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### RESEARCH ARTICLE

#### IN VIVO ASSESSMENT OF ANTI-ARTHRITIC ACTIVITY OF *WITHANIA COAGULANS* DUNAL FRUITS.

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#### Manuscript Info

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##### Key words:-

Anti-arthritis, *Withania Coagulans*, Complete Freund's adjuvant, arthritis index, dorsal flexion.

#### Abstract

The aim of this study was to determine *in-vivo* anti-arthritis activity of methanolic and hydro alcoholic extract of *Withania Coagulans* Dunal fruits i.e. *Withania Coagulans* Methanolic Extract (WCME) and *Withania Coagulans* Hydro alcoholic Extract (WCHAE). Phytochemical analysis has indicated presence of alkaloids, flavonoids, glycosides, steroids, saponins, fixed oil, carbohydrates. Both the extracts have showed anti-arthritis activity by Complete Freund's adjuvant induced arthritis (CFA) in rats method. Treatment with WCME-200mg/kg, WCME-500mg/kg, WCHAE-450 mg/kg caused significant ( $p < 0.01$ ) inhibition of the arthritis index score, paw edema volume, joint area and pain perception parameters like dorsal flexion and motility along with rise in stair climb score in comparison with disease control (CFA induced) groups on 28<sup>th</sup> day. Both the extracts showed the good anti-inflammatory activity but WCHAE showed the most significant anti-arthritis activity than WCME. The findings of the present study suggested that *Withania Coagulans* dunal fruits could be a potential natural source of anti-arthritis activity which is very important against treatment of rheumatoid arthritis disease.

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#### Introduction:-

RA is chronic inflammatory and autoimmune diseases affects millions of people across the world leading to suffering, economic loss and premature death. Common inflammatory diseases include asthma, chronic obstructive pulmonary diseases (COPD), allergic rhinitis, osteoarthritis, inflammatory bowel diseases (IBD) and psoriasis. Inflammatory diseases including different types of rheumatic diseases are a major cause of mortality of the working force throughout the world. This has been called the 'king of the human miseries.'

Inflammation is the reaction of living tissues to injury, infection or irritation of foreign substance. Lysosomal enzyme released during the inflammation leads to the tissue injury which is responsible for pathological condition of rheumatoid arthritis. Inflammation manifest in the form of painful swelling associated with some changes in skin covering at the site. It can be classified either acute or chronic. Acute inflammation is initial response of body to harmful stimuli and prolonged inflammation is known as chronic inflammation.<sup>[1]</sup>

The immune system is a well-organized and well-regulated system. The deregulation of the immune system may lead to the development of RA with destructive inflammatory polyarticular joint potentially resulting in progressive

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destruction of articular and periarticular structure.<sup>[2]</sup> Autoimmune diseases occur when the body loses the ability to discriminate self proteins from non-self proteins. This loss of tolerance ultimately results in the destruction of self tissues by the immune system. Typically, autoimmune diseases are characterized by the presence of auto-antibodies and auto reactive T lymphocytes acting against specific self proteins. Within joints, the autoimmune mediated course of RA is characterized by four stages: (I) inflammation of the synovial membrane and joint capsule, (II) formation of a pannus (granulation tissue) that first covers and then invades cartilage and bone, (III) fibrous invasion of the pannus, (IV) calcification of the fibrous tissue.<sup>[3]</sup>

The therapy for rheumatism includes medications with steroids, non-steroidal anti-inflammatory drugs (NSAIDs) used to suppress the symptoms and disease modifying anti-rheumatic drugs (DMARDs) and immunosuppressant drugs required to inhibit or halt the immune process.<sup>[4]</sup> Unfortunately, although these drugs have shown to improve signs and symptoms alter the natural history of the disease and improve quality of life but there is still no cure. In addition, these available therapies are associated with potential risks of death/ irreversible organ damage.<sup>[5]</sup> Owing to these shortcomings, there is need to have a more effective and safe therapeutic strategy to treat RA. By virtue of its safety and efficacy, plants still hold their own unique place. The Ayurvedic systems of treatment are being increasingly recognized as an alternate approach to arthritic treatment. In India Herbal medicine has a traditional name "Ayurvedic". Ayurveda is derived from two Sanskrit words, namely, "Ayus" and "Veda," meaning life and knowledge, respectively. Major formulations used in ayurveda are based on herbs. Hence, there is need of systematic approach to find out the herbs which possess anti-inflammatory, immunomodulatory and anti-arthritic potential.

*Withania Coagulans* dunal fruits family *Solanacea* have many medicinal and therapeutic benefits although many of them have yet to be proven by science. Hence, current research project was undertaken with an objective to validate the claims of the medicinal use of *Withania Coagulans* dunal fruits as an anti-inflammatory, immunomodulatory and anti-arthritic agent.

## Material And Methods:-

### Material:-

Freund's Complete Adjuvant (Difco Lab. USA) as inducing agent, SGOT (Randox Laboratories Ltd) SGPT (Randox Laboratories Ltd) ALP (Transasia Bio-Medical Ltd) used as diagnostic kit, Indomethacin (Ipca) as used as standard drug.

Chemicals: Anesthetic ether (Molychem), Formic acid (Molychem).

Instruments: Centrifugation machine (Remi), Semi- Auto analyzer (Randox Laboratories Ltd), Mersap Digital Plethysmometer (IITC Life Sciences, USA), Micrometer Screw Gauge (Aerospace), Stair climb apparatus.

All the above chemicals and instruments were obtained from the local suppliers.

### Collection And Authentication of Plant Material:-

The fruits of *Withania Coagulans* Dunal belong to the family *Solanacea* is distributed throughout India. For the present study fruits were collected in the month of August from local suppliers in Mumbai (Maharashtra). The dried fruits was identified, confirmed and authenticated by A. S. Upadhye, Scientist, Plant Drug Authentication Service, Botany group, Plant Sciences Division, Agharkar Research Institute, Pune for further work. The dried fruit material was then pulverized by a mechanical grinder. The resulting coarse powder was then used for extraction.

### Processing of the WC Fruits to Obtain an Extract:-

#### Preparation of Extract:-

Dried fruits of *Withania Coagulans* Dunal were coarse powdered and packed into Soxhlet column and extracted with methanol for methanolic extract.<sup>[6]</sup> Whereas the water: methanol (40:60) is used for preparation of hydro alcoholic extract.<sup>[7]</sup> The extract was concentrated under reduced pressure to get dried powder. The dried extract was stored in airtight container in refrigerator below 10°C. Whereas the hydro alcoholic extract is prepared by using water and methanol in ratio of 40:60. To convert into powder, distillation process have been followed to recover solvent and then dried.

### Analysis of Extract:-

#### a) Preliminary Phytochemical Studies

Phytochemical studies of methanolic extract and hydro alcoholic extract of fruits part of *Withania Coagulans* Dunal was done. It is subjected to the different chemical tests in ordered to identify the presence of various phytoconstituents. Dragendorff's test, Mayer's test, Wagner's test, Hager's test for alkaloids, Molish's test, Fehling's test for carbohydrates, Legal's Test for glycosides, Liebermann burchard's test for steroidal compounds, Lead acetate Test, Reaction with Sodium Hydroxide for flavonoids, Spot test for fixed oil, foam test for saponin glycosides was carried out for their presence.<sup>[8]</sup>

#### b) TLC Profile

TLC profile was developed for WCME and WCHAE.

#### Procedure:-

Thin layer chromatography of extract was performed on silica gel plates. A TLC plate of width 4 cm and height 9 cm was taken and origin was marked at 1.2 cm from one end of the plate. The solvent system chloroform: methanol in the ratio of 9:1 was prepared. 10 µl of samples were spotted on the TLC plate, air dried and placed in chromatographic chamber previously saturated with the solvent system. The run was performed until the solvent reaches the top of the plate. Dried and sprayed the plate with 10 % sulphuric acid and air dried. Spot were developed by heating in hot air oven. The spot developed were recorded and R<sub>f</sub> value was calculated. The R<sub>f</sub> value can be determined as follows:

$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by the solvent}}$
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#### Methods:-

##### Animals:-

Sprague Dawley rats of either sex weighing 150- 200 gm were used in the study. They were procured from Bharat Serum, Thane, India. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 22±1°C, under a 12 h light/dark cycle, with a free access to commercial diet and water ad libitum throughout the study. The care, use and experimental procedures conducted in accordance with guidelines and procedures for animal experimentation as prescribed by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

##### Determination of Acute Oral Toxicity Study (LD50):-

The acute oral toxicity study of methanolic and hydro-alcoholic extract of *Withania Coagulans* Dunal fruits were determined in female Sprague Dawley rats in accordance with OECD (Organization for Economic Co-operation and Development) guideline 425 to evaluate safety profile of these plant extract.

##### In Vivo Assessment of Anti-arthritis Activity

##### Complete Freund's Adjuvant Induced Arthritis in Aats (CFA) <sup>[9, 10]</sup>

##### Procedure

Arthritis was induced by injecting a 0.1 ml (0.1 % w/v) suspension of killed mycobacterium tuberculosis bacteria homogenized in liquid paraffin i.e. CFA into the right hind paw.

Animals were divided into 6 groups of 6 rats each.

Group I: The Disease Control group was administered with normal saline.

Group II: The Normal Control group was administered with normal saline

Group III: The standard group received Indomethacin (10 mg/kg) orally.

Group IV and Group V: The test group was administered with methanolic extract of WC fruits at the dose of 200 mg/kg and 500 mg/kg rats orally.

Group VI: The test group was administered with hydro-alcoholic extract of WC fruits at the dose of 450 mg/kg rats orally

Drug treatment was started from the initial day from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 28th day. Paw edema and joint thickness (perimeter) was measured on 7th, 14th, 21st and 28th day by using Mersap digital plethysmometer and micrometer screw gauze respectively. The mean changes in injected paw edema and joint thickness, were calculated on respective days.



**Figure-1:-** Mersap Digital plethysmometer



**Figure-2:-** Micrometer Screw Gauge

### **Evaluation:-**

#### **A) Assessment of Arthritic Parameters**

##### **i) Arthritis Index**

Assessment of arthritis index was done by visual observation of level of inflammation found on left hind paw in terms of edema and redness. The scoring of arthritis index was done on day 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and on 28<sup>th</sup> day. Scoring is given in following manner.

1. No sign of edema or redness    2. Redness without edema    3. Redness with mild edema
4. Redness with severe edema    5. Redness, severe edema and stiffness in movement

##### **ii) Paw Volume**

Paw edema was measured on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day by using Mersap digital plethysmometer. The mean changes in injected paw edema were calculated on respective days.

##### **iii) Joint Thickness**

Joint thickness (perimeter) was measured on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day by using micrometer screw gauge. The mean changes in joint thickness, were calculated on respective days.

#### **B) Assessment of Pain Perception Parameter**

##### **i) Arthritic Dorsal Flexion Pain Test**

Ankle joint was gently flexed dorsally until the toes touched the front of the leg for 5 times with an inter-test interval of 5 seconds. Pain was scored zero when the animal showed neither squeaking nor quick leg-withdrawal. The scores was 1 when either reaction appeared, and scored 2 when both reactions appeared. All the groups were evaluated in this manner on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and on 28<sup>th</sup> day.

##### **ii) Stair Climbing Activity**

Overnight fasting animals were trained for one week to climb a staircase with steps at 5, 10 and 15 cm having water at second step and food at the third step. Climbing ability of the rats in the above groups scored 0, if the rats did not climb ; 1, if the rats climbed onto step-1; 2, if the rats climbed onto step -2 and 3, if the rats could climb all the three steps. All the groups were evaluated in this manner on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and on 28<sup>th</sup> day.

##### **iii) Motility Test**

The motility patterns of rats was observed for a period of 5 minutes on plane surface and score 0, if the rat walked easily, score 1, if rat walked with more difficulty and scored 2, if rat walked with more difficulty and avoided touching the toes of the inflamed paw to the floor. All the groups were evaluated in this manner on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and on day 28<sup>th</sup>. [11, 12]

#### **C) Hematological Determination in Arthritic Rats**

On 28<sup>th</sup> day blood was withdrawn through retro-orbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters like Hemoglobin (HB) content, total White blood cells (WBC) count, Hematocrit value (HCT), Red blood cells (RBC), Erythrocyte sedimentation rate (ESR), Platelet (PLT), Neutrophil (NEU), Lymphocytes (LYM) were analyzed. [9, 13, 14]

**D) Histopathological Analysis of Arthritic Rats**

On 28<sup>th</sup> day all animals were sacrificed and their hind limbs were removed surgically and fixed in 10 % buffered formalin. Then limbs were decalcified in 15 % formic acid for histopathological study.<sup>[15]</sup>

**E) Radiological Analysis of Arthritic Rats**

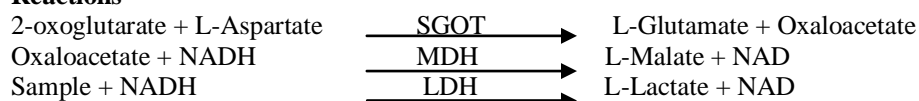
Before sacrificing the animals, X- rays were taken on 14<sup>th</sup> and 28<sup>th</sup> day at the joint of hind paw of the animals for evaluating the bone and cartilage damage. Radiographs were taken using X- ray apparatus (Siemen- 60 MA, Germany) and industrial X-Ray film (Fuji photo film, Japan). The X- ray apparatus was operated at 220 V with a 40 V peak, 0.2 second exposure time, and a 60 cm tube to film distance for anterior-posterior projection.<sup>[15]</sup>

**F) Analysis of Serum Transaminases**

The blood was collected after sacrificing the animals of all groups on 28<sup>th</sup> day. It is then centrifuged (REMI) and serum was then analyzed for lysosomal enzymes such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) by using SGPT, SGOT and ALP analysis kit.<sup>[16]</sup>

**i) Estimation of Serum Glutamate Oxaloacetate Transaminase Activity (SGOT/AST) [U.V. Kinetic method]****Principle**

Serum Glutamate Oxaloacetate Transaminase (SGOT or AST) catalyzes the reversible transfer of an amino group from L-aspartate to 2-Oxoglutarate forming L-Glutamate and oxaloacetate. The rate of this reaction is monitored by an indicator reaction coupled with malate dehydrogenase (MDH). The oxaloacetate produced is reduced to malate ion by MDH in the presence of NADH (Nicotinamide adenine Dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGOT activity. The rate of decrease in concentration of NADH measured spectrophotometrically is proportional to the catalytic activity of AST present in the serum sample.

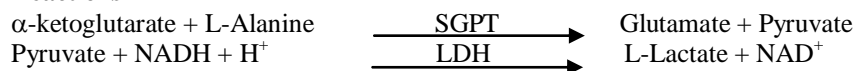
**Reactions****Procedure****Table 1:-** Preparation of Blank, STD and Sample for SGOT Test

Pipette	Sample (μl)
Working reagent	500
Sample	50

As mentioned in the above table blank, standard and sample was prepared by considering 500 μl of working reagent and 50 μl each of distilled water, standard and sample respectively, later all the samples were aspirated individually and absorbance was recorded at 340 nm.

**ii) Estimation of Serum Glutamate Pyruvate Transaminase Activity (SGPT/ALT) [U.V. Kinetic Method]****Principle**

Serum Glutamate Pyruvate Transaminase (SGPT or ALT) catalyses the reversible transfer of amino group from L-alanine to 2-oxoglutarate forming pyruvate and L-glutamate. The pyruvate produced is reduced to L-Lactate by lactate dehydrogenase (LDH) and NADH. The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm which is proportional to the SGPT activity. The rate of decrease in concentration of NADH measured by UV kinetic method is proportional to catalytic activity of ALT present in the serum sample.

**Reactions**

**Procedure****Table 2:- Preparation of Blank, STD and Sample for SGPT test**

Pipette	Sample (μl)
Working reagent	500
Sample	50

As mentioned in the above SGOT, the same procedure had been followed.

**iii) Estimation of Alkaline Phosphatase Activity (ALP)****Principle**

Phosphatase catalyses the hydrolysis of phosphates. Alkaline and acid phosphatases are differentiated by pH range of their optimum activities. The serum alkaline phosphatase hydrolyses p-nitro phenyl phosphate in the presence of oxidizing agent  $Mg^{+2}$

In the phosphatase determination, p-nitrophenylphosphate is used as substrate, which is dissociated by the enzyme into p-nitrophenolate and phosphate. The phenolate liberation occurs as yellow anion, which is then determined by using UV method. The amount of p-nitrophenolate liberated in unit time is directly proportional to the phosphatase activity.

**Reaction****Procedure****Table 3:- Preparation of Blank, STD and Sample for ALP**

Pipette	Sample (μl)
Working reagent	500
Sample	10

As mentioned in the above blank, standard sample was prepared by considering 500 μl of working reagent and 10 μl of each of distilled water, standard and sample respectively, later all the samples were aspirated individually and absorbance was recorded at 405 nm.

**Statistical Data Analysis**

Data obtained were subjected to one way analysis of variance (ANOVA) followed by *Dunnet's test* \*p < 0.05, \*\*p < 0.01 was considered significant

**Results:-****5.1 Preliminary Phytochemical Evaluation**

Phytochemical studies of methanolic extract and hydro alcoholic extract of fruits part of *Withania Coagulans* Dunal showed the presence of carbohydrates, saponin glycosides, flavonoids, alkaloids, glycosides, steroidal compounds, fixed oils.

**TLC Profile**

$R_f$  value found to be 0.6 for WCME and 0.65 for WCHAE which is similar to that of  $R_f$  value of Withaferin A (i.e.0.6-0.65) hence it shows the presences of Withaferin A in the extracts.

**5.2 Acute Toxicity Studies**

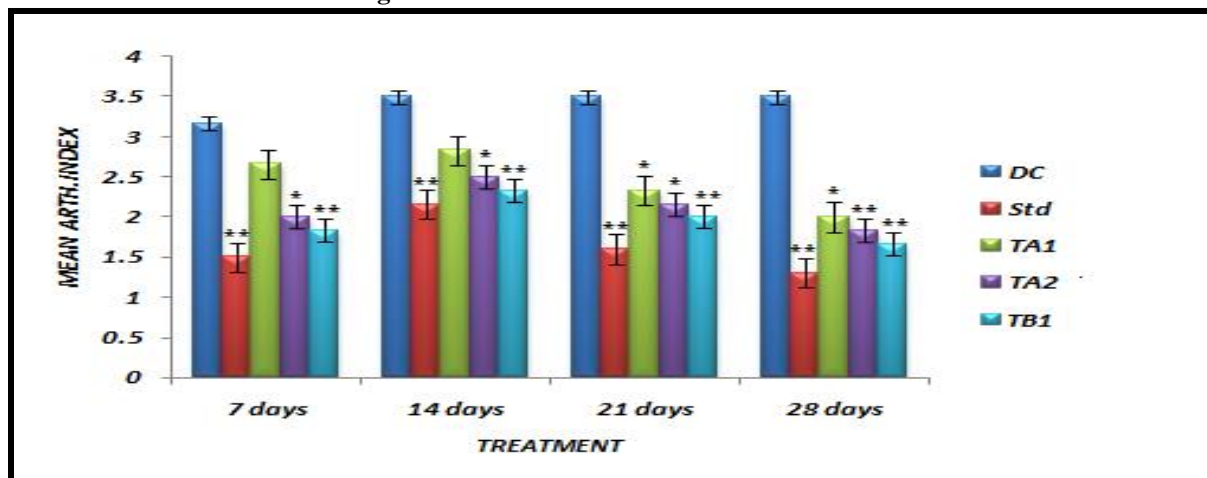
WCME and WCHAE did not show any toxic or deleterious effects up to 2000 mg/ kg oral dose. As the rats were administered up to maximal possible dose, the  $LD_{50}$  value of WCME and WCHAE could not be determined.

**a) Effect of WCE on Arthritis Index Score in FCA Induced Arthritis**

Effect of WCE on arthritis index was summarized in Table 4. Significant increased in arthritis index was observed in FCA treated group of animal when compared with normal animals. FCA increased arthritis index were significantly (p<0.05, p<0.01, p<0.01 and p<0.01) reduced by treatment with WCME-200, WCME-500, WCHAE-450 and

indomethacin (10 mg/kg) on 28<sup>th</sup> day in comparison with disease control group in FCA induced arthritis model respectively.

**Figure 3:- Effect of WCE on Arthritis Index Score**



Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

#### b) Effect of WCE on Paw Edema in FCA Induced Arthritis

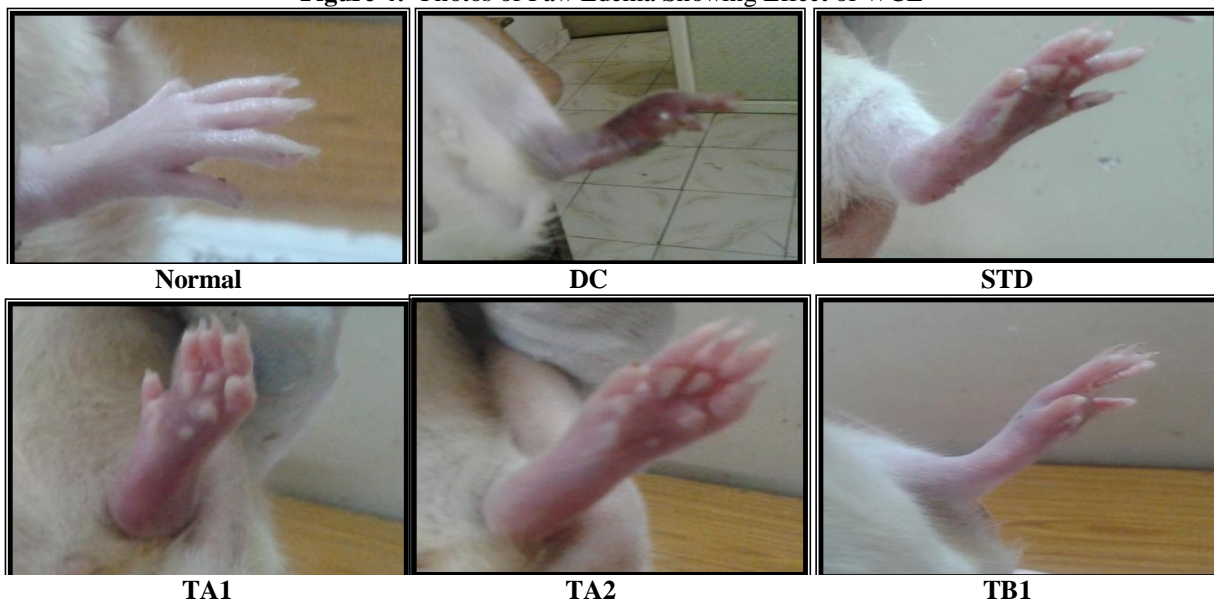
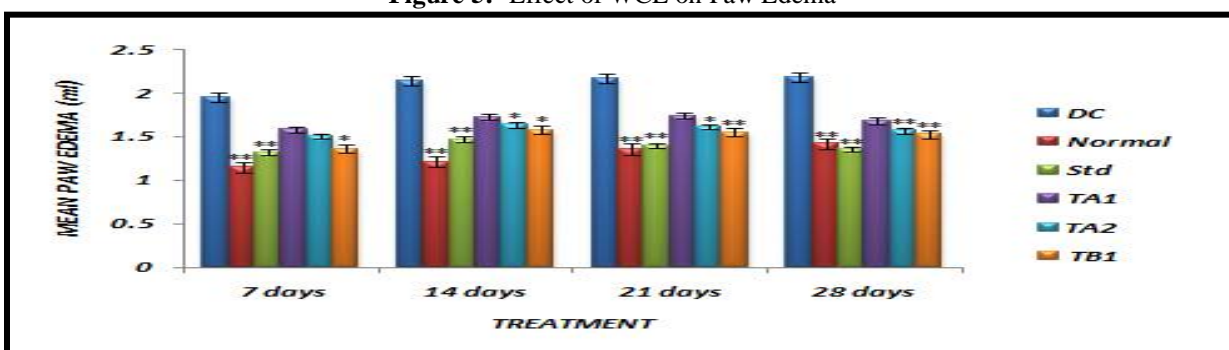
Effect of WCE on paw edema was summarized in Table 4. In present study it was observed that treatment of WCME-500 and WCHAE-450 significantly (p<0.01) reduce the paw edema volume (ml) on 28<sup>th</sup> day in comparison with disease control group in FCA induced arthritis model. Here, in agreement with other studies, WCME and WCHAE effectively inhibited the development of edema, a characteristic sign of inflammation, in arthritic hind of arthritic rats.

**Table 4:- Effect of WCE (200 mg/kg, 500mg/kg and 450 mg/kg) on Arthritis parameters i.e. Arthritis Index and Paw Edema**

Experimental Group ↓ Days →	ARTHRITIC PARAMETERS							
	ARTHRITIS INDEX				PAW EDEMA			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
DC	3.17± 0.167	3.5± 0.224	3.5± 0.224	3.5± 0.224	1.95± 0.101	1.22± 0.054	1.36± 0.039	1.42± 0.049
N	0	0	0	0	1.15± 0.044**	2.14± 0.152**	2.17± 0.15**	2.19± 0.108**
STD	1.5± 0.342**	2.17± 0.307**	1.67± 0.211**	1.33± 0.333**	1.32± 0.129**	1.47± 0.116**	1.40± 0.17**	1.36± 0.171**
TA1	2.67± 0.211	2.83± 0.167	2.33± 0.211*	2± 0.258*	1.58± 0.141	1.73± 0.193	1.74± 0.159	1.68± 0.164
TA2	2± 0.258*	2.5± 0.224*	2.17± 0.167*	1.83± 0.447**	1.50± 0.134	1.63± 0.069*	1.62± 0.044*	1.57± 0.050**
TB1	1.83± 0.307**	2.33± 0.211**	2± 0.516**	1.67± 0.333**	1.36± 0.181*	1.58± 0.144*	1.55± 0.13**	1.53± 0.156**

Values are expressed in Mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg



**Figure 4:-** Photos of Paw Edema Showing Effect of WCE**Figure 5:-** Effect of WCE on Paw Edema

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

### c) Effect of WCE on Joint Diameter (thickness) in FCA Induced Arthritis

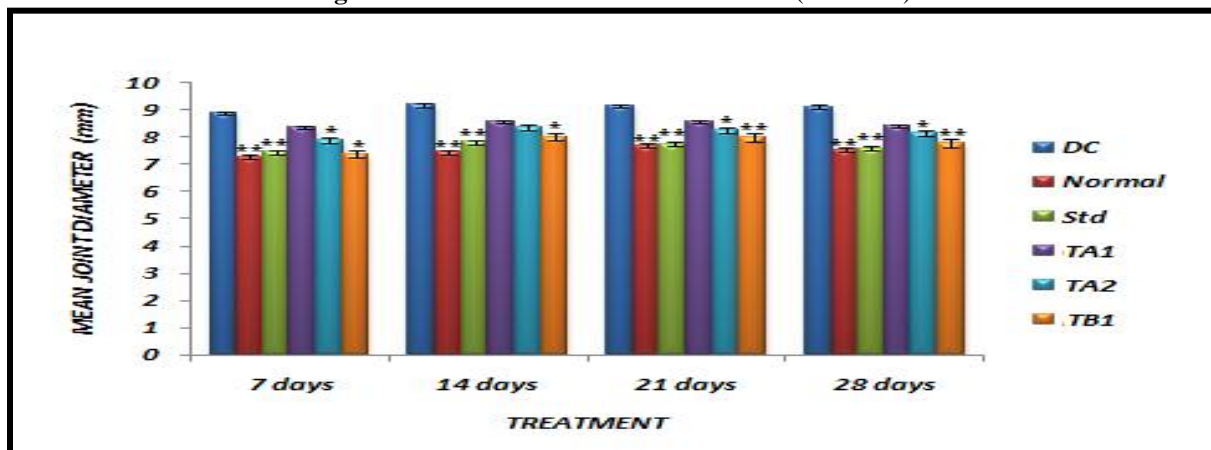
In present study it was observed that treatment of WCME-500 and WCHAE-450 significantly (p<0.05 and p<0.01) reduce the paw edema volume (ml) on 28<sup>th</sup> day respectively in comparison with disease control group in FCA induced arthritis model.

**Table 5:-** Effect of WCE (200 mg/kg, 500mg/kg and 450 mg/kg) on Joint Thickness

Experimental Group	JOINT THICKNESS			
	7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>	28 <sup>th</sup>
DC	8.885 $\pm$ 0.2067	9.173 $\pm$ 0.2154	9.142 $\pm$ 0.2532	9.103 $\pm$ 0.2785
N	7.263 $\pm$ 0.3200**	7.458 $\pm$ 0.2666**	7.685 $\pm$ 0.2680**	7.538 $\pm$ 0.3279**
STD	7.453 $\pm$ 0.1810**	7.812 $\pm$ 0.2381**	7.74 $\pm$ 0.2398**	7.57 $\pm$ 0.2930**
TA1	8.353 $\pm$ 0.3199	8.542 $\pm$ 0.3290	8.555 $\pm$ 0.2104	8.398 $\pm$ 0.2464
TA2	7.888 $\pm$ 0.2012*	8.367 $\pm$ 0.2532	8.253 $\pm$ 0.2393*	8.14 $\pm$ 0.2267*
TB1	7.838 $\pm$ 0.2527*	8.018 $\pm$ 0.2213*	7.988 $\pm$ 0.1935**	7.785 $\pm$ 0.1016**

Values are expressed in Mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME-. Treatment200 mg/kg, TA2: WCME- 500 mg/kg, TB1: WCHAE-450 mg/kg

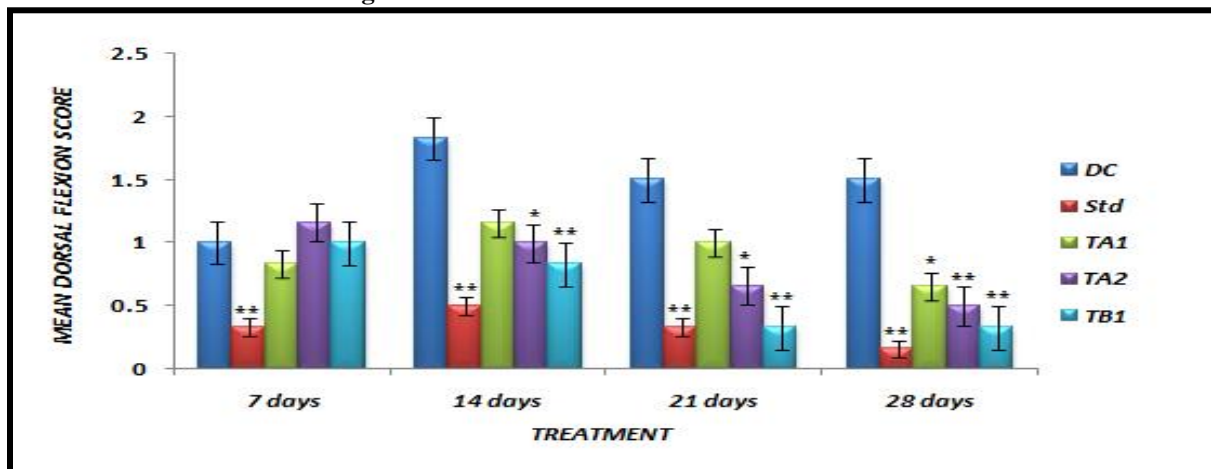


**Figure 6:- Effect of WCE on Joint Diameter (thickness)**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

#### d) Effect of WCE on Dorsal Flexion Score in FCA Induced Arthritis

In present study it has been observed that FCA induced increased dorsal flexion score were significantly (p<0.05, p<0.01, p<0.01 and p<0.01) reduced by treatment with WCME-200, WCME-500, WCHAE-450 and indomethacin (10 mg/kg) on 28<sup>th</sup> day in comparison with disease control group in FCA induced arthritis model respectively. As reduction in response given by arthritic animals on dorsal flexion was observed, hence it may be concluded that the level of inflammation is decreased with the progression of WCE treatment.

**Figure 7:- Effect of WCE on Dorsal Flexion Score**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

#### e) Effect of WCE on Motility Score in FCA Induced Arthritis

In present study it has been observed that FCA induced increased motility score were significantly (p<0.05, p<0.01, p<0.01 and p<0.01) reduced by treatment with WCME-200, WCME-500, WCHAE-450 and indomethacin (10 mg/kg) on 28<sup>th</sup> day in comparison with disease control group in FCA induced arthritis model respectively. It has been observed in radiological study that ankle joint space of arthritic animals is retained effectively by continuous WCE treatment which may be responsible for enhanced motility behavior of arthritic animals which may lead to less

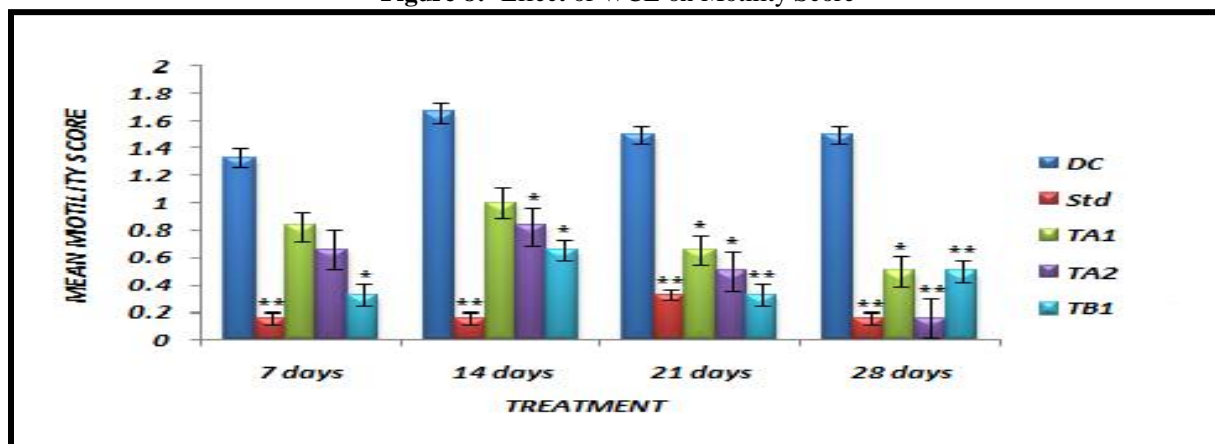
stiffness in joints. Hence it can be proposed that level of motility increases with progression of WCE treatment in FCA induced arthritis.

**Table 6:-** Effect of WCE (200 mg/kg, 500mg/kg and 450 mg/kg) on Pain Perception Parameters

Exp. Grp ↓ Day →	PAIN PERCEPTION PARAMETER										
	DORSAL FLEXION SCORE				MOTILITY SCORE				STAIR CLIMB SCORE		
	7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>	28 <sup>th</sup>	7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>	28 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>	28 <sup>th</sup>
DC	1.16± 0.167	1.833± 0.167	1.50± 0.224	1.50± 0.224	1.33± 0.211	1.667± 0.211	1.50± 0.224	1.50± 0.224	0.833± 0.167	0.667± 0.307	1.00± 0.365
STD	0.33± 0.21*	0.50± 0.22**	0.333± 0.21**	0.167± 0.17**	0.167± 0.17**	0.167± 0.17**	0.333± 0.21**	0.167± 0.17**	2.333± 0.21**	2.667± 0.21**	2.667± 0.21**
TA1	1.00± 0.26	1.167± 0.17	1.00± 0.25	0.667± 0.21*	0.833± 0.17	1.00± 0.26	0.667± 0.21*	0.50± 0.34*	1.50± 0.34	1.667± 0.21	2.00± 0.26*
TA2	0.833± 0.17	1.00± 0.26*	0.667± 0.21*	0.50± 0.22**	0.667± 0.33	0.833± 0.31	0.50± 0.22*	0.333± 0.21**	1.833± 0.31*	2.00± 0.37*	2.333± 0.21**
TB1	0.667± 0.21	0.833± 0.17**	0.333± 0.21**	0.333± 0.21**	0.333± 0.21*	0.667± 0.33*	0.333± 0.21**	0.167± 0.17**	2.00± 0.26*	2.166± 0.31**	2.50± 0.22**

Values are expressed in Mean ± SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, DC: Disease Control, STD: Standard, TA1: WCME-200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-4500 mg/kg

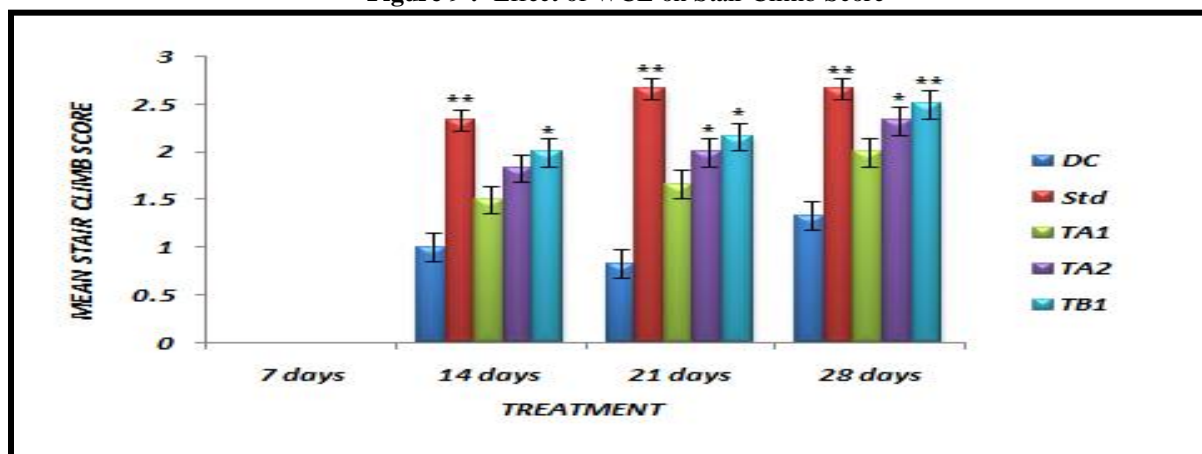
**Figure 8:-** Effect of WCE on Motility Score



Values are expressed in mean ± SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

#### f) Effect of WCE on Stair Climb Score in FCA Induced Arthritis

In present study it has been observed that FCA induced decreased motility score were significantly (p<0.05, p<0.01 and p<0.01) reduced by treatment with WCME-500, WCHAE-450 and indomethacin (10 mg/kg) on 28<sup>th</sup> day in comparison with disease control group in FCA induced arthritis model respectively. As it has been observed in radiological study that ankle joint space of arthritic animals is retained effectively by continuous WCE treatment which may be responsible for enhanced motility behavior of arthritic animals which may lead to less stiffness in joints and more climbing capacity.

**Figure 9 :- Effect of WCE on Stair Climb Score**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

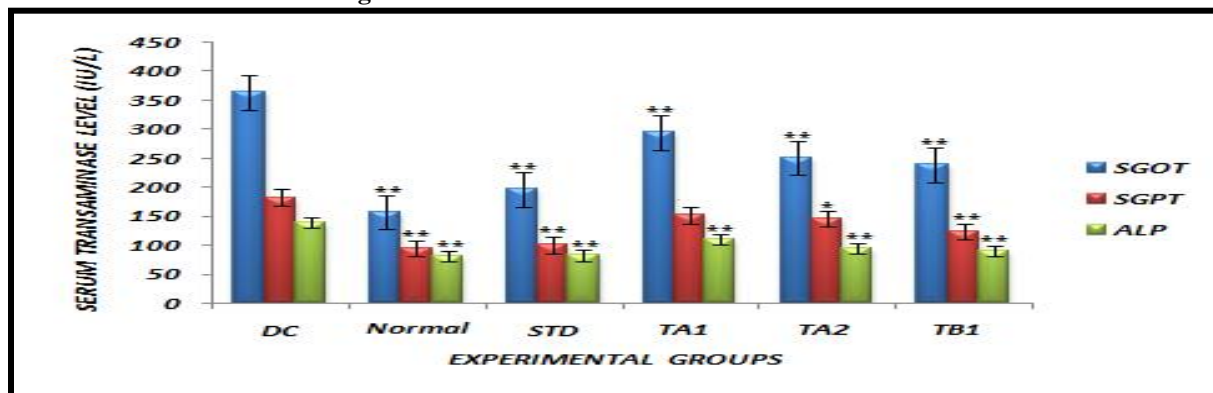
#### g) Effect of WCE on Serum Transaminases in FCA Induced Arthritis

In present study it has been observed that treatment of WCE significantly decreased the raised level of serum transaminases like SGOT, SGPT and ALP. On 28<sup>th</sup> day WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.01, p<0.01, p<0.01 and p<0.01) decrease the increased level of SGOT respectively. WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.01 and p<0.01) decrease the elevated and standard significantly (p<0.05, p<0.01 and p<0.01) decrease the elevated level of SGPT respectively. WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.01, p<0.01, p<0.01 and p<0.01) decrease the increased level of ALP in comparison to disease control group in FCA induced arthritis respectively.

**Table 7:- Effect of WCE (200 mg/kg, 500mg/kg and 450 mg/kg) on Serum Transaminases Level**

Experimental Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
DC	363.956 $\pm$ 7.057	182.706 $\pm$ 8.068	139.852 $\pm$ 14.323
N	156.950 $\pm$ 7.540**	94.291 $\pm$ 1.859**	80.50 $\pm$ 3.222**
STD	196.505 $\pm$ 7.642**	100.370 $\pm$ 5.909**	82.583 $\pm$ 4.286**
TA1	294.587 $\pm$ 23.318**	158.400 $\pm$ 14.423	110.966 $\pm$ 4.295**
TA2	251.066 $\pm$ 10.357**	146.766 $\pm$ 10.075*	95.00 $\pm$ 12.362**
TB1	238.900 $\pm$ 10.488**	123.333 $\pm$ 7.797**	90.85 $\pm$ 3.629**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

**Figure 10:-** Effect of WCE on Serum Transaminases

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

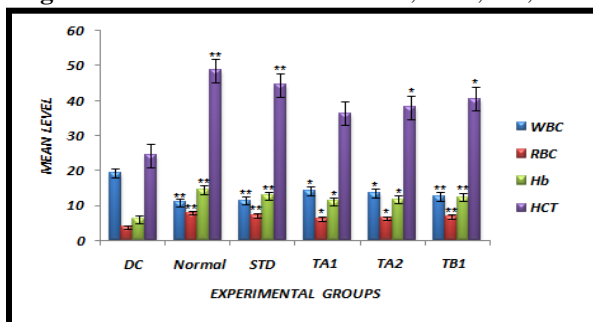
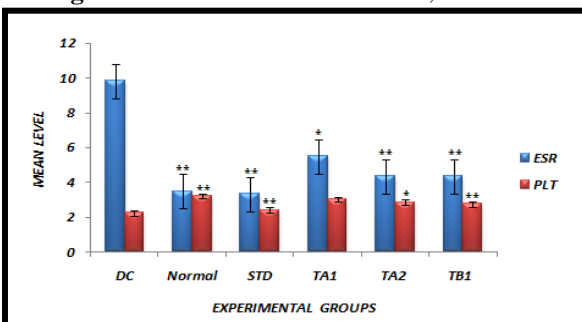
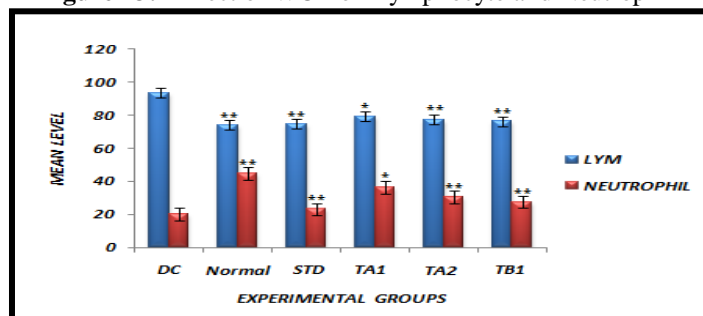
#### **h) Effect of WCE on Hematological Parameters in FCA Induced Arthritis**

In present study it has been observed that treatment of WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.05, p<0.01 and p<0.01) decreased the raised level of WBC(White Blood Cells) count. WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.01, p<0.01 and p<0.01) decreased the raised level of ESR (Erythrocyte Sedimentation Rate). WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.01) decreased the raised level of Platelets(PLT). WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.01, p<0.01 and p<0.01) decreased the raised level Neutrophil (NEU) and Lymphocyte(LYM) to normal level. Whereas treatment of WCME-200, WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.05, p<0.01 and p<0.01) increased the decreased level of RBC (Red Blood Cells) and HB (Hemoglobin) respectively. Treatment of WCME-500, WCHAE-450 and standard significantly (p<0.05, p<0.05, p<0.01) increased the decreased level HCT in comparison to disease control group in FCA induced arthritis.

**Table 7:-** Effect of WCE (200 mg/kg, 500mg/kg and 450 mg/kg) on Hematological Parameters

Exp. Grp	Hematological Parameters							
	WBC (thousand/ cu.mm)	RBC (million/ cu.mm)	HB (gm/dl)	HCT (%)	ESR (mm/hr)	PLT (Lakhs/ ml)	NEU (%)	LYM (%)
DC	19.283 $\pm$ 1.542	3.88 $\pm$ 0.254	6.166 $\pm$ 0.619	24.333 $\pm$ 2.722	9.833 $\pm$ 1.352	3.205 $\pm$ 0.057	43.76 $\pm$ 2.216	93.55 $\pm$ 4.542
N	10.866 $\pm$ 1.263**	7.945 $\pm$ 1.207**	14.55 $\pm$ 2.187**	48.65 $\pm$ 4.29**	3.50 $\pm$ 0.56**	2.233 $\pm$ 0.10**	19.37 $\pm$ 1.057**	73.95 $\pm$ 5.404**
STD	11.48 $\pm$ 1.412**	7.18 $\pm$ 6.193**	12.82 $\pm$ 0.493**	44.52 $\pm$ 4.93**	3.33 $\pm$ 0.49**	2.395 $\pm$ 0.103**	23.41 $\pm$ 2.141**	74.70 $\pm$ 3.266**
TA1	14.11 $\pm$ 1.603*	6.193 $\pm$ 0.302*	11.246 $\pm$ 0.821*	36.33 $\pm$ 2.502	5.50 $\pm$ 4.333*	3.015 $\pm$ 0.085	35.51 $\pm$ 1.547*	79.37 $\pm$ 1.135*
TA2	13.633 $\pm$ 1.076*	6.425 $\pm$ 0.719*	11.80 $\pm$ 1.771*	38.05 $\pm$ 3.831*	4.333 $\pm$ 1.41**	2.85 $\pm$ 0.059*	30.145 $\pm$ 1.893**	77.22 $\pm$ 2.390**
TB1	12.633 $\pm$ 1.171**	6.811 $\pm$ 0.254**	12.366 $\pm$ 0.931**	40.55 $\pm$ 2.440*	4.333 $\pm$ 1.17**	2.756 $\pm$ 0.05**	26.168 $\pm$ 1.319**	76.383 $\pm$ 1.520**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

**Figure 11:- Effect of WCE on WBC, RBC, HB, HCT****Figure 12:- Effect of WCE on ESR, Platelets****Figure 13:- Effect of WCE on Lymphocyte and Neutrophil**

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

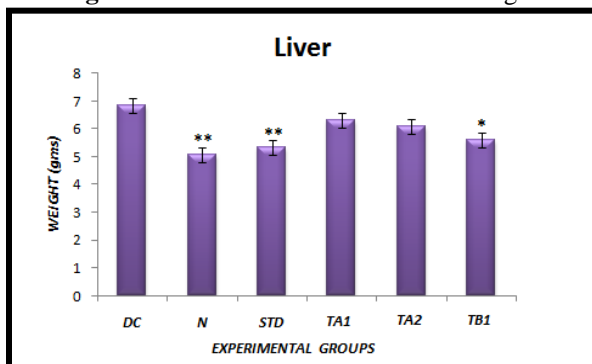
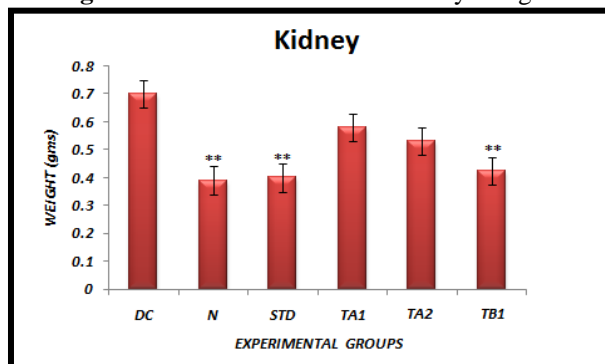
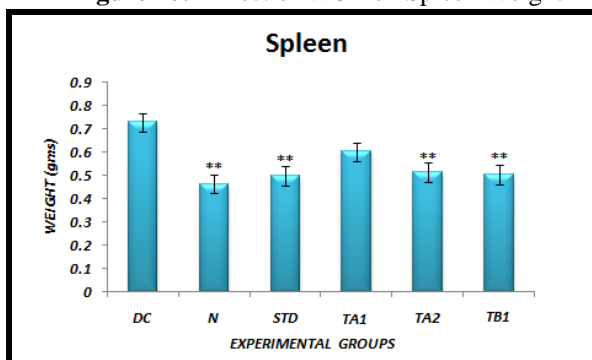
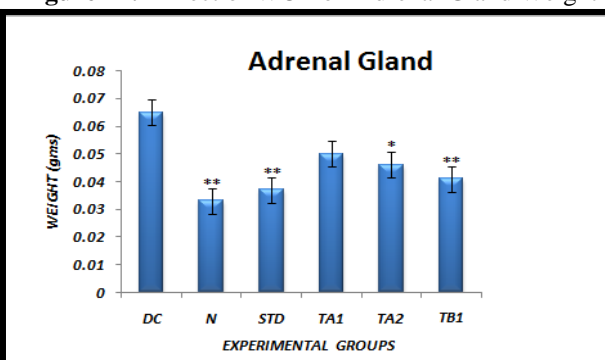
#### i) Effect of WCE on Different Organ Weight in FCA Induced Arthritis

In case of liver arthritic rat shows increase in weight of liver whereas the Indomethacin and WCHAE-450 mg/kg shows significant (p<0.01 and p<0.05) decreased the weight. In case of increased weight of kidney, Indomethacin and WCHAE-450 mg/kg shows significant (p<0.01 and p<0.01) decrease in weight. In case of spleen Indomethacin, WCME-500 and WCHAE-450 mg/kg shows significant (p<0.01, p<0.01 and p<0.01) decrease in weight. Indomethacin, WCME-500 and WCHAE-450 mg/kg showed significant (p<0.01, p<0.05 and p<0.01) decrease in elevated weight of adrenal gland respectively. There were not significant changes observed in liver, kidney, spleen and adrenal gland weight in case of WCME-200 mg/kg.

**Table 8:- Effect of WCE on Different Organ Weight in FCA Induced Arthritis**

Experimental Group	Organ Weight			
	Liver	Kidney	Spleen	Adrenal Gland
DC	6.848 $\pm$ 0.4163	0.70 $\pm$ 0.0318	0.728 $\pm$ 0.0329	0.065 $\pm$ 0.0086
N	5.075 $\pm$ 0.1018**	0.396 $\pm$ 0.0492**	0.463 $\pm$ 0.0449**	0.0333 $\pm$ 0.0042**
STD	5.3183 $\pm$ 0.1742**	0.408 $\pm$ 0.0379**	0.4983 $\pm$ 0.6016**	0.0373 $\pm$ 0.0034**
TA1	6.318 $\pm$ 0.2493	0.585 $\pm$ 0.0592	0.6016 $\pm$ 0.0267	0.050 $\pm$ 0.0025
TA2	6.102 $\pm$ 0.0675	0.533 $\pm$ 0.0323	0.5133 $\pm$ 0.0575**	0.0466 $\pm$ 0.0033*
TB1	5.613 $\pm$ 0.4464*	0.425 $\pm$ 0.0802**	0.5033 $\pm$ 0.0549**	0.0416 $\pm$ 0.0047**

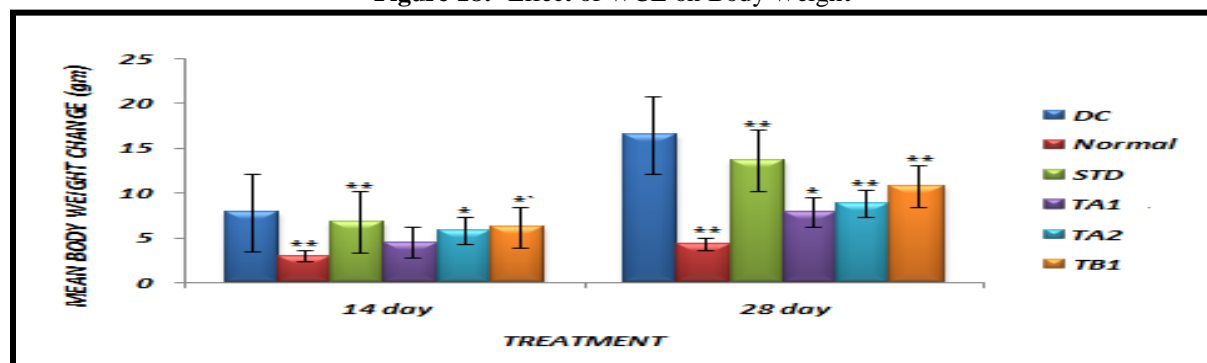
Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

**Figure 14:- Effect of WCE on Liver Weight****Figure 15:- Effect of WCE on Kidney Weight****Figure 16:- Effect of WCE on Spleen Weight****Figure 17:- Effect of WCE on Adrenal Gland Weight**

Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME, TA2: WCHAE

#### j) Effect of WCE on Body Weight in FCA Induced Arthritis

In present study, it is clear from the data obtained that there is close relationship between the extent of joint inflammation and the degree of weight loss. In the first week after adjuvant injection, the arthritic rats showed marked weight loss, followed by normal weight gain in subsequent weeks whereas the WCME, WCHAE and standard drug treated groups did not show any weight loss. The significant ( $p < 0.01$ ) weight gain in the Indomethacin treated rats on 14<sup>th</sup> and 28<sup>th</sup> day. WCME-200 mg/kg treated group rats shows significant ( $p < 0.05$ ) weight gain on 28<sup>th</sup> day whereas the WCME-500 mg/kg and WCHAE-450 mg/kg treated group rats shows significant ( $p < 0.05$  and  $p < 0.01$ ) weight gain on 14<sup>th</sup> and 28<sup>th</sup> day respectively.

**Figure 18:- Effect of WCE on Body Weight**

Values are expressed in mean  $\pm$  SEM (n=6), \* $p < 0.05$ , \*\* $p < 0.01$  vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg



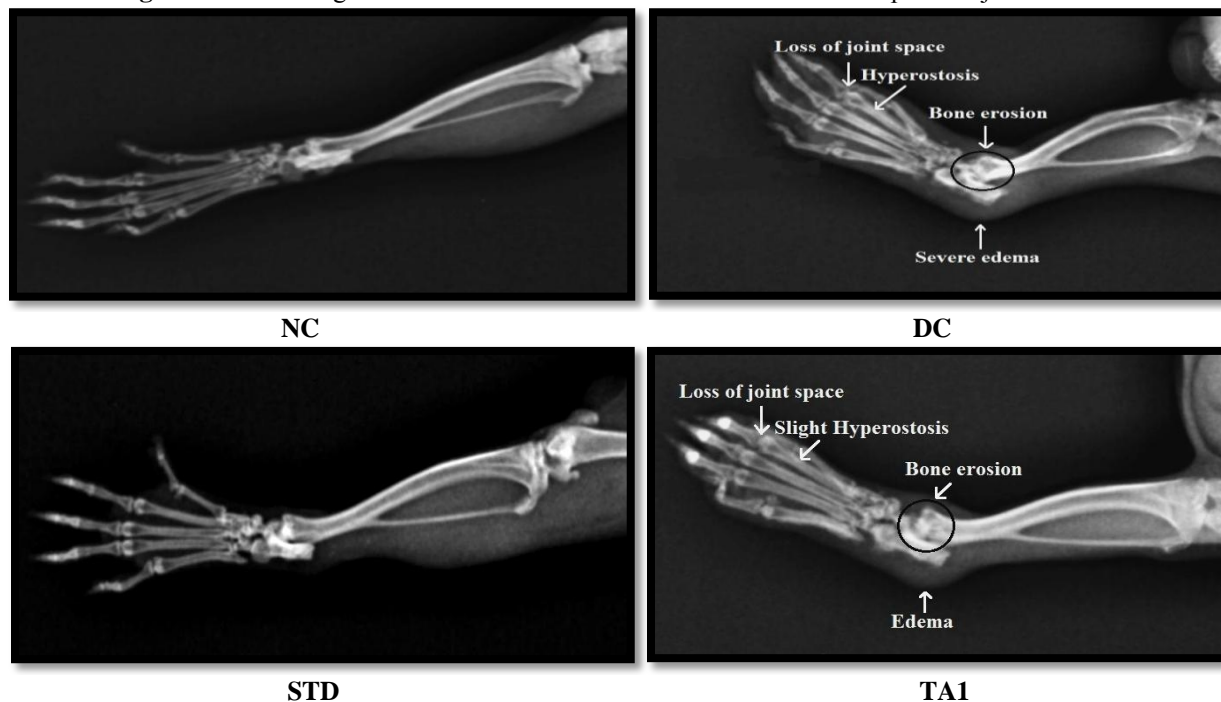
**Table 9:-** Effect of WCE on Body Weight in FCA Induced Arthritis

Experimental Group	Body Weight (gm)				
	0 Day	14 Day	28 Day	Mean Changes 0 Day - 28 Days	Mean Changes 14 <sup>th</sup> day-28 <sup>th</sup> day
DC	184.33± 3.190	187.333± 3.630	190.33± 4.104	4.333± 0.667	3.00± 0.5774
N	185.5± 3.106	194.166± 2.845	202.0± 2.921	16.500± 0.7638**	7.833± 0.3073**
STD	183.0± 3.337	189.83± 3.833	196.66± 3.694	13.666± 0.8819**	6.833± 0.3073**
TA1	183.16± 3.114	186.5± 3.128	191.0± 2.933	7.833± 0.6009*	4.50± 0.6708
TA2	184.33± 3.169	187.33± 3.303	193.16± 3.135	8.833± 0.6009**	5.833± 0.6009*
TB1	182.83± 3.027	187.5± 2.895	193.66± 2.486	10.833± 1.558**	6.166± 1.400*

Values are expressed in mean  $\pm$  SEM (n=6), \*p<0.05, \*\*p<0.01 vs Disease Control group, Data analyzed by One way ANOVA test followed by Dunnet's multiple test for comparison. Treatment of various groups is as follows, N: Normal, DC: Disease Control, STD: Standard, TA1: WCME- 200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

#### k) Radiographic Evaluation in FCA Induced Arthritis

In present study on 14<sup>th</sup> and 28<sup>th</sup> day, radiological assessment of rat paw through X-rays showed significant reduction in various imaging parameters like spur formation, bone erosion, soft connective tissue swelling around ankle joint, calcium deposition and increase in interspacing between the bones in case of ankle joint, in Standard Indomethacin, WCME-200, WCME-500 and WCHAE-450 groups respectively in comparison with disease control group.

**Figure 19:-** Radiological Assessment of Arthritic Joint in Freund's Complete Adjuvant Arthritis





TA2  
NC-Normal control, DC- Disease Control, STD- Standard, TA1- WCME-200 mg/kg, TA2-WCME-500 mg/kg, TB1-WCHAE-450 mg/kg

**Table 10:-** Effect of WCE on Radiographic Evaluation

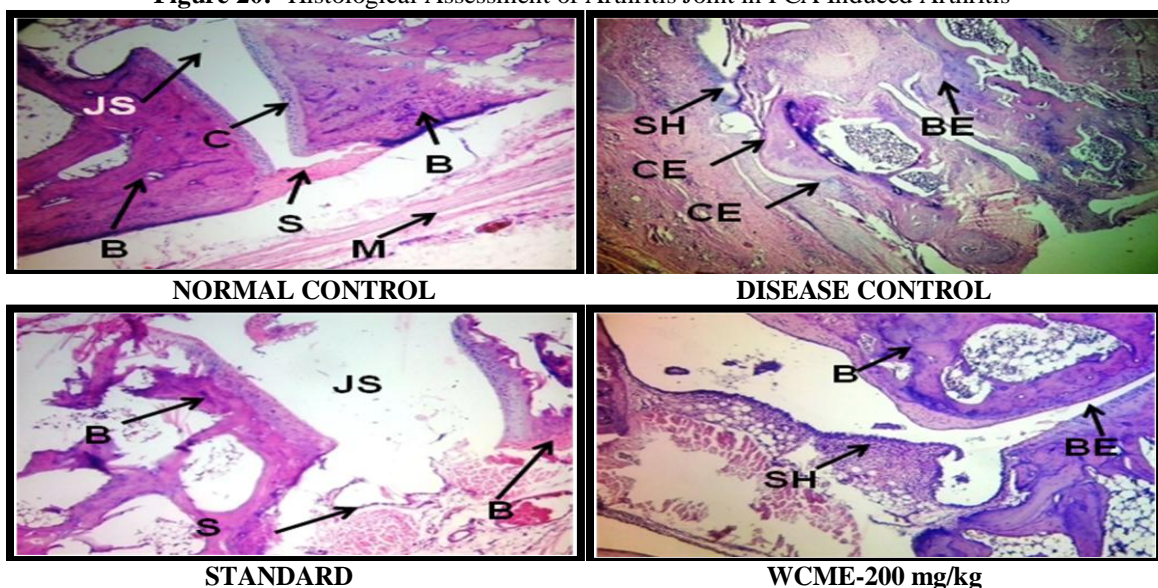
Groups	Dose	X-ray
Normal	Distilled water	No soft tissue swelling with normal joint space
Disease control	CFA 1% w/v	Soft tissue swelling with bone destruction
Standard	Indomethacin 10 mg/kg	Prevented bone destruction and soft tissue swelling
TA1	200 mg/kg	Soft tissue swelling and bone destruction
TA2	500 mg/kg	Less bone destruction and soft tissue swelling
TB1	450 mg/kg	Prevented bone destruction and soft tissue swelling

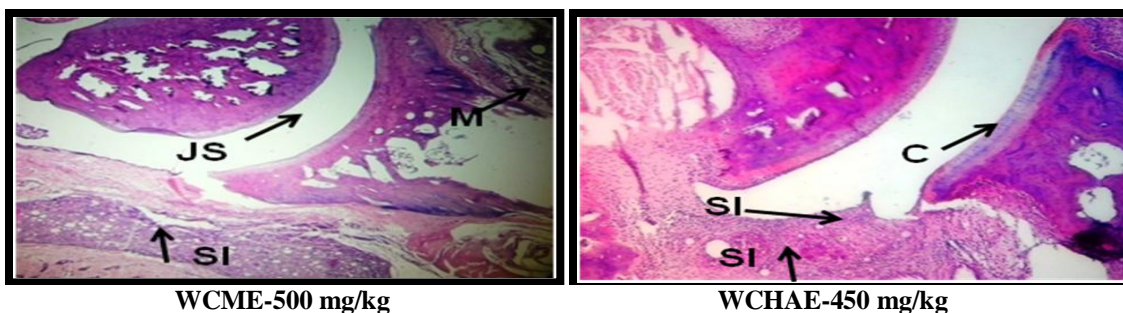
Therefore from above study it was observed that both WCME and WCHA extract treatment shows significant anti-arthritic activity.

#### I) Histological Evaluation in FCA Induced Arthritis

Histopathological studies of left hind paw joints in normal control rats shown intact articular cartilage and normal synovial lining. Distorted articular cartilage, bone erosion, synovial membrane lining cells hyperplasia, and infiltration of the inflammatory cells like lymphocytes and eosinophils were abundant in synovium and joint space in disease control rats. It was found that standard, WCME-500 mg/kg and WCHAE-450 mg/kg showed better retention of joint parameters like very less disruption of synovial cartilage, reduction in synovial hyperplasia, bone erosion, tissue infiltration with lymphocytes and eosinophils. While in case of treatment of WCME-200 mg/kg group, above parameters were found to have slightly more effect in comparison with disease control group.

**Figure 20:-** Histological Assessment of Arthritis Joint in FCA Induced Arthritis





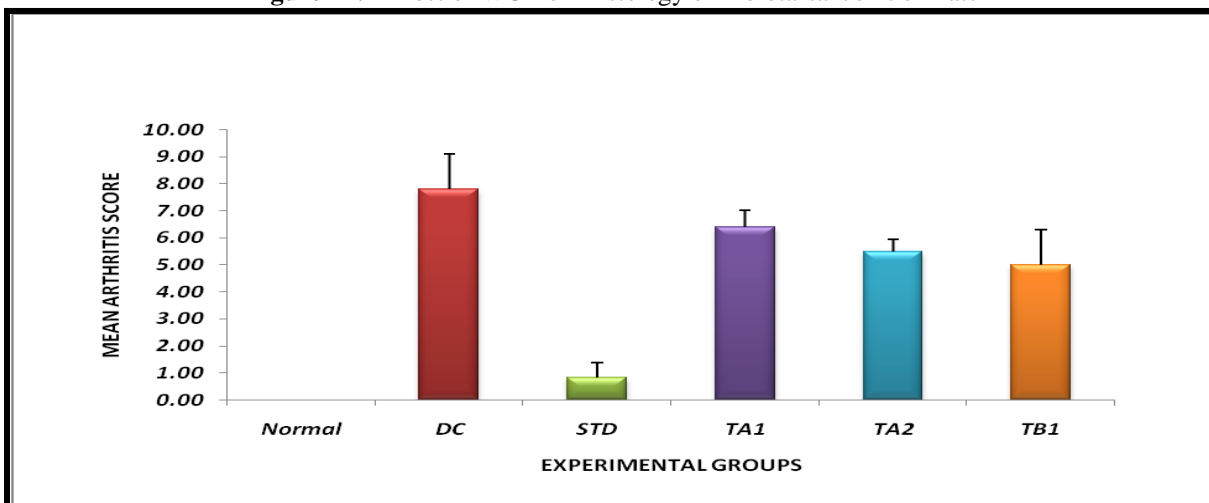
S: Synovium; B: Bone; C: Cartilage; JS: Joint Space; SH: Synovial Hyperplasia; SI: Synovial Infiltration of Inflammatory Cells; BE: Bone Erosion; CE: Cartilage Erosion; I: Infiltration.

**Table 11:-** Effect of WCE on Histology of Tibiotarsal Joint of Rats in FCA Induced Arthritis

Sr. No.	Group	Mean arthritis score ± S.E.M.
1	N	0
2	DC	7.8±1.3
3	STD	0.83±0.5
4	TA1	6.40±0.6
5	TA2	5.50±0.4
6	TB1	5.00±1.3

Values are expressed as Mean ± SEM where n=6. NC-Normal control, DC: Disease Control, STD: Standard, TA1: WCME-200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

**Figure 21:-** Effect of WCE on Histology of Tibiotarsal Joint of Rats



Values are expressed as Mean ± SEM where n=6. NC-Normal control, DC: Disease Control, STD: Standard, TA1: WCME-200 mg/kg, TA2: WCME-500 mg/kg, TB1: WCHAE-450 mg/kg

### Discussion:-

In spite of discovery of several newer agents, the search for better anti-inflammatory drugs continues because of their existing side effects and none of them is suitable for prolonged use. These side effects of the anti-inflammatory drugs are one of the major problems in developing medicine today. Therefore there is a need for development of new and more powerful drugs with fewer side effects. Therefore there are various alternatives like drugs from herbal and animal origin were discovered with few side effects, herbal drugs like *Vertex Negunda*, *Terminalia Arjuna* [17], *Cassia Auriculata* [18] had shown effective anti-inflammatory activity in experimental animal models of inflammation and arthritic condition. In this research work we have primarily focused on the investigation of *Withania Coagulans* dunal fruits extracts against inflammatory and immune system conditions associated with RA.

Rheumatoid arthritis is chronic, relapsing inflammatory and autoimmune multisystem illness that affects the joints. It is characterized by inflammation of the synovial membrane, pain and restricted joint movement, stiffness and swelling of joints.<sup>[19]</sup> The process produce an inflammatory response of synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid and the development of pannus in the synovium. The pathology of disease process often leads to the destruction of articular cartilage and ankylosis of the joints. In RA immune system activates the lymphocytes and chemical messenger like cytokine, TNF, IL-1 and IL-6 are expressed in inflamed area. This deregulation of the immune system may lead to the development of rheumatoid arthritis. Autoimmunity plays a pivotal role in both its chronicity and progression.

#### **In Vivo Anti-Arthritic Potential of WCE :-**

##### **Complete Freund's Adjuvant Induced Arthritis in Rats:-**

The evaluation of WCE for anti-arthritic potential was done with CFA model which has been used to induce an arthritic immunopathological condition that displayed many pathological features of human RA.<sup>[19]</sup> Freund's adjuvant arthritis can be produced in rats by administration of *Mycobacterium Tuberculosis* suspended in oil in sub-plantar region of rat paw. Bacterial peptidoglycan and muramyl dipeptides are responsible for arthritic condition. It occurs through cell mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycan in rats.<sup>[20]</sup> Liberation and overproduction of bradykinin, prostaglandin, and kinin in paw tissue. This model is also involves pannus formation, neutrophil infiltration and bone erosion. Arthritis typically persists during the first two week after its appearance.<sup>[21]</sup> Severely affected animals eventually develop chronic deforming joint lesion.

In FCA induced arthritis it was observed that WCHAE than WCME significantly inhibited the arthritis index score, paw edema volume, joint area and pain perception parameters like dorsal flexion and motility along with rise in stair climb score in comparison with disease control for 28 days. After 14<sup>th</sup> day in FCA prominent inhibition was observed in all parameters. The swelling has been found to be increasing in the initial phase of inflammation and then becomes constant in two weeks. The change in paw volume has been found to be associated with an increase in granulocyte and monocytes. Because, the activation of macrophages results in the production of several cytokines including IL-1, IL-6, interferon- $\gamma$  and TNF- $\alpha$  which have been implicated in immune arthritis.

Tissue damage was assessed by measuring the activity of enzyme in the serum. Liver impairment is also one of the features of adjuvant arthritis. The increase in the aminotransferase is due to their release from the cells of the damaged organ. Alkaline phosphatase activity has been reported to increase during the morphological and functional development of tissue. Thus ALP, SGOT, SGPT are good indices of liver and kidney impairment which is also considered as feature of adjuvant arthritis. To evaluate liver function, SGOT and SGPT levels were estimated.<sup>[22]</sup> SGOT and SGPT has been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinin in inflammatory process confirmed a positive correlation between the increased activity of serum alkaline phosphatase and disease activity of RA.<sup>[23]</sup> Amino transferase and alkaline phosphatase were significantly reduced by WCHAE than WCME in arthritic rats. This reducing effect may be related to their anti-inflammatory and anti-arthritic effect. The decreased enzyme levels on WCME and WCHAE treatment emphasized decreased bone loss and organo protective role of WCME and WCHAE in CFA induced rats.

As the severity of arthritic condition increases level of inflammation rises in patient of RA. Along with rise in inflammation, level of WBC's also increases by virtue of autoimmunity. Hence the measurement of WBC's is an effective tool to assess the severity of disease state in RA patients. As a part of WBC's, B cells in patients with rheumatoid arthritis are usually secrete large amount of auto-antibodies which can enhance tissue destruction and release of auto-antigens, indicating that B-cells are also critically important in the severity and length of disease.<sup>[24]</sup> The rise in WBC count is due to release of IL-1B increase the production of monocyte and colony stimulating factor.<sup>[25]</sup>

WBC count increase in arthritic rat to kill invading pathogenic microorganism. 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.<sup>[26]</sup> It was observed that WCHAE than WCME significantly restored the increased WBC count to the normal in comparison to disease control group which may be possibly due to inhibition of matured bone marrow dendritic cell growth which might be responsible for innate immunity. This is probably due to inhibition of matured bone marrow dendritic cells, responsible for development of innate immunity and T-cell cytotoxicity by PLA<sub>2</sub>. The increase in WBC count is an indication of increased

autoimmunity and level of inflammation along with anemic condition of animal due to decrease in RBC, HB and HCT as iron is deposited in connective tissue.

Anemia arises due to abnormal storage of iron in reticuloendothelial system and synovial tissue due to which RBC count, HB count and HCT value decreases below normal level.<sup>[27]</sup> It was observed that RBC, Hb and Hematocrit values significantly increased in WCHAE than WCME, which might be due to the matured bone marrow cells respond to anemic condition by preventing abnormal deposition of iron in reticuloendothelial system and synovial tissue.

The increase in platelet count might be due to the stimulation of immune system against the invading pathogenic microorganism. This is evident by the infiltration of inflammatory mononuclear cells in the joint of arthritis rat.<sup>[28]</sup> T-lymphocytes have been reported to play a central role in the pathogenesis of rheumatoid arthritis. These cells comprise the majority of the lymphoid cells found in the rheumatoid synovium. In arthritic condition there is a moderate elevation in lymphocyte count. In all treated groups the lymphocyte count was suppressed significantly as compared to control. ESR is an estimate of the suspension stability of RBC's in plasma, related to the number of size of red cells and to the relative concentration of plasma proteins especially fibrinogen and the  $\alpha$  and  $\beta$  globulins. The acute phase proteins in ESR share the property of showing elevations in the concentration in response to stress or inflammation that occurs like infection, injury, and surgery and tissue necrosis. So in arthritic condition, ESR is elevated.<sup>[13]</sup> All the treated group significantly reduced the elevated level of ESR in comparison to disease control group.

In a chronic inflammatory process, such as RA, large numbers of neutrophils are attracted across the synovial membrane, and become activated. Neutrophils possess a range of potent proteinases and hydrolases, and have the ability to generate a series of reactive oxygen intermediates (ROI) via the combined activities of NADPH (reduced form) oxidase and myeloperoxidase (MPO).<sup>[29,30]</sup> If the neutrophils are not efficiently depleted, their production of inflammatory mediators, such as ROI, could prolong the inflammatory reaction. In fact, inappropriate release of ROI from activated neutrophils is responsible for joint damage observed in RA.<sup>[31]</sup> Beside ROI and inflammatory mediators, neutrophils are also an important source of proteolytic enzymes which play a role in degradation of articular structures. In present study all the treated group significantly reduced the elevated level of neutrophils in comparison to disease control group.

The changes in the body weight were monitored as apparent indicator of arthritic symptoms and the loss of body weight usually began to appear at the onset stage of arthritis. In the present study, the body weight decreased in arthritis induced animals might be linked to the systemic or local action of cytokines such as TNF-  $\alpha$ , since TNF-  $\alpha$  has been closely related to the loss in body weight occurring in animals suffering from chronic inflammation. The loss of body weight observed in CFA induced arthritic rats may be due to the reduced absorption of  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -leucine in the rat intestine. WCHAE than WCME showed significant increase in body weight reveals the restoration of absorption capacity of the intestine in the arthritic animals.<sup>[32]</sup>

In adjuvant treated rats an increase in lipid peroxide level was seen in liver and kidney. Liver and kidney weight were increased in AIA rats. WCHAE than WCME alter these values near to normal. Spleen is a vital organ serves as the reservoir for the cells and antibody formation which involved in the immune response. In adjuvant arthritis, spleen Increased in the weight of spleen is associated with the splenomegaly, generalized lymphadenopathy and altered hepatic function. In disease control group weight of spleen was significantly increased.<sup>[15]</sup> In case of spleen and adrenal gland weight both of this result were significant in WCHAE than WCME treated rats.

Radiological changes in RA condition are useful diagnostic measure which indicates the severity of disease. Soft tissue swelling is the earlier radiographic sign, whereas the prominent radiographic changes like bone erosion narrowing of joints spaces can be observed only in the developed stages of arthritis. WCHAE than WCME groups X- rays showed significant reduction in various imaging parameters like hyperostosis, bone erosion, soft connective tissue swelling around ankle joint and increase in interspacing between the bones in case of ankle joint in comparison with disease control group.

In present study histological assessment of joint showed very less disruption of synovial cartilage, reduction in synovial hyperplasia, bone erosion, tissue infiltration with lymphocytes and eosinophils in case of WCME, WCHAE and standard treatment groups in comparison with disease control group in FCA induced arthritis.

Thus, to summarize, WCME and WCHAE of fruits showed significant anti-arthritic and immunosuppressive activities probably by virtue of an underlying antioxidant activity.

### Conclusion:-

- WCME oral treatment at both the doses- 250 mg/kg and 500 mg/kg demonstrated significant anti-arthritic activity.
- WCME-500 mg/kg provides more significant anti-arthritic activity and is comparable to the standard drug indomethacin.
- Whereas the WCHAE-450 mg/kg shows highly significant anti-arthritic activity and is comparable to the standard drug indomethacin.

Phytochemical constituents like flavonoides, alkaloids, saponin, glycosides, steroids and withanolides were already reported to anti-inflammatory, immunosuppressant and anti-arthritic activity and these constituents were present in both WCME and WCHAE. Hence these chemical constituents can be accounted for the observed anti-arthritis activity.

The present study aims to bring about value addition to the health benefits of *Withania Coagulans* dunal fruits establish it as a potent “functional food” and promote its use in people’s diets. Besides, it is cheap, readily available to all strata of society, with medicinal properties attributed to it. Future research may be carried out to isolate the active constituents and bioactivity evaluation may be carried out.

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