Screening therapeutically efficiency of Capsicum Annum and Capsicum Frutescens

Thenmozhi, M., Hemalatha, N., Kavitha Shree, RK , P. Dhasarathan

Abstract: Medicinal plants serve as the therapeutic alternatives, safer choices or in some cases as the only effective treatment. In the present investigation, the serum collected from normal control mice (without treatment) administered with rheumatoid antigen was treated with agglutination reaction and it showed positive result. The butanol extracts of Capsicum annum treated mice suppressed swelling of the mice paws and the recovery effect was good compared to other extracts treated mice of Capsicum annum and Capsicum frutescens. The production of antinuclear antibody was reduced in C.annum treated animal compared to the C.frutescens treated animal. The butanol extract of Capsicum annum produced profound enhancement in the production of CRP. The extracts of hexane, ethanol, chloroform and aqueous extracts of Capsicum annum slightly enhanced the production of CRP of the mice.

Index Terms: Antigen, Anti nuclear antibody, Catabolic reactive protein, Capsicum annum, Capsicum frutescens, Immunomodulation, Rheumatoid arthritis,

1 INTRODUCTION

The fruit of the capsicum is used to make medicine red chili peppers (C. frutescens) have been used for several thousand years as food additives and variety of clinical applications in Indian, Native American, African and Chinese medical traditions (Szallasi et al., 1999). Capsaicin is used for various problems with digestion including upset stomach, intestinal gas, stomach pain, diarrhoea and cramps. It is also used to help people who have difficulty swallowing. This realization was there since time immemorial and great promises and prospects of natural wealth for health care were enumerated in ancient Indian literature (Agarwal and Singh, 1999). Capsicum is a medicinal and nutritional herb; it is the purest and most certain stimulant. Despite the tremendous progress in medicine, infectious diseases caused by bacteria, fungi, viruses and parasites continue to possess a threatening challenge to public health (Cos et al., 2006). Capsicum may be valuable in the prevention and the treatment of blood clots. Capsicum is a specific and very effective remedy for yellow fever as well as other fevers. There is an ever increasing interest on research of different plant species to find out their therapeutic applications all over the world. The main factors that make natural products attractive for human use include their ease of availability, cost effectiveness and presumed safety. Effects are now being made to unravel the mechanism of action to these natural products (Mediratta et al., 2002).

Rheumatoid arthritis (RA) is an auto immune disease that causes chronic inflammation in synovial tissues and joints which leads to impaired joint function, severe pain and reduced life expectancy (Tenney et al., 2004). This disease affects about 1% of the human populations.

- *Thenmozhi, M – Department of Biotechnology, Prathyusha Engineering College, Tiruvallur – 602025*
- *Hemalatha, N –*
- *Kavitha Shree, RK - Department of Biotechnology, Prathyusha Engineering College, Tiruvallur – 602025*
- *P. Dhasarathan – Corresponding Author, Department of Biotechnology, Prathyusha Engineering College, Tiruvallur – 602025 E mail: hod.biotech@prathyusha.edu.in*

The etiology and pathogenesis of this disease are not yet fully understand but it seems likely that an autoimmune mediated attack on joints plays a crucial role in rheumatoid arthritis (Jawaheer et al., 1994). Capsaicin in capsicum softens the arteries, dilates the circulatory system, and strengthens the heart. It protects against blood clot formation by causing an increase in fibrinolytic activity of red blood cells. CRP is a member of the class of acute phase reactants, as its level rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages (Pepys and Hirschfield, 2003) as well as adipocytes (Lau et al., 2005). The modern medicines have also started admitting that Ayurveda and herbal medicine, yoga and pranayama have a lot of positive influence on the treatment of arthritis. There are a number of herbs that work synergistically to reduce join inflammation in cases such as osteo arthritis, rheumatoid arthritis and other types of arthritis. In the Ayurvedic system of medicine, Capsicum annum / Capsicum frutescens is used in the treatment of rheumatoid arthritis. Hence, in the present study, an attempt has been made to evaluate the anti arthritis activity of the different extracts of Capsicum annum / Capsicum frutescens using killed pathogen induced arthritis in mice.

2 MATERIALS AND METHODS

In recent years there has been renewed interest in the field of medicine regarding the use of medicine regarding the use of naturally occurring substances as remedies for disease. Much of this interest has focused on plants/ animals in what is commonly known as herbal/animal medicine. Hence the present study programmed to study the therapeutic valuable components obtained in chilli samples. The test and control chilli samples were collected from Nagercoil and local market of Virudhunagar, Tamilnadu, India and transported to the laboratory and stored in room temperature.

Preparation of extracts and treatment: Ten gram of powdered chilli was taken in clean sterile Soxhlet apparatus and extracted with 100 ml of different solvents (low polar to high polar) like as hexane, butanol, chloroform, ethanol and water. After extraction the extracts were dried in room temperature until extract reach into solid form. Required quantities of the Capsicum annum / Capsicum frutescent

swere dissolved in sterile distilled water and the concentration of 100 mg/Kg/day was prepared. The chili extracts were dissolved in water and fed to mice with drinking water using special feeding bottle for treatment.

Rheumatoid arthritis: Freunds adjuvant induced arthritis model was used to assess the anti arthritic activity in albino mice (Newbould, 1963). Three groups of mice were separated, each group having six animals. Normal control (without any treatment) mice directly treated with RA antigen. RA antigen injected to other two groups of animals (Capsicum annum/ Capsicum frutescens treated mice). Arthritis was induced by injecting 0.1 ppm of rheumatoid antigen into the left hind paw of three groups of animals. Drug treatment was started from the initial day i.e from the day of adjuvant injection and continued till 21st day. Paw volume was measured on 7th, 14th and 21st day with the help of pethysoometer.

Anti nuclear antibody (ANA) factor assay: Animals were divided into three groups of six animals each. Group I served as normal control, group II and III are Capsicum annum / Capsicum frutescens treated mice. ANA antigen (0.1 ppm) directly injected to the normal control mice. The same concentration was also injected the remaining two groups of animals. After 1st, 2nd and 3rd week incubation period, blood sample was collected from three groups of animals and checked the ANA by agglutination reaction.

Catabolic reactive protein (CRP) assay: In the present experiment, CRP was estimated using Monozyme Immunoscreen CRP kit (Monozyme India Ltd., Chennai). The serum from normal and capsicum samples (C. annum / C. frutescens) treated mice was collected after SRBC challenge. Test serum was inactivated at 56ºC for 30 minutes. The serum was placed on one side of a divided slide using a capillary tube and undiluted serum was placed on the other side. Later, one drop of anti CRP reagent was added to each side. The reaction mixture was mixed (diluted sample first) and spread over using a wooden applicator. The slide was tilted slowly from side to side for 1-2 minutes and observed for macroscopic clumping. Visible flocculation in either or both sides of the slide indicates the presence of CRP. Serum devoid of this abnormal protein gives a smooth suspension with no visible flocculation on either side. Interpretation of results may be made as shown below.

Undiluted serum	*Diluted serum*	*Interpretation*
0 to 2+	*2+ to 4+*	*Strongly positive*
3+ to 4+	*0 to 2+*	*Positive*
1+ to 2+	*Negative*	*Weakly positive*
Negative	*Negative*	*Negative*

3 RESULTS AND DISCUSSION

In the present investigation, the serum collected from normal control mice (without treatment) administered with RA antigen was treated with agglutination reaction and it showed positive result (Table 1 and 2). RA antigen caused swelling in this group of animals. It indicated that the mice synthesized maximum level of antibody against the RA antigen. But that antibody is not enough to inactivate maximum concentration of RA antigen. So the result is strongly positive. At the same time RA antigen was injected into the different solvent extracts of Capsicum annum and Capsicum frutescens treated mice. The butanol extracts of Capsicum annum treated mice suppressed swelling of the mice paws and the recovery effect was good compared to other extracts treated mice of Capsicum annum and Capsicum frutescens. The extracts of hexane, ethanol and chloroform moderately suppressed the swelling of the mice paws and the recovery effect was found to be less in water extracts treated mice. Similar results were obtained in animals administered with Premna serratifolia (Rajendran and Krishnakumar, 2010). From the results observed in the current investigation, it may be concluded that the butanol extract of Capsicum annum at the dose of 250 mg/Kg body weight displays a significant antiarthritis activity which may be due to the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds and glycosides. Several studies indicated the phytoconstituents possess antiarthritis activity (Elmali et al., 2005, Choi et al., 2009 and Paulsi and Dhasarathan, 2011). In the present study, the mice were selected to induce arthritis that leads to development of chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid arthritis (Sing and Majumdar, 1996). The determination of mice paw swelling was apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutics effects of drugs. The chronic inflammation involved the release of number of mediators like cytokines, granulocyte macrophages colony stimulating factors (GMCSF), interferons and polymorph granulocyte differential factors (PGDF). These mediators were responsible for pain reduction on bone and cartilage that lead to severe disability (Eric et al., 1996). Rajendran and Krishnakumar (2010) obtained the same result in mice treated with Premna serratifolia and Mangifera indica stem bark. Similar changes were observed in mice due to administration of rheumatoid antigen in the present study. The formation of antinuclear antibody analysis also observed and recorded in table 3 and 4. The production of antinuclear antibody was reduced in test chili treated animal compared to the control chili treated animal. Receptor modified CRP uptake was associated with decreased nitric oxide bioavailability in human endothelial cells (Devaraj et al., 2005) and induced plasminogen activation inhibitor which in turn reflected no production or decrease in reactive oxygen species that may be related to the reduction in CRP. Epidemiological studies suggest that regular physical activity can reduce inflammation (Liu et al., 2002). In the present investigation, Capsicum annum administered mice showed predominant amount of CRP production (Table 5 and 6). The butanol extract of Capsicum annum produced profound enhancement in the production of CRP. The extracts of hexane, ethanol, chloroform and aqueous extracts of Capsicum annum slightly enhanced the production of CRP of the mice. Different solvent extracts of Capsicum frutescens promoted the production of CRP and it was less than that produced by the extracts of Capsicum annum. The acute phase protein CRP shares the property of showing elevations in the concentrations in response to stress or inflammations like injection, injury, surgery and tissue necrosis (William, 1996). Libby et al., 2002 reported that a marked increment in CRP (40%) within 2 weeks, a finding unrelated to CRP instability. Badr Al-Dahmesh (2011) reported that chili has no significant effect on the number of erythrocyte in rabbits, while leukocytic counts were significantly increased. The percentage of both neutrophils and lymphocytes in blood of rabbits

administered with chili gradually increased. Dietary capsaicin enhances lymphocyte proliferation and serum immunoglobulins level as reported by Fawcett et al., (1986). Vacuolization of some spleenic cells were detected in mice treated with RA alone. Butanol extract of capsicum treated mice after administration of RA, showed a histological structure similar to that of normal structure of mice spleen. From this study, it is confirmed that therapeutical effects of butanol extract of test capsicum is very effective against the activity of RA in mice. In this study suggests plant based drugs useful for treatment of autoimmune diseases. The emerging antibiotic strain may be control with help of the plant based drugs.

4 SUMMARY AND CONCLUSION

In the present studies indicate that chili samples may suppress autoimmune disorders in animal model because of its immunostimulant potential. It is worthy to mention here that the butanol extract of test capsicum suppresses rheumatoid arthritis. It is assumed that the active component identified in the plant extracts with present investigation might have made positive variation in the catabolic reactive protein. Moderate levels of significant in vivo effects were found in all kind of analysis. It is clear that the system is regulated by active compounds of plant based drugs in the following conditions of injury or infection. Therefore, more work is necessary to better understand the broad consequences of pharmcological approaches that modulate immune levels.

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Table 1. *RA factor assay in mice maintained as control and mice exposed to various extracts of Capsicum annum*

Group	*Extract concentration (ppm)*	*RA agglutination*		
		I week	*II week*	*III week*
Control	*0*	+	++	+++
RA treated	*0.1*	+	+	+
Hexane	*5*	+	+	++
RA treated	*0.1*	-	-	+
Butanol	*5*	+	++	+++

RA treated	*0.1*	-	+	+
Ethanol	*5*	-	+	+
RA treated	*0.1*	-	-	+
Chloroform	*5*	+	+	+
RA treated	*0.1*	-	+	+
Water	*5*	-	+	+
RAtreated	*0*	+	+	+

+++ Highly positive, ++ Positive, + Moderate, - Negative

Table 2. *RA factor assay in mice maintained as control and mice exposed to various extracts of Capsicum frutescens*

Group	*Extract concentration (ppm)*	*RA agglutination*		
		I week	*II week*	*III week*
Control	*0*	+	++	+++
RA treated	*0.1*	-	-	+
Hexane	*5*	+	+	+
RA treated	*0.1*	-	-	+
Butanol	*5*	+	+	++
RA treated	*0.1*	-	+	+
Ethanol	*5*	+	+	+
RA treated	*0.1*	-	+	+
Chloroform	*5*	+	+	+
RA treated	*0.1*	-	+	+
Water	*5*	-	+	+
RAtreated	*0*	-	+	+

+++ Highly positive, ++ Positive, + Moderate, - Negative

Table 3. *ANA factor assay in mice maintained as control and mice exposed to various extracts of Capsicum annum*

Group	*Extract concentration (ppm)*	*ANA-agglutination*		
		I week	*II week*	*III week*
Control	*0*	+	++	+++
ANA treated	*0.1*	+	+	+
Hexane	*5*	++	++	++
ANA treated	*0.1*	+	+	+
Butanol	*5*	++	++	++
ANA treated	*0.1*	+	++	+
Ethanol	*5*	+	++	++
ANA treated	*0.1*	+	+	+
Chloroform	*5*	+	++	++
ANA treated	*0.1*	+	+	+
Water	*5*	+	++	++
ANA treated	*0.1*	+	+	+

+++ Highly positive, ++ Positive, + Moderate, - Negative

Table 4*. ANA factor assay in mice maintained as control and mice exposed to various extacts of C. frutescens*

Group	*Extract concentration (ppm)*	*ANA-agglutination*		
		I week	*II week*	*III week*
Control	*0*	+	++	+++
ANA treated	*0.1*	+	+	+
Hexane	*5*	+	+	++
ANA treated	*0.1*	+	+	+
Butanol	*5*	+	++	+++
ANA treated	*0.1*	+	+	+
Ethanol	*5*	+	++	+++
ANA treated	*0.1*	+	+	+
Chloroform	*5*	+	++	++
ANA treated	*0.1*	+	+	+
Water	*5*	+	++	+++
ANA treated	*0.1*	+	+	+

+++ Highly positive, ++ Positive, + Moderate, - Negative

Table 5*. Changes in serum CRP in mice maintained as control and mice exposed to various extracts of C. annum*

Group	*Extract conc. (ppm)*	*CRP formation*		
		I week	*II week*	*III week*
Control	*0*	++	++	++
RA treated	*0.1*	-	-	-
Hexane	*5*	+	++	+++
RA treated	*0.1*	-	+	+
Butanol	*5*	++	+++	+++

RA treated	*0.1*	-	-	+
Ethanol	*5*	++	++	+++
RA treated	*0.1*	-	+	+
Chloroform	*5*	++	++	+++
RA treated	*0.1*	-	-	+
Water	*5*	++	+++	+++
RA treated	*0.1*	-	-	-

+++ Highly positive, ++ Positive, + Moderate, - Negative

Table 6. *Changes in serum CRP in mice maintained as control and mice exposed to various extracts of C. frutescens.*

Group	Extract conc. (ppm)	CRP formation		
		I week	II week	III week
Control	0	++	++	++
RA treated	0.1	-	-	-
Hexane	5	+	+	++
RA treated	0.1	+	+	+
Butanol	5	++	+++	+++
RA treated	0.1	-	+	+
Ethanol	5	++	++	+++
RA treated	0.1	+	+	+
Chloroform	5	++	++	+++
RA treated	0.1	-	+	+
Water	5	++	+++	+++
RA treated	0.1	+	+	+

+++ HIGHLY POSITIVE, ++ POSITIVE, + MODERATE, - NEGATIVE