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Antiarthritic and antioxidant activities of *Gossypium herbaceum* plant (cotton plant) leaves

Narahari Srinivas Reddy[1] and Akondi Butchi Raju*

Ibn Sina National College for Medical Studies,
Jeddah, Saudi Arabia.
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ABSTRACT

Gossypium herbaceum commonly known as cotton plant cultivated world over and the main produce is used by the textile industries. Other parts of this plant including leaves can also be explored for their best utilization, hence in this direction we have evaluated efficacy of cotton plant leaf extract against an inflammatory condition *i.e* rheumatoid arthritis. Freund's complete adjuvant (FCA) induced arthritis model was selected for this study and. All the rats received treatments of plant extract from 10th day of the induction of arthritis and continued up to 28 days of the study. Once in every 7 days, the arthritic assessments *i.e*. paw volume; body weight and paw erythema were assessed. Arthritic rats showed severe paw swelling, erythema, reduced body weight, abnormal changes in haematological, biochemical and antioxidant parameters ($p<0.05$) compared with normal control. Studies showed that treatment with MEGH (200mg/kg) was effective in reversing the symptoms and restore the normalcy by restoring the elevated parameters. Over all the plant proved useful in treating Rheumatoid Arthritis in rodents and further studies in this direction are necessary to understand its mechanism.

Key words: Rheumatoid arthritis, Freund's complete adjuvant, *Gossypium herbaceum*, Methotrexate.

INTRODUCTION

Gossypium herbaceum (GH) Linn, commonly known as cotton plant and cultivated in India and other parts of the world. (Kokate *et al.*, 2007). The leaf of GH plant was screened and proved to be effective as diuretic, haemitnic, astringent, drug to treat bronchitis, antidysenteric, expectorant, wound healing agent, and agent for prevention of excessive bleeding during menstruation, anti-inflammatory(Kalyana Narasimha *et al.*,2008), analgesic agent (Nadkarni *et al.*, ,2011) and antioxidant (Saifuddin Khalid *et al.*,2011). The principle constituent of plant is gossypol. The leaves and flowers contain gossypetin 8-rhamnoside, gossypin, gossypetin (Kalyana Narasimha *et al.*, 2008) respectively. As the plant was already proved to be effective for its anti-inflammatory activity, we tried to establish its activity against rheumatoid arthritis, which is also a chronic autoimmune inflammatory disorder. We have selected proven and suitable animal model *i.e*. Freund's complete adjuvant (FCA) induced arthritis model in rats for the this experiment.

MATERIALS AND METHODS

Collection of plant material: The fresh leaves of *Gossypium herbaceum* Linn were collected from Dharmasagar village, Warangal district of Andhra Pradesh-India in the month of March, 2012. The Plant was identified and authenticated (Voucher No. 1864) by Dr. Vatsavaya S. Raju, Senior Professor, Deportment of Botany, Kakatiya University, Warangal, Andhra Pradesh, India.

Preparation of extract: The shade dried powder of the leaves was packed in Soxhlet apparatus and was subjected to continuous hot extraction with methanol. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccator till experimentation.

Preliminary phytochemical screening: Preliminary phytochemical screening of methanol extract of *Gosssypium herbaceum* was carried out according to standard procedures out lined by Kalyana Narasimha *et al.*, (2008).

Chemicals: Freund's complete adjuvant (FCA**)** was procured from Sigma Aldrich, St. Louis, U.S.A and methotrexate was purchased from SUN Pharmaceuticals, India. All other chemicals and reagents used for study were of analytical grade.

Animals: Female Wistar rats (Weight, 150 - 200g), age of 6 weeks were procured from the Sanzyme Ltd., Hyderabad, India. The animals were housed as per standard guidelines in poly acrylic cages with not more than six animals per cage. Rats have free access to standard diet and drinking water *ad libitum*. The research protocol was approved by the Institutional Animal Ethical Committee (IAEC, 10/

*Corresponding author's e-mail: drraju2020@gmail.com
[1]Department of Pharmacology, St. Peters Institute of Pharmaceutical Sciences, Hanamkonda, Warangal, A. P-506001, India.

SPIPS/IAEC/2012). The entire study was conducted in St. Peters Institute of Pharmaceutical Sciences, Warangal, India.

Acute toxicity study: Acute toxicity test was conducted according to OECD guidelines. The extract is diluted with water and orally administered to overnight- fasted, healthy rats (n=5). The dose of the extract was 2000mg/kg body weight and the animals were monitored up to 14 days.

Induction of arthritis and treatment protocol: Arthritis induced by Freund's complete adjuvant was used and this model is found to be very similar to human rheumatoid arthritis (Sharad Mali M *et al.,* 2011). Arthritis was induced by 0.1mL of FCA into sub-plantar region of rat right hind paw, on day one under anesthesia.

Group-I : Normal control (Normal saline oral)
Group-II : Arthritic control (FCA induced)
Group-III : Standard dose of methotrexate (MTX) 2 mg/kg/oral to FCA induced rats.
Group-IV : Methanol extract of *Gossypium herbaceum* (MEGH) 100 mg/kg per day was orally administered to FCA induced rats.
Group-V : Methanol extract of *Gossypium herbaceum* (MEGH) 200 mg/kg per day was orally administered to FCA induced rats.

All animals received treatments from 10th day of the induction of arthritis and continued up to 28 days of the study. Initially before starting the experiment, the rat body weight was measured, and then once in every 7 days paw volume and arthritic index were measured.

Arthritic assessments:

Paw volume: Paw volume was one of the parameter for assessment of inflammation in arthritic conditions. Paw volume was measured by using digital plethysmometer(Manufactured by INCO Limited, Ambala, India) before giving injection on day '0', after then once in every '7' days and continued up to 28th day. The differences in change of initial and final paw volumes were compared. (Sharad Mali *et al.,* 2011).

Arthritic score: After injecting FCA, symptoms of arthritis like redness, swelling and erythema were recorded. Paws were examined and graded for severity and loci of erythema, swelling and indurations using a 5-point scale: 0 = no signs of disease, 1 = signs involving the ankle/wrist, 2 = signs involving the ankle plus tarsal of the hind paw and/or wrist plus carpals of the forepaw, 3 = signs extending to the metatarsals or metacarpals, and 4 = severe disease involving the entire hind or fore paw. The maximum arthritic score per rat was set at 16 (4 points×4 paws) (Lixing Lao *et al.,* 2009).

Body weight: Body weight was measured before giving injection on day '0'and once in every '7' days and continued up to 28th day.

Determination of haematological parameters and estimation of biochemical parameters: On day 28, blood was withdrawn by retro-orbital puncture. Hematological parameters were determined using various techniques. The parameters analyzed were white blood cell (WBC) number, red blood cell (RBC) number, hemoglobin (Hb) concentration, packed cell volume (PCV), differential cell count (granulocytes, granulocytes), erythrocyte sedimentation rate (ESR) (David Banji *et al.*,2011).

Biochemical parameters like Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), rheumatoid factor (RF), C- reactive protein (CRP), creatinine, total protein, alkaline phosphatase (ALP), catalase, superoxide dismutase were tested using standard procedures (Gomes *et al.*,2010).

Histological analysis: The animals were sacrificed on day 28 by CO_2 inhalation method. Ankle joints were separated from the hind paw, weighed and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, embedded in paraffin, cut into tissue sections. The sections were stained with haematoxylin and eosin and evaluated under light microscope (Sharad Mali *et al.,* 2011).

Statistical analysis: All data is expressed as mean ± S.E.M. Statistical significance (P) calculated by one-way ANOVA followed using Graph pad-5 software. $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity studies: The test animals were given single dose (2000 mg/kg) of methanol extract of *Gosssypium herbaceum* and observed for 14 days. No abnormal changes were observed in battery of tests conducted including behavioral tests. Hence the dose up to 2000mg/kg is declared as safe.

Arthritic assessments

Effect of methanol extract of *Gossypium herbaceum* (MEGH) on Paw volume: Paw volume has significantly increased in Freund's complete adjuvant (FCA) treated group. Test drug MEGH 200 mg/kg treated group and methotrexate 2 mg/kg treated groups has shown reduced paw volume when compared with arthritic control group; MEGH 100mg/kg treated group has not shown any beneficial effect. (Fig 1).

Effect of MEGH on arthritic score: MEGH 200mg/kg group has shown a significant activity ($p<0.05$) when compared with arthritic control group. Arthritic score was significantly increased ($p<0.05$) in the rats treated with FCA compared with normal control group. MEGH 100mg/kg treated group has not shown much beneficial effect. Methotrexate 2mg/kg treated group shown a significant activity ($p<0.05$), arthritic score has reduced to normal. MEGH 200mg/kg has shown a significant activity starts from 21st day of the study. (Fig 2)

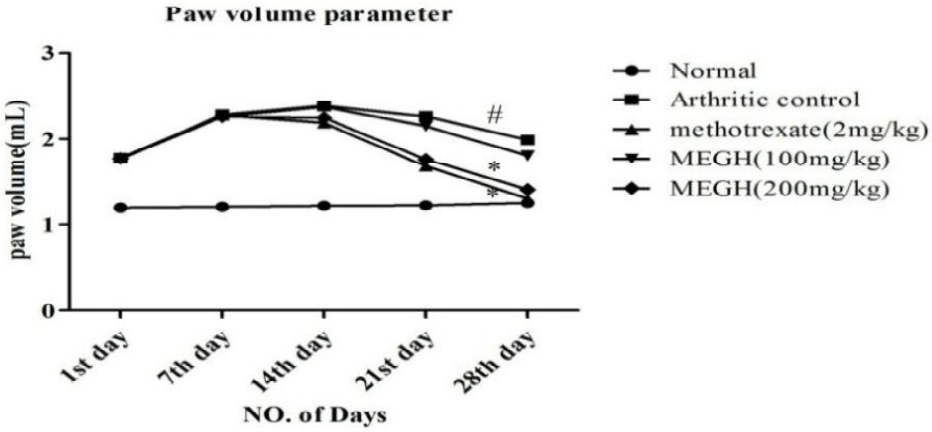


Fig 1: Effect of *gossypium herbaceum* extracts on paw volume of Rheumatoid arthritis induced rats.

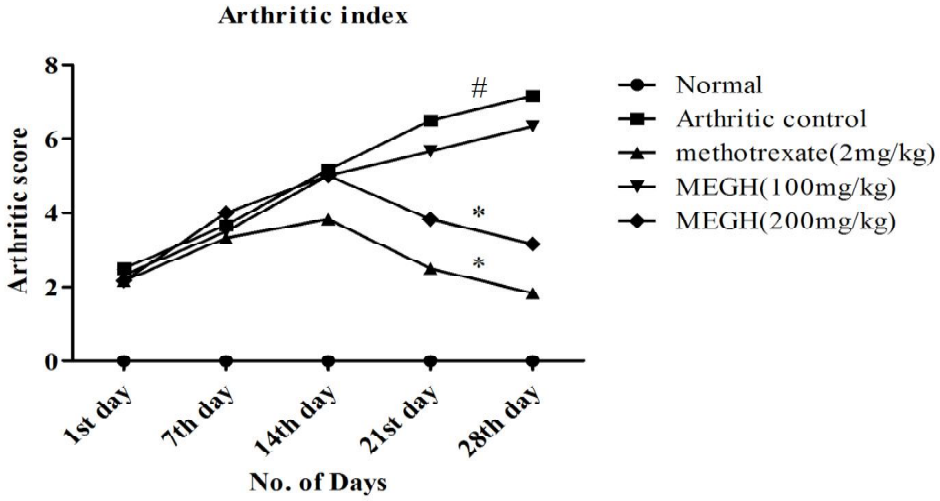


Fig 2: Effect of *gossypium herbaceum* extracts on arthritic index of Rheumatoid arthritis induced rats.

Effect of MEGH on body weight: Body weight was significantly reduced ($p<0.05$) in the rats treated with FCA compared with normal control group. Body weight was unaltered in MEGH 200mg/kg group and methotrexate treated groups ($p<0.05$) (Fig 3).

Effect of MEGH on hematological parameters: Hematological parameters of arthritic rats on day 28th day were found to be significantly changed from normal group. MEGH 200mg/kg, treatment group has shown a significant activity ($p<0.05$) when compared with arthritic control group. Few parameters like ESR, PCV, lymphocyte and neutrophil have shown significant improvement with 100mg/kg also. Methotrexate 2mg/kg treated group has shown a significant activity ($p<0.05$) in FCA induced rats. Both the test drug, MEGH (200mg/kg) and standard drug, methotrexate (2mg/kg) treatments restored the altered haematological profile. (Fig 4).

Effect of MEGH on biochemical parameters: In arthritic conditions biochemical estimations were abnormally changed, there were significant changes in SGPT, SGOT, ALP, creatinine, total protein, CRP and RF levels in arthritic control animals compared with normal control animals. Both the test drug, MEGH (200mg/kg) and standard drug, methotrexate (2mg/kg) treatments have restored the normalcy. (Table 1).

Effect of MEGH on antioxidant activity: In the arthritis induced rats the SOD and Catalase levels were reduced when compared to normal group. In MEGH (200mg/kg) treated groups, we found dose dependent antioxidant activity of the plant extract. (Table 1).

Effect of MEGH on histology of inflamed joints: Histological slides were stained with haematoxylin and eosin dyes and evaluated under light microscope (Magnification 40X). In FCA injected animals, the histopathological evaluation of the tibiotarsal joint showed prominent inflamed degenerative connective tissue associated with cellular inflammation edema and granuloma formation. In MEGH (100mg/kg and 200mg/kg) treated group animals and

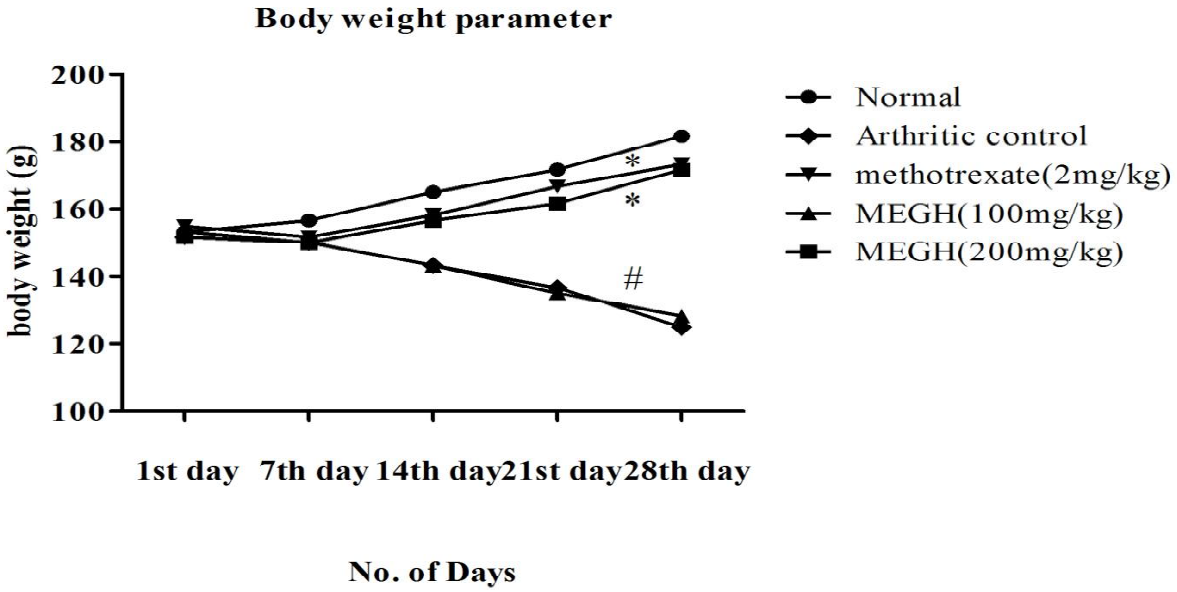


Fig 3: Effect of *gossypium herbaceum* extracts on body weight of Rheumatoid arthritis induced rats.

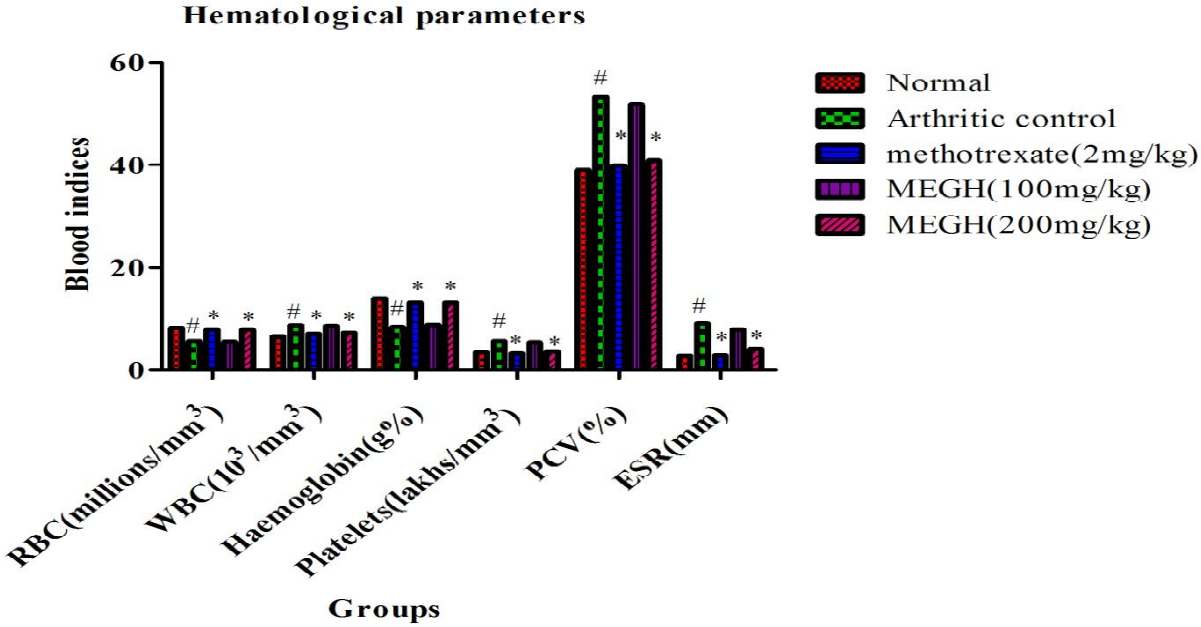


Fig 4: Effect of *gossypium herbaceum* extracts on hematological parameters of Rheumatoid arthritis induced rats

methotrexate (2mg/kg) treated group of animals, there was clear reduction in the inflammatory signs and leukocyte infiltration. (Fig 5-A, 5-B,5-C,5-D,5-E).

Pharmacological Studies on *Gossypium herbaceum* plant (cotton plant) were conducted previously and indentified it as an Anti-inflammatory and antioxidant agent. Rheumatoid arthritis (RA) is an auto immune disorder, causes inflammation of joints, restricts the movements and causes moderate to severe pain. *Gossypium herbaceum* leaf extract was explored for its activity against inflammatory condition of rheumatoid arthritis using a standardized mice model. Disease-modifying anti-rheumatic drugs and steroids are current treatment options available for RA, but majority of them have serious adverse drug reactions and considered toxic for long term usage. Freund's complete adjuvant (FCA) model was used in this study and it is a very similar to human rheumatoid arthritis (David Banji *et al.*, 2011) as cell mediated autoimmunity is involved in induction of the arthritis. (Van Eden *et al.*, 1985). Secondary lesions, cartilage distraction and bone erosion were also observed after 7 days.



Fig 5A: Histological architecture of tibiotarsal joint histology in normal rat. H & E staining, 40X

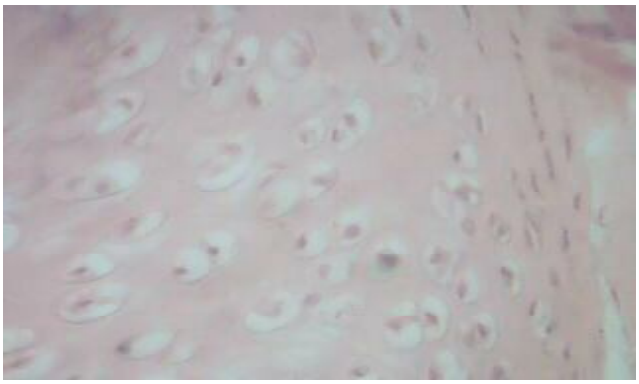


Fig 5 B: Histological architecture of tibiotarsal joint histology in FCA induced rheumatoid arthritis rat. H & E staining, 40X. Prominent inflamed degenerative connective tissue associated with cellular inflammation edema and granuloma formation can be observed here.



Fig 5 C: Histological architecture of tibiotarsal joint histology in Methotrexate treated FCA induced rheumatoid arthritis rat. H& E staining. 40 X. Reduction in cellular inflammation is evident.

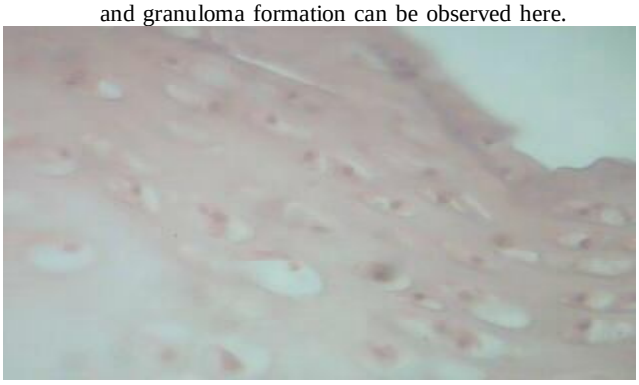


Fig 5 D: Histological architecture of tibiotarsal joint histology in MEGH (100mg/kg) treated FCA induced rheumatoid arthritis rat. H &E staining. 40 X. Reduction in cellular inflammation can be observed.

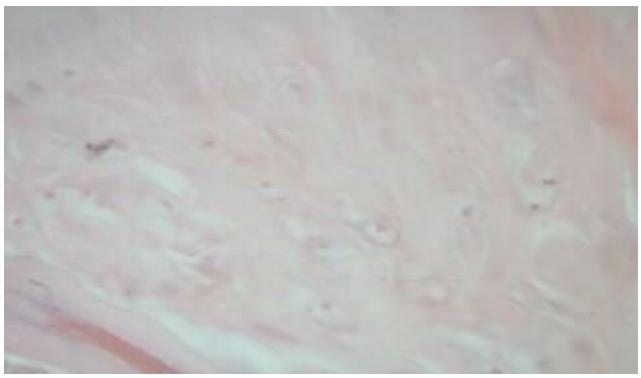


Fig 5 E: Histological architecture of tibiotarsal joint histology in MEGH (200mg/kg) treated FCA induced rheumatoid ar thritis rat. H &E staining. 40 X. Almost normal tissue can be observed with re duction in leukocyte infiltration

Fig 5: Effect of *gossypium herbaceum* extracts on histological parameters of Rheumatoid arthritis induced rats. H & E staining, 40X

Table 1 : Effect of methanol extract of *Gossypium herbaceum* on Biochemical parameters of rats.

Parameters	Group-I Normal	Group-II Arthritic control	Group-III Methotrexate(2mg/kg)	Group-IV MEGH(100mg/kg)	Group-V MEGH(200mg/kg)
ALT	27.83±3.48	58.67±1.22#	38.33±1.35*	58.00±1.52	41.00±1.80*
AST	85.33±5.00	143.70±3.35#	103.5±6.73*	137.7±5.04	114.2±5.72*
ALP	49.83±1.57	81.83±1.95#	52.67±1.05*	78.17±1.84	57.33±1.10*
Creatinine	0.70±0.08	2.11±0.16#	1.03±0.13*	2.06±0.15	1.31±0.08*
Total protein	6.38±0.11	3.40±0.03#	5.30±0.09*	3.85±0.14*	4.96±0.08*
CRP	0.80±0.25	28.80±4.29#	3.00±0.60*	18.40±4.70	14.40±2.14*
RF	13.33±6.66	533.30±67.40#	146.70±38.18*	480.00±71.55	240.00±87.64*
Catalase	15.68±0.10	0.83±0.03#	4.75±0.07*	2.15±0.16*	4.60±0.09*
SOD	25.47±0.03	4.54±0.03#	19.23±0.08*	8.22±0.04*	18.25±0.07*

Arthritic rats showed severe paw swelling, erythema, redness, reduced body weight, abnormal changes in haematological, biochemical and antioxidant parameters. Studies show that treatment with MEGH (200mg/kg) and methotrexate (2mg/kg) were effective in reducing arthritis symptoms as well as prevention of fall in the body weight. Tumor necrosis factor - alpha (TNF-α levels, haematological, biochemical and antioxidant parameters levels were reduced to normal in MEGH treated groups when compared to FCA induced arthritis rats.

Treatment with MEGH (200mg/kg) has reduced secondary lesions; MEGH also reduced the arthritic score along with secondary paw swelling. Reduced body weight was observed in the arthritic rats, it might be due to the increased levels of TNF-α. In the present study, it was found that MEGH exhibited antiarthritic effect, treatment with MEGH (200mg/kg) has shown prevent the reduction of body weight, increase in body weight as compared with arthritic control animals. Methotrexate (2mg/kg) group has shown protected activity.

Raised white blood cell (WBC) count in arthritic conditions were reduced to normal by MEGH and methotrexate treatments. The arthritic rats showed reduced red blood cells (RBC) count (Cai *et al.*,2006, Helen *et al.*,2012). Activation of polymorphonuclear neutrophils (PMNs) reflects a primary immunological response to invading pathogens (Lau *et al.*, 2005). In the present study, elevated levels of neutrophil count, decreased levels of RBC, Hb, ESR count was observed. Treatment with MEGH (200mg/kg), methotrexate (2mg/kg) in arthritic animals, neutrophil count was reduced to normal, RBC, Hb, ESR levels were also maintained at normal levels. MEGH (200mg/kg) and methotrexate (2mg/kg) treatments restored the altered haematological profile.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were found to be increased in arthritic induced groups. Liver impairment is a typical feature in adjuvant arthritis Van Eden *et al.*, 1985). Tissue damage in adjuvant arthritis was measured by the enzyme levels in the serum. We observed an elevated level of aminotransferase in the FCA treated group which might be released from the damaged cells of the liver. Treatments with MEGH (200mg/kg) and methotrexate (2mg/kg) in arthritic animals SGOT, SGPT, and ALP levels were reduced to normal.

C-reactive protein (CRP) is a protein and important biomarker in various inflammatory, degenerative and neoplastic diseases (Rainsford *et al.*, 1982). Elevated levels of CRP in blood are seen in the animals infected with various diseases associated with active inflammation or tissue destruction, particularly in rheumatoid arthritis (Andersen *et al.*, 1950) and it was clearly observed in our arthritic control animals also. In the present study, treatments with MEGH (200mg/kg) and methotrexate (2mg/kg) in arthritic animals CRP levels were brought back to normal.

Rheumatoid factor is very important biomarker in rheumatoid arthritis. Auto-antibodies are produced against self antigens. “Rheumatoid factors” (RF) can be detected which are immunoglobulins of the class IgM, IgG, IgA and IgE. IgM class RF which are specific to IgG (Fc) is the most useful prognostic marker of RA (Singer *et al.*, 1956) and it was clearly observed in our arthritic control animals also. In the present study, treatments with MEGH (200mg/kg) and methotrexate (2mg/kg) in arthritic animals RF levels was reduced to normal. MEGH (200mg/kg) has shown a protected activity.

In the present study, elevated creatinine level and decreased total protein levels in FCA treated animals was observed. Treatments with MEGH (200mg/kg) and methotrexate (2mg/kg) in arthritic animals, creatinine level reduced to normal and prevent the reduction of total protein levels were observed. MEGH (200mg/kg) has shown a protected activity.

In arthritic state, the affected articulations are infiltrated by blood-derived cells, mainly neutrophils, macrophages and dendritic cells; these activated cells generate free radicals, which are released in large amounts in the surrounding tissue (Aghdassi *et al.*, 2000). Released

reduction of superoxide dismutase (SOD) and catalase levels. In a study published in 2011, the *Gossypium herbaceum* has shown antioxidant activity (Sharma Pravesh Kumar *et al.*, 2011). MEGH at 200mg/kg dose has shown a maximum protection activity.

From the histopathological studies of the tibiotarsal joint, it was evident that the inflammation of the connective tissue was protected by treatment with MEGH (200 mg/kg).

Previous studies reported that *Gossypium herbaceum* plant have an anti-inflammatory activity (Nadkarni AK and Nadkarni KM,2011)and in the present study, it was found that methanol extract of *Gossypium herbaceum* exhibited antiarthritic effect, evident from the various parameters studied above. In conclusion, methanol extract of *Gossypium herbaceum* (MEGH) in higher test dose is proved to be effective in treating rheumatoid arthritis significantly.

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