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EFFECT OF ARUMUGA CHENDOORAM IN HEMATOLOGICAL PROFILE ON FREUND'S ADJUVANT INDUCED ARTHRITIC RATS

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ABSTRACT

The aim of the study to investigate the effect of Arumuga chendooram in hematological profile on freund's adjuvant induced arthritic rats. Group I served as normal rats. On day zero, group II to IV rats were injected into the sub plantar region of the left hind paw with 0.1ml of Freund's complete adjuvant. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 5mg/ml (This dose confirmed in our lab followed by different concentrations (1 to 10mg/ml)). Administration of standard indomethacin (3 mg/Kg body weight) and Arumuga chendooram treated to group III and Group IV rats respectively were started on the first day and continued for 21 days. At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected and analysed various hematological parameters. Rats treated with Arumuga Chendooram showed significant ($p<0.001$) increase in Hb, RBC MCH, MCHV and MCV and decreased in WBC count and ESR when compared to arthritis rats. Rats treated with Arumuga Chendooram showed significant ($p<0.001$) restored in Total and Differential count of leucocytes when compared to arthritis rats. The results of the present study concluded that significantly restored the hematological profile on treatment with Arumuga Chendooram than the standard.

INTRODUCTION

Rheumatoid arthritis (RA) is a kind of chronic inflammatory autoimmune diseaseof joints that results in joint pain, swelling and destruction (Arend and Dayer, 1990). It affects an estimated 1% of the adult population throughout the world. RA progresses in three stages first stage is the swelling of synovial lining causing pain, warmth, stiffness, redness and swelling around the joints, second is the rapid division and growth of cells or pannus which causes the synovium to thicken, in the third stage the inflamed cells releases enzyme that may digest bone and cartilage, often causing the involved joint to lose its shape and alignment resulting pain and loss of movement [1]. Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immune-suppressants used in the treatment of RA have been developed over the past few decades, there is still an urgent need for more effective drugs with lower side effects [2]

By appraising the significance of various complementary alternative medical systems (CAM); The five major CAM systems used in India i.e. AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy) and their use in the field of rheumatology. The practicable role of Siddha systems in management of inflammatory diseases have been already published [3]. In the current research we have tried to explore the role of Siddha system of medicine (SSM) in arthritis rats. The SSM is primarily concerned with the development of high potency herbal drugs, which have long life. It also focuses to initiate the generation of cells and to prolong the longevity [4]. Therefore, the present study was to the effect of Arumuga chendooram in hematological profile on freund's adjuvant induced arthritic rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-220 were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatization to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Preparation of Arumuga Chendooram

The Siddha medicine Arumuga chendooram was prepared as per the procedures of IMCOPS, Chennai. In the first stage of the preparation of Arumuga chendooram. Five parts of purified mercury (Suththi seitha rasam), nine parts of purified sulphur (Suththi seitha kanthakam), seven parts of purified lode stone (Suththi seitha kantham), twelve parts of purified iron filing (Suththi seitha ayapodi), four parts of rock salt (Induppu) and eight parts of desiccated borax (Poriththa venkaram) were ground with sufficient quantity of aloe juice (*Kumari charu* for five days continuously. This was then made into small cakes and dried. It was then sealed in discs and burnt for 24 hours. If the colour of the chendooram does not appear as dark purple the grinding and burning are usually repeated. Equal to pH and then attractive particle interactions predominate which may influence the drug delivery.

Experimental Design:

Freund's Complete Adjuvant induced Arthritic Model

Adult Wistar male rat with an initial body weight of 180 to 220g were taken, and divided into four groups each containing six animals. Group I served as normal rats. On day zero, group II to IV rats were injected into the sub plantar region of the left hind paw with 0.1ml of Freund's complete adjuvant. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 5mg/ml (This dose confirmed in our lab followed by different concentrations (1 to 10mg/ml)). Administration of standard indomethacin (3 mg/Kg body weight) and Arumuga chendooram treated to group III and Group IV rats respectively were started on the first day and continued for 21 days. Group II rats served as control rats (arthritis rats). The degree of inflammation was measured by a mercury displacement method. The edema formation and the percentage of inhibition were calculated as follows.

$$\text{Percentage of inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vc is the edema volume of the control group and Vt is the edema volume of the treated group.

Collection of blood sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected from the tail vein into a micro centrifuge tube containing 50mM ethylenediamine tetra acetic acid (EDTA) for the determinations of hematological profile.

Hematological estimations

Haemoglobin was estimated by Cyanmethaemoglobin method [5]. RBC and WBC counted by the method of Ochei and Kolhatkar [6]. ESR sedimentation rate measured by the method of Ochei and Kolhatkar [6]. PCV counted by the method of Ochei and Kolhatkar [6]. Differential leukocyte were counted by the method of Srikumar *et al*., [7]. Total leukocyte were counted by the method of Srikumar *et a*l. [7].

Statistical Analysis

Statistical analysis is performed using SPSS. Data are expressed as mean ± SD and statistically assessed using one-way ANOVA followed by Tukey test; $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effect of Arumuga Chendooram on hematological changes in experimental rats

A significant reduce in the levels of Hb and RBC was observed in control rats (group II) when compared to normal rats (group I). Administration of Indomethacin and Arumuga Chendooram to diseased rats (group III and group IV) enhanced the levels of RBC and Hb to close normal levels. The raise in WBC count and ESR were significantly overcome in the Indomethacin and Arumuga Chendooram treated groups (group III and group IV) (Table 1). Rats treated with Arumuga Chendooram showed significant ($p<0.001$) increase in Hb, RBC and decreased in WBC count and ESR when compared to arthritis rats A significant reduce in MCH, MCHV and MCV was observed in control rats (group II) when compared to normal rats (group I). Administration of Indomethacin and Arumuga Chendooram to diseased rats (group III and group IV) enhanced the levels of MCH, MCHV and MCV to close normal levels. Rats treated with Arumuga Chendooram showed significant ($p<0.001$)increase in MCH, MCHV and MCV when compared to arthritis rats (Table 1).

Table 1 Hematological changes in Freund's adjuvant induced arthritis in experimental rats

	Group I	Group II	Group III	Group IV
Hb (gm/dl)	15.34± 1.04	8.52±0.57#	10.79±0.73*	14.77±1.00*
RBC (Million/cu.mm)	10.20±0.69	7.00±1.08#	13.00±0.88*	11.00±0.74*
WBC (cu.mm)	3.1±0.21	4.6±0.31#	3.8±0.25*	3.2±0.21*
ESR (mm)	15.30±1.04	22.30±1.51#	19.12.4±1.38**	17.6±1.19*
PCV (%)	43±2.92	53±3.60#	47±3.19***	45±2.78**
MCH (pg/cell)	15.03±1.02	5.32±0..36#	8.30±0.56*	13.42±0.91*
MCHC (%)	35.67±2.42	16.07±1.03#	22.95±1.56*	32.82±2.44*
MCV (cubic micron)	42.15±2.86	33.12±2.38#	36.15±2.45*	38.13±2.5*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II #*p< 0.001; ** p< 0.01; *** p< 0.05

Significantly different from Group I

Total and Differential count of leucocytes were significant differences were observed and represented in table 2. A significant increase in total count was observed in control rats (group II) when compared to normal rats (group I). Administration of Indomethacin and Arumuga Chendooram to diseased rats (group III and group IV) restored the total count to close normal levels. A significant increased in Monocyte Lymphocyte and decreased in Neutrophil Eosinophil and Basophil were observed in control rats (group II) when compared to normal rats (group I). Administration of Indomethacin and Arumuga Chendooram to diseased rats (group III and group IV) restored the total count to close normal levels. Rats treated with Arumuga Chendooram showed significant ($p<0.001$) restored in Total and Differential count of leucocytes when compared to arthritis rats.

Table 2 Total and Differential count of leucocytes on Freund's adjuvant induced arthritis in experimental rats.

Groups	Total count (TLC) (Cu.mm/ml)	Differential count (DLC) (%)				
		Neutrophil	Eosinophil	Monocyte	Basophil	Lymphocyte
Group I	4628.33 ± 80.37	41.33 ± 2.04	1.43 ± 0.21	1.31 ± 0.12	1.12 ± 0.19	50.63 ± 1.82
Group II	5926.66 ± 182.56[#]	38.83±1.97 [a]	1.38± 0.19[#]	1.64± 0.18[#]	0.93 ± 0.16[a]	68.66± 2.13[#]
Group III	5596.63±137.60**	35.65±1.63 [a]	1.40±0.20 [a]	1.28±0.11*	0.61 ± 0.19 [a]	62.63±1.85*
Group IV	5313.30 ± 101.54*	42.66±2.21***	1.53±0.24 [a]	1.10±0.08*	1.06 ± 0.21[a]	52.66±2.15*

Values were expressed as mean ± SD for six rats in each group.

*Significantly different from Group II

[#] Significantly different from Group I

[a] Non-Significant different from Group I

[#] *P < 0.001; **P < 0.01; ***P < 0.05

Most chronic inflammatory rheumatic diseases are complicated by hematologic abnormalities, including anemia; disorders of leukocytes, platelets, and the coagulation system; and hematologic malignancies [8] (Hamilton, 1983)]. Two major factors appear to be important: trapping of iron in macrophages, making it relatively unavailable for new hemoglobin synthesis; and inability of the morphologically normal marrow to increase erythropoiesis in response to the anemia [9, 10]. Inflammatory mediators, particularly tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, IL-6, IL-10, and interferon gamma, contribute to these changes [11]. Hepcidin, an acute phase reactant produced by the liver, may play a key role in cytokine-mediated anemia, as this protein decreases intestinal absorption and iron release from macrophages [12].

Arthritic animals (Group II) exhibited significant increase in WBC and ESR and significant decrease in the levels of hemoglobin, RBC counts and Packed Cell Volume. The hematological changes produced during arthritic condition were altered significantly upon

administration of Arumuga Chendooram in CFA induced rats. The WBC count was increased in arthritic rats to kill invading pathogenic microorganisms and was also associated with an increase in platelet count and ESR [13]. Barvalia *et al*., [14] observed significant increase in total white and neutrophil cell count. A large number of leucocytes that generate free radicals are present in the region of inflammation. Hence, free radical measurement reflects the number of leucocytes present in the inflammatory condition.

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients, oxygen and transports of waste products away from those of same cells. Blood is the most important body fluid that governs vital functions of the body like respiration, circulation, excretion, osmotic balance and the transport of metabolic substance. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs [12]. Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition [15]. The importance of haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals [16].

In this model the decreased levels of Hemoglobin (Hb) and Red blood cell count (RBCs) associated with the reduced erythropoietin levels caused due to decreased response of the bone marrow erythropoietin and destruction of premature RBCs in FCA induced arthritis. Erythrocyte sedimentation rate (ESR) is an index of suspension stability of RBC's in plasma. The number and size of RBC is associated with ESR. It also involved in the accelerated formation of endogenous proteins including plasma proteins such as fibrinogen, alpha and beta globulins. ESR is elevated during the inflammation, stress and cell necrosis. In elevated level of the IL-1α inflammatory response in FCA induced arthritis results in increase in granulocyte and macrophages colony stimulating factors which is associated with elevated level of White blood cell count (WBC) which plays a major role in body defense mechanism [17]. MCV, MCH and MCHC showed significant decrease in the present investigation due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic hypo chromic anemia. Thus, the increase in the Hb and RBC count brought about by ultrasound and phonophoresis treatment further support its anti-arthritic effect.

Pronounced anaemia and increased leucocytosis are found in rats with adjuvant-induced polyarthritis. The anaemia is hypochromic and macrocytic and is accompanied by rather high reticulocytosis. Leucocytosis is due mainly to an increase in polymorphonuclear granulocytes, but the absolute number of lymphocytes is also augmented, in contrast to the lymphopenia observed in mice in the early stages ofadjuvant-induced disease [18] (Morton and Siegel, 1966).

The significant increase in total and differential leukocytes count (Table 2) has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue. Such lymphocyte response might be due to the presence of toxic substances may be associated with the pollutant induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of leukocytes and the respective decrease in ultrasound and phonophoresis treated groups showed its immunomodulation effect. The total and differential leukocytes count which significantly decreased in arthritic control group has been remarkably counteracted by ultrasound and phonophoresis restoring back to near normal thus justifying its significant role in arthritic conditions

Kullich *et. al.* [19], reported that in the arthritic condition the hemoglobin level decreased which has been associated with anemia. Anaemia may be due to reduced erythrocyte deformability. The reduced deformability leads to a shortened life span of erythrocyutes, which results in depression of RBC a marker of rheumatoid disease. The erythrocyte sedimentation rate was monitored on day 51 and our reports showed the extracts of Rheumatocure-2004 reduced the increased ESR caused in arthritic rats. Our results of hematological parameters were in agreement with the study of Kweifio *et al.* [20]; Ramesh and Vinod, [214] and Singh *et al*., [22].

CONCLUSION

Rats treated with Arumuga Chendooram showed significant ($p<0.001$) increase in Hb, RBC MCH, MCHV and MCV and decreased in WBC count and ESR when compared to arthritis rats. Rats treated with Arumuga Chendooram showed significant ($p<0.001$) restored in Total and Differential count of leucocytes when compared to arthritis rats. The results of the present study concluded that significantly restored the heamatological profile on treatment with Arumuga Chendooram than the standard.

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