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Research Article

To Carry Out Anti-Arthritic Activity of Combination of Quercetin and Curcumin Using FCA (Freund's Complete Adjuvant) Induced Arthritis in Laboratory Rats

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Abstract

To investigate anti-arthritic activity of Quercetin and Curcumin in Freund's complete adjuvant (FCA)-induced arthritis in rats. AA was induced by injecting with Freund's complete adjuvant (FCA). Rats were randomly divided into six groups with 10 mice in each group: (1) Normal group (saline), (2) Vehicle control, (3) Leflunomide (LF, 10 mg/kg), (4) Curcumin (80), (5) Quercetin (40), (6) Curcumin (80) + Quercetin (40). Male SD rats were subjected to treatment with Lut at 10 mg/kg from days 18 to 24 after immunization. Arthritic scores, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), paw histopathology and the proteins of P2X4 pathway were assessed at the end of the experiment. Lut reduced the severity of arthritic scores during the experimental period as compared with positive control (RA). Lut significantly suppressed TNF-α and IL-1 as compared with RA group. Histopathological examination indicated that Lut alleviated infiltration of inflammatory cells and synovial hyperplasia as well as protected joint destruction. Lut significantly suppressed inflammatory cells, and Caspase-1p10. Quercetin and Curcumin may be a potential preventive or therapeutic candidate for the treatment of inflammation and arthritis.

Keywords: Rheumatoid arthritis, anti-arthritic activity, Quercetin, Curcumin, Freund's complete adjuvant (FCA), Leflunomide, histopathology Etc.

INTRODUCTION:

Rheumatoid arthritis is a chronic, inflammatory, autoimmune disease, the pathology of which is primarily and symmetrically localized in diarthrodial joints. The pathogenesis of RA is characterized by an inflamed synovium (lining the joint cavity), degradation of articular cartilage and erosion of sub-chondral bone. The systemic ramifications of the disease, with their attendant morbidity and mortality, include cardiopathy, nephropathy, vasculopathy and pulmonary and cutaneous disorders. It is important to note that arthritis is not a single ailment, but rather a collection of medical issues collectively known as "Arthritis". In the United States alone, around 47 million adults and 300,000 children are impacted by this condition. If timely and appropriate treatment is not provided, it can result in permanent disability. On a global scale, arthritis places a significant financial burden in terms of lost wages and medication expenses.[1-4]

The therapeutic approach affords symptomatic relief but the process of degeneration of the cartilage is not arrested by the drugs like NSAIDS. There is paucity of disease modifying drugs. However, in the traditional system of medicine large number of plants have been reported to afford relief in the symptoms of rheumatoid arthritis. The polyphenols from plants are now investigated due to their antioxidant properties for Ant arthritic activity. With respect to this and as per need, we decided to carry out screening activity of combination of Curcumin and Quercetin along with Leflunomide. Documented studies by number of researchers have well established adjuvant-induced arthritis as one of the important experimental models to assess the efficacy of various molecules. For this screening activity, we studied number of parameters including *In-vivo* and *Ex-vivo* parameters.[5-9]

MATERIALS AND METHODS:

Animals:

Female Sprague Dawley rats weighing 180-220 g were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal – Bhor, Pune. The animals were housed in polypropylene cages and maintained under environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under 12h light: 12 dark cycle. The animals had free access to food pellet (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Loknete Shri Dadapatil Pharate College of Pharmacy.

Mandavgaon Pharate constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is **2168/PO/Re/S/22/CPCSEA**.

Materials:

Curcumin, Quercetin, Freund's Complete Adjuvant, ATP & NADPH were purchased from Sigma-Aldrich, India.

Preparation of drug solution, storage, volume, and route of administration:

Curcumin and Quercetin:

Preparation of test drug solution:

Drug solution of Curcumin was prepared by using distilled water a vehicle

Storage of drug solution:

Curcumin and Quercetin powder was stored in a desiccator. A fresh drug solution was prepared for each day's work. The solution was kept in airtight amber-colored bottles and stored at room temperature until ready for use.

The volume of drug administration:

The volume of Curcumin and Quercetin solution to be administered was calculated based upon the body weight of animals.

Route of administration:

The solution of Curcumin and Quercetin was administered per oral (p.o.) route.

Leflunomide:

Preparation of test drug solution:

Drug solution of Leflunomide was prepared by using distilled water as a vehicle

Storage of drug solution:

Leflunomide powder was stored in a desiccator. A fresh drug solution was prepared for each day's work. The solution was kept in airtight amber-colored bottles and stored at room temperature until ready for use.

The volume of drug administration:

The volume of Leflunomide solution to be administered was calculated based upon the body weight of animals.

Route of administration:

The Leflunomide solution was administered per oral (p.o.) route.

FCA-induced arthritis in laboratory animals:

Experimental designs:

The animals were divided randomly into groups with six rats per group as follows:

Group I: Normal group

The rats treated with vehicle (distilled water, 15 mg/kg, p.o.) for 30 days and received saline (100 mg/kg, i.p.) on 29th and 30th day

Group II: Vehicle control

FCA (complete fraction of *Mycobacterium butyricum* suspended in oil) was injected (0.1 ml) into the sub plantar region of the left hind paw (100 mg/kg, i.p.) of rats then they were treated with vehicle (distilled water, 15 mg/kg, p.o.) for 28 days

Group III: Leflunomide (10) treated group

FCA (complete fraction of *Mycobacterium butyricum* suspended in oil) was injected (0.1 ml) into the sub plantar region of the left hind paw (100 mg/kg, i.p.) of rats then they were treated with Leflunomide (10 mg/kg, p.o.) for 28 days

Group IV: Curcumin (80) treated group

FCA (complete fraction of *Mycobacterium butyricum* suspended in oil) was injected (0.1 ml) into the sub plantar region of the left hind paw (100 mg/kg, i.p.) of rats then they were treated with curcumin (80 mg/kg, p.o.) for 28 days

Group V: Quercetin (40) treated group

FCA (complete fraction of *Mycobacterium butyricum* suspended in oil) was injected (0.1 ml) into the sub plantar region of the left hind paw (100 mg/kg, i.p.) of rats then they were treated with quercetin (40 mg/kg, p.o.) for 28 days

Group VI: Curcumin (80) + Quercetin (40) treated group

FCA (complete fraction of *Mycobacterium butyricum* suspended in oil) was injected (0.1 ml) into the sub plantar region of the left hind paw (100 mg/kg, i.p.) of rats then they were treated with curcumin (80 mg/kg, p.o.) + quercetin (40 mg/kg, p.o.) for 28 days

Treatment of Curcumin, Quercetin and Leflunomide

The different doses of curcumin (80 mg/kg), quercetin (40 mg/kg) and Leflunomide (10 mg/kg) were calculated based on the animal's body weight were administered per oral for 28 days.

Parameter for assessment of the effect of curcumin and quercetin FCA-induced arthritis in rats:

- Body weight
- Paw volume
- Joint diameter
- Paw withdrawal latency
- ALP
- AST
- ALT
- Albumin
- Serum CRP
- Anti-oxidant activity in hepatic tissue
  - ✓ Total protein
  - ✓ MDA
  - ✓ GSH
  - ✓ SOD
  - ✓ Nitric oxide
- Histopathology of Tibiotarsal joint

Statistical Analysis:

Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, USA). Statistical comparisons were made between drug-treated groups and disease control animals (vehicle control). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

Table 1- Effect of curcumin, quercetin and their combination on FCA-induced alteration in body weight:

Time (in days)	Body weight (gm) - Mean ± SEM					
	Normal	AIA Control	Leflunomide (10 mg/kg)	Curcumin (80 mg/kg)	Quercetin (40 mg/kg)	Curcumin (80mg /kg) + Quercetin (40 mg/kg)
0	213.83 ± 1.74	213.17 ± 1.90$^{\#}$	212.17 ± 1.60	213.67 ± 1.26	210.67 ± 0.76	213.67 ± 1.58
6	223.67 ± 1.74	210.33 ± 1.58$^{\#\#\#}$	214.67 ± 1.43	214.83 ± 1.22	217.5 ± 1.23	217.5 ± 1.15
12	225.50 ± 0.56	209.00 ± 1.46$^{\#\#\#}$	215.33 ± 2.32**	213.33 ± 1.74	216.17 ± 2.33	217.83 ± 1.49
16	228.17 ± 3.12	206.33 ± 1.38$^{\#\#\#}$	220.50 ± 1.67***	213.83 ± 1.80	212.17 ± 1.78	217.50 ± 1.50**
20	233.33 ± 1.82	199.17 ± 1.08$^{\#\#\#}$	227.17 ± 1.62***	217.00 ± 1.29***	215.67 ± 0.80***	220.33 ± 1.31***
28	240.00 ± 1.32	192.83 ± 1.54$^{\#\#\#}$	233.17 ± 1.62***	214.67 ± 0.92***	212.83 ± 1.30***	226.17 ± 1.45***

Data were analyzed by Two-Way ANOVA followed by Bonferroni's post-hoc test. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with AIA Control group on respective days.

There was no significant difference in the body weight of normal rat, AIA Control and treatment groups rat before induction of arthritis on day 0. However, FCA administration resulted in significant decreased ($P < 0.05$) in the body weight of AIA Control rats on day 6 as compared to normal rats. The body weight of AIA Control rat was still significantly reduced ($P < 0.001$) up to 28 days when compared with normal rats. Body weight of leflunomide (10 mg/kg) treated rats also significantly increased ($P < 0.001$) from 16 days onwards. Rat treated with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone showed a significant ($P < 0.001$) inhibition in FCA-induced decrease in body weight from 20 day onwards. Treatment with combination of curcumin (80 mg/kg) and quercetin (40 mg/kg) for 28 days significantly inhibited FCA-induced decreased ($P < 0.001$) the body weight as compared to AIA Control rats from 12 days onwards.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in paw morphology:

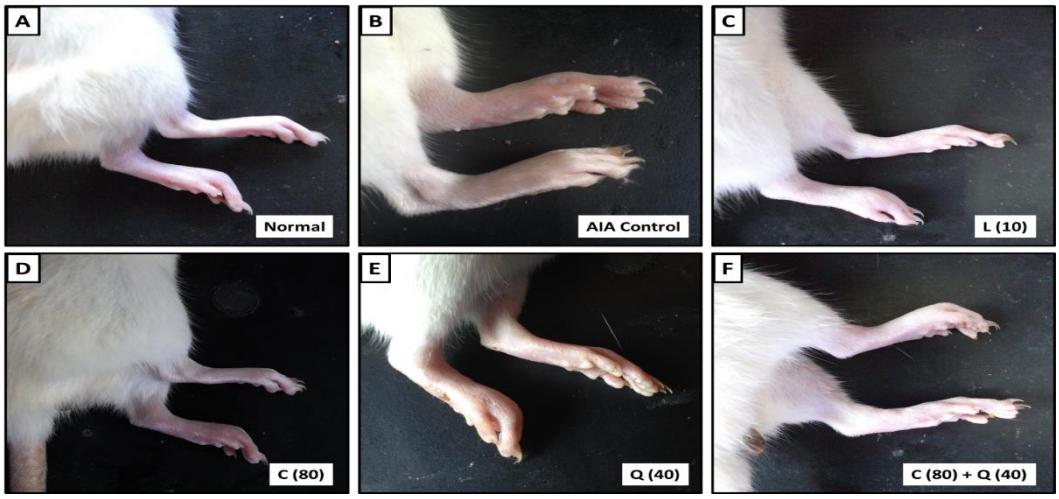

Figure 1: Morphological representation of rat paw from various treatment groups.

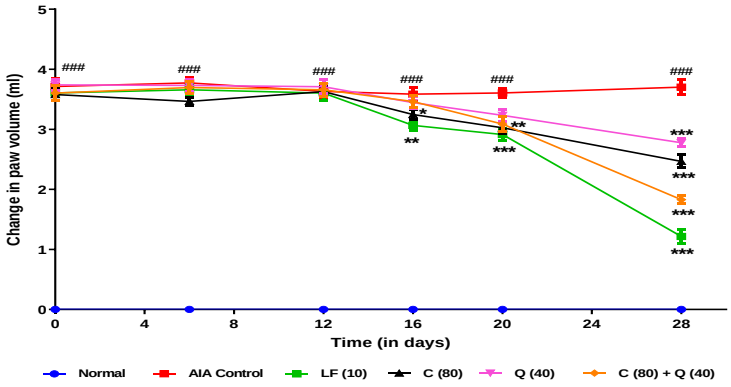

Figure 2 Effect of curcumin, quercetin and their combination on FCA-induced alteration in change in paw volume

Data were analyzed by Two-Way ANOVA followed by Bonferroni's post-hoc test. $^{\#\#\#}P < 0.001$ as compared with normal group and $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with AIA Control group on respective days.

Paw volume in the AIA Control rats significantly increased ($P < 0.001$) as compared to normal rat post administration of FCA day 0. This increased in the paw volume after sub-plantar administration of FCA remains significant ($P < 0.001$) decreased till 28th day in arthritis control rats as compared to normal rats. The 28 days treatment of curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) showed the significant decreased ($P < 0.001$) in the paw volume as compared to AIA Control rats from 16 days onwards. FCA-induced increased paw volume was significant attenuated ($P < 0.05$) in leflunomide (10 mg/kg) treatment on 16 days onwards as to compared to AIA Control rats. Treatment with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone also significantly attenuated ($P < 0.001$) this increased in the paw volume on 16st and 24th day compared to AIA Control rats.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in change in joint diameter

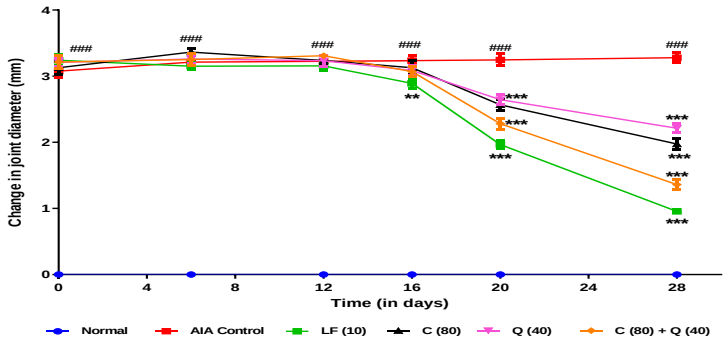

Figure 3 Effect of curcumin, quercetin and their combination on FCA-induced alteration in change in joint diameter

Data were analyzed by Two-Way ANOVA followed by Bonferroni's post-hoc test. ###$P < 0.001$ as compared with normal group and **$P < 0.01$, ***$P < 0.001$ as compared with AIA Control group on respective days.

The joint diameter or thickness in the FCA treated significantly increased ($P < 0.01$) after sub-plantar FCA administration in AIA Control rats as compared to normal rats and remains significant ($P < 0.001$) for next 28 days in arthritis control rats. The 28 days treatment of curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) showed the significant decreased ($P < 0.001$) in the joint diameter as compared to AIA Control rats from 20 days onwards. The joint diameter in leflunomide (10 mg/kg) treatment was significantly decreased ($P < 0.001$) from 16 day onwards as compared to AIA Control rats. This inhibition in the decreased in the joint diameter was also significant ($P < 0.001$) in the curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated rats from 20 days onwards compared to AIA Control rats.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in paw withdrawal latency i.e., thermal hyperalgesia (Plantar test):

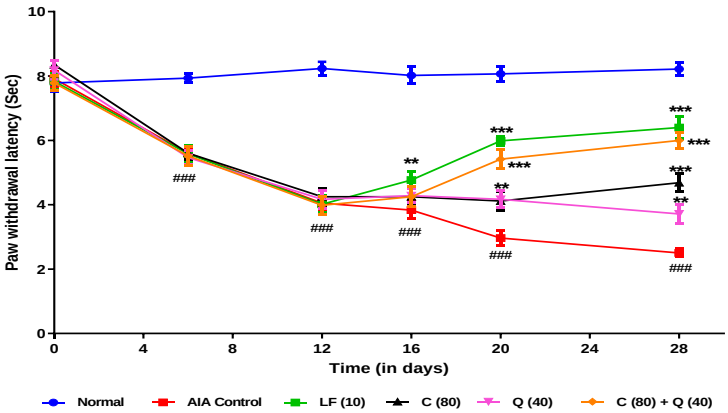

Figure 4 Effect of curcumin, quercetin and their combination on FCA-induced alteration in paw withdrawal latency i.e., thermal hyperalgesia (Plantar test)

Data were analyzed by Two-Way ANOVA followed by Bonferroni's post-hoc test. ###$P < 0.001$ as compared with normal group and **$P < 0.01$, ***$P < 0.001$ as compared with AIA Control group on respective days.

The paw withdrawal latency in the AIA Control rats did not significantly differ than normal rats on day 0 before induction of arthritis. FCA administration caused significant decreased ($P < 0.01$) in the paw withdrawal latency in AIA Control rats as compared to normal rats from day 6 onwards. This decreased in the paw withdrawal latency was more significant ($P < 0.001$) in on 28th day in AIA Control rats as compared to normal rats. Treatment with curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) showed the significant attenuation ($P < 0.001$) in these decreased paw withdrawal latency as compared to AIA Control rats from 20 days onwards. Leflunomide (10 mg/kg) treatment treated rats showed the significant inhibition ($P < 0.01$) in decrease in paw withdrawal latency on 16 days onward compared to AIA Control rats. Treatment with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone also showed the significant increase in the paw withdrawal latency form 20 days onwards ($P < 0.01$ and $P < 0.001$) as compared to AIA Control rats.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in AST and ALT levels:

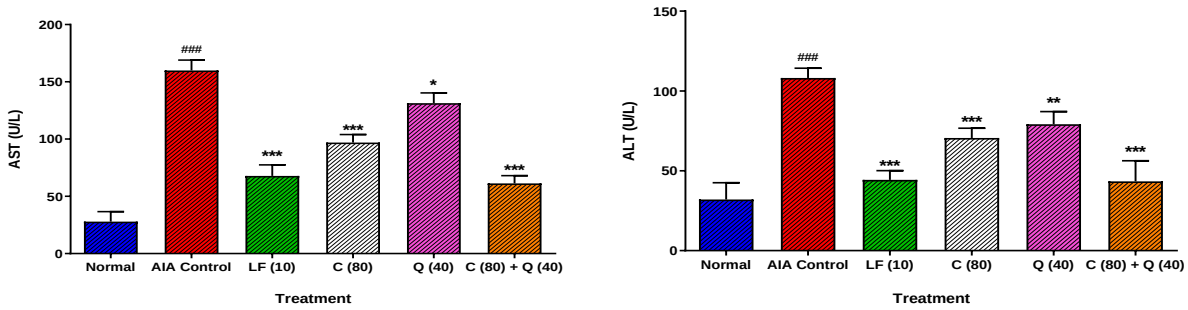

Figure 5: Effect of curcumin, quercetin and their combination on FCA-induced alteration in CK-MB and LDH levels

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###$P < 0.001$ as compared with normal group and *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared with AIA control group.

The levels of AST and ALT were significantly ($P < 0.001$) increased in AIA control group when compared to normal group. On the other hand, treatment with leflunomide (10 mg/kg) significantly ($P < 0.001$) decrease the AST and ALT levels compared to AIA control group. Treatment with curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) decreased the AST and ALT significantly ($P < 0.001$) compared to AIA control group. There was significant decrease in levels of AST ($P < 0.001$ and $P < 0.05$) and ALT ($P < 0.001$ and $P < 0.01$) by curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated groups compared to AIA control group.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in ALP and Albumin level:

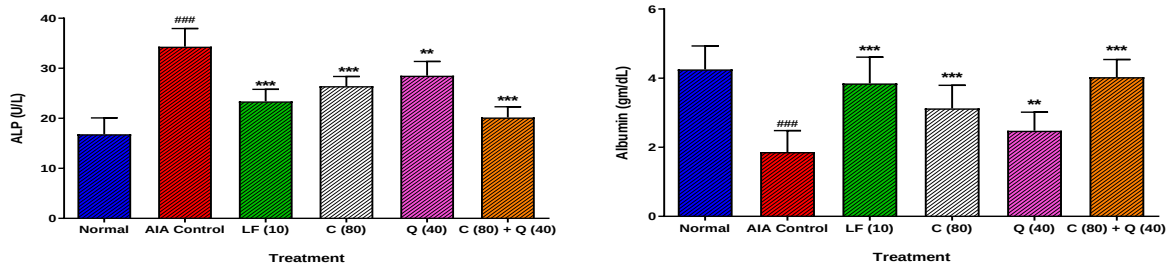

Figure 6: Effect of curcumin, quercetin and their combination on FCA-induced alteration in ALP level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###$P < 0.001$ as compared with normal group and **$P < 0.01$, ***$P < 0.001$ as compared with AIA control group.

The significant increased ($P < 0.001$) in ALP and decreased albumin level was found after sub-plantar administration of FCA in AIA control rats as compared to normal rats. This increased level of ALP and decreased albumin was significantly attenuated ($P < 0.001$) by leflunomide (10 mg/kg) as compared to AIA control rats. Treatment with curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) also significantly ($P < 0.001$) decreased the ALP and increased albumin level compared to AIA control rats. Rats treated with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated significantly decreased the ALP ($P < 0.001$ and $P < 0.01$) and increased albumin level ($P < 0.001$ and $P < 0.01$) as compared to AIA control rats.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in CRP level:

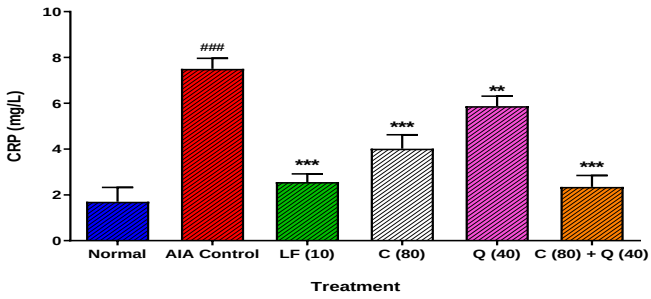

Figure 7: Effect of curcumin, quercetin and their combination on FCA-induced alteration in CRP level

There was a significant increase ($P < 0.001$) in serum CRP level in AIA control group when compared to normal group. Administration of leflunomide (10 mg/kg) for 28 days significantly ($P < 0.001$) decrease serum CRP level compared to AIA control rats. Treatment with curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) also significantly ($P < 0.001$) decreased the serum CRP level compared to AIA control rats. Rats treated with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated significantly decreased the serum CRP ($P < 0.001$ and $P < 0.01$) as compared to AIA control rats.

Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic total protein level:

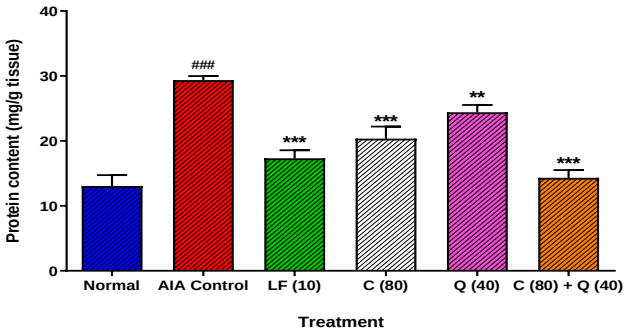

Figure 8: Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic total protein level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with AIA control group.

There was a significant increase (P < 0.001) in hepatic total protein level in AIA control group when compared to normal group. Administration of leflunomide (10 mg/kg) for 28 days significantly (P < 0.001) decrease total protein level in hepatic tissue compared to AIA control rats. Treatment with curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) also significantly (P < 0.001) decreased the hepatic total protein level compared to AIA control rats. Rats treated with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated significantly decreased the hepatic total protein level (P < 0.001 and P < 0.01) as compared to AIA control rats.

Table 2: Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic SOD and GSH level:

Parameter	Hepatic SOD (U /mg of protein) and GSH µg/mg of protein) levels - Mean ± SEM					
	Normal	AIA Control	Leflunomide (10 mg/kg)	Curcumin (80 mg/kg)	Quercetin (40 mg/kg)	Curcumin (80 mg/kg) + Quercetin (40 mg/kg)
SOD (U /mg of protein)	5.34 ± 0.22	2.85 ± 0.11###	4.51 ± 0.20***	4.64 ± 0.19***	4.00 ± 0.18**	5.24 ± 0.20***
GSH (µg/mg of protein)	88.65 ± 4.11	43.51 ± 4.89###	73.35 ± 6.15***	64.45 ± 2.8***	56.59 ± 3.05**	75.00 ± 5.29***

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###P < 0.001 as compared with normal group and **P < 0.01, ***P < 0.001 as compared with AIA control group.

The hepatic SOD and GSH level in the AIA control rats was significantly decreased (P < 0.001) as compared to normal rats. The SOD and GSH level in the hepatic tissue of leflunomide (10 mg/kg), curcumin (80 mg/kg) alone and curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) treated rats was significantly increased (P < 0.001) as compared to AIA control rats. The 28 days treatment of quercetin (40 mg/kg) significantly attenuated (P < 0.01) this FCA-induced decreased level of SOD and GSH as compared to AIA control rats.

Table 3: Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic MDA and NO level:

Parameter	Hepatic MDA (nM/mg of protein), nitric oxide (µg/ml) - Mean ± SEM					
	Normal	AIA Control	Leflunomide (10 mg/kg)	Curcumin (80 mg/kg)	Quercetin (40 mg/kg)	Curcumin (80 mg/kg) + Quercetin (40 mg/kg)
MDA (nM/mg of protein)	0.97 ± 0.10	2.38 ± 0.10###	1.26 ± 0.07***	1.41 ± 0.10***	1.93 ± 0.07**	1.17 ± 0.10***
Nitric oxide (µg/ml)	7.81 ± 0.8	17.75 ± 0.55###	8.39 ± 0.61***	12.31 ± 0.84***	15.78 ± 0.64**	7.90 ± 0.78***

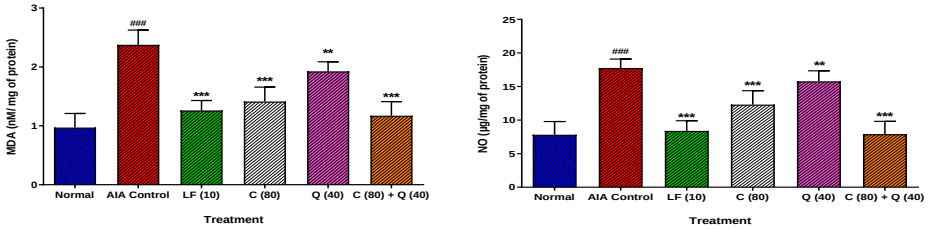

Figure 10: Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic MDA and NO level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###$P < 0.001$ as compared with normal group and **$P < 0.01$, ***$P < 0.001$ as compared with AIA control group.

There was significant increase in hepatic MDA and NO levels in AIA control rats as compared to normal rats. When compared to AIA control rats, the MDA and NO level in hepatic tissue of leflunomide (10 mg/kg), curcumin (80 mg/kg) alone and curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) was significantly deceased ($P < 0.001$). The quercetin (40 mg/kg) alone treatment also produce significant decrease ($P < 0.01$) in MDA and NO level compared to AIA control rats.

Effect of FCA on histopathological alteration in tibiotarsal joint:

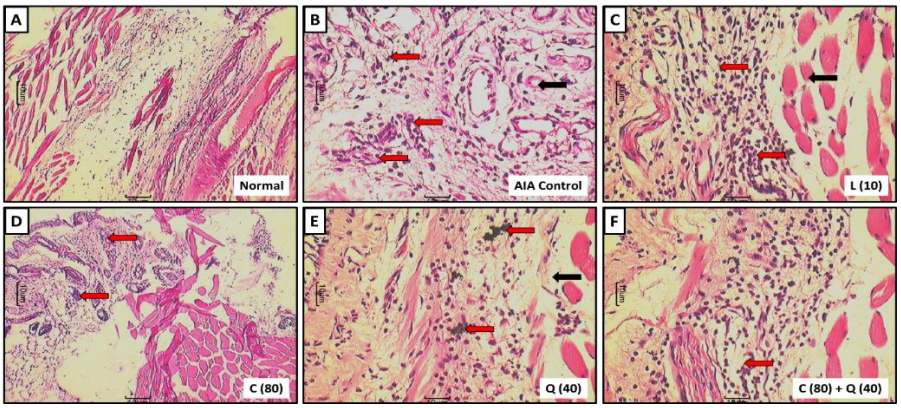

Figure 11: Histopathological representation of tibiotarsal joint stained with H&E (at 100 X). Infiltration of neutrophils (red arrow), Cartilage erosion (black arrow)

Histopathological observations of the tibiotarsal joint from normal group revealed well maintain architecture with infiltration of neutrophils, cartilage erosion, synovial proliferation and necrotic changes (Figure A).

Table 4: Drug compare with control group

Treatment	Infiltration of neutrophils	Cartilage erosion	Synovial proliferation	Necrotic Changes
Normal	-	-	-	-
AIA Control	++++	++++	+++	+++
Leflunomide (10 mg/kg)	+	+	-	+
Curcumin (80 mg/kg)	+	+	+	+
Quercetin (40 mg/kg)	++	++	++	+
Curcumin (80 mg/kg) + Quercetin (40 mg/kg)	+	+	-	+

Note:

-: no abnormality detected
+: damage/ active changes up to less than 25 %
++: damage/ active changes up to less than 50 %
+++: damage/ active changes up to less 75 %
++++: damage/ active changes up to more than 75 %

The tibiotarsal joint from the AIA control rats showed severe infiltration of neutrophils (++++), cartilage erosion (++++), synovial proliferation (+++) with and necrotic changes (+++) (Figure B).

Administration of leflunomide (10 mg/kg) showed protection against FCA-induced synovial proliferation (-) with mild infiltration of neutrophils (+), cartilage erosion (+) and necrotic changes (+) (Figure C).

Tibiotarsal joint section from the curcumin (80 mg/kg) treated rats showed presence of mild infiltration of neutrophils (+), cartilage erosion (+), synovial proliferation (+) with necrotic changes (+) (Figure D).

However, administration of quercetin (40 mg/kg) showed presence of moderate infiltration of neutrophils (++), cartilage erosion (++), synovial proliferation (++) with mild necrotic changes (+) (Figure E).

Administration of curcumin (80 mg/kg) in combination with quercetin (40 mg/kg) showed reduction in infiltration of neutrophils (+), cartilage erosion (+) and necrotic changes (+) without synovial proliferation (-) when compared with AIA control group (Figure F).

CONCLUSION:

On the basis of the result, we can conclude that curcumin in combination with quercetin was an effective agent in attenuating the adjuvant-induced arthritis rats in a concentration-dependent manner, and therefore it could be scrutinized as a probable treatment for human chronic arthritis condition. Additionally, curcumin in combination with quercetin gave beneficiary results in all the studied parameters. The results suggest that curcumin in combination with quercetin is beneficial in the treatment of painful inflammatory and chronic arthritic condition.

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