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INTEGRATING PHARMACOLOGICAL APPROACHES TO STUDY ANTI-OXIDANT AND ANTI-ARTHRITIC ACTIVITY: ARTHRUM PLUS CAPSULE

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ABSTRACT

Rheumatoid arthritis is a chronic multi-system disease of unknown cause. It affects people in their prime of life, predominantly between the ages of 20-50 years with unpredictable cause. Arthrum plus capsule is a polyherbal formulation, used for the treatment of rheumatoid arthritis. The present study was planned to evaluate efficacy of Arthrum plus capsule as antioxidant activity and anti-arthritis activity. Healthy albino rats of Wistar strain use for the study of Freund's adjuvant induced arthritis model. Paw volume was measured on 4th day, 7th day, 14th day and 21st day. At 22nd day blood was withdrawn through retroorbital vein and the biochemical parameters like haemoglobin content, WBC count, ESR, RBC, Neutrophil, RA factor were analyzed. Antioxidant activity was performing by DPPH radical scavenging and Scavenging of Hydrogen peroxide activity. Arthrum plus capsule has potent antioxidant activity. Arthrum plus capsule were decrease the inflammation, ESR and WBC count and RA factor that indicate Arthrum plus capsule gives significant anti-arthritis effect. The results indicate that Arthrum plus capsule possesses potent antioxidant and anti-arthritis activity.

KEYWORDS: Arthrum plus capsule, anti-arthritis, complete Freund's adjuvant, antioxidant.

INTRODUCTION

Today's medicine is based on traditional medicine. Traditional medicines exist in every continent of the globe and in every cultural area of the world. The most famous ones are traditional Ayurvedic medicine in India, Chinese medicine in East Asia and formerly galenic medicine in Europe. Herbal medicine is the oldest form of healthcare known to mankind.^[1]

The use of traditional medicine is wide spread and plants still present a large source that might serve as leads for development of novel drugs. Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints and causing inflammation (arthritis). The disease is characterized by articular inflammation and by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints. Pharmacological treatment of Rheumatoid arthritis can be divided into disease-modifying anti-rheumatic drugs (DMARDs), anti-inflammatory agents and analgesics, immunomodulator and biological agents.^[2]

Arthrum plus capsule is a polyherbal formulation believed to have the potential for providing relief in rheumatoid arthritis (RA). Many of the ingredients from Arthrum plus capsule are reported to have a potential antioxidant, anti-arthritic and anti-inflammatory effect. The antioxidant and anti-arthritic properties of Arthrum plus capsule have not been scientifically investigated yet. Therefore, present research work being carried out using rat as an experimental model, to assess the antioxidant and anti-arthritic potential of polyherbal Arthrum plus capsule. Tacking these facts in to consideration, the present study deals with the evaluation of antioxidant and anti-arthritic activity and its changes in haematological and biochemical parameters of the formulation in Freund's adjuvant induced arthritic rats.

MATERIALS AND METHODS

Drugs, Chemicals, Instrument and Reagents

Arthrum plus capsule is ayurvedic proprietary polyherbal formulation as test drug of Vital care Pvt Ltd., Vadodara, India. The ingredients, botanical name and quantity of the Arthrum plus capsule are tabulated in Table.1. Dexamethasone as standard (Dexona, Cadila Healthcare Ltd., India), carboxymethyl cellulose as a suspending agent. Complete Freund's adjuvant was obtained from Sigma Aldrich (Saint Louis, Missouri, USA), α - α diphenyl β picrylhydrazyl (DPPH), Methanol. UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D), Hydrogen peroxide and Phosphate buffer saline. All chemicals used for the study are purchased from SD-fine chemicals; India and all other reagent used were of analytical grade.

Table.1 Ingredients of Arthrum plus capsule

Ingredients	Botanical name	Part Used	Quantity(mg)
Shallaki	<i>Boswellia serrata</i>	Oleogum-resin	250
Yograj guggulu	Classical formulation	---	100
Rasna	<i>Pluchea lanceolata</i>	Root	100
Nirgundi	<i>Vitex negundo</i>	Leaves	50

IN-VITRO ANTIOXIDANT STUDY

Preparation of Arthrum plus capsule solution

Alcoholic extract of the formulation was prepared at the concentration of 1000 μ g/ml in methanol. From the stock solution different concentrations were prepared in methanol and used for antioxidant studies.

Preparation of Standard stock solution of Ascorbic acid:

Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1000 µg/ml in methanol. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz. 10, 20, 40, 60, 80, 100 µg/ml were prepared in methanol and used for antioxidant studies.

DPPH radical scavenging activity [3, 4, 5, 6]**Procedure**

Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100 µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517nm, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Hydrogen peroxide scavenging activity [7]**Procedure**

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of product extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80, 100 µg/ml in methanol (1ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A0 is the absorbance of the control and A1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC50. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

IN-VIVO ANTI-ARTHRITIC STUDY**Freund's adjuvant induced arthritis** [8, 9, 10, 11]**Preparation of drug sample**

Powder of Arthrum plus capsule which was weighing 1500 mg. Add 0.6ml CMC to it 60 ml of water was added and triturated to make fine solution. Dose of Arthrum plus Capsule was calculated on the basis of human dose.

Animals

Healthy albino rats of Wistar strain, weighing 150-200 gm of either sex were used for the study. The animals were housed under controlled condition of temperature ($22\pm 2^{\circ}\text{C}$), relative humidity: 50+-70%, and 12-hr/12-hr light/dark cycle. Animals had free access to standard pellet diet and purified drinking water ad libitum. All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Treatment protocol for the study of Anti-Arthritic activity

The experimental animals were divided into five group, six animals in each group and drug were given in following order: Group 1: Normal control animals was given 1% CMC, 1ml/kg p.o. Group 2: Disease Control animal was given 1% CMC, 1ml/kg p.o. and Group 3, 4 and 5 were given aqueous extract of Arthrum plus capsule dose 100, 200, 300 mg/kg respectively. 0.1ml CFA administered in Group 2, 3, 4 and 5 Along with their respected drugs.

Procedure

Adjuvant induced arthritis was induced in animals by intra dermal injection into the footpad of the right hind paw with 0.1 ml Freund's adjuvant containing 1 mg/ml mycobacterium tuberculosis suspension. Drug treatment was started from the initial day from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 21th day. Paw volume was measured on 4th day, 7th day, 14th day and 21st day by using plethysmometer. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days.

$$\text{Paw edama} = \frac{\text{Volume on day 28} - \text{Volume before adjuvant injection}}{\text{Volume before adjuvant injection}} * 100$$

The change in body weight was recorded on 4th day, 7th day, 14th day and 21st day. At 22nd day blood was withdrawn through retroorbital vein puncture of all groups by anaesthetizing the animal with diethyl ether and the biochemical parameters like haemoglobin content, total WBC count, ESR, RBC, Neutrophil, RA factor were analysed.

Statistical analysis

Results are presented as Mean \pm SEM of six animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by

Dunnett test using graph pad prism software. The significance difference if any among the groups at $p \leq 0.01$ was considered statistically significant.

RESULT

Antioxidant activity of Arthrum plus capsule

The antioxidant activity was carried out using DPPH radical scavenging assay and H_2O_2 radical scavenging assay by in-vitro models. Antioxidant activity of Arthrum plus capsule was carried out using ascorbic acid as standard antioxidant. Results are given in Table.2 and graphical presentation is given in Fig.1&2.

Table.2 DPPH radicals and H_2O_2 scavenging activity of alcoholic extract of Arthrum plus capsule

Sr.No.	Antioxidant Model	IC ₅₀ value(μ g/ml) Ascorbic acid	IC ₅₀ value(μ g/ml) Alcoholic extract
1	DPPH radicals scavenging activity	39.35	69.99
2	H_2O_2 scavenging activity	40.145	69.59

Result indicated the significant reduction in the concentration of DPPH and H_2O_2 radicals due to the scavenging ability by increasing the dose of ascorbic acid, as a reference standard and alcohol extract of Arthrum plus capsule. The IC₅₀ values in DPPH and H_2O_2 radical scavenging models were 39.35 μ g/ml and 69.99 μ g/ml and 40.145 μ g/ml and 69.59 μ g/ml for ascorbic acid and alcohol extract of Arthrum plus capsule respectively.

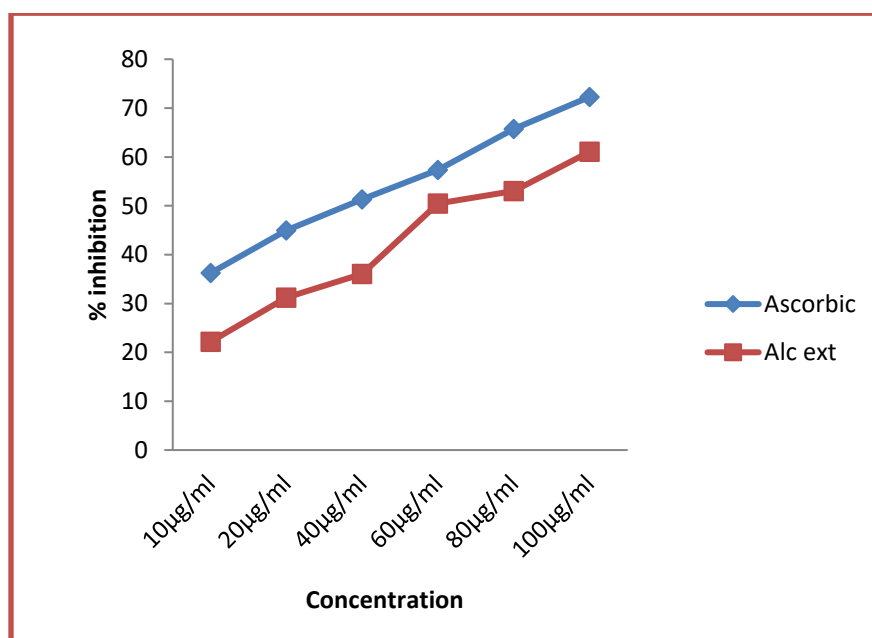


Fig.1 DPPH radical scavenging activity of alcoholic extract of Arthrum plus Capsule

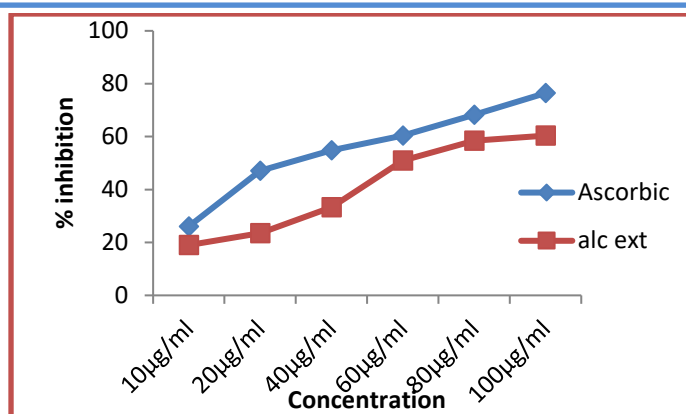


Fig.2 H₂O₂ scavenging activity of alcoholic extract of Arthrum plus Capsule

Anti-arthritic activity of Arthrum plus capsule

Rat Paw Volume

Effect of Arthrum plus capsule on rat paw volume in Freund's adjuvant induced arthritis shown in Table.3

Table.3 Effect of Arthrum plus capsule on paw volume

Treatment	0day	4th day	7thday	14th day	21th day
Normal control	0.51±0.01	0.508±0.01	0.503±0.02	0.505±0.02	0.488±0.02
Arthritis control	0.515±0.02	1.7±0.04	1.55±0.07	1.48±0.06	1.32±0.07
APC 100mg/kg	0.513±0.04	1.616±0.03	1.44±0.07	1.38±0.08	1.3±0.08
APC 200mg/kg	0.515±0.01	1.52**±0.03	1.34±0.04	1.18*±0.07	0.98**±0.05
APC 500mg/kg	0.5±0.01	1.42**±0.03	1.23**±0.03	1.1**±0.05	0.96**±0.01

APC-Arthrum plus capsule. All values represented as mean±S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test Comparisons were made between disease control Vs treatment and normal control * denotes significance at the level of $p \leq 0.05$. **denote the significance at the level of $p \leq 0.01$.

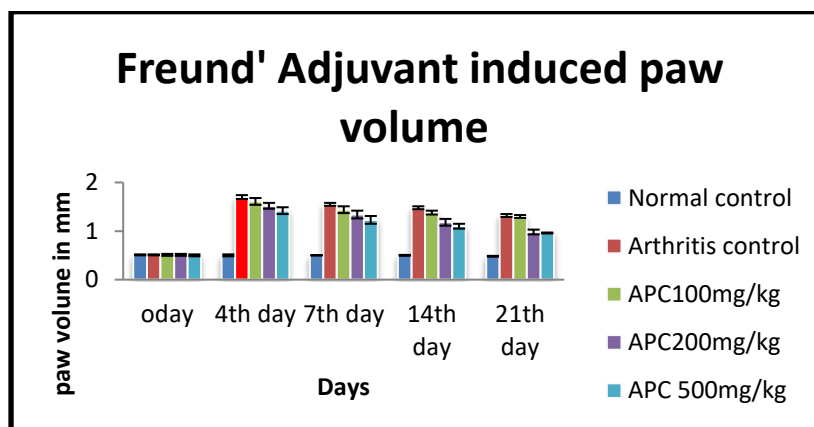


Fig.3 Anti arthritic Effect of Arthrum plus capsule on Rat paw volume

In Freund's adjuvant induced arthritis, Disease control group showed highest inflammation on 4th day and reduced afterward. Arthrum plus capsule significantly decreased in rat paw volume. That was indicating Arthrum plus capsule having anti-arthritic activity.

Rat Body Weight

Effect of Arthrum plus capsule on rat body weight in Freund's adjuvant induced arthritis was shown in Table.4.

Table.4 Effect of Arthrum plus capsule on rat body weight

Treatment	0 day	4 th day	7 th day	14 th day	21 th day
Normal control	190±3.66	190±3.66	190±2.36	186.6±2.11	180±2.11
Arthritis control	186±4.96	186±4.96	185±3.1	185±3.75	188.3±4.02
APC 100mg/kg	183±6.17	183.3±6.17	183.3±3.05	183.3±3.34	185±3.42
APC 200mg/kg	168.3±1.67	168.3±1.67	166.6±1.93	171.6±1.67	175±2.24
APC 500mg/kg	185*±3.42	185*±3.42	184.1*±2.99	185*±3.42	186.6*±4.23

APC: Arthrum plus capsule. All values represented as mean±S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test; comparisons were made between: disease control against treatment and normal control. *denotes significance at the level of $p \leq 0.05$.

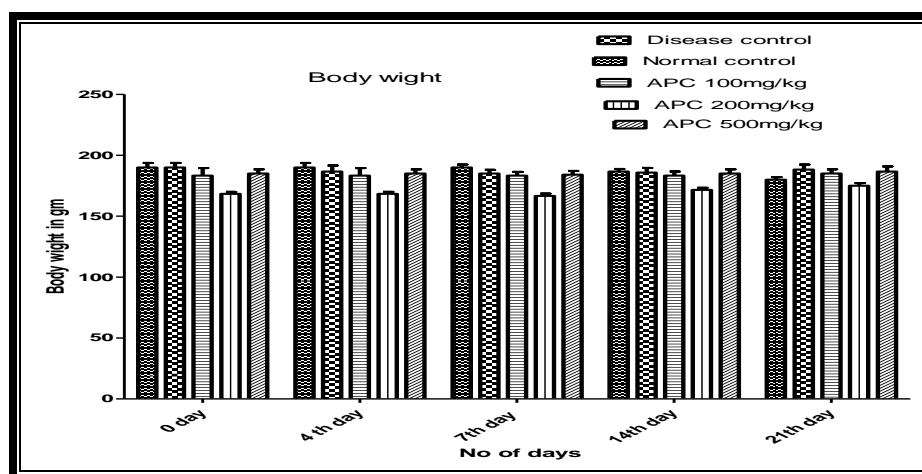


Fig.4 Effect of Arthrum plus Capsule on rat body weight

Treatment with Arthrum plus capsule produced slightly increased in body weight than normal control. This effect was not significant indicating body weight in treated and untreated rat was not much change suggesting body weight is not consistent parameter in anti-arthritic studies.

Radiological Analysis

Photograph of Radiological analysis on bone destruction after the treatment of Arthrum plus capsule shown in Figure. 5

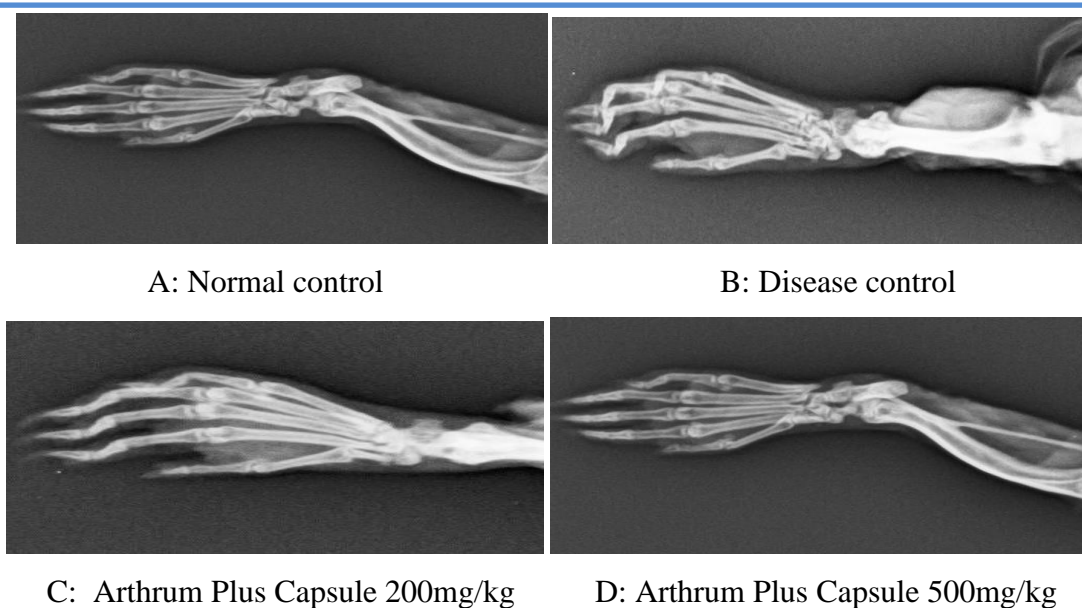


Fig.5 Radiological analysis on bone destruction after the treatment of Arthrum plus Capsule

Freund's adjuvant induced treated group of rats showed the narrowing of metatarsal and phalangeal joint space, diffused joint in phalangeal region, deformity in shape, soft tissue swelling and bending of metatarsal and phalangeal joints can be seen. These changes were normalized in Arthrum plus Capsule treated animals and the joint space of metatarsal and phalanges have been regenerated indicating protective effect of Arthrum plus capsule on Arthritic condition.

Diseases development Parameters

Effect of Arthrum plus capsule on disease development parameters like Gait test, Mobility Test, Joint Stiffness in Freund's adjuvant induced arthritis rats shown in Table.5

Table.5 effect of Arthrum plus capsule on disease development parameters

Parameter	Normal Control	Arthritis control	APC+ 100mg/kg	APC+ 200mg/kg	APC+ 500mg/kg
Gait Test	2	1	1	1*	2*
Mobility Test	6	2	3	4**	5**
Joint Stiffness	2	1	1	1*	2**

APC+: Arthrum plus capsule. All values represented as mean \pm S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test; comparisons were made between: control and treated animals. * denotes significance at the level of $p \leq 0.05$.

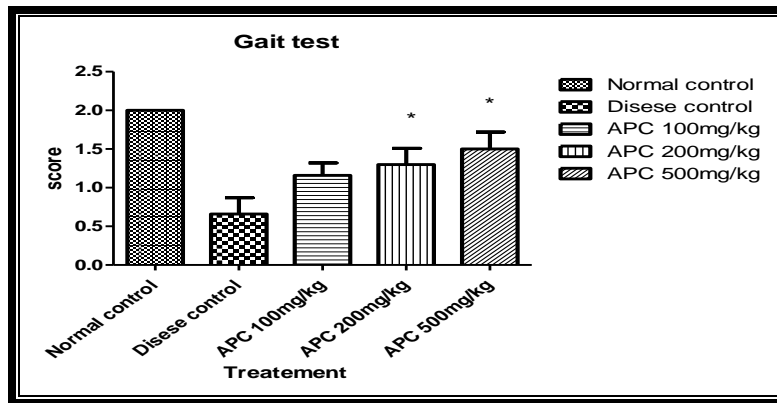


Fig.6 Effect of Arthrum plus capsule on Gait test

In Freund's adjuvant induced arthritis Gait score was decreased than normal. Arthrum plus capsule treated rats showed significant increase in gait score in dose dependent manner.

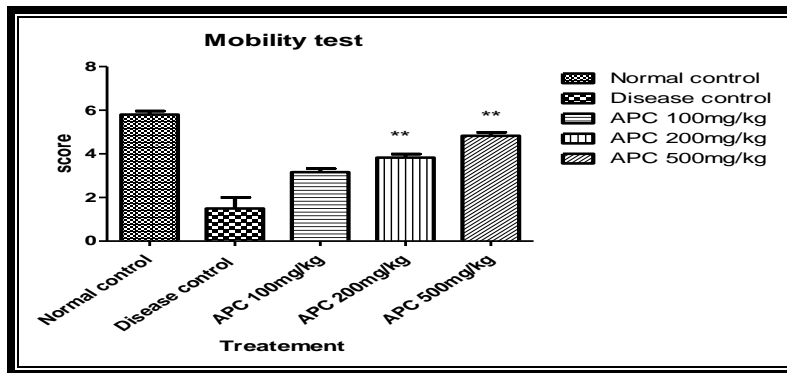


Fig.7 Effect of Arthrum plus capsule on mobility

In Freund's adjuvant induced arthritis rats the mobility score was decreased. There was restriction on the full-range movement of joints in FAI rats, and the joints of the Arthrum plus capsule treated rats showed no restriction throughout the observation period. Nearly maximal improvement in mobility was seen in the rats treated with Arthrum plus capsule produced significant increase in dose dependent manner compare to normal.

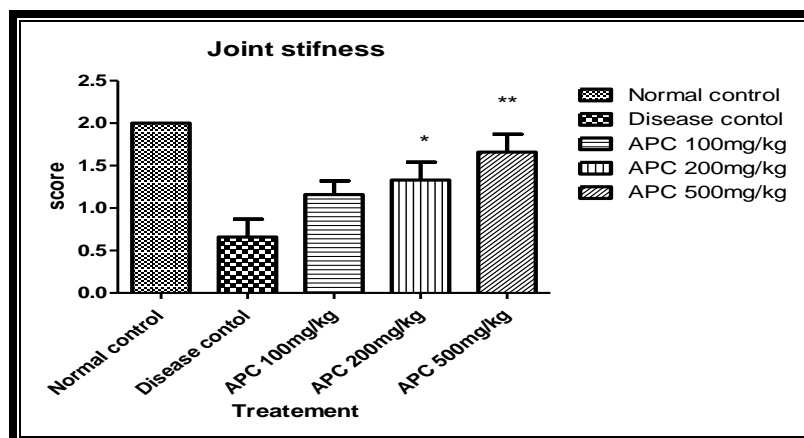


Fig.8 Effect of Arthrum plus capsule on joint stiffness

In Freund's adjuvant induced arthritis rat joint stiffness score was decreased than normal. Arthrum plus capsule treated rats showed significant increase in joint stiffness score in dose dependent manner.

Hematological Parametrs

Effect of Arthrum plus capsule herbal formulation on haematological parameters shown in Table.6

Table.6 Effect of Arthrum plus capsule on hematological parameters

Parameter	Herbal formulation treatment (mg/kg.p.o)				
	Normal control	Arthritis control	+APC 100mg/kg	+APC 200mg/kg	+APC 500mg/kg
ESR(mm) (After 1hr)	3.6±0.21	4.6±0.21	3.5±0.1	2.9**±0.2	2.7**±0.04
Total WBC (cu.mm)	7916.6±114.21	8166.6±167	4100±36.65	5083.3±30.85	6000±129.6
Lymphocyte (%)	42±0.42	53±0.22	41±1.88	35**±1.05	31**±0.88
Neutrophil (%)	59±1.05	60±0.4	51±0.4	54*±1.05	55**±1.08
Haemoglobin gm%	13.26±0.08	14.31±0.07	11.93±0.28	12.41*±0.13	13.16**±0.24
RBC (million/cm)	4.4±0.03	3.45±0.02	4.43±0.03	5.45**±0.07	6.33**±0.05

+APC: **Arthrum plus capsule** all values represented as mean±S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test; Comparisons were made between: disease control Vs treatment and normal control. * denotes significance at the level of $p \leq 0.05$. ** denotes the significance at the level of $p \leq 0.01$.

RA Factor

Effect of Arthrum plus capsule on Rhumatoid arthritis Factor was shown in Table.7

Table.7 Effect of Arthrum plus capsule on RA factor

Treatment & dose	Normal Control	Arthritis control	APC+ 100 mg/kg	APC+ 200 mg/kg	APC+ 500 mg/kg
Score	6.9	7.7	7.5	7*	6.7*

+APC: Arthrum plus capsule. All values represented as mean \pm S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test; comparisons were made between: control and treated animals. * denotes significance at the level of $p\leq 0.05$. ** denotes the significance at the level of $p\leq 0.01$.

DISCUSSION

Increased oxidative stress has been suggested to play a major role in pathogenesis of variety of diseases resulting from defective natural antioxidant; hence antioxidant therapy should include either enzyme or natural antioxidant agent, which is capable of augmenting the function of oxidative free radical scavenging enzyme.^[12]

The IC₅₀ values in DPPH and H₂O₂ radical scavenging model were 39.35 μ g/ml and 69.99 μ g/ml and 40.145 μ g/ml and 69.59 μ g/ml for ascorbic acid and alcohol extract of Arthrum plus capsule respectively. Results indicated the antioxidant activity may be due to presence of active Phytochemicals.

The purpose of the study is to screen and evaluate anti-arthritic activity using modern scientific, internationally approved standard experimental procedure. Evaluation of anti-arthritic activity of Arthrum plus capsule was studied on Freund's adjuvant induced arthritis in Wistar strain albino rats. The choice of the animal strain has been found to be very important for the performance of this test. Wistar-Lewis rats have been proven to be very suitable in contrast to other sub strains.^[13]

In Freund's adjuvant-induced arthritis model, rats developed a chronic swelling in hind paw with influx of inflammatory cells, erosion of joint cartilage, bone destruction and remodelling which have close similarities to human rheumatoid arthritis.^[14] These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal. The Freund's adjuvant administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as oedema of the particular tissues.^[15]

In present study, the test drug Arthrum plus capsule significantly suppressed the swelling of the paws. Reduction of paw swelling in the Arthrum plus capsule treated rats observed from the 3rd week onwards may be due to immunological protection rendered by the formulation effect. In the study, there was an increase in erythrocyte sedimentation rate (ESR) level in Freund's adjuvant treated group which is a common diagnostic feature in patient in chronic arthritis.^[16] Increase in the ESR is an indication of active but obscure disease process which gets elevated during response to stress, inflammation and cell necrosis.^[17] Arthrum plus capsule treated rat showed significantly increase ESR and haemoglobin count in dose dependent manner. WBC

count which plays a major role in body defense mechanism is mild to moderately increased in arthritic condition; An increase in WBC count may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor.^[18]

The WBC count was decreased in Arthrum plus capsule treated rat.

In Freund's adjuvant-induced arthritis rats gait score, mobility score, joint stiffness score was decreased than normal. Arthrum plus capsule treated rats showed significant increase in gait score and mobility score in dose dependent manner.

In Freund's adjuvant-induced arthritis RA factor was increased than normal. Arthrum plus capsule treated rat showed significantly decrease in RA factor.

CONCLUSION

Arthrum plus capsule gives good antioxidant activity that helps in prevention of further progression during Rheumatoid arthritic diseases condition. Arthrum plus capsule exhibited significant decrease in paw swelling, ESR and WBC count and RA index that shows potent anti-arthritic activity on complete Freund's adjuvant induced arthritis model in rats. The detail study reveals to Arthrum plus Capsule work on all outcome measures of Rheumatoid arthritis such as disability in movement, joint pain and swelling.

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