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EVALUATION OF *IN- VITRO* RHEUMATOID ARTHRITIC ACTIVITY OF POLYHERBAL ETHANOLIC EXTRACT CONTAINING FORMULATIONS FOR SELECTED POTENTIAL INDIAN HERBS

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Abstract:

Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response.[3] One fifth of the world's elderly suffer with arthritis .In the anti-arthritic activity the production of auto antigen in certain arthritic disease may be dueto denaturation of protein, membrane lysis and proteinase action. The mechanism of denaturation probably involves electrostatic hydrogen,hydrophobic and disulphide bonding. Anti denaturation study which includes the albumin denaturation is performed by usingBovine serum albumin (BSA). When BSA is heated it undergoes denaturation and express antigens associated with type 3 hypersensitivity reactions and that is related to diseases such asserum sickness, glomerulonephritis, rheumatic arthritis, and lupus erythromotosus.

Key words: *Inflamatory,Arthritis,Medical response,Lysis*

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INTRODUCTION:

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.[1] 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. [2] Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response.[3] One fifth of the world's elderly suffer with arthritis.[4] The current treatment of arthritis includes minimization of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs.[5,6] Due to adverse reactions of the NSAIDs and disease modifying anti-rheumatic drugs, the arthritic patients tend to search for other treatments that are effective and less toxic. Therefore, complementary and alternative medicines are commonly preferred by such patients.[7]

Polygonumglabrum: the tribes of Chhattisgarh use the root paste as a medicine for snake bite [20]. In some areas the root stock is used for the treatment of jaundice and piles. [8] The leaves are used as an antimalarial agent in Sudan. [9] In south India *Polygonumglabrum* leaves are used for the treatment of dysentery. [10] A decoction of the leaves and seeds are used as cardio tonic, astringent and anthelmintic.[11]The whole plant decoction is used as a remedy for colic pain, pneumonia and the boiled paste is applied in cuts and wounds.[12]Apart from medicinal use, the whole plant is powdered and used as bait for fishing. Peels from stem are used for treating rheumatism.[13]

Ochnaobtusata DC. (FamilyOchnaceae).is a small tree up to 8 m tall. The family is characterized by the presence of secondary metabolites like flavonoids, terpenoids. [14]And 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. [15] Several studies conducted on *Ochna*species revealed the presence of glycosides, saponins, steroids, flavones and fatty acids. [16] The leaves and roots of *O. obtusata*are used for ulcer, asthma and bronchitis and also possess anti-ulcer genic activity. [17]

Canthiumdicoccum Ethanolic extract of whole plant of *Canthiumdiococcum* for anti-inflammatory activity in Wister albino rats in various models of anti-inflammatory activity viz. Carrageenan induced paw edema, Formalin induced paw edema, fresh egg white induced paw edema and cotton pellet induced granuloma model. Results showed the extract with anti-inflammatory activity and suggests a potential alternative to NSAIDS like diclofenac.[18]ethanolic extract of *Canthiumdiococcum* for anti-diabetic in an alloxan induced diabetic rat model. Results showed a significant drop in fasting blood sugar in a dose-dependent manner, with an effect on the beta-cell population in the pancreas. The extract showed almost equipotent anti-diabetic activity compared to standard drug Glibenclamide. [19] Ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against Egg-albumin induced arthritis model.[20]

In the recent studiesof the author ethanolic extract of the above plants and the polyherbal formulations with different fractions of ethanolic extract showed good anti-oxidant activity.The present study is deigned to evaluate the ant arthtric activity for the different polyherbal formulations

MATERIALS AND METHODS:

Plant source and authentication:

Polygonumglabrum, Ochnaobtusata DC,and Canthiumdicoccum were collected from Tirumala Hills, Tirupati, and Chittoor district of Andhra Pradesh, near Seshachalam and Tirumala Hills. The plant specimen was verified to be of the correct species by Dr. MadhavaSetty, a botanist from the Department of Botany, S. V. University, Tirupati .

Chemicals and reagents:

All the chemical are used analytical grade and the Egg Albumin ,Bovine Serum And Diclofenac sodium obtained from sigma Aldrich

Preparation of poly herbal extract:

Aerial parts of *Polygonumglabrum, Canthiumdicoccum,Ochnaobtusata* were collected and dried. Then the material was blended to form a fine powderand extracted Ethanol using Soxhlet apparatus for 6 hrs at 50ºC and water by maceration The solvent was completely removed by rotary evaporator (Rotavapor® R-210, BUCHI Corporation) and respective extracts preserved for various investigations.

Preparation of ethanol extract of four selected polyherbal formulations using different portions

The above extract used for the preparation of five different poly herbal formulations with varying proportions and working formula given in the table.1

	FORMULATIONS	PGEE	CDEE	OOEE
1	FORMULATION 1	1	1	1
2	FORMULATION 2	2	1	1
3	FORMULATION 3	1	2	1
4	FORMULATION 4	1	1	2

Preliminary phytochemical studies: [28-30]:
Previously various preliminary phytochemical tests were performed 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.

Evaluation of in-vitro anti-arthritis of polyherbal formulations:
Inhibition of protein denaturation method using bovine serum [31 -34]
Preparation of the standard solution: The standard solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5 % w/v aqueous solution) and 0.05 ml of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test solution: The test solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test control solution: This solution (0.5 ml) was prepared using of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.

Experimental procedure:
All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

Inhibition of protein denaturation method using egg albumin [35-38]
Preparation of the standard solution: The standard solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test solution: The test solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of different concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test control solution: This solution prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of distilled water.

Experimental procedure:
All above solutions were incubated at 37 ± 2°C in incubator for 15 min and then heated at 70°C for 5 min. After cooling their absorbance were measured at 660 nm by using vehicle as a blank. The percentage inhibition of protein denaturation was calculated by using the following formula
Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

HRBC membrane stabilization method [39-45]:
The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and was mixed with equal volume of sterilized alsevers solution. The blood solution was centrifuged in a centrifugation

machine at 3,000 rpm for 15 min and the upper layer was carefully removed with a syringe or sterile pipette. The packed cells remained at the bottom were separated and washed with isosaline solution and a 10% v/v suspension was made with isosaline. Human red blood cells suspension was used for the study.

Preparation of the standard solution: The standard solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of distilled water. Preparation of the test solution: The test solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of extract solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of distilled water. Preparation of test control: The test control solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline, 2.5 mL of distilled water.

Experimental procedure:
All the assay mixtures were incubated at 37ºC for 30 min and centrifuged at the rate of 3,000 rpm. The supernatant liquid was poured out and the hemoglobin content was estimated by UV spectrophotometer at 560 nm. The percentage of human red blood cell membrane stabilization or protection against hypotonicity induced hemolysis was calculated by using the following formula.

Percentage protection = 100- [(optical density sample/optical density control) × 100]

RESULTS:

Table no .2 Inhibition of protein denaturation method using bovine serum

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	51.92±0.4	37.12±0.4	44.82±0.24	34.22±0.12	39.36±0.34
50	57.81±0.5	43.24±0.6	51.91±0.3	42.91±0.24	46.18±0.4
100	63.14±0.9	49.42±0.6	54.64±0.6	45.82±0.19	69.26±0.8
200	79.12±1.5	57.12±2.5	71.24±1.3	53.64±0.9	61.92±1.4
400	85.46±0.8	65.46±0.4	78.36±0.6	67.74±0.25	64.82±0.6
800	92.16±0.9	74.24±0.2	84.29±0.9	72.92±0.16	76.19±0.8
1000	94.28±1.0	81.26±0.5	89.16±0.6	79.29±0.24	82.28±0.28

Table no .3 Inhibition of protein denaturation method using egg albumin

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	52.32±0.8	46.12±0.25	49.42±0.24	42.12±0.5	46.89±0.5
50	58.24±0.6	48.24±0.62	53.91±0.3	44.24±0.2	49.12±0.12
100	65.04±1.2	51.42±0.24	57.64±0.6	48.42±0.4	52.22±0.4
200	73.24±1.4	56.12±2.5	69.24±1.3	51.12±1.5	58.25±2.15
400	76.08±1.18	63.46±0.4	74.36±0.6	59.46±1.4	64.14±0.41
800	79.16±1.5	72.24±0.2	76.29±0.9	68.24±1.2	73.43±0.12
1000	86.14±1.2	79.26±0.5	83.16±0.6	74.26±0.5	81.12±0.15

Table no .4 HRBC membrane stabilization method

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	56.92±0.4	41.56±0.46	44.56±1.2	39.56±0.4	39.96±0.14
50	62.81±0.5	49.21±0.42	51.21±1.5	45.21±0.6	50.21±1.3
100	89.14±0.9	82.13±1.3	84.13±0.9	82.13±0.25	81.13±1.6
200	91.59±1.5	85.14±0.2	89.14±1.4	78.14±1.2	84.14±1.2
400	94.26±0.8	89.46±1.4	92.46±0.4	84.46±0.6	86.46±0.9
800	96.59±0.9	90.49±1.2	94.49±0.5	89.49±1.3	92.49±0.5
1000	98.98±1.0	92.98±0.25	96.98±0.26	93.98±0.4	93.98±0.6

DISCUSSION:

The Ethanolic extracts of *Polygonumglabrum, Canthiumdicoccum ,Ochnaobtusata*, are formulated into four ormulation with different portion as given in the above formula and evaluated for *in -vitro* anti-arthritis activity with concentrations varying from 10 µg/ml to 800 µg/ml.by the *in –vitro* Inhibition of protein denaturation method using bovine serum, Inhibition of protein denaturation method using egg albumin and HRBC membrane stabilization method comparing with standard drug as Diclofenac sodium.in the five formulation F2 and F4 having the significantly more inhibition of protein denature and membrane stabilization which are concentration dependent.

CONCLUSION:

The above result gives a conclusion that the polyherbal formulations with the different concentration have the anti-arthritis activity and the F2 and F4 having significantly more potential. Further investigation is required to use the two formulations in the treatment of Rheumatoid Arthritis

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Conflict of interest:

No conflict of interest

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