How to Cite:
Barik, S., Soni, P., & Kharia, A. A. (2022). Evaluation of anti-arthritic activity of tofacitinib citrate controlled released formulation in complete Freund's adjuvant induced arthritis experimental model. *International Journal of Health Sciences*, *6*(S6), 2959–2969. https://doi.org/10.53730/ijhs.v6nS6.10528

Evaluation of anti-arthritic activity of tofacitinib citrate controlled released formulation in complete Freund's adjuvant induced arthritis experimental model

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***Abstract*---**In an animal model of complete Freund's adjuvant-induced arthritis, the tofacitinib citrate controlled-release formulation (TCRF) was examined. We examined the impact of TCRF on FCA-induced arthritis in rats, as well as weight alterations and changes in haematological and biochemical indicators. In contrast to the control group, the paw volume reduced and the animals' overall weight increased normally in the TCRF-treated animals, despite the fact that their body weight increased in the FCA-induced arthritis rats. An effective treatment for arthritis-induced haematological and biochemical abnormalities in arthritic rats was found to be TCRF at doses of 2.5, 5, 7, and 10mg/kg/p.o. At both the early and late stages of FCA-induced arthritis, the effects of treating arthritis-prone rats with TCRF showed significant reductions in rat paw edoema volume as well as a restoration of normal haematological and biochemical parameters.

***Keywords*---**tofacitinib, arthritis, freund's complete adjuvant.

Introduction

Systemic autoimmune rheumatoid arthritis (RA) is a complex illness, involving elements of genetics, endocrinology, ecology, and physiology. Excessive T lymphocyte production is a hallmark of rheumatoid arthritis, which causes macrophages, PMN's, and synoviocytes to become activated. The degradation of articular cartilage in RA patients leads to bone deformities and a loss of joint function, resulting in intense discomfort (Qasim et al., 2021). Phospholipase denatures cartilage phospholipids in arthritis (Bustamante et al., 2017). Rheumatoid arthritis can cause persistent inflammation, a primary cause of death worldwide (Furman et al., 2019). This multisystem autoimmune disorder affects 1-2% of the world's population.

TNF alpha or IL-1 factors, for example, are pro-inflammatory cytokinins that may contribute to the production of proteolytic enzymes during inflammation (Kany et al., 2019). In the RA synovium and fluids, a vital cytokine involved with the disease's development, TNF alpha, has been reported to be present at biologically noteworthy levels. RA synovial TNF alpha levels seem to correlate with the degree of inflammation and bone degradation in RA (Farrugia and Baron, 2016). With the direct impact on many tissues and the activation of additional cytokines, such as TNF alpha, further worsening the disease condition are the biological results linked with TNF alpha stimulation. 30 percent of RA patients fail to react to treatment despite the availability of a wide range of choices (Bullock et al., 2018).

Peptic ulcers, small intestinal and colon issues, perforation, haemorrhage, stricture, and chronic anaemia owing to iron deficiency and protein loss are just some of the major side effects associated with long-term use of these medicines. There is a pressing need to find a treatment plan that is both successful and cost-efficient because of the harmful effects and higher costs of present medicines. Drugs like analgesics (painkillers), anti-inflammatory drugs (steroids), and newer therapies such as DMARDs (disease-modifying antirheumatic drugs) are used to control the symptoms of inflammation and pain (Li et al., 2017). All of these drugs, however, come with a long list of undesirable side effects. Researchers are continually looking for ways to improve the bioavailability of medications while minimising negative effects in their formulations.

One or more TNF inhibitors have not worked for individuals who have rheumatoid arthritis (a disorder where the body attacks its own joints, resulting in swelling and loss of function). Tofacitinib can be used alone or in combination with other drugs to treat this illness (Kucharz et al., 2018). JAK inhibitors are the class of medications that it belongs to. Biochemical processes such as blood production and the immunological response that produces pain, soreness, and swelling are controlled by enzymes (proteins) known as JAKs. Several cell types, including bone and joint stem cells, include JAKs. Tofacitinib, a JAK inhibitor, reduces inflammation and tissue damage in rheumatoid arthritis patients (Scott et al., 2013). Tofacitinib was given the go light in November of last year. Preparation of controlled-release formulations using nanoparticles with a diameter of between 10 and 1000 nanometers allows for sustained release of medications, which reduces the frequency of drug administration and increases patient compliance (Adepu and Ramakrishna, 2021). Over time, a medicine is released slowly and steadily

through an oral controlled release drug delivery device. Tofacitinib citrate controlled release formulation (TCRF) was therefore developed for the treatment of RA in an experimental rat model.

Material and Methods

Drugs and Chemicals

Tofacitinib citrate was taken as free sample from Aurobindo Pharma Ltd. and Complete Freund's adjuvant were procured from Sigma-Aldrich. The laboratory chemicals used in this study were of analytical reagents grade.

Tofacitinib citrate controlled release formulation (TCRF)

Manufacturing method adopted as controlled release matrix tablets with hydrophilic polymer with high viscosity grade to retains the drug release. Selected polymer was Polyethylene oxide, Hypromellose (Methocel K100premium LV CR) in core tablets composition. To retain the drug in initial stage Ethyl cellulose was selected as hydrophobic polymer and Hydroxy propyl cellulose (Klucel LF) used as pore former with minimum concentration in coating composition. The formulation was manufactured with non-aqueous high shear granulation technique. Manufacturing unit operation involves sifting, mixing, granulation, drying, milling, blending, compression and coating.

Experimental Animals

The Institutional Animal Ethics Committee (IAEC) has given its approval to the experiment reported in this article. Animals (36 male albino rats (150–230g) of the wistar strain) were obtained from the animal house of the institute. The rats were housed in typical settings of relative humidity and temperature for an animal shelter. They have access to water and a pellet food that is the same every day. The rats were acclimated to their new surroundings in the animal house for seven days prior to the start of the experiment. The experimental protocols utilised in the study have been approved, and the instructions are closely adhered to throughout the investigation.

Acute toxicity study

There were only five animals in each group used in this study, in accordance with OECD standards 423, to ensure that sufficient data could be gathered on the test sample. Before the oral delivery of the test sample, the experimental animals are fasted for 3-4 hours. After administering the medication, the animals are checked every 30 minutes for the first 4-6 hours of their lives. As a result, data will be provided in a tabular form, including the number of animal participants, animal deaths, the number of animals showing toxicity signs, the time course of toxic effects and their reversibility, and necropsy findings.

Anti-arthritic Activity of TCRF on experimental animals
Induction of Arthritis

Sub-plantar injection of Freund's full adjuvant into the left hind paw was performed. On the day of the adjuvant injection, a daily dose of medication was taken orally for the next 12 days. The paw volume plethysmographically was measured on days 0, 7, 14, 21 and 28 after injection of Complete Freund's adjuvant to examine the change in inflammatory reactivity (CFA).

Experimental design

There were 36 Wistar-breed male albino rats (150–230 g) utilised in the investigation. Seven groups of six animals each were formed. Rats were fed TCRF via oral gavage rather than the conventional Tofacitinib Citrate (10mg/kg, p.o). Tofacitinib Citrate, the standard anti-arthritic drug, was administered to Group III (as a positive control, 10 mg/kg, p.o.), and Groups IV and VII were treated with TCRF at doses of 2.5, 5, 7.5, and 10 mg/kg/p.o. for 28 days starting on the induction day. Groups I and II served as controls (without treatment). Group II served as an arthritic control (0-28 days treatment). It was discovered that the body weight fluctuated on a weekly basis.

Biochemical Analysis

On day 29 of the experiment, all animals were decapitated and blood was taken in plain and EDTA-containing tubes. It measured RA (Rheumatoid Arthritis Factor). Protein, albumin, globulin, and fibrinogen Plasma/serum and homogenised samples were tested for ceruloplasmin.

Statistical Analysis

The Mean ± SEM of six animals was presented. Following a one-way analysis of variance (ANOVA) and the Dunnett multiple comparison test, statistical significance was determined. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ were regarded statistically significant, if any difference was found between the groups, respectively.

Results

FCA induced rat paw edema

Compared to normal control rats, FCA-induced arthritic rats exhibited larger paws. TCRF therapy reduced rat paw edema volume relative to the arthritic group (Table1). On day 28^{th} the paw edema in TCRF 4 group was significantly ($p<0.001$) reduced from 0.25 mm to 0.21 mm, which was highest among the groups as compared to the control group. The other treated groups were also reduced paw edema in rats in a significant ($p<0.01$) manner. However, reduction in paw edema by the standard drug was 0.48 mm on 28^{th} day.

Body weight changes

Data from this study show a link between joint inflammation and weight loss. Adjuvant injection caused significant weight loss in arthritis rats in the first week, followed by weight gain. At this point in the experiment, neither the TCRF nor the standard drug-treated groups showed any weight loss.

Hematological parameters

Figure 1 to 4 indicate haematological changes in adjuvant-induced arthritic rats. Administration of TCRF returned edema to normal at both stages of adjuvant-induced arthritis. All the treatment groups significantly restored the normal levels of hematological parameters in FCA-induced arthritic rats. TCRF 4 treat group normalized RBC (Figure 1), WBC (Figure 2), Hb (Figure 3) and ESR (Figure 4) level to 5.87 million/mm3 ($p<0.001$), 8.96 thousands/mm3 ($p<0.001$), 14.44 g/dL ($p<0.001$) and 5.12 mm/hr ($p<0.01$) as compared to the control group. Other treated groups were also significant in restoring the normal levels of blood profile. However, standard group was less significant ($p<0.05$) among other treated groups.

Blood urea and serum creatinine

Adjuvant-induced arthritic animals have greater blood urea and serum creatinine levels ($p<0.001$) than normal rats (Figure 5). TRCF reduced these changes. Urea and creatinine levels were increased tremendously after induction of arthritis, which was significantly restored by TCRF treated groups. The reduction in increased urea and creatinine levels by TCRF 4 groups were 23.27 ($p<0.001$) and 1.65 mg/dL ($p<0.01$) as compared to the control group.

Total protein, albumin, globulin and A/G ratio

Table 2 demonstrates differences in protein concentrations. Compared to control rats, arthritic rats had lower total protein, albumin, and A/G ratios, but higher globulin levels ($p<0.001$). TCRF reversed all these changes. Protein level was restored to 5.35 g/dL, albumin to 3.08 g/dL, globulin to 2.55 g/dL, cerruloplasmin to 23.48 mg/dL, fibrinogen to 85.42 mg/dl and A/G ratio to 1.20 as compared to the control group. The standard group was also effective in restored the biochemical parameters, however, less significant to TCRF treated groups.

Acute phase proteins

There are two acute phase proteins that are considered fibrinogen and ceruloplasmin in the body. It was found that these two acute phase markers were considerably higher in arthritis-induced rats than they were in arthritis-free rats ($p <0.001$) (Table 3). In arthritic rats, TCRF treatment reduced acute phase proteins considerably ($p <0.001$). Multiple-component auto-immune disorder Rheumatoid arthritis (RA) has been described as a joint impairment illness (Perveen et al., 2013; Bashir and Niazi, 2020). There are several elements that contribute to RA development, but TNF alpha has been found to be the most

important one. The Freund complete adjuvant-induced arthritis (FCAIA) model of chronic inflammatory arthritis has been extensively studied. Animals inoculated with FCA have shown a severe systemic inflammatory response characterised by significant edoema, joint remodelling, and synovial penetration. Paw edoema, weight loss, joint discomfort, and cartilage degeneration are all hallmarks of RA in humans, and these symptoms are strikingly comparable (Thurston et al., 2010; Quirke et al., 2011). To engage the immune system, CFA injection in the hind paw results in aberrant leukocyte proliferation. Paw edoema and the arthritic index were markedly reduced in animals treated with TCRF.

TCRF lowered paw volume and arthritic index, as may be seen from our current findings. It is also possible that restoring biochemical parameters, such as COX-2 and PGE2, inhibits inflammation and, thus, reduces paw volume and the arthritic index. This can be hypothesised (Mo et al., 2011). Additionally, the measurement of body weight and the haematological profile have been used to estimate the antiarthritic effects of CFA-induced arthritis. Cachexia has long been associated with RA. Cachexia, the muscle loss associated with RA, can be caused by a number of reasons, but elevated levels of TNF alpha have long been known to play a role (Shen et al., 2014). Blood parameters (CBC, liver, and kidney parameters) have been linked to RA pathobiology because of its inflammatory and immunological characteristics. It would be really beneficial to look at the influence of any test agent on resolving these issues before coming up with a new treatment plan for RA.

Anemia is produced by low erythropoietin levels, early erythrocyte destruction, low plasma iron, and aberrant iron loading in the reticuloendothelial system and synovium. The bone marrow is unable to reverse anaemia due to this combination of variables (Tracey et al., 1988). TCRF boosted Hb and RBC levels, preventing anaemia by promoting erythropoiesis, possibly by decreasing TNF alpha production from monocytes and macrophages. TCRF has been shown to reduce ESR and RF (important haematological markers) in arthritic rats, notably in the serum of arthritic rats, which indicates its protective effect in alleviating the symptoms of rheumatoid arthritis (Frank et al., 2013). As a result, it can be said that TCRF treatment has restored normal blood parameters altered by arthritis. As a result of all of the experiments, it can be concluded that TCRF is effective in the treatment of RA; nevertheless, further mechanistic investigations are needed to identify the primary signalling pathways implicated.

Table 1
Effect of TCRF on rat paw edema in FCA arthritis in rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
NC	0.22 ± 0.05	0.25 ± 0.09	0.21 ± 0.05	0.22 ± 0.09	0.21 ± 0.08
AC	0.27 ± 0.03	0.82 ± 0.05	0.80 ± 0.09	0.71 ± 0.12	0.77 ± 0.09
Standard	0.26 ± 0.01	0.53 ± 0.03 **	0.40 ± 0.05 **	0.42 ± 0.05 **	0.48 ± 0.07
TCRF 1	0.24 ± 0.08	0.62 ± 0.09 **	0.52 ± 0.09 **	0.52 ± 0.07 **	0.40 ± 0.04 **

TCRF 2	0.28 ± 0.09	0.54 ± 0.05	0.50 ± 0.09 **	0.49 ± 0.04	0.35 ± 0.11 **
TCRF 3	0.26 ± 0.06	0.58 ± 0.04 **	0.48 ± 0.08 **	0.46 ± 0.09 **	0.30 ± 0.02 ***
TCRF 4	0.25 ± 0.04	0.25 ± 0.08 *	0.26 ± 0.07 *	0.26 ± 0.05 *	0.21 ± 0.01***

For each group, there were n = 6 animals, and the values are expressed as the mean standard error of the mean (SEM). Significantly different from the usual control group at *p < 0.05, **p < 0.01, ***p < 0.001. NC = Normal control, AC = Arthritic control, Standard = Tofacitinib citrate, TCRF = Tofacitinib citrate controlled release formulation (2.5, 5, 7.5 and 10 mg/kg).

Table 2
Effect of TCRF on biochemical parameters in FCA arthritis in rats

Groups	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Cerruloplasmin (mg/dl)	Fibrinogen (mg/dl)
NC	6.62±0.20	3.58±0.06	2.30±0.08	1.60±0.10	19.65±2.65	42.65±2.28
AC	4.28±0.25	2.36±0.09	2.86±0.06	0.79±0.10	52.74±2.84	162.45±4.93
Standard	5.32±0.18 **	3.02±0.10**	2.54±0.04**	1.15±0.08**	22.12±1.3*	88.04±3.2**
TCRF 1	5.30±0.20 *	2.98±0.08*	2.58±0.08*	1.18±0.07*	26.81±2.4*	92.66±5.6*
TCRF 2	5.24±0.15 *	2.89±0.09*	2.62±0.09*	1.12±0.09*	26.24±3.6*	98.53±6.8*
TCRF 3	6.52±0.19 *	3.64±0.07*	2.24±0.04**	1.61±0.04*	18.63±2.9*	42.20±7.8*
TCRF 4	5.35±0.16 **	3.08±0.06**	2.55±0.06**	1.20±0.08**	23.48±2.4***	85.42±8.6**

For each group, there were n = 6 animals, and the values are expressed as the mean standard error of the mean (SEM). Significantly different from the usual control group at *p < 0.05, **p < 0.01, ***p < 0.001. NC = Normal control, AC = Arthritic control, Standard = Tofacitinib citrate, TCRF = Tofacitinib citrate controlled release formulation (2.5, 5, 7.5 and 10 mg/kg)

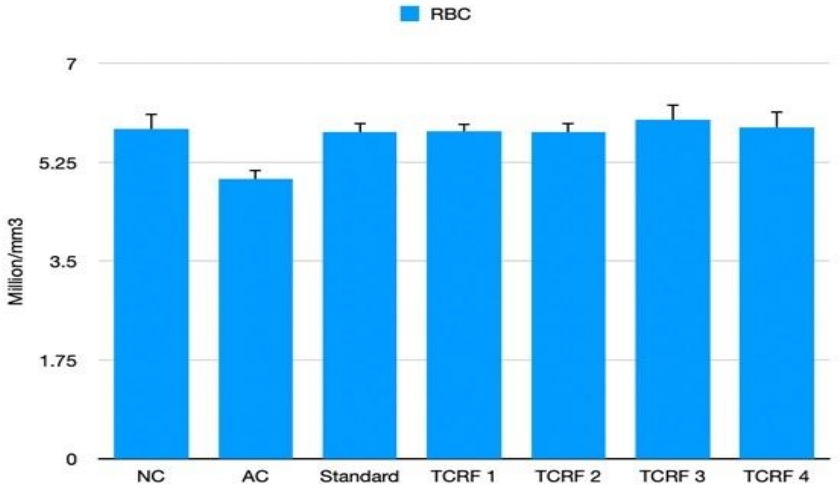

Figure 1. Effect of TCRF on RBC of FCA-induced arthritis animals

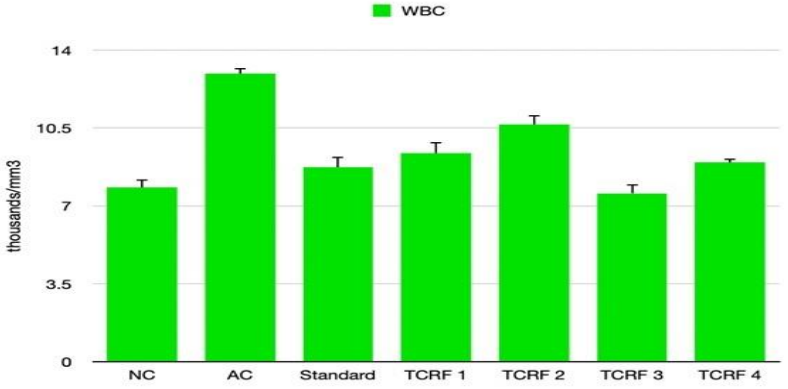

Figure 2. Effect of TCRF on WBC of FCA-induced arthritis animals

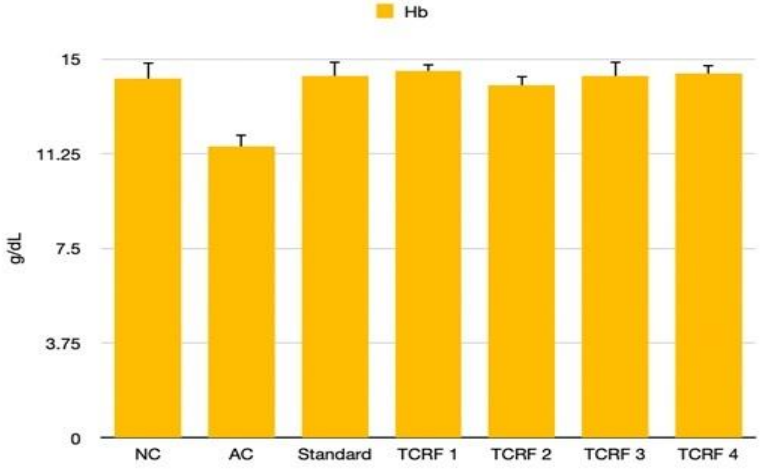

Figure 3. Effect of TCRF on Hb of FCA-induced arthritis animals

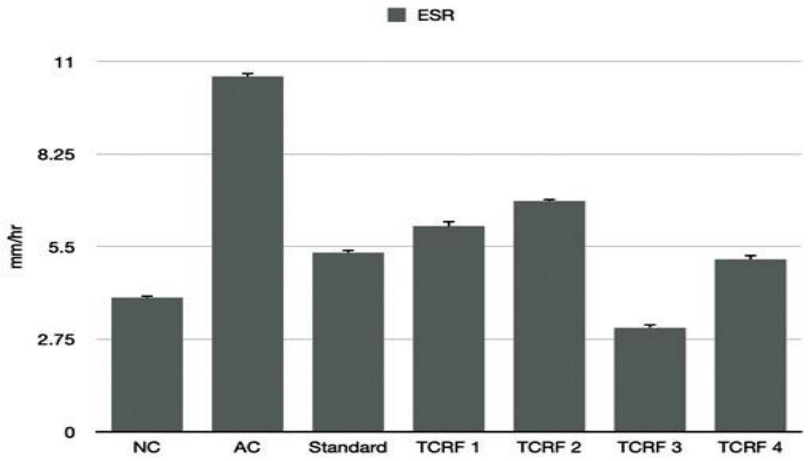

Figure 4. Effect of TCRF on ESR of FCA-induced arthritis animals

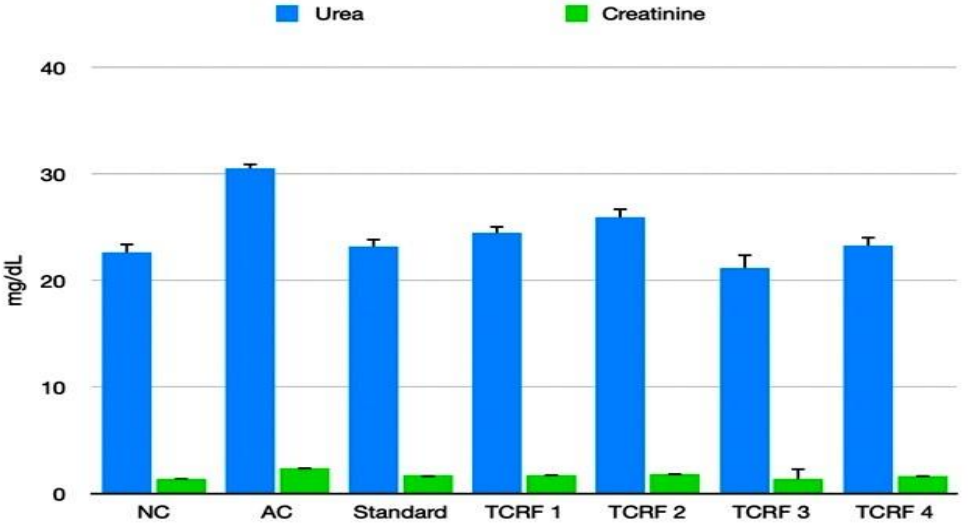

Figure 5. Effect of TCRF on ESR of FCA-induced arthritis animals

Conclusion

This suggests that TCRF may have antiarthritic properties, given the studies discussed above. TCRF inhibits inflammation and has a considerable antiarthritic effect that is concentration dependent. TCRF, on the other hand, greatly reduced paw edoema in animals with arthritis. Additional evidence of its ability to reduce inflammation in CFA-induced arthritis may be provided by a decrease in mRNA expression levels of TNF alpha in the paws, weight loss, and haematological profile. TCRF antiarthritic effects may, in part, be attributable to the compound's anti-inflammatory properties. As a result, it's reasonable to assume that TCRF could be used to treat RA. The specific mechanism of action of this medication has to be elucidated through additional, more thorough mechanistic screening.

Declaration

The authors declared no conflict of interest.

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