



Haematological Assessment of Methanolic Stem Extract of *Cissus quadrangularis*Linn. against Freund's Complete Adjuvant induced Female Albino Arthritic Rats

S. Shamina^{*1}, Ms. Anjana²and S. Suja³

¹Head and Associate Professor, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India.

²II M.Sc Biochemistry, Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India

³Professor and Head, Department of Biochemistry, Bharathiar University, Coimbatore-641046, Tamilnadu, India.

Abstract: Rheumatoid arthritis is a chronic, inflammatory and autoimmune disorder. Rheumatoid arthritis is a joint destruction which occurs due to increased expression of cytokines and transcription factors. *Cissus quadrangularis* belongs to the Vitaceae family. The plant shows anti-osteoporotic, anti-inflammatory and anti-microbial properties. Soxhlet method was used for the extraction of the plant using methanol as a solvent. The anti-arthritis activity of methanolic extract of *Cissus quadrangularis* was evaluated by in vivo study. The present study assess the haematological parameters in female albino rats after the induction of Freund's Complete Adjuvant. Dexamethasone was used as the standard reference drug. The study showed varied changes in the haematology after the arthritic induction by 0.1 ml FCA which were analyzed before and after the treatment period. The inhibition of secondary inflammation in adjuvant arthritic rats by methanolic extract of *Cissus quadrangularis* was studied by haematological parameters. The total haemoglobin, total RBC, total WBC, ESR and RF values were assessed before initiation of the treatment and after for 28 days of the treatment. The results showed significant incline or decline in each parameter in the study period of 28 days. While their changes were rectified with the oral administration of methanolic extract of the plant at a dose of 250mg/kg. The methanolic extract boosted the levels of Hb, RBC and simultaneously reduced WBC, ESR levels along with the serological factor RF. The plant extract showed effective results in the haematological parameters, when compared to the standard reference drug Dexamethasone. The results from the study suggest that the plant has potential effect in curing arthritis.

Keywords: *Cissus quadrangularis*, arthritis, FCA, Haematological parameters, Dexamethasone.

*Corresponding Author

S. Shamina , Head and Associate Professor, Department of Biochemistry, RathnavelSubramaniam College of Arts and Science, Coimbatore- 641402, Tamilnadu, India.



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1. INTRODUCTION

Rheumatoid arthritis is a systemic, inflammatory and chronic autoimmune disorder, with the characteristics of articular inflammation and rheumatoid pannus which leads to the bone destruction and cartilage damage^{1,2}. Early reports states that 1% of the world's adult population is affected with this disorder and it is predominant in menthan in women within the age group of 30-55. The main symptoms of this disorder are joint pain, joint inflammation, and synovial tissue proliferation³. The major causative agents for the disorder is still unknown. The main problem in rheumatoid arthritis is joint destruction which occurs due to increased expression of cytokines and transcription factors^{4,5}. Genetic, epigenetic, gender, environment and lifestyles are some of the factors which are considered as precursors for the disease⁶. The development of auto-antibodies, immunoglobulin G (IgG) such as rheumatoid factor (RF) and citrullinated proteins are characterized for RA development⁷. The pathogenic changes are induced in the synovial area, by increasing the activation of macrophages. The increased macrophage activity releases pro-inflammatory cytokines, chemokines and collagen-degrading proteases (matrix metalloproteinases)^{8,9}. *Cissus quadrangularis* is a tender herb which belongs to Vitaceae family. It is fleshy and cactus in nature¹⁰. It is known as adamant creeper, square stalked vine, veldt grape, devil's backbone, asthisamharaka, hadjod, pirandai, sannalam, nalleru, vajravelli and mangaravalli¹¹. It is a native plant India, Bangladesh and Sri Lanka. The whole plant is used for various medicinal purposes¹². It is known for being cultivated in plains, coastal areas, jungles and wastelands mainly up to 500m altitude. The plant always flowers from the month of June-December. The stem occurs in varying sizes, in a quadrangular shape. The leaves are simple, long and at times round. The plant can be propagated using cuttings¹³. *Cissus quadrangularis* is used as an alternative



Fig 1. (*Cissus quadrangularis*)

2.3 Preparation of plant extract

The fresh stems of the plant were collected as shown in Fig.1 and prepared into thin slices and were shade dried. The thin slices of the stem were ground to a fine powder using a mixer grinder as shown in Fig.2 and stored in neat and clean plastic containers without any moisture content in it. The powdered material of the plant was extracted through Soxhlet method with methanol as a solvent. 20grams of plant powder was taken in the Soxhlet apparatus and 200ml of methanol was taken for the extraction procedure. The setup was maintained in 50-60°C for 2 hours. Once the

medicine for treating disorders like piles, anorexia, indigestion, chronic ulcers, otorrhoea, and wounds. The plant is considered to have the curative property of fracture healing and has an abundant source of calcium¹⁴. In Ayurveda, it is known as bone setter, due to its potential to join bones. The plant also shows properties like anti-osteoporotic, anti-inflammatory and anti-microbial¹⁵. The plant is used for the treatment of venereal diseases, gout, piles, leucorrhoea and syphilis in the traditional medicinal system. While in Siddha, the use of the plant is believed to heal broken bones, as an analgesic and as a tonic¹⁶. The present study was carried out to examine the antiarthritic efficiency of the methanolic extract of *Cissus quadrangularis* against the Freund's complete adjuvant induced female albino rats. The activity was studied by examining the animal behaviour during the period of study along with the haematological parameters.

2. MATERIALS AND METHODS

2.1 Chemicals and instruments

Methanol, Freund's complete adjuvant, Sodium lauryl sulphate, Diethyl ether, EDTA and all the required chemicals were purchased from Himedia, India. Soxhlet apparatus was used for the extraction of the methanolic extract of *Cissus quadrangularis*. The haematological parameters were assessed using Blood Cell Counter CD1700 and Nephelometry.

2.2 Collection of the plant

The fresh stem of *Cissus quadrangularis* was collected from Kalampalayam Coimbatore, Tamil Nadu during September and October, 2019. The plant was identified and confirmed by the Department of Botany, Kongunadu Arts and Science College, Coimbatore.



Fig 2. Stem powder (*Cissus quadrangularis*)

extract of the plant is prepared, it is kept in a boiling water bath under 70-80°C for 15mins to evaporate the solvent from the extract. The dried extract was stored in clean and sterile capped tubes in the refrigerator for further use.

2.4 Experimental animals

Healthy adult female albino rats weighing about 150 to 200 grams of 3 months age were being purchased from the Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The ethical approval was got for the animals, the Ref:RVSCOPS/CPCA/IAEC/Ph.D/

22/2018-19. Rats that were kept in animal room in RVS College of pharmaceutical sciences, Sulur, Tamilnadu, India. The rats were placed in large polypropylene cages with the stainless steel top grill that was given with the pelleted food and water. The paddy husk was used as the bedding material and it was changed twice in a week. The animals were

maintained in 12 hrs, light and dark cycle at 28°C ±2° C in a ventilated animal house under natural conditions and they were acclimatized to the laboratory conditions for about 10 days prior to the commencement of the experiment. The animals were fed with a standard pellet diet given water and maintained under standard laboratory conditions.

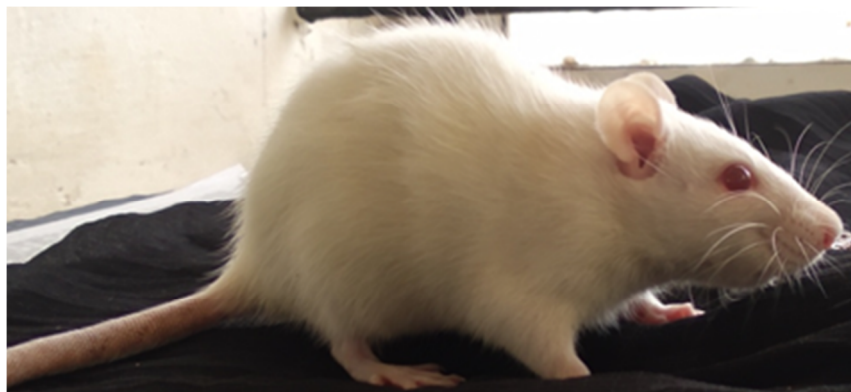


Fig 3. Abino Rat

2.5 Experimental Design

The animals weighing 150 grams were randomly divided into four groups with six animals each, serving as normal (non-arthritic), arthritic control, arthritic treated with plant

extract and arthritic reference control, that is dexamethasone treated. Dexamethasone was given at a dose of 0.5mg/kg of body weight¹⁷. The oral administration of methanolic extract of *Cissus quadrangularis* (250 mg/b.w) was continued once daily at the same time for 28 days.

Table I: Experimental Groups	
GROUP I	Control group received a normal pellet diet with 1 ml of 1% sodium lauryl sulphate (SLS) for 28 days.
GROUP II	Arthritis was induced by Freund's Complete Adjuvant (0.1ml) and those rats received a normal pellet diet with 1.0 ml of 1% sodium lauryl sulphate (SLS) for 28 days.
GROUP III	Arthritis induced rats by Freund's Complete Adjuvant (0.1ml) were treated with methanolic extract of <i>Cissus quadrangularis</i> (250mg/Kg body weight) prepared in 1% of sodium lauryl sulphate (SLS) were administered orally at the rate of 1.0ml/rat/day for 28 days.
GROUP IV	Arthritis induced rats by Freund's Complete Adjuvant (0.1ml) were treated with Dexamethasone at a dose of (0.5 mg/kg) were administered orally prepared in 1 % of sodium lauryl sulphate (SLS) at the rate of 1.0 ml/rat/day for 28 days.

2.6 Collection of blood sample

Blood samples were collected from the animals via retina puncture by first anesthetizing with diethyl ether. The blood was collected into the centrifuge tubes. 2ml of blood was collected from each rat per group, from which 1ml of blood

was collected in tubes containing EDTA and the other 1ml was collected to separate serum from it. The blood was collected on the 28th day. The blood samples collected without EDTA were centrifuged at 4000 rpm for 20 minutes at 22°C and the plasma samples were frozen up at -4°C until biochemical estimations.



Fig 4. Blood Sample



Fig 5. Blood collection

2.7 Hematological Assays

The estimation of hematological parameters like RBC, Hb, WBC, and ESR were performed by automatic hemocytometer (Blood Cell Counter CD1700). While the serological factor RF level was measured using Nephelometry method.

3. STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM for six rats in each group (n=6). The above estimations were analyzed by

one-way ANOVA followed by post hoc Duncan multiple range test. P<0.05 was considered as statistically significant.

4. RESULTS

The efforts to develop safer and effective treatments for rheumatoid arthritis are based on an improved understanding of the role of inflammatory mediators that are being investigated with the development of biologic agents of therapeutic approach to RA and inflammatory diseases. The antiarthritic effect of *Cissus quadrangularis* against Arthritic induction with FCA in animal model shows the effectiveness of *Cissus quadrangularis* against Rheumatoid arthritis.

Group/Treatment	Before Treatment	After Treatment(28 th day)
Group 1	12.73±0.40	12.92±0.40 ^{b,c,d}
Group 2	13.21±0.30	10.25±0.32 ^{c,d}
Group 3	13.64±0.33	11.08±0.35
Group4	12.70±0.36	11.44±0.34

Values are expressed as mean ± SEM, n = 6 and were estimated by one-way ANOVA followed by post hoc DMRT. P<0.05 was considered as statistically significant. The statistical comparison a refers to Group 1, b refers to Group 2, c refers to Group 3 and d refers to Group 4.

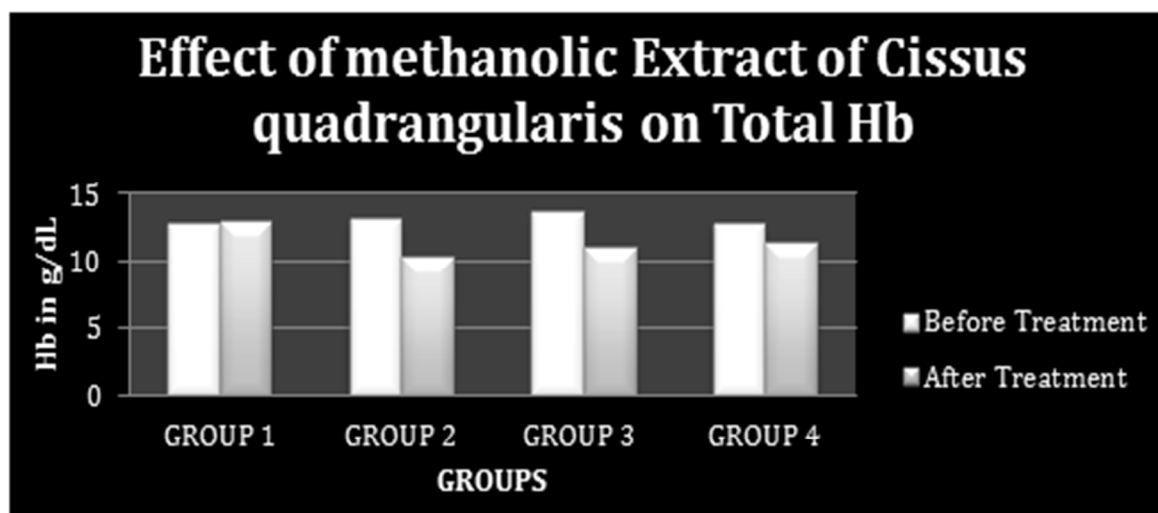


Fig 6. Effect of methanolic extract of *Cissus quadrangularis* on Total Hb

The values of Table 2 gives the total Hb level in the groups 1, 2, 3 and 4 before the treatment. Whereas, after the treatment period, there is a statistically significant difference between the groups, where the plant treated group is similar to the standard drug as shown in Table 2 and Fig.6. When the Total Hb values were compared on the treatment basis, the group 2 rats showed a significant decrease in Hb level compared to that of group 1 rats, While the group 3 treated with the methanolic extract showed a significant decrease from group 1 rats. Similarly group 4 administered with standard drugs showed a decrease compared to group 1. The FCA induced rats showed a significant decrease in the hemoglobin levels is due to the toxicity increased after the induction of FCA the levels of Hb significantly decreased in all

the group 2, 3 and 4 rats. The toxicity was reduced by administering methanolic extract of *Cissus quadrangularis* in group 3 and standard drug dexamethasone in group 4. The treatment values showed the drug has the ability to rectify the toxic effect of FCA by improving the Hb values in the rats significantly. It was reported that in the arthritic condition, the hemoglobin level decreased which is associated mainly with anemia. Anemia is due to reduced erythrocyte deformability. The reduced deformability leads to shortened life span of RBC, a marker of rheumatoid arthritis, upon treatment the level reverted nearing normal, which is in concordance with Anti-inflammatory effect of stevioside that when stevioside lead to rise in haemoglobin levels which was decreased by FCA induction.¹⁸

Group/Treatment	Before Treatment	After Treatment(28 th day)
Group1	7.48±0.41	8.11±0.34
Group2	7.64±0.40	6.37±0.58 ^{a,c,d}

Group3	8.08±0.35	7.37±0.40 ^a
Group4	7.99±0.41	7.63±0.35

Values are expressed as mean ± SEM, n = 6 and were estimated by one-way ANOVA followed by post hoc DMRT. P<0.05 was considered as statistically significant. The statistical comparison a refers to Group 1, b refers to Group 2, c refers to Group 3 and d refers to Group 4.

The total RBC values of group 1, 2, 3 and 4 are shown in the Table 3 that were found before treatment. After treatment the group's statistical difference seems to differ significantly. Here group 4 is similar to group 3 rats. When these values are compared on the basis of treatment the total RBC level shows a significant decline in the group 2 rats compared to group 1 rats. While, the group 3 and group 4 rats shows decrease in the levels of RBC compared to Group 1. The total red blood cell count seemed to decline in the significant manner after the induction of arthritis by FCA in groups 2, 3

and 4. The effect of FCA increased the inflammation in the rats which caused the significant decrease in the RBC levels of the rats. This decrease was treated with the plant extract and the standard drug in the group 3 and group 4 respectively. The initial decrease in the RBC levels were significantly increased by the treatment in group 3 and nearing normal in group 4 rats. The results were similar with subsequent reduction in the number of red blood cells in Anti-inflammatory effect of stevioside abates Freund's complete adjuvant (FCA)-induced adjuvant arthritis in rats.¹⁸

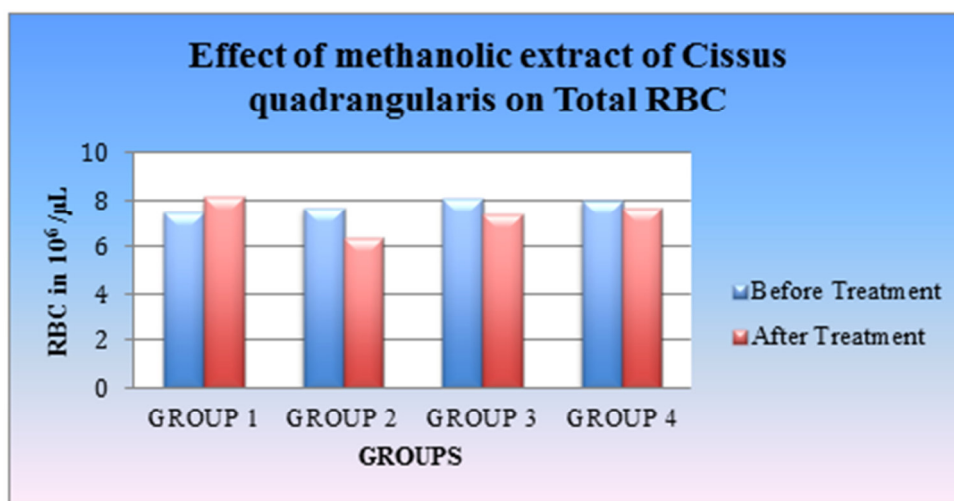


Fig 7. Effect of methanolic extract of Cissus quadrangularis on Total RBC

Group/Treatment	Before Treatment	After treatment(28 th day)
Group1	5.05±0.32	5.49±0.22 ^{b,c}
Group2	4.98±0.28	6.54±0.35 ^{c,d}
Group3	5.41±0.24	6.04±0.22 ^d
Group4	5.50±0.21	5.50±0.33

Values are expressed as mean ± SEM, n = 6 and were estimated by one-way ANOVA followed by post hoc DMRT. P<0.05 was considered as statistically significant. The statistical comparison a refers to Group 1, b refers to Group 2, c refers to Group 3 and d refers to Group 4.

The total WBC values of group 1, 2, 3 and 4 are shown in the Table 4 that were found before treatment. The animals after treatment category showed a significant difference in the groups where group 4 is similar to group 1 as shown in Table 4 and Fig 8. Comparison based on the treatment showed a significant increase in the WBC count after the FCA induction in the group 2 rats compared to group 1 rats. Whereas, the group 3 rats showed an increase compared to group 1. The group 4 level was rectified back to the initial level after the treatment. The total white blood cell count is observed to increase in all the rats in group 2, 3 and 4 after the induction of FCA with increase in inflammation. The

group 2 rats show increase in the WBC levels, whereas the other two groups control the increase with the administration of the plant extract and standard drug. The treatment shows the control of the significant reduction in the WBC count in group 3 and the values reverted normal in group 4 rats compared to group 1. The biomarker for defense, white blood cell count is distinctly increased in arthritis induced models was reduced by the Leaves and stems extracts of Capparis erythocarpos,¹⁹The results were similar to the results obtained for Cissus quadrangularis on Total WBC.

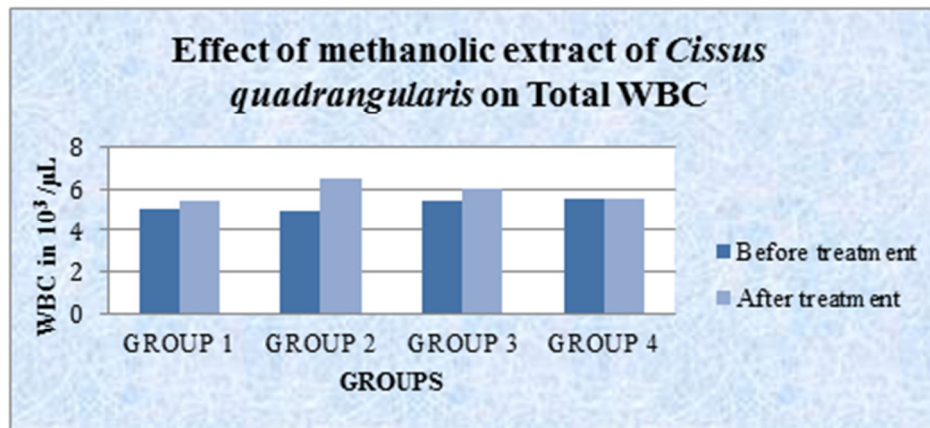


Fig 8. Effect of methanolic extract of *Cissus quadrangularis* on Total WBC

GROUP/Treatment	Before treatment	After treatment(28th day)
GROUP1	13.30±0.37	13.53±0.14
GROUP2	13.47±0.22	15.39±0.40 ^{a,c,d}
GROUP3	13.59±0.19	13.48±0.37
GROUP4	13.44±0.33	13.49±0.19

Values are expressed as mean ± SEM, n = 6 and were estimated by one-way ANOVA followed by post hoc DMRT. P<0.05 was considered as statistically significant. The statistical comparison a refers to Group 1, b refers to Group 2, c refers to Group 3 and d refers to Group 4.

Erythrocyte sedimentation rate levels of group 1,2,3 and 4 are shown in Table 5 in the before treatment category. Table 5 and Fig.9 states that the groups in the after treatment category shows a significant difference in the rats of group 3 and 4 compared to group 2 rats. The treatment basis comparison shows that the ESR level inclined in the group 2 compared to group 1. In group 3 the levels showed a slight decline compared to group 2 and group 4 showed a minute variation in the level compared to rats of group 2. The ESR levels were observed to incline in a rapid way after the FCA induction with the increase in toxicity level. This was

observed in the group 2 rats as the ESR levels were increasing rapidly. The groups 3 and 4 undergone with treatment rectified this increase by reducing the toxic effect of FCA thereby reducing the ESR levels. The results coincide with Anti-inflammatory and Anti-Arthritic Activity of Nanocurcumin that indicated that ESR level significantly amplified in the arthritic key group, that proves the consequence to quickened configuration of protein like fibrinogen. It determines the flow in ESR as a powerful step that indicates the complicated disease manner, concluding ESR as an lab index of an inflammatory performance.²⁰

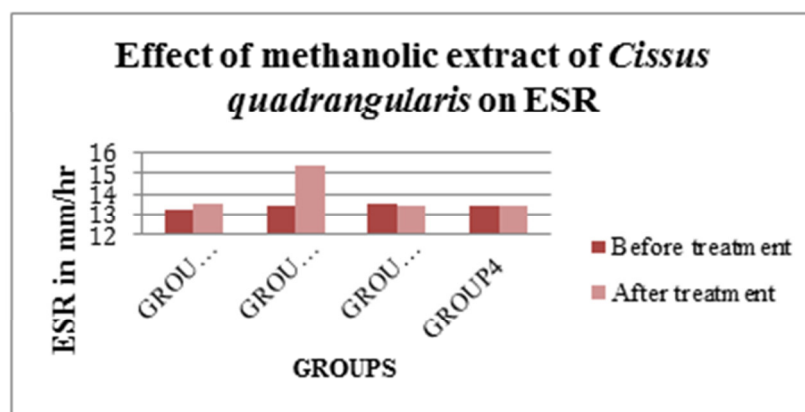


Fig 9. Effect of methanolic extract of *Cissus quadrangularis* on Erythrocyte sedimentation rate (ESR)

GROUP/Treatment	Before Treatment	After treatment(28 th day)
GROUP1	11.11±0.39	11.37±0.26 ^{b,c,d}
GROUP2	11.43±0.25	18.53±0.34 ^{c,d}
GROUP3	11.54±0.31	13.88±0.25 ^d
GROUP4	11.97±0.24	12.92±0.29

Values are expressed as mean ± SEM, n = 6 and were estimated by one-way ANOVA followed by post hoc DMRT. P<0.05 was considered as statistically significant. The statistical comparison a refers to Group 1, b refers to Group 2, c refers to Group 3 and d refers to Group 4.

The RF value found before the treatment for the rats of group 1,2,3 and 4 are shown in Table 6. While after treatment, the category group showed a significant difference between the groups as shown in Table 6 and Fig10. The RF compared on the basis of treatment showed a rapid increase in the group 2 when compared to group 1 rats. The group 3 and group 4 rats showed an increase in RF level compared to group 1 rats. The induction FCA in the group 2, 3 and 4 rats increased the inflammatory response which in turn increased

the levels of rheumatoid factor in the rats. Thus the arthritic rats showed a continuous increase in the RF levels and the group 3 and 4 rats controlled this increase with the treatment of the plant extract and standard drug, which inhibited the continuous incline in the level. The result is similar to that of Anti-arthritic property of crude extracts of *Piptadeni astrumaffricanum* which showed significant increase in RF level due to arthritic induction.²¹

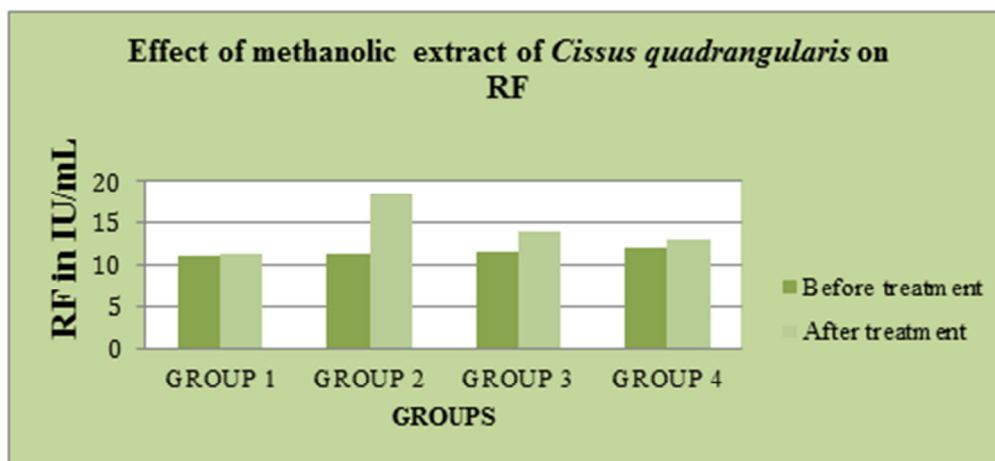


Fig 10. Effect of methanolic extract of *Cissusquadrangularis* on Rheumatoid Factor (RF)

5. DISCUSSION

In the study, the methanolic extract of *Cissus quadrangularis* exhibited significant anti-arthritic activity in a dose-dependent manner. The present study, showed that 250 mg/kg of methanolic extract of *Cissus quadrangularis* could significantly reduce the development of rheumatoid arthritis in treatment group animals²². The rats were induced for arthritis with 0.1ml FCA in the left hind paw of the rats in group 2, 3 and 4. The methanolic extract of *Cissus quadrangularis* was administered orally to group 3 rats and Dexamethasone to group 4 rats in a dosage of 0.5mg/kg. The inhibition of secondary inflammation in adjuvant arthritic rats by methanolic extract of *Cissus quadrangularis* was studied by haematological parameters. In the arthritic condition, the haemoglobin level decreased which has been associated with anemia. Anemia may be due to reduced erythrocyte deformability²³. The reduced deformability leads to shortened life span of RBC, which results in decreased RBC, a marker of rheumatoid disease. These levels were boosted after the oral administration of methanolic extract^{18,20,24}. Changes in haematological parameters reflects antigen induced arthritis, studies on B cell hyperactivity revealed a primary role of B cell complement engagement by circulating immune complexes in the production of antibodies and diffuse infiltrative lymphocytes which acts as a determining factor in host immunogenicity. Increased WBC count in arthritic rats was significantly suppressed by the treated group indicating its immune suppressant nature. Arthritis condition generally results in accumulation of leukocytes and release of lysosomal enzymes, the main mediators in arthritis. In the present study, the migration of leukocytes in the inflamed area is significantly suppressed by the methanolic extract of *Cissusquadrangularis* as seen from the significant decrease in total WBC count^{25,26}. The ESR

level which drastically increased in the arthritic group has been remarkably counteracted by the drug, restoring it to near normal, to justifying its significant role in arthritic condition^{27,28}. The serological factor-Rheumatoid Factor signifies their increased levels after the induction of FCA with the inflammation and swelling in the toxic rats and this was reduced with the treatment of methanolic extract of *Cissus quadrangularis* in the study period²⁹.

6. CONCLUSION

The in vivo studies conducted in the period of 28 days with the methanolic extract of *Cissus quadrangularis* at a dose of 250mg/kg has a significant therapeutic effect on FCA induced rats. The plant extract showed effective results in the haematological parameters, when compared with the standard reference drug dexamethasone. These results support future studies in order to develop plant-derived alternatives for conventional anti-arthritic drugs such as dexamethasone.

7. AUTHOR CONTRIBUTION STATEMENT

Mrs. Shamina S conceptualized and gathered the data with regard to this work. Mrs. Shamina S, Ms. Anjana and Dr. S. Suja analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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