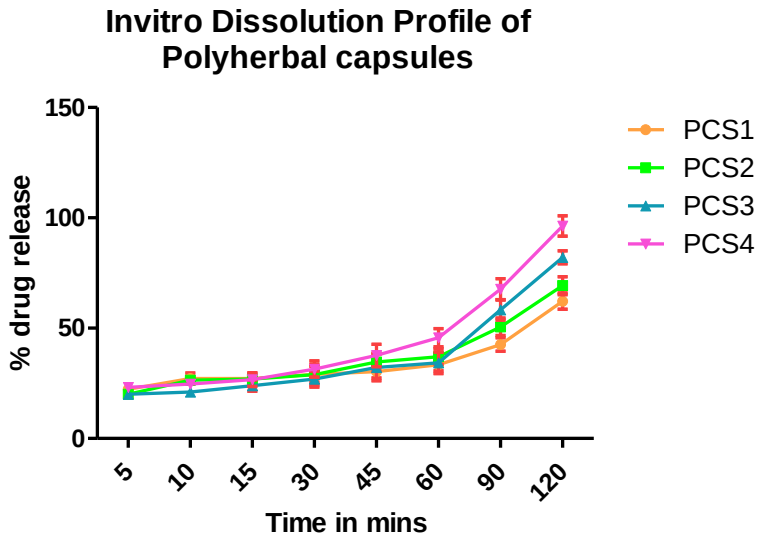

Figure 3:In-vitro Dissolution profile of all the six formulations

Stability studies

The formulations F6 were selected for stability studies on the basis of their high cumulative % drug release and also results of in vitro disintegration time and wetting time. The stability studies were carried out at 40^0C at 75% RH for the selected formulations up to 3 months shown in Table 8. For every 1 month time interval the capsules were analyzed for drug content uniformity, hardness, in vitro disintegration time, friability and wetting time up to 30 days. These formulations showed not much variation in any parameter. From these results it was concluded that, formulation F6 was stable and retained its original properties.

Table 7: Stability Studies Report

Intervals of Testing	Appearance	Drug content (99-110%)
0 day	White colour, circular, bio convex capsules	100.5
15 days	White colour, circular, bio convex capsules	99.61
30 days	White colour, circular, bio convex capsules	101.87
45 days	White colour, circular, bio convex capsules	100.93
60 days	White colour, circular, bio convex capsules	100.86

Conclusion:

Polyherbal capsules incorporating herbal extracts were prepared and analyzed subjecting them to various tests like drug content, invitro drug release, stability studies etc. The results showed that PCS4 had best release and even though the variations in drug content uniformity was higher than other formulations; it can be adjusted though using additives. Over all it can be said that the best formulation

in terms of pharmaceutical parameters is PCS4. This also opens up path to investigate the formulations for antiarthritic activity invivo and comparing them to the marketed formulations to establish the formulations as better choices for treatment of arthritis. Also standardization is required to ensure the quality and repeatability of results.

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