Research Article

A Study on Anti-Arthritic Activity of Methanolic Extract of *Cypraea Arabica*

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ABSTRACT

Arthritis is one of the most common autoimmune disease characterized by systemic inflammation of joints, damaging cartilage and bone around the joints. It can affect the whole body and internal organs. Apart from allopathy the alternative medicine to treat arthritis includes the natural products with less or no side effects, hence the present study is undertaken to elucidate the mechanism of anti-arthritic activity of methanolic extract of the marine gastropod *Cypraea arabica*. The anti-arthritic activity of *Cypraea arabica* was evaluated using Freund's adjuvant induced arthritis in rats. There is a significant increase in paw volume of FCA injected rats and control rats when compared to the standard. The methanolic extract of *Cypraea arabica* treatment at the dose of 200mg/kg and 400 mg/kg showed significant reduction in rat paw oedema (42.58% and 30.68%) when compared to the control group. The SGOT, SGPT levels of the methanol extract treated groups for which the levels were found to be 114.56±3.73, 90.60±1.84 and 100.64±3.16, 84.91±2.27 IU/L for low (200 mg/kg) and high (400mg/kg) dose groups respectively. The total protein, creatinine and ALP were also studied and their levels were recorded as 4.13±0.12, 0.99±0.01 and 17.33±1.04 respectively at 200mg/kg dose and 4.97±0.24, 0.92±0.01 and 16.87±1.001 respectively at 400mg/kg dose levels. The changes in haematological parameters were studied and there was a decrease in RBC count and haemoglobin content, increase in WBC and ESR count respectively.

Keywords: *Cypraea arabica,* methanol, Freund's adjuvant arthritis, SGOT, SGPT.

INTRODUCTION

The ocean, the "mother of origin of life", wrap over 70% of the earth's surface and contains highly ecological, chemical and biological diversity starting from microorganisms to vertebrates. Natural products have great economic and ecological importance, and many of natural products are yet to be discovered. Over 60% of natural products can be considered as drugs in the pharmaceutical industry[1]. Searching of foods and therapeutic remedies from animal origin invertebrates have long been practice, since the time of human civilization[2]. Among the molluscs, the class gastropod constitutes an important group and most molluscan medicines are derived from shelled gastropods and bivalves[3].

Typically arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement. Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic progressive systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction which is responsible for the deformity and disability[4].

Through conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs ($NSAID_S$), the disease modifying anti-rheumatoid drugs ($DMARD_S$) and corticosteroids have all been associated with adverse effects. Hence, it may an urgent need to develop new safer and effective anti-arthritic drugs from natural origin. Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain, they are associated with certain undesirable side effects. Animal derived natural products contributed a major part in traditional medicine to prevent inflammation related diseases. Hence an attempt is made to evaluate the anti-arthritic activity of methanolic extract of *Cypraea arabica* on the Freund's adjuvant induced arthritis in rats.

MATERIALS AND METHODS

Sample collection

The gastropod *Cypraea arabica* was collected from Tuticorin coast (8°45'N ; 78°46'E) along Gulf of Mannar region. The Gulf of Mannar is an established Marine National Park located between India and Srilanka on the South east Coast of India. The Specimens of *Cypraea arabica* were collected during low tides from the sea in their natural habitat that is intertidal zone and from reefs by divers, brought to the laboratory and maintained under laboratory conditions for further observations.

Sample preparation

The shells were broken and the soft tissues were cut into pieces, and dried in hot air oven at 56^{o} C for 5 days. The tissues were powdered and macerated with methanol and used for anti-arthritic study.

Experimental animals

Adult Wistar albino rats of either sex weighing between 150 and 180 gm maintained in the animal house of S.B College of Pharmacy, Sivakasi and were used for further studies. The selected animals were housed under

standard environmental conditions (temperature of 22±1°C) maintained by giving uniform pellet diet, water ad libitum with an alternating 12 hrs light dark cycle and relative humidity of 60±5%.

Prior approval of Institutional Animal Ethics Committee (IAEC) was obtained.

All the animals were acclimatized to laboratory conditions prior to experiment, 2ml of 1% vanillin was used as a flavouring agent to enhance the acceptability of the extract.

Freund's adjuvant induced arthritis

The male albino rats were divided into 4 groups (i.e) control, standard, drug treated (2 groups of methanolic extract of *Cypraea arabica* at low and high dose treated 200, 400mg/kg).

Group I served as control (received 10% Tween - 80).

Group II was the standard group and received the diclofenac sodium 10mg/kg suspended in CMC, group III was the 1st test group receiving methanolic extract of *Cypraea arabica* at dose of 200mg/kg orally and Group IV was the 2nd test group receiving methanolic extract of *C.arabica* at a dose of 400mg/kg.

Rats were injected with 0.1ml of FCA into planter region of left hind paw.

The paw volume of both hind paws were measured using a plethysmometer and body weight was recorded on the day of adjuvant injection.

The methanolic extract of *Cypraea arabica* (200 and 400mg/kg) and diclofenac sodium 10mg/kg doses were administered orally for 14 days from the day of FCA injection.

The changes in the paw thickness was measured using mercury plethysmometer on 1st, 7th, 14th and 21st days from the day of adjuvant's injection.

The animals were weighed using digital weighing balance on 1st, 7th, 14th, and 21st day from the day of adjuvant injection.

At the end of experiment on the 21st day all animals were anaesthetized and blood was withdrawn by retro-orbital puncture and collected in plain and EDTA containing tubes respectively for serum separation.

The homogenized samples were subjected to biochemical examinations like SGOT, SGPT, Total protein, Creatinine and ALP.

The haematological parameters like RBC, WBC, ESR and Haemoglobin content were also estimated in all the 4 groups by following the standard methods[5].

Statistical analysis

The results were expressed as Mean ± standard error of mean. The data was analyzed statistically using one way analysis of variance (ANOVA) and Dunnett's t – test. P values less than 0.05 were considered as significant.

RESULTS

There is a significant increase in rat paw volume of FCA injected control rats when compared to the standard and drug treated rats. Methanolic extract of *Cypraea arabica* treatment at the dose of 200mg/kg and 400mg/kg showed significant reduction in rat paw oedema when compared with control group. After 21days it was found that methanolic extract of *Cypraea arabica* significantly shows dose dependent inhibition in paw thickness. Standard diclofenac sodium decrease the paw thickness (i.e.) 65.33% after the induction of FCA whereas methanol extract of *Cypraea arabica* at high dose (400mg/kg) significantly decrease the paw thickness (30.68%) as compared to the low dose which was found to be 42.58% (Table 1).

In the present study it is clear that there is a dose dependent relationship between the extract, the degree of weight loss. The control group when compared to the standard and both of the test treated groups found that, weight of the rats was highest in case of standard group 171±1.11gm. Standard drug and methanol extract of *C.arabica* at high and low doses significantly increased the body weight of animal compared to that control group (162±0.17) which was recorded to be 167±0.71 and 168±0.61gm for low and high dose group respectively (Table 2).

The SGOT, SGPT, total protein, creatinine and ALP levels of all these groups were elevated and compared with that of control. The SGOT, SGPT levels of the standard groups were found to be 73.79±1.92 and 56.12±2.49 IU/L respectively, which were least as compared to methanol extract treated groups for which the levels were found to be 114.56±3.73, 90.60±1.84 and 100.64±3.16, 84.91±2.27 IU/L for low (200 mg/kg) and high (400mg/kg) dose groups respectively. However, the treated groups were able to reduce SGOT, SGPT levels better as compared to control (119.28±3.69 and 94.88±2.38) proving its anti-arthritic efficacy. Similarly the total protein, creatinine and ALP were also studied and their levels were recorded as 4.13±0.12, 0.99±0.01 and 17.33±1.04 respectively at 200mg/kg dose and 4.97±0.24, 0.92±0.01 and 16.87±1.001 respectively at 400mg/kg dose levels of methanolic extract of *C.arabica* and the respective results were statistically significant (Table 3).

The changes in haematological parameters in adjuvant induced arthritic rats are shown in table 4.

There was a decrease in RBC count and haemoglobin content, increase in WBC and ESR of arthritic rats, when compared with control rats.

The drug treatment (methanolic extract of *Cypraea arabica*) significantly brought back the altered haematological changes of adjuvant induced arthritis.

Table 1: Effect of methanolic extract of *Cypraea arabica* on paw oedema volume against FCA induced arthritis in albino rats

Dose mg/kg p.o	Mean changes in paw volume				% inhibition of oedema
	1st day	7th day	14th day	21st day	
Control	0.73±0.01	0.86±0.03	0.90±0.02	1.07±0.02	-
Standard (10mg/kg)	0.66±0.1	0.58±0.01	0.32±0.01	0.30±0.01	65.33
MECA (200 mg\kg)	0.72±0.01	0.78±0.02	0.71±0.002	0.69±0.02	42.58
MECA (400 mg\kg)	0.69±0.00	0.74±0.02	0.68±0.02	0.63±0.01	30.68

Values are expressed as mean ± SEM; MECA – Methanolic extract of *Cypraea Arabica;* % inhibition of paw volume = (Vc-Vt/Vc) 100
Where Vt = Mean paw volume of each treated group; Vc = Mean paw volume of control group

Table 2: Effect of methanolic extract of *Cypraea arabica* on body weight against FCA induced arthritis in albino rats

Treatment days	Control	Standard	200mg/kg	400mg/kg
1	150.3±1.02	151.1±1.13	152.0±1.19	151.03±0.38
7	152.0±1.01	156.2±1.16	157.6±0.03	158.8±1.03
14	156.7±0.04	165±0.79	164.0±0.68	165.3±0.89
21	162±0.17	171±1.11	167.0±0.71	168.0±0.61

Values are expressed as mean ± SEM

Table 3: Biochemical parameters of FCA induced arthritis of methanolic extract of *Cypraea arabica*

Group	Dose mg/kg p.o	SGPT (U/L)	SGOT (U/L)	Total protein	Creatinine (mg/dl)	ALP
I	Control	94.88 ± 2.38	119.28 ± 3.69	4.49 ± 0.15	1.09 ± 0.04	20.33 ± 1.01
II	Standard (10mg/kg)	56.12 ± 2.49	73.79 ± 1.92*	6.65 ± 0.24*	0.83 ± 0.02*	18.07 ± 1.02*
III	MECA 200mg/kg	90.60 ± 1.84*	114.56 ± 3.73	4.13 ± 0.12*	0.99 ± 0.01	17.33 ± 1.04*
IV	MECA 400mg/kg	84.91 ± 2.27	100.64 ± 3.16	4.97 ± 0.24*	0.92 ± 0.01*	16.87 ± 1.001*

Values are expressed as mean ± SEM; *p < 0.05 compared with control; MECA – Methanolic extract of *Cypraea arabica*

Table 4: Effect of methanolic extract of *Cypraea arabica* on haematological parameters against FCA induced arthritis in albino rats

Group	Dose mg/kg p.o	RBC (millions/mm3)	WBC (millions/mm3)	Haemoglobin (g/dl)	ESR (mm/hr)
I	Control 5mg/kg	8.56 ± 0.1	9.6 ± 0.23	12.35 ± 0.37	2.46 ± 0.19
II	Formaldehyde 2% intradermal	5.38 ± 0.14	14.55 ± 0.29*	7.83 ± 0.36*	7.36 ± 0.16
III	Standard	8.62 ± 0.12	9.75 ± 0.16	13.53 ± 0.31*	3.36 ± 0.17
IV	MECA 200mg/kg	4.57 ± 0.25*	14.4 ± 0.36*	8.03 ± 0.18	6.24 ± 0.15
V	MECA 400mg/kg	6.41 ± 0.07	10.43 ± 0.34*	8.65 ± 0.27*	5.23 ± 0.07

Values are expressed as mean ± SEM; *p < 0.05 compared with control; MECA – Methanolic extract of *Cypraea arabica*

DISCUSSION

Marine invertebrates, which develop in a different environment from terrestrial animals, are the source of a broad range of pharmacological substances.

They either express constitutively or the expression is induced by exposure to pathogens[6]. By the virtue of such excellent property marine organisms are of interest in terms of pharmaceutical potentials particularly invertebrates[7].

A lot of structurally and pharmacologically important substances have been isolated from marine gastropods with novel antimicrobial, antitumour and anti-inflammatory properties[8].

Evaluation of anti-arthritic activity of *Cypraea arabica* was studied on Freund's adjuvant induced arthritis in wistar albino rats.

Most of the investigators have reported that inhibition of adjuvant induced arthritis in rats is one of the most suitable test procedures to screen anti-arthritic agents since it closely resembles human arthritis. Freund's adjuvant induced arthritis is thought to occur through all mediated autoimmunity structural mimicry between mycobateria and cartilage proteoglycan in rats. It activates macrophages and lymphocytes by adjuvant inoculation or their products like monokines, cytokines and chemokines may be involved in abnormal lipid and protein metabolism. The FCA administered rats showed soft tissue swelling around the ankle joints during the development of arthritis which was considered as oedema of the particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities[9]. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The Freund's adjuvant model is chosen as it develop chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction[10]. On the 21st day, a significant decrease in edema volume was observed in methanol treated group as compared to the Freund's adjuvant injected control rats (Table 1).

In the present study the FCA induced rats developed chronic swelling in multiple joints as a result of inflammation. Assessment of the levels of SGOT, SGPT, ALP, creatinine and total protein provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The activities of these enzymes ESR and WBC counts were significantly increased in arthritic rats. These are good indicators of liver and kidney, impairment, which are also considered to be features of adjuvant arthritis[11,12]. Increased WBC counts are a common feature of inflammatory reactions, especially those induced by microbial infection. So in arthritic group an increase in WBC count was found. Erythrocyte sedimentation rate was decreased in control rats whereas in case of treated groups, the ESR level was significantly increased. The similar results were observed from the methanolic extract of *Glycyrrhiza glabra* and *Costus speciosus*[13,14]. Many anti-arthritic works have been carried out from plant materials and this is the first report of anti-anthritic activity from marine gastropod *Cypraea arabica*. The methanolic extract of *Cypraea arabica* is having a promising anti-arthritic agent of animal origin in the treatment of rheumatoid arthritis and inflammatory disorders.

CONCLUSION

Marine life is fascinating and is considered to have great potential for its intrinsic value as well as development of new drugs.

It is well known that traditional system of medicines always played important role in meeting the global health care needs. The presently available synthetic drugs in the market are associated with adverse effects. So we conclude that *Cypraea arabica* can serve as a potential source for this anti-arthritic activity. Further research on clinical side and mechanism of actions are needed.

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