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ANTI-ARTHRITIC POTENTIAL OF BIOPOLYMERIC FRACTION OF SEEDS OF *TRIGONELLA FOENUM* GRACEUM ALONE AND ITS COMBINATION WITH GLYCYRRHIZIN AS A BIO-ENHANCERS IN ARTHRITIC RATS

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Keywords:

Rheumatoid arthritis, Dexamethasone, Polysaccharides, Glycyrrhizin

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ABSTRACT: Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disorder. It is characterized by inflammation of the synovial joints and concomitant destruction of cartilage and bone. However, rheumatoid arthritis is typically a progressive illness that has potential to cause joint destruction and functional disability. In the present research, GSH content were significantly decreased in arthritic control group when compared to normal control group. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, causes increase in GSH content, which was comparable with standard drug Dexamethasone. Change in body weight is in response to the incidence and severity of arthritis and used to assess disease onset. Weight loss is associated with increased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1. Adjuvant arthritis is characterized by reduced body weight. The body weight of arthritic control rats decreased compared to the normal control group due to FCA administration. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, recovered the body weights, significantly which was comparable with standard drug Dexamethasone.

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic, autoimmune disease that is considered a foremost health setback worldwide because approximately about 0.75% in India and 1% adult population in the world are exaggerated with this disease [1]. RA is most commonly seen in male population as compared to females, while the disease onset can occur between the ages of 30 and 55.

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Some of the symptoms of RA involve extreme joint pain, mostly due to joint inflammation and proliferation of synovial, which eventually leads to the disability of joints. However, other problems such as gastrointestinal tract disorders, immune deficiency, hormonal disturbances and complications associated with the cardiovascular system has previously been reported [2].

The mechanism of RA for joint destruction includes increased expression of cytokines and transcription factors. Interleukins, namely IL-6, tumour necrosis factor (TNF)-a, IL-1b and IL-1 are the cytokines involved in progression of arthritis. IL-6 stimulates the growth in blood vessels, which promotes inflammation. TNF-a, amplifies inflammation *via* stimulation of synovial

fibroblasts that express cellular adhesion molecules and increase the leukocytes migration into the joints resulting in damage, while IL-1 helps in bone resorption, cartilage destruction and in turn can modify the production of nitric oxide (NO) and prostaglandin (PGE2). PGE2 can stimulate pain receptors and thus induce fever. Due to this imbalance which occurs between proinflammatory and anti-inflammatory state, it has resulted in synovial membrane inflammation and damage in the joints [3].

Four Classes of Drugs are Available for RA Treatment: steroid hormones, biological agents, immunosuppressants, anti-rheumatic drugs and anti-inflammatory drugs. Although anti-arthritic drugs have various advantages in treatment, criteria such as high costs, side effects, and efficacy towards specific target sites limit their clinical use [4].

In pre-clinical research, animal models of arthritis continue to play an important role, particularly in identifying and validating drug targets. Out of the many available animal models, CIA, SCW and more recently, he K/BxN model has proven useful in understanding some of the mechanisms involved in RA. Among other models of arthritis in rodents, Complete Freund's Adjuvant (CFA) induced have shown various similarities with that of human arthritis, which makes it the most suitable model for inducing arthritis [5]. Currently, available therapy is hampered by several disadvantages, such as a failure to cure, symptomatic relief and cost.

Thus, the therapeutic approach for arthritis requires the drug to be cost-effective, long-lasting, and minimal less side effects. The drugs should also inhibit inflammation and pro-inflammatory cytokines expression thereby preventing damage of joints [6]. Due to this, apparently there are several reasons to explore plant-based anti-arthritic medicines. In traditional medicine, various herbal drugs are used as in the treatments of RA from ancient times. During the current time, interest in the biopolymer constituents of medicinal plants has arisen because of the observations that the therapeutic effect, in particular immune-related activity, of a large number of plants is due to their biopolymer constituent. These constituents have emerged as an important class of natural products that act as biological response modifiers (BRMs) through modulation of the immune system, resulting in various therapeutic effects [7]. Add name, bs, family of drug The seed of *Trigonella foenum-graecum* also contain polysaccharides. And seeds have used in inflammation and inhibition of inflammatory mediator but it has no clinical and scientific evidence in treatment of rheumatoid arthritis.

This herbs have a wide margin of safety and are highly effective. Antioxidant agents [8-10] *Glycyrrhiza glabra* is another plant which is previously evaluated for its anti-inflammatory antiepileptic, antiasthmatic, antiulcerogenic, anxiolytic, anti-viral, hepatoprotective activity, antioxidant actions and also acts as bioenhancer [11-12]. Hence, the study is undertaken to evaluate scientifically the therapeutic potential of *Trigonella graceum foenumin* animal model of RA.

MATERIALS AND METHODS:

Collection of Plant Material: The Seeds of *Trigonella foenum graceum* was collected from the local market during January 2012. The plant is authenticated from P.R.E.S.'s, PVP College, Pravaranagar, Maharashtra (PVP/2012/729)

Extraction of Plant Materials: The seeds of the plant *Trigonella foenum graceum* were soaked in a 2N-aquoeous Sodium Hydroxide and kept overnight. The resultant extract was filtered and the alkaline solution was centrifuged at 6000-7000 rpm. Above process was repeated and the alkaline solution was pooled with first extract.

This combined extract was diluted with alcohol (1:6) and kept overnight undisturbed. The resultant precipitate was collected by centrifugation at 6000-7000 rpm. The precipitate was dissolved in distill water and now acidified with an equal volume of 15% trichloroacetic acid and kept overnight. The precipitate obtained after centrifugation was suspended in warm distilled water and centrifuged. The percentage yield of each extract was calculated [13].

Detection of Carbohydrate: Extract were treated with α-napthol and conc. H_2SO_4, if produced purple colour shows the presence of carbohydrates (Molish test) [14].

Chromatographic Study:

TLC Study of Biopolysaccharides Extracted from *Trigonella foenum graceum* are as follows:

TLC of Polysaccharides: TLC Studies of ethanolic extract and different fractions were performed by using precoated plates. On precoated plates place a drop of the ethanolic extract and different fractions in the center with the help of capillary tube and appropriately mark its position with a pencil.

In TLC chamber mixture of petroleum ether: ethyl acetate in the proportion of (7:3) were taken and inserting filter paper to saturate the chamber for 20min and then place the one end of TLC plate in close to the container developing solvent travels up the plate. After the developer transversed two-thirds of the plate's length, the plate was removed from the container and dried. The positions of the components were then determined by sprayed with 1% vanillie – sulphuric acid reagent and heated for 5 minutes at 110 °C.

Pharmacological Investigation:

Animals: Male albino rats of Wistar strain weighing around 100-180g were procured from Animal house, Department of Pharmacology, College of Pharmacy; allanimals were housed in cages. The animals were appropriately fed with standard rat diet and tap water throughout the experiment. These animals were used for anti-inflammatory as well as locomotor activity. The experiment were designed and conducted in accordance with the ethical norms approved by Institutional Animal Ethical Committee Guidelines. The animal experimentation was carried out in accordance to the guidelines mentioned in the CPCSEA (Protocol No: CPCSEA/77/2011),

Experimental Setup: Before any treatment rats were divided into five groups, each group contains six animals as followed for analysis of histological and biochemical parameters.

Group I: Normal control, rats were injected intradermally saline 0.1 ml.

Group II: Arthritic control, (Injected 0.1 ml FCA, containing 10 mg of heat-killed *Mycobacterium tuberculosis* into right hind paw of the rats on day 0).

Group III: CFA induced arthritic rats treated with treated with TGF, (75mg/kg/day) orally

Group IV: CFA induced arthritic rats treated with treated with TGF (75mg/kg/day) and 25mg/kg Glycyrrhizin administered orally.

Group V: Arthritic rats induced with CFA were treated with Dexamethasone (0.7 mg/kg/day) by I.P. administration. No treatment was given in the normal control group. In every treated group, drugs were administered orally as a suspension in normal saline. Adjuvant was given to each animals of each group other than normal control and treatment begins on day 0 and continues until day 14 [15].

The standard drug, Dexamethasone used in the experiment, was purchased from the market, having a dose of 2mg/ml in the form of liquid injection. At the end of the experimental period, rats were fasted overnight and anaesthetized. These anesthetized rats were sacrificed by cervical decapitation, and the blood was collected into tubes by cardiac puncture prior to sacrifice. Blood samples were immediately centrifuged at 3000 rpm for 10 min., and plasma or serum samples were stored under freezer until assayed [15].

Body Weight Measurement: Every rats used in the experiment weighed at the beginning (Baseline) and at the end of the experiment, which is the final day after adjuvant injection (day 25) [16].

Body weight – Based on pre-study values

0 = < 5 % decrease, 1 = 6 -10 % decrease, 2 = 11-20 % decrease, 3 = 21-25 % decrease, 4 = > 25 % decrease

Paw volume (Plethysmometer): Paw volume were examined after every 3-4 days. The right hind paw volume was measured with plethysmometer (basic value, day 0) and repeated on days 0, 3, 7, 10, 14, 17, 20, 24 and 28.

The intensity of edema in the paw was determined by measuring the paw volume of the entire inflamed paw (right hind paw) with the help of a mercury plethysmometer equipped for accurate measurement of the rat's paw swelling through dislocation of fluid volume. The change in volume of the affected paw was evaluated on before the

induction of inflammation or arthritis ($V_{b.i.}$), 14 days after induction (V_{14}) and 24 days after induction (V_{24}) and paw volume index was calculated by following formula [17].

$$\text{Paw volume index (\%)} = V_{24} - V_{14} / V_{b.i.} - V_{14} \times 100$$

$$\text{Paw volume inhibition (\%)} = 1 - V_{24} - V_{14} / V_{b.i.} - V_{14} \times 100$$

Here, $V_{b.i.}$ = Paw volume before arthritis induction, V_{14} = Paw volume after 14 days, V_{24} = Paw volume after 24 days.

Ankle Diameter Measurement: Ankle diameters were examined after every 3-4 days. The ankle diameter was measured with screw gauge (basic value, day 0) and repeated on days 0, 3, 7, 10, 14, 17, 20, 24 and 28. By measuring the ankle diameter, which gives information of the intensity of the swelling of the joint.

The change in ankle diameter of the affected paw was evaluated on before the induction of inflammation or arthritis ($V_{b.i.}$), 14 days after induction (V_{14}) and 24 days after induction (V_{24}) and index of ankle diameter was calculated by following formula [18]

$$\text{Index of ankle diameter (\%)} = (\text{Ankle}_r - \text{Ankle}_{14}) / (\text{Ankle}_{bef.ind.} - \text{Ankle}_{14}) \times 100$$

Arthritic Score (Polyarthritic Index): For clinical evaluation of AA, the polyarthritis severity was graded on a 0-4 scale, each paw was graded and 4 grades were summed to a maximum possible score of 16.Rats of each group were evaluated daily for arthritis using a macroscopic scoring system explain as follows: [19].

0 = Normal or no sign of arthritis or no swelling, 1 = Swelling and/or redness in one joint, 2 = Swelling and/or redness in more than one joint, 3 = Swelling and/ or redness in the entire paw, 4 = Deformity and/ or ankylosis.

Index of Immune Organ (Spleen): In the course of the experiment, the body weight of rats was measured. The animals were killed on day 28 after immunization, and the spleen was promptly removed and weighed. The index of spleen was expressed as the percentage (%) of spleen wet weight Vs. body weight, respectively[20].

$$\text{Index} = \text{Organ weight of rat} / \text{Body weight of rat} \times 100$$

Assessment of Pain Behavior:

Hot Plate Paw Withdrawal Latency Method: The arthritic rat's hind paw has become inflamed so the heat sensitivity of the right hind paw has been changed. The heat sensitivity of the paw has been measured in arthritic rats using the hot-plate test and paw withdrawal latency. As the inflammation progresses in the paw, the sensitivity to the heat of the rat paw decreases. Thus the paw withdrawal latency decreases. The rat was kept on the hot plate with a temperature maintained at 55±°C and the maximum exposure time to the hot plate was 15 sec. For the evaluation of heat sensitivity, reaction time (paw licking or paw withdrawal) was recorded for each animal of each group [21].

Radiographic Analysis (Evaluation of Bone Destruction by X- rays): At the end of the experiment on day 25 after adjuvant injection, rats were anesthetized by inhalation of anaesthetic ether and imaged on Fuji HR-Fast film (Fuji photo film), using a siemens X-rays tube assembly (Siemens AG, Munich, Germany). Whole bodies were X-rayed using a 90° projection from the dorsal-ventral aspect. Radiographs of each rat were evaluated for soft tissue swelling, bone matrix resorption, periosteal new bone formation and bone erosion, and were scored in a blind fashion by two independent observers graded as follows:

0 = Normal No change, 1 = Slight change, 2 = Moderate change, 3 = Severe change

The total radiological scores were calculated from the sum of both hind paws, with a maximum possible score of 6 for each radiological parameter per rat [22].

Biochemical Assays:

Aspartate Transaminase (AST): One millilitre of the buffered substrate was incubated for 10 min at 37°C. Then 0.2 ml of the enzyme was added and incubation was continued for an hour. To the control tubes, the enzyme was added after arresting the reaction with 1.0 ml of DNPH and the tubes were kept at room temperature for 20 min. Then 5.0 ml of 0.4 N NaOH was added. A set of standard pyruvate was also treated in a similar manner. The colour developed was read at 540 nm. The enzyme activity was expressed as micromoles of pyruvate liberated per milligram of protein per minute [23].

Alanine Transaminase (ALT): One millilitre of the buffered substrate was incubated for 10 min at 37°C. Then 0.2 ml of the enzyme was added. The tubes were incubated at 37°C for 30 min. To the control tubes, enzymes were added after arresting the reaction with 1.0 ml of DNPH and the tubes were kept at room temperature for 20 min. Then 5.0 ml of 0.4 N NaOH was added. A set of standard pyruvate were also treated in similar manner. The colour developed was read at 540 nm. The enzyme activity was expressed as moles of pyruvate liberated per milligram of protein per minute [23].

Measurement of Inflammatory Mediators:

Lipid Peroxidation: Lipid peroxidation in erythrocytes was estimated by measuring thiobarbituric acid reacting substances (TBARS). The method is based on spectrometric measurement of purple colour generated by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA).

The procedure involved 2.5 ml of TCA solution (10% w/v) being added to 0.5 % supernatant of the tissue preparation in each centrifuge tube and tubes were placed in a boiling water bath for 15 min.

After cooling to room temperature, the tubes were centrifuged at 1000X g for 10 min and 2 ml of each sample supernatant was transferred to a test tube containing 1 ml of TBA solution (0.67 % w/v). Each tube was placed in a boiling water bath for 15 min after cooling to room temperature; the absorbance was measured at 532 nm. The concentration of MDA was calculated based on the absorbance coefficient of the TBA-MDA complex ($\varepsilon = 1.56 \times 10^5$/cm/M) and it was expressed as nmol/mg protein [24].

Reduced Glutathione (GSH): The liver was quickly removed from sacrificed rat and was homogenized in 5 ml of distilled water. The homogenate was centrifuged at 6000 rpm for 10 min. 0.5 ml of the above solution was mixed with 0.5 ml of 10 % TCA, and a protein-free supernatant was obtained by centrifugation. 0.5 ml of TCA was mixed with 1 ml of 0.6 M Na_2HSO_4 and 0.5 ml of DTNB reagent. The absorbance of this solution was measured at 410 nm. The absorbance was read from the Concentration vs Absorbance standard graph of pure glutathione [25].

Statistical Analysis: The experimental results were expressed as Mean ± S.E.M. of n = 6 rats per group. Their 95% Confidence intervals (95% CI) were calculated by linear regression analysis. Software Graph Pad Prism 5.01.336 was used for data analysis. Statistical analysis was evaluated by one-way ANOVA followed by Tuckey's test.

RESULT AND DISCUSSION:

Pharmacological Investigation:

Arthritic Score (Polyarthritic Index): The arthritic score was found to be significantly increased in arthritic group as compared to the normal group

Effect on Inflammation Parameter:

Paw Volume: Injection of FCA on the right hind paw of rat produced a significant increase in the swelling of the right paw of rats in arthritic group as compared to the normal group.

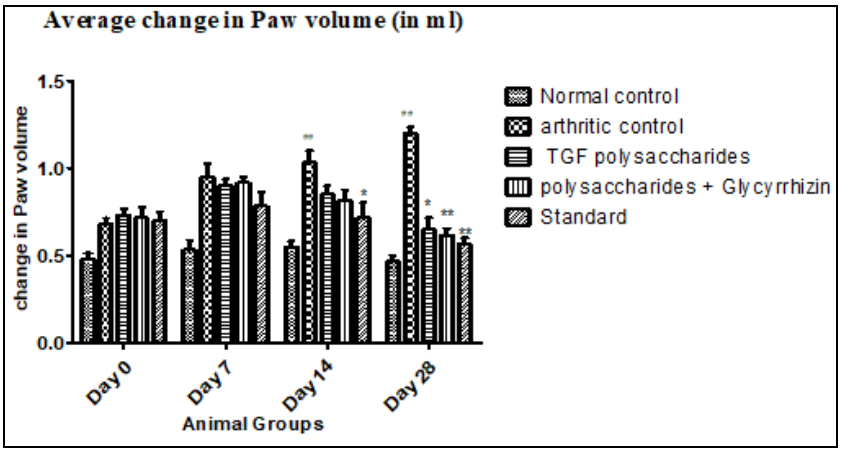

FIG. 1: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM GRACEUM* AND GLYCYRRHIZINON PAW VOLUME. ##p < 0.01 when compared with normal control, *p < 0.05, **p < 0.01 when compared with arthritic control.

Measurement of Ankle Diameter: Injection of FCA on right hind paw of rat produced an increase in ankle diameter in arthritic group as compared to normal group.

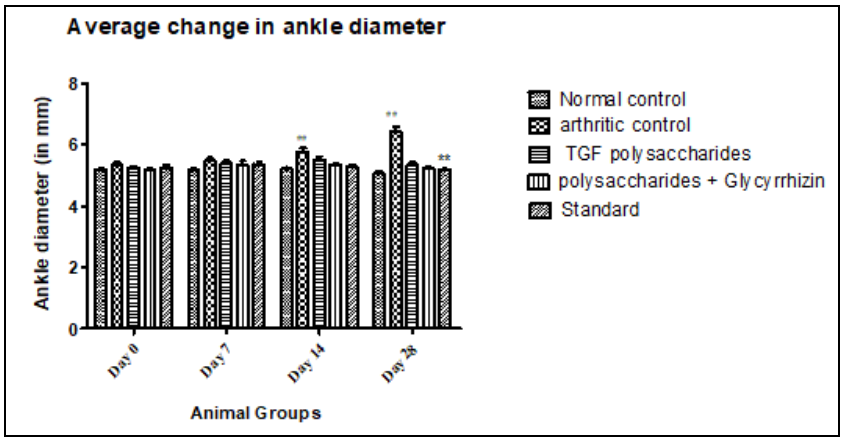

FIG. 2: MEASUREMENT OF ANKLE DIAMETER

Radiographic Analysis (Evaluation of Bone Destruction by X-rays): The radiographic pictures of the joints of arthritic animals show the joint's erosion. Drug treated with TGF polysaccharides, TGF polysaccharides + glycyrrhizin and Dexamethasone shows the marginal erosion of joint and the changes were normalized in these groups

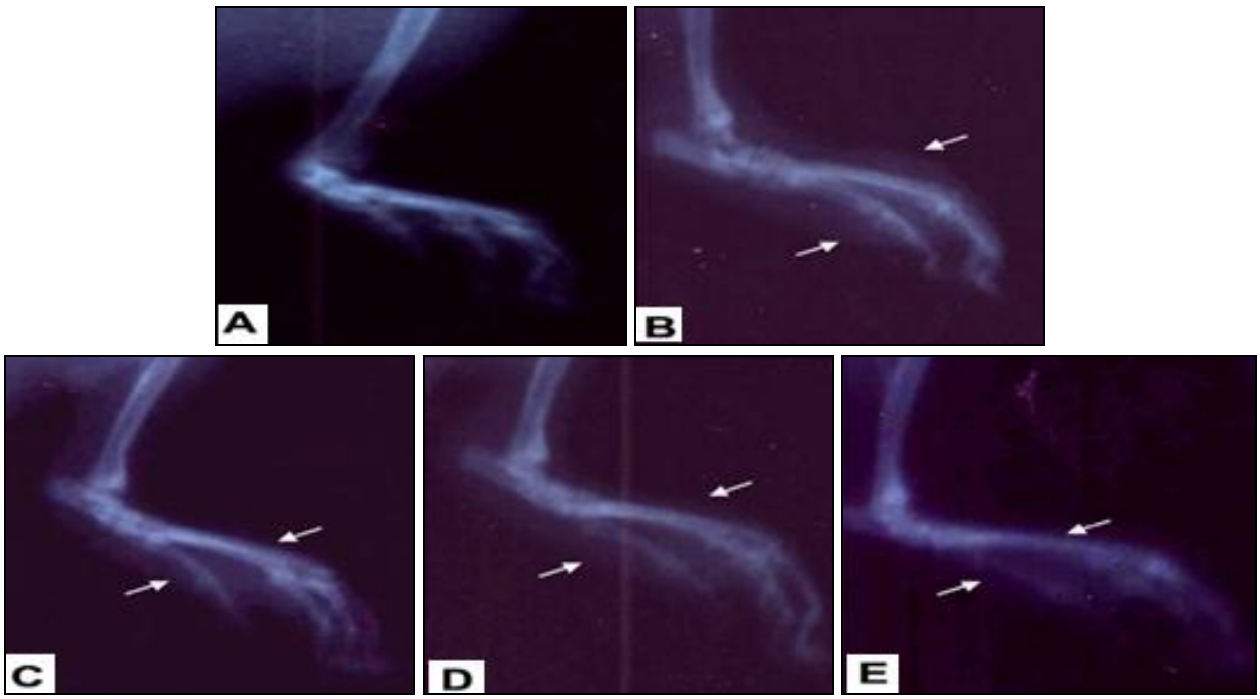

FIG. 3: X-RAYS PICTURES OF THE JOINTS OF CONTROL AND EXPERIMENTAL ANIMALS

Control: Showing normal pictures of joint.

Arthritic Rat: Showing the diffused joint and narrowing of joint space.

Treatment Group [TGF]: Shows clear and minimal narrowing of joint spaces.

Treatment group [TGF + Glycyrrhizin]: Shows clear and minimal narrowing of joint spaces.

Treatment Group [Dexamethasone]: Shows clear and minimal narrowing of joint spaces.

Biochemical Assays:

Aspartate Transaminase (AST): A significant increase in the activity of AST (membrane marker enzymes) was observed in the liver tissue homogenate, of arthritic group when compared to normal control rats on 28th day.

Arthritic rats treated with TGF polysaccharides show a significant decrease whereas treatment with TGF polysaccharides + glycyrrihizin shows very significant results compared to arthritic rats.

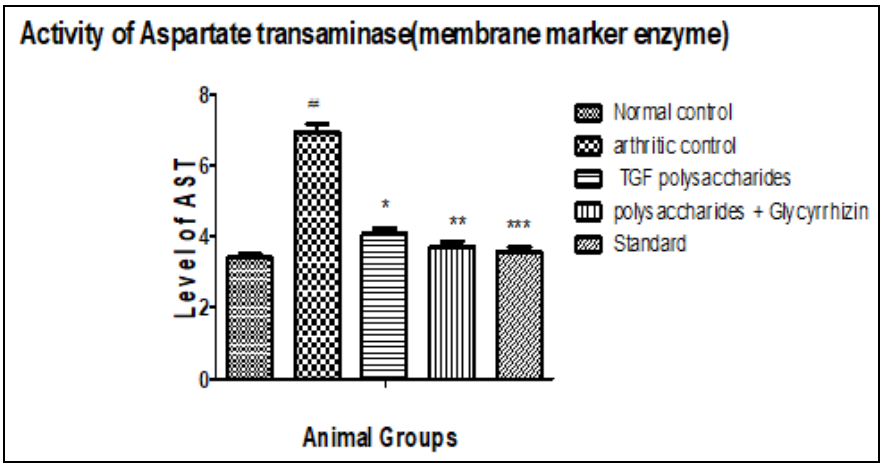

FIG. 4: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM GRACEUM* ON AST LEVEL: BARS SHOWING AST LEVEL IN ARTHRITIC RATS AFTER THERAPEUTIC ADMINISTRATION OF TGF POLYSACCHARIDES AND TGF POLYSACCHARIDES + GLYCYRRIHIZIN AND TGF+GLYCYRRHIZIN AND STANDARD DRUG DEXAMETHASONE. $^{\#\#}p < 0.01$ when compared with normal control, *p < 0.05, **p < 0.01, ***p < 0.001 when compared with arthritic control.

Alanine Transaminase (ALT): A marked increase in the activity of ALT (membrane marker enzymes) was observed in the liver tissue homogenate of arthritic group when compared to normal control rats on 28th day. Arthritic rats treated with TGF polysaccharides show a significant decrease whereas treatment with TGF polysaccharides + glycyrrihizin shows very significant results compared to arthritic rats. The standard drug dexamethasone shows a highly significant decrease in the activity of ALT enzymes.

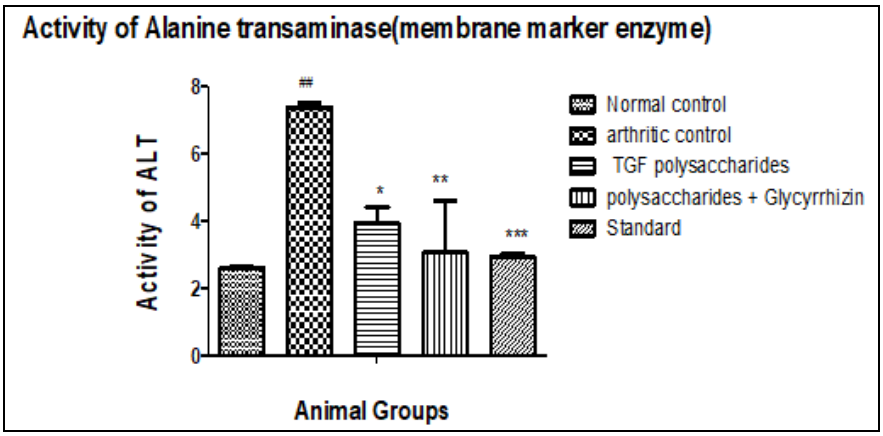

FIG. 5: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM GRACEUM* ON ALT LEVEL: BARS SHOWING ALT LEVEL IN ARTHRITIC RATS AFTER THERAPEUTIC ADMINISTRATION OF TGF POLYSACCHARIDES AND TGF POLYSACCHARIDES + GLYCYRRIHIZIN AND TGF+GLYCYRRHIZIN AND STANDARD DRUG DEXAMETHASONE. $^{\#\#}p < 0.01$ when compared with normal control, *p < 0.05, **p < 0.01, ***p < 0.001 when compared with arthritic control.

Measurement of Biochemical Parameters:

Lipid Peroxidation in Erythrocytes (TBARS) in Blood Homogenate: FCA injection produced an increase in the level of tissue TBARS (Thiobarbituric acid reacting substances) in blood homogenate of arthritic rats when compared to normal rats on both 14th and 28th day. Arthritic rats treated with TGF polysaccharides + glycyrrihizin shows highly significant results on 14th day whereas treatment with TGF polysaccharides shows significant result on 28th day.

Standard drug dexamethsone shows highly significant better reduction of tissue peroxidase on 28th day.

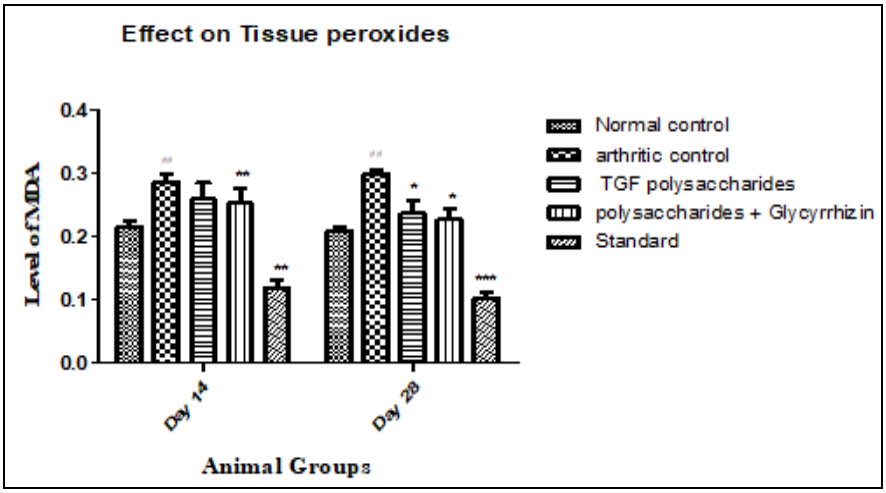

FIG. 6: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM GRACEUM* AND GLYCYRRHIZINON TBARS LEVEL: BARS SHOWING TBARS LEVEL IN ARTHRITIC RATS AFTER THERAPEUTIC ADMINISTRATION TGF POLYSACCHARIDES AND TGF POLYSACCHARIDES + GLYCYRRIHIZIN AND STANDARD DRUG DEXAMETHASONE. $^{\#\#}p < 0.01$ when compared with normal control, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared with arthritic control

Determination of Reduced Glutathione (GSH) on Liver Tissue: Oxidative stress associated with FCA-induced polyarthritis was evaluated by measuring level of GSH in blood homogenate. A marked decrease in the GSH level, which is naturally occurring antioxidant in body was observed in arthritic rats as compared to normal rats The arthritic rats treated with TGF polysaccharides + glycyrrhizin shows significant results as compared to arthritic rats treated with TGF polysaccharides alone. Standard drug dexamethsone shows significant increase in the GSH level.

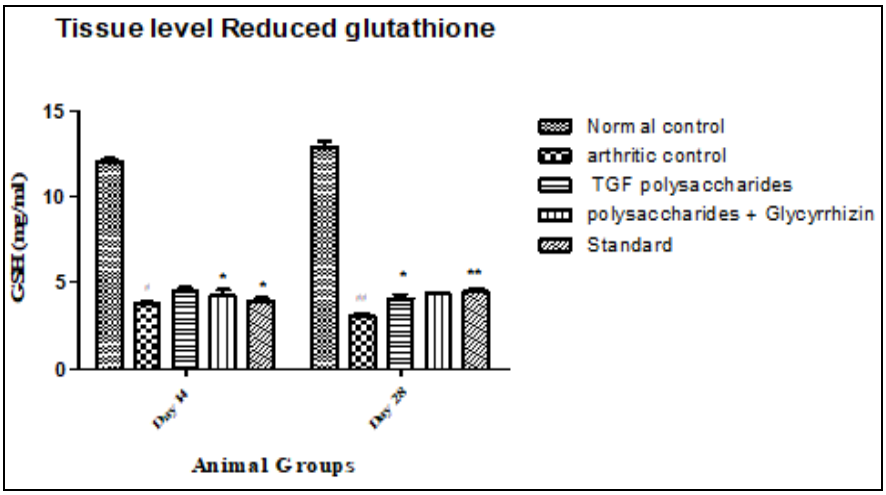

FIG. 7: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM GRACEUM* AND GLYCYRRHIZINON GSH LEVEL:-BARS SHOWING GSH LEVEL IN ARTHRITIC RATS AFTER THERAPEUTIC ADMINISTRATION TGF POLYSACCHARIDES AND TGF POLYSACCHARIDES + GLYCYRRIHIZIN AND STANDARD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared with normal control, $^{*}p < 0.05$, $^{**}p < 0.01$when compared with arthritic control

Body Weight Measurement: The increase in the body weight was comparable to arthritis-induced rats and normal rats. Beginning on day 7, the body weight increase in arthritis induced rats was significantly less than the normal rats, and this trend continued throughout the experiment. After administration of TGF polysaccharides and TGF polysaccharides + Glycyrrihizin rats exhibited a significant weight gain. The standard drug dexamethsone causes a significant increase in body weight.

TABLE 1: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM* ON CHANGES IN BODY WEIGHT IN GRAMS

Groups	Treatment	Dose (mg/kg)	Body weight		Change in Body weight (gm)	
Control	Vehicle	-	138.00±8.68	10.3±2.3	14.7±1.6	27.5±4.3
Arthritic control	Vehicle	-	136.7±14.06	6.8±2.2##	8.4±2.1##	14.8±3.2##
TGF polysaccharides	Biopolymeric fraction	75 mg/kg	146.7±9.45	7.2±2.1	10.5±2.3*	17.3±1.5*
TGF polysaccharides + Glycyrrhizin	Biopolymeric fraction + Glycyrrhizin	75mg/kg +20mg/kg	154.7±10.85	8.0±2.4	12.3±1.7**	18.3±2.6*
Standard	Dexamethasone	0.7 mg/kg	146.7±8.43	8.5±2.3*	14.1±1.5**	21.2±1.5**

#p < 0.05, ##p < 0.01 when compared with normal control, *p < 0.05, **p < 0.01when compared with arthritic control. Values are expressed as mean ± SEM, n = 6 rats in each group. The Stastical difference was evaluated by one way ANOVA followed by Tuckey's test.

Index of Immune Organ (Spleen): The index of spleen of the adjuvant arthritic rat was determined at day 28 after immunization. It was found that there was a decrease of spleen weight in arthritic rats. The administration of TGF polysaccharides and TGF polysaccharides + Glycyrrihizin evidently increased the weight of the spleen of adjuvant arthritic rats. This is a significant increase in the weight of the spleen of Dexamethasone treated group.

TABLE 2: EFFECT OF BIOPOLYMERIC FRACTION OF *TRIGONELLA FOENUM* ON WEIGHT MEASUREMENT INDEX OF IMMUNE ORGAN (SPLEEN)

Groups	Treatment	Dose (mg/kg)	Index (100%)
Normal Control	Vehicle	-	0.53 ± 0.14
Arthritic control	Vehicle	-	0.29 ± 0.02#
TGF polysaccharides	Biopolymeric fraction	75 mg/kg	0.37 ± 0.1*
TGF polysaccharides + Glycyrrhizin	Biopolymeric fraction + Glycyrrhizin	75mg/kg +20mg/kg	0.31 ± 0.15
Standard	Dexamethasone	0.7 mg/kg	0.42 ± 0.09**

#p < 0.05, when compared with normal control, *p < 0.05, **p < 0.01when compared with arthritic control. Values are expressed as mean ± SEM, n = 6 rats in each group. The Stastical difference was evaluated by one way ANOVA followed by Tukey's test

DISCUSSION: Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disorder [4]. It is characterized by inflammation of the synovial joints and concomitant destruction of cartilage and bone. However, rheumatoid arthritis is typically a progressive illness that has the potential to cause joint destruction and functional disability [5]. Edema and pain are characteristic signs of an inflammatory response where the present study demonstrates that Freund's complete adjuvant (CFA) containing killed M. tuberculosis-induced AA in rats. Adjuvant-induced arthritis (AA) is thought to occur through cell- mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats. Thus, activated macrophages and lymphocytes by adjuvant inoculation or their product, monokines, cytokines, chemokines, may be involved in abnormal lipid and protein metabolism. The role of prostaglandins and histamine is well established. Prostaglandins are endogenous mediators of inflammation and are generated from arachidonic acid by the action of cyclooxygenase (COX) that exist in two isoforms, COX-1 and COX-2. Cox-1 is constitutive, whereas COX-2 is the cytokine-inducible form of the enzyme. The ability of the drug to reduce edema formation may thus be related to its inhibitory action on prostaglandin synthesis. FCA causes an increase in polyarthritic score, paw volume, ankle diameter and causes decrease in paw withdrawal latency. Treatment with TGF polysaccharides alone causes a significant decrease in the polyarthritic score, paw volume, ankle diameter and an increase in paw withdrawal latency. Still, better results are obtained in arthritic rats treated with TGF polysaccharides + glycyrrhizin, which was comparable with standard drug Dexamethasone. The diagnosis of RA is usually obvious clinically. In arthritic rats, erosions representing bony destruction were evident on bone unprotected by cartilage, since they are exposed directly to cytokines and enzyme mediators in

synovial tissue. Narrowing of the joint space secondary to articular cartilage is diffuse within joints. The radiographic pictures of the joints of arthritic animals show high joint erosion compared to the normal control group. Treatment with TGF polysaccharides alone causes a significant reduction in erosions. Still, better results are obtained in arthritic rats treated with TGF polysaccharides + glycyrrhizin, in which these changes were normalized, which was comparable with the standard drug Dexamethasone. Cellular enzymes, such as aspartate transaminase (AST), alanine transaminase (ALT) are an indicator of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions. Increased activities of these enzymes were observed in arthritic control group when compared to normal control group. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, causes a significant decrease in the activity of these enzymes, which was comparable with standard drug Dexamethasone.

Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids found primarily in cell membranes. An increase in the level of lipid peroxides in tissues, therefore, reflects membrane damage. In many diseases, especially rheumatoid arthritis, membrane damage occurs in some organ or tissue, which provokes lipid peroxidation in the membrane and accelerates the disorder in the structure and function of these membranes. The lack of an antioxidant defence leads to an increase in lipid peroxidation and subsequent deleterious effects. In the present research, lipid peroxides were significantly increased in arthritic control group when compared to the normal control group. The increased lipid peroxide level under arthritic condition might be due to poor antioxidant defence and inactivation of antioxidant systems. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, causes decrease in lipid peroxidation, which was comparable with standard drug Dexamethasone. Although the mechanism underlying the decline in GSH content in the drug treated animal is not well understood. As adequate concentrations of GSH are required for various immune functions, it has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immune deficiency. The lymphoid organs spleen and thymus are affected during arthritic conditions, which may be the result of phagocytosis. The spleen weight of arthritic control rats decreased compared to the normal control group. Treatment with TGF poly-saccharides alone causes a significant increase in spleen weights compared to treatment with TGF polysaccharides + glycyrrhizin. Highly significant results were obtained in treatment with standard drug Dexamethasone group.

CONCLUSION: In the present research GSH content were significantly decreased in arthritic control group when compared to normal control group. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, causes increase in GSH content, which was comparable with standard drug Dexamethasone. Change in body weight is in response to the incidence and severity of arthritis and used to assess the onset of disease. Previous researchers have reported that the weight loss is associated with increased production of pro-inflammatory cytokines such as tumor necrosis factor alpha and interleukin-1. Adjuvant arthritis is characterized by reduced body weight. The body weight of arthritic control rats decreased compared to the normal control group due to FCA administration. Treatment with TGF polysaccharides and TGF polysaccharides + glycyrrhizin, regained body weights, significantly which was comparable with standard drug Dexamethasone.

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