ORIGINAL ARTICLE

Evaluation of *in vivo* rheumatoid arthritis activity of formulated capsule with different portions polyherbal ethanolic extract from selected potential Indian herbs

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Abstract

Objective: Rheumatoid arthritis (RA) is a systemic disorder which involves the activation of immune system against the self-tissues. The main targets of this disease are the joints. Being systemic, the development of this disease involves different mechanisms, and thus, the exact cause of this disease remains unknown. Although different drugs have been developed, none has been found to be the cure for this disease. The present study was commenced to evaluate the *in vivo* anti-arthritic effect of polyherbal formulation of selected plants *Polygonum glabrum, Canthium dicoccum, Ochna obtusata,* and *Argyreia nervosa.* **Materials and Methods:** *In vivo* anti-arthritic activity of the ethanolic extract of different portions capsule formulation F4 investigated orally was assessed using complete Freund's adjuvant-induced arthritis. **Results:** In complete Freund's adjuvant-induced arthritis models, the polyherbal extract formulations significantly ($P < 0.001$) reduced joint and paw swelling and markedly improved body weight, hematology profile, and parameters in complete Freund's adjuvant model. **Conclusion:** It could be concluded that the ethanolic extract of two different formulations holds anti-arthritic potential, supporting its traditional use in the treatment of RA.

Key words: *Argyreia nervosa*, *Canthium dicoccum*, ethanolic extract, Freund's adjuvant-induced arthritis, *in vivo* rheumatoid, *Ochna obtusata*, *Polygonum glabrum*, polyherbal

INTRODUCTION

Herbal medicine is the oldest form of health care known to humankind. It is an integral part of the development of modern civilization. In herbal medicine, plant-based formulation is used to alleviate diseases. However, the most important challenges faced by these formulations arise due to their lack of complete evaluation. Hence, evaluation is necessary to ensure the quality and purity of the herbal product. It is very important to establish a system of evaluation for every plant medicine in the market since the scope for variation in different batches of medicine is enormous.[1]

Inflammation is a normal protective response to tissue injury which involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair.[2] It is characterized by redness, swelling, pain, stiffness of joint, and loss of joint function. Inflammation is associated with membrane alterations, increase in vascular permeability, and protein denaturation.[3] Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune-mediated response.[4] One-fifth of the world's elderly suffer with arthritis.[5] The current treatment of arthritis includes minimization of this associated pain and inflammation using nonsteroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs.[6] Due to adverse reactions of the NSAIDs and disease-modifying antirheumatic drugs, the arthritic patients tend to search for other treatments that are effective and

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Received: 01-06-2018
Revised: 23-03-2019
Accepted: 04-04-2019

less toxic. Therefore, complementary and alternative medicines are commonly preferred by such patients.[7]

Polygonum Glabrum

The tribes of Chhattisgarh use the root paste as a medicine for snake bite[8] and also having different uses such as jaundice and piles,[9] antimalarial agent in Sudan,[10] dysentery,[11] and anthelmintic.[12] The whole plant decoction is used as a remedy for colic pain, pneumonia, and the boiled paste is applied in cuts and wounds.[13] Peels from stem are used for treating rheumatism.[14] *Ochna obtusata* DC. (Family Ochnaceae) is a small tree up to 8 m tall. The family is characterized by the presence of secondary metabolites such as flavonoids and terpenoids.[15] Moreover, it is extensively used in Indian traditional medicine for the treatment of epilepsy, menstrual complaints, lumbago, asthma, ulcers, and as an antidote to snake bites[16] and having glycosides, saponins, steroids, flavones, and fatty acids.[17] It is also ulcer, asthma, and bronchitis and also possesses antiulcer genic activity.[18] *Canthium dicoccum* ethanolic extract of whole extract showed almost equipotent antidiabetic activity compared to standard drug glibenclamide.[19] Moreover, also, egg albumin-induced arthritis model.[20] *Argyreia nervosa* seeds are found to possess hypotensive and spasmolytic activity which was due to the mixture of ergot alkaloids, isolated, and analyzed by ultraviolet. Due to instability only, one constituent was identified as ergometrine. Other constituents such as caffeic acid and ethyl caffeate were identified.[21,22] Apart from ergoline alkaloids, N-formylloline alkaloids, flavonoid sulfates, steroids, and triterpenoids were isolated from other parts of *A. nervosa*.[23,24] Para-hydroxy-cinnmate, scopeltin, and argyroside[25,26] isolated oil from the seed of *A. nervosa* and evaluated the antibacterial effect.[27]

In the previous studies, the author noticed ethanolic extract of the above plants and with polyherbal formulations with different fractions of ethanolic extract showed good antioxidant activity as well as *in vitro* antiarthritis activity.[28] By considering above facts, the present study is aimed at developing formulations from crude plant extract of the above plants and several antirheumatoid constituents which act by several modes of action to influence multiple biological pathways and thereby producing more effective through oral route. The study was also designed to produce formulation which is safe, cheaper and which can reduce rheumatoid, thereby providing multifaceted benefits.

MATERIALS AND METHODS

Plant Source and Authentication

P. glabrum, O. obtusata DC., *C. dicoccum,* and *A. nervosa* were collected from Tirumala Hills, Tirupati, and Chittoor district of Andhra Pradesh, near Seshachalam and Tirumala Hills (Rayalaseema region, Andhra Pradesh, India), areas that are geographically located in the South Eastern Ghats, are recognized for their rich flora and fauna. The plant specimen was verified to be of the correct species by Dr. Madhava Setty, a botanist from the Department of Botany, S. V. University, Tirupati, Specimen Voucher no:1972,1220,1012,2162 preserved for further reference at our laboratory.

Drugs and Chemicals

Diclofenac sodium obtained as a generous sample from Meditech Pharma Pvt. Ltd., Mumbai, ethanol (Sigma-Aldrich, USA), and complete Freund's adjuvant (Sigma-Aldrich, USA).

Experimental Animals

Swiss Albino rats of either sex weighing from 200 to 300 g were used. The rats were housed under standard conditions of temperature (23–25°C), relative humidity (55%) with 12 h light and 12 h dark cycle. They were fed with standard pellet diet and tap water *ad libitum*. The experiment was designed and carried according to norms of ethical committee (CPSCEA) and approved by the institutional animal ethical committee (1987/PO/Re/S/17/CPCSEA).

Preliminary Phytochemical Studies[29-31]

Previously, various preliminary phytochemical tests were performed for the extract used for capsule formulations using standard procedures and the above formulations showed the presence of mainly carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids, and saponins which majorly responsible for the desired activity.

Preparation of Polyherbal Granules

Polyherbal granules were prepared by wet granulation method. Polyherbal extract was mixed well with lactose monohydrate, add required quantity of starch to obtain a smooth mass then passed through # 12 to produce granules. Prepared granules were gently subjected to drying (<60°C) in an oven. Dried granules were passed through # 16/44 to get uniform sized granules. Separate the fines. 15% of fines were mixed with granules and remaining excipients talc and magnesium stearate were added in required quantities. Granules were also prepared containing croscarmellose sodium (CCS) as superdisintegrant. After addition of lactose to the extract, CCS was incorporated at variable amounts (3%, 4%, and 5% with respect to avg. weight) separately and granulations were carried out similar manner as above. Quantities for formulation trails are presented in Table 1.[32-35]

Prepared granules were subjected for various flow property measures such as determination of Carr's index, Hausner ratio, and angle of repose.[36,37]

Table 1: Composition of different ingredients used for formulation

Name of the ingredient	Quantity (mg)			
	F_1	F_2	F_3	F_s
Herbal extract	25	25	25	25
Lactose monohydrate	227	218	215	212
Starch paste	30	30	30	30
Croscarmellose sodium	-	9	12	15
Talc	9	9	9	9
Magnesium stearate	9	9	9	9
Total weight	300	300	300	300

Formulation of Polyherbal Capsules

Prepared granules were packed into hard gelatin capsule (size 2) using hand-operated capsule filling machine such that each capsule contains 300 mg of granules. Polyherbal capsules without CCS were labeled as F1 and capsules containing 3%, 4%, and 5% of CCS were labeled as F2, F3, and F4, respectively, and quantities for formulation trails are presented in Table 1.

Animals were housed in polypropylene cages, maintained under standardized condition (12 h light/dark cycle, 24°C, and 35–60% humidity) and provided free access to standard palate diet and purified drinking water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout.

Acute Toxicity Study

For acute toxicity study on mice, "Fixed-dose" method of the organization for economic cooperation and development guideline 420 was followed.[38,39] The formulation was suspended in distilled water and administered by gavages (orally) at single doses of 2000 mg/kg. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the formulation. The general behavior of the rats was continuously monitored for 3 h, and then every 30 min for next 3 h till 24 h and then daily for a total of the 14 days. Changes in the normal activity of rats, their body weights, sign and symptoms of toxicity, and mortality were monitored and recorded.

In vivo Evaluation Selected Polyherbal Capsule

Complete Freund's adjuvant-induced arthritis in rats

The male Swiss albino rats were divided into five different groups of six animals each as follows:

- Group I: Normal control.
- Group II: Arthritic control.
- Group III: Capsule formulation (F_4).
- Group IV: Diclofenac sodium (10 mg/kg b.wt orally).

Before the experiment, paw volume (baseline) of each animal at 0 day was measured. In complete Freund's adjuvant (5 mg of heat-killed, powdered *Mycobacterium tuberculosis* cell was suspended with liquid paraffin to get a 5 mg/ml suspension) was used to induce arthritis in rats. The rats were anesthetized with intraperitoneal injection of 40 mg/kg thiopentone sodium. Mineral oil was injected in the right ankle joint of normal group of animals. Adjuvant arthritis was induced by subcutaneous injection of Freund's complete adjuvant (FCA) (0.1 ml) into subplantar tissue of the right hind paw of each rat. The test groups consisted of FCA-injected rats challenged with the respective doses of the test drugs administered orally 24 h before FCA injection, while the vehicle control rats were injected with 0.1 ml of liquid paraffin (incomplete Freund's adjuvant) only. The drug treatments were continued once daily on the same time after the challenge for 20 more days. The swelling in the injected and contralateral hind paws of the rats was monitored daily using liquid displacement plethysmometer. Increase in the extent of erythema and edema of the tissues shows the severity of the inflammation. The change in body weight and paw edema was recorded at desired frequent intervals.[40,41]

At the end of the study, blood samples were withdrawn from all groups through retro-orbital plexus puncture, and whole blood was used for hematological analysis and serum was used for biochemical analysis.[42] Hematological parameters such as the hemoglobin (Hb) level, the red blood cell (RBC) count, the white blood cell (WBC) count, and the erythrocyte sedimentation rate (ESR) were estimated manually using fresh blood. Serum samples were collected after centrifugation of whole blood at 3000 rpm for 20 min. Liver markers such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatinine were analyzed using an autoanalyzer (Vital Scientific N.V., the Netherlands). The liver enzyme levels were estimated using Lab Kit enzymatic kits. The C-reactive protein (CRP) and serum copper CRP levels estimated using the enzyme-linked immunosorbent assay kit (obtained from Alpha Diagnostic Intl., USA) and the colorimetric bathocuproindisulfonate method of Zak and Landers, respectively.[43,44]

Statistics

All values are shown as mean ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test. $P < 0.05$ was considered statistically significant and $P < 0.001$ was considered statistically highly significant, as compared to control group.

RESULTS

Polyherbal granules were prepared from extract along with formulation additives by moist granulation technique and composition for formulation trails is presented in Table 1. Prepared granules were subjected for various flow property measures such as determination of Carr's index, Hausner ratio,

and angle of repose and from the results of preformulation studies clear that all blends were possessed good flow characteristics prepared granules were packed in capsule shells (2) with the help of hand-operated capsule filling machine. Formulae for trails are summarized in Table 1.

All capsule formulations were subjected to various pharmacopoeial tests and results of them like weight variation were found to be within limits, drug content was founded to be within the range, disintegration time was founded to be within the range.

The *in vitro* dissolution study was performed using USP Type-II dissolution test apparatus. The operating conditions were 900 ml of phosphate buffer pH 6.8 as dissolution fluid, paddle rotated at a speed of 100 rpm at 37 ± 0.5°C. From the results of *in vitro* dissolution study, it reveals that marker component rutin was released from the capsules. Percentage cumulative drug release for rutin from formulation F1 and F4 was found to be within the range of 52.86 ± 0.05–98.99 ± 0.01 at 12 h.[45] From the results, polyherbal capsule formulation F4 showed good physical properties such as disintegration, hardness, and dissolution rate. After the comparative study of different formulation having different excipient yielded a conclusion that CCS 15 mg (5%) is better suitable. Hence, F4 was selected and evaluated through *in vivo* in this article.

Clinical Signs of Intoxication, Body weight, and Mortality

In the preliminary acute toxicity study, formulation seems to be safe at 2000 mg/kg. There were no toxic or deleterious effects [Table 2] observed immediately in 24 h and up to 14 days of observation period. There was no major change in body weight showed in Table 3 and no mortality which is recorded in Table 4.

Table 2: Cage-side observations of animals (general behavior)

Parameters	Observations (2000 mg/kg)
Condition of fur	Normal
Skin	Normal
Subcutaneous swelling	Nil
Eyes dullness	Nil
Eyes opacities	Nil
Color and consistency of feces	Normal
Condition of teeth	Normal
Breathing abnormalities	Nil

DISCUSSION

In the preliminary acute toxicity study, prepared capsule seems to be safe at 2000 mg/kg. There were no toxic or deleterious effects observed immediately in 24 h and up to 14 days of observation period. There was no major change in body weight and no mortality found in any animal [Tables 3 and 4].

The preliminary phytochemical screening of polyherbal formulation the formulated capsules showed the presence of alkaloids, flavonoids, and tannins.[46] These compounds have well-known anti-inflammatory and antiarthritis activity. The effects observed with formulated capsules could possibly be due to the synergistic actions of these compounds. In the present study, formulated capsules demonstrated a highly significant ($P < 0.001$) antiarthritis activity at different formulations in rat model of antiarthritis activity results showed in Table 5.

Animal model used for *in vivo* evaluation of antiarthritis activity complete Freund's adjuvant-induced arthritis animal model in which clinical and pathological alterations are akin to those seen in human rheumatoid arthritis (RA).[47,48] Complete Freund's adjuvant is a mixture of heat-killed *M. tuberculosis* with liquid paraffin which stimulates cell-mediated immunity, thus potentiating the production of certain immunoglobulins in body.[49] Adjuvant-induced arthritis in the rat can be alienated into three distinctive phases; first, the induction phase without the manifestation of synovitis, followed by early synovitis, and finally, late synovitis accompanied by unremitting cartilage and joint tissue destruction. In this method, arthritis model offers an opportunity to examine the pathological changes in a variety of tissues other than joints.[50] Anemia is the most common extracellular manifestation in RA and may be caused by the decreased level of plasma iron due to sequestration of iron in the reticule endothelial system and synovial tissue ultimately failure of bone marrow to counter anemia.[51] IL-1 in association with the acute phase response also decreases plasma iron content and it is challenging to speculate that the sequestration of less deformable erythrocytes by endothelial

Table 3: Mean body weight and percentage body weight gain

Group	Dose (mg/kg body weight)	Body weight		% body weight gain	Body weight	% body weight gain	% body weight gain
		Day 1	Day 7	Day 1–7	Day 14	Day 7–14	Day 1–14
Control 1	-	22.47	23.69	5.43	25.62	8.14	14.02
I	2000	22.85	24.44	6.96	26.25	7.40	14.87

cells in the spleen also plays a causative role in shortened half-life of erythrocytes thus, resulting in anemia.[52] Alternatively, a rise in both WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism and it is evident by the influx of inflammatory mononuclear cells in the joints of arthritic rats.[53,54]

In the present experimental study, the herbal formulation-treated groups had considerably increased level of Hb and RBC, while the level of WBC and platelets was significantly reduced in contrast to arthritic control group but comparable to normal control group [Table 6]. Similarly, ESR is an imperative hematological index for the diagnosis as well as prognosis of infectious and inflammatory diseases. With reference to the standard drug and herbal treatment together its fractions remarkably decreased ESR count in arthritic rats, thus justifying its significant role in arthritic conditions. Rheumatoid factor (RF), a key serologic marker, is an autoantibody directed against the Fc (Fragment, crystallizable) portion of IgG and form immune complexes that contribute toward the succession of RA. A noteworthy decrease in RF level in the serum of arthritic rats treated with treated with polyherbal extract in specific ratio unveil the protective against RA [Table 6]. From these hematological findings, it can be proposed that polyherbal formulation changes the alterations in blood parameters toward normal by inhibiting the inflammatory response which might be due to its blocking action on pro-inflammatory cytokines and cyclooxygenase enzyme as well as suppressing the immune response as supported by the previous studies.

Table 4: Mortality record

Group	Dose (mg/kg body weight)	Mortality	
		Male	Female
I	2000	0/3	0/3

CONCLUSION

The result of the acute toxicity test, for oral preparation of capsule formulation, indicates that it is relatively safe and non-toxic to rats. The above polyherbal extract with different portions *P. glabrum* ethanolic extract, *C. dicoccum* ethanolic extract, *O. obtusata* ethanolic extract, and *A. nervosa* ethanolic extract (2:1:1:1) in F4 capsule is proven with good *in vivo* antiarthritis activity which is medicinally valuable plant and its antiarthritic effect might be due to its anti-inflammatory, antioxidant, and immunosuppressant actions, although, actual mechanism is not known.

ACKNOWLEDGMENT

We are thankful to the management and staff of Vels University, Chennai, Department of Pharmacognosy, for providing facilities and encouragement.

Table 5: Results for complete Freund's adjuvant-induced arthritis in rats for selected capsule formulations (F4)

Group	Treatment	Effect of paw edema (*n*=3)			
		Days			
		1	5	10	15
Group I	Normal control	4.3±0.1[a]	4.5±0.2[a]	4.4±0.1[a]	4.6±0.1[a]
Group II	Arthritic control	7.6±0.0	16.6±0.0	22.5±0.0	25.7±0.0
Group III	F_4	5.45±0.0[a]	6.93±0.0[a]	6.2±0.0[a]	4.85±0.0[a]
Group IV	Diclofenac sodium	6.1±0.0[a]	7.4±0.0[a]	6.5±0.0[a]	5.9±0.0[a]

Values are expressed as mean±standard error of the mean *n*=5; One-way analysis of variance followed by Dunnett's test. *P*<0.05 was considered statistically significant and *P*<0.001 was considered statistically highly significant, as compared to control group

Table 6: Results for hematological parameters in arthritic rats

S. No	Group	Treatment	Hematological parameters in arthritic rats (*n*=3)					
			Hb (g/dL)	RBCs 106/µL	WBCs 103/µL	Platelets 103/µL	ESR mm/1st h	RF IU/mL
1	Group I	Normal control	14.2±0.2[a]	7.4±0.2[a]	5.2±0.1[a]	311±3.2[a]	3.0±0.5[a]	14±0.0[a]
2	Group II	Arthritic control	9.3±0.1	4.9±0.0	9.4±0.2	1225±105.3	20.3±0.8	48.3±2.0
3	Group III	F_4	10.6±0.1[a]	5.7±0.1[a]	7.7±0.2[a]	454.3±18.7[a]	13.3±0.8[a]	26.0±1.1[a]
4	Group IV	Diclofenac sodium	12.8±0.1[a]	6.9±0.1[a]	7.9±0.2[a]	734.3±4.6[a]	12.5±1.2[a]	25.5±2.3[a]

RBC: Red blood cell, WBC: White blood cell, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, RF: Rheumatoid factor. Values are expressed as mean±standard error of the mean *n*=5; One-way analysis of variance followed by Dunnett's test. *P*<0.05 was considered statistically significant and *P*<0.001 was considered statistically highly significant, as compared to control group

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Source of Support: Nil. **Conflict of Interest:** None declared.