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Original Article

EFFECT OF CAFFEINE IN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS IN RATS

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ABSTRACT

Objective: Primary objective of this study was to evaluate anti-inflammatory effect of caffeine in complete Freund's adjuvant model of rheumatoid arthritis. Secondary objective was to compare the topical anti-inflammatory action with systemic action of caffeine and to minimize many psychotropic effect of caffeine in normal individual or arthritic patient due to systemic administration and more emphasis on topical use of caffeine as an anti-inflammatory (TNF-α blockers).

Methods: Arthritis was induced by a single sub-plantar injection (0.1 ml) of CFA into the left hind paw. Rats were treated with dexamethasone (0.05 mg/kg, p. o.), caffeine (20 and 50 mg/kg, p. o.) and caffeine gel (3% and 7% topical) from day 0 to day 12. Efficacy was evaluated by change in paw volume, serum C-reactive protein (CRP), estimation of serum rheumatoid factor (RF), arthritis index, and body weight and by histopathology of synovial joint.

Results: CFA showed significantly ($p < 0.001$) higher paw volume, CRP, RF and arthritic index as compared to caffeine 20 mg/kg, caffeine 50 mg/kg, caffeine gel 3% and caffeine gel 7% treated animals. It was observed that topical caffeine gel (3% and 7%) suppressed paw volume, CRP, RF and arthritic index in a more statistically significant manner compared to oral caffeine solutions (20 mg/kg and 50 mg/kg).

Conclusion: Topical caffeine gel (3% and7%) shows more significant anti-inflammatory effect as compared to oral caffeine solution (20 mg/kg and 50 mg/kg).

Keywords: Rheumatoid arthritis, Caffeine.

INTRODUCTION

RA typically manifest with signs of inflammation and affected joints becomes swollen, warm, painful and stiff early in the morning on waking or following prolonged inactivity [1]. Cytokines play a central role in joint inflammation and destruction in RA [2]. A cascade of events starting with vasodilatation and increased vascular permeability induced in part by the release of histamine from ruptured or activated mast cells, circulating basophiles and platelets, and increased vascular permeability leads to local edema [3]. In particular, the pro inflammatory cytokines includes interleukin (IL-1, IL-6, IL-8, IL-10), tumor necrosis factor alpha (TNF-α) and granulocyte–macrophage colony stimulating factor (GM-CSF) [4]. TNF-α is produced mainly by monocytes and macrophages and also by B-cells, T-cells and fibroblasts [5]. The primary role of TNF-α is the regulation of immune cells and induction of apoptotic cell death and inflammation. TNF-α also stimulates mesenchymal cells, to release substances for tissue degradation like matrix metalloproteinase (MMP-1,2,3,9,13) that leads to the damage of the cartilage thereby increasing the production of super oxide radicals and prostaglandin [6].

Methylxanthine derivatives are non-selective adenosine antagonists responsible for inhibition of adenosine receptor leading to the inhibition of enzyme phosphodiesterase (PDE) that ultimately increases cAMP activates PKA thereby suppressing the production of proinflammatory cytokine TNF-α [7]. Caffeine is a methylxanthine derivative. Some methylxanthine derivatives including Lisofylline and Pentoxifylline possesses anti-inflammatory effects which inhibit the secretion of the TNF-α and IL-1β from stimulated monocytes and also increases the production of anti-inflammatory IL-10 from lymphocytes [8]. Other possible mechanisms reported that xanthine derivative also reduces the platelet aggregation and are effective in reduction of blood viscosity and thrombus formation [9]. Caffeine is used orally and is associated with various psychiatric side effects [10].

Thus, the aim of our study was to evaluate the anti-inflammatory effect of caffeine in Complete Freund's adjuvant model of rheumatoid arthritis and compare the topical anti-inflammatory action with the systemic action of caffeine.

MATERIALS AND METHODS

Drugs and chemicals

Caffeine and Freund's complete adjuvants (CFA) were purchased from Sigma Aldrich (St. Louis, USA). Dexamethasone was purchased from Zydus Cadila Ltd (Ahmedabad, India), CRP and RF kit were purchased from Beacon Diagnostics Ltd (Navsari, India).

Test animals and experimental design

After approval from Institutional Animal Ethics Committee (IAEC), 48 female wistar albino rats weighing 150-210 g were used for the study. Animals were randomly divided into 8 groups (n=6) like control (Group I), disease control (Group II, distilled water), dexamethasone 0.05mg/kg, p. o treated (Group III), caffeine 20mg/kg, p. o. treated (Group IV), caffeine 50 mg/kg, p. o. treated (Group V), placebo gel (Group VI), caffeine gel 3% treated (Group VII) and caffeine gel 7% treated (Group VIII). Arthritis was induced in all animals by sub plantar injection of Complete Freund's adjuvant 0.1 ml in sub plantar region of left hind paw. Drugs were administered from day 0 to day12. Changes in paw volume (on day 0, 1, 12, 21), CRP (on day 0, 12, 21), RF (on day 0, 12, 21), arthritic index (on day 21) and percentage change in body weight (on day 12, 21) were measured to evaluate the efficacy of different treatments.

Preparation of caffeine gel

Ingradiants	Quantity for 3%	Quantity for 7%
Carbopol 974	2 g	2 g
Caffeine	3 g	7 g
Distilled water	Up to 100 ml	Up to 100 ml

Required quantity of caffeine was weighed and dissolved in 100 ml of warm water. It was allowed to cool and then after 2 g of Carbopol 974 was added and allowed to stand overnight. Next day tritethanolamine was added to adjust pH-7 with constant stirring.

Characterization of caffeine gel

(1) pH Measurement: pH of gel was around 6-7

(2) Color of gel: Clear transparent gel

(3) Odor: No characteristic odor was found

(4) Spread ability: Gel was easily applied without any staining on application site.

(5) Grittiness: No suspended particles were observed.

Statistical analysis

The data were expressed as Mean ± SEM. Statistical analysis was performed by one way ANOVA followed by Tukey test; p <0.05 were considered as statistically significant.

RESULTS

Paw volume

The challenge with CFA in rats resulted in significant increase ($p < 0.001$) in paw volume in Group II when compared with Group I at day 1. Fig. 1 shows changes in paw volume measurement after the administration of 0.1 ml CFA injection in sub-plantar region of left hind paw. Paw volume in all groups showed significant ($p < 0.001$) increase when compared with Group I. The treatment with Group IV and Group V from day 0 to day 12 showed significant ($p < 0.001$) inhibition of the paw volume when compared with Group II at day 12 as shown in fig. 2.

From day 13 to day 21 no treatment were given to Group IV and Group V and inhibition of paw volume were significantly ($p < 0.05$) reduced when compared with Group II at day 21 shown in fig. 3. Similar treatments were started with Group VII and Group VIII from day 0 to day 12 but it was applied topically. From day 13 to day 21 no treatment were given to Group IV and Group V. Paw volume when evaluated on day 21, was significantly ($p < 0.05$) lower in both groups as compared to CFA treated animals. Topical caffeine in dose of 3 % and 7 % was applied on knee joint in group VII and group VIII from day 0 to 12. Paw volume in Group VII and Group VIII were significantly ($p < 0.001$) lowered when compared to Group II on day 12 and day 21. Interestingly statistically significant ($p < 0.001$) reduction in paw volume were observed in Group VII and VIII when compared with Group IV and Group V on day 12 and day 21 as shown in fig. 2 and 3 respectively.

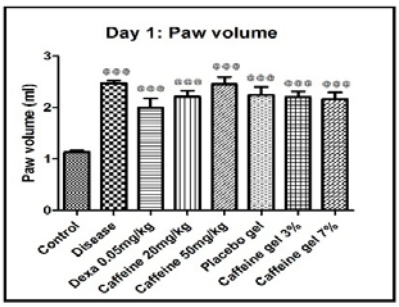

Fig. 1: It shows Paw volume at day 1

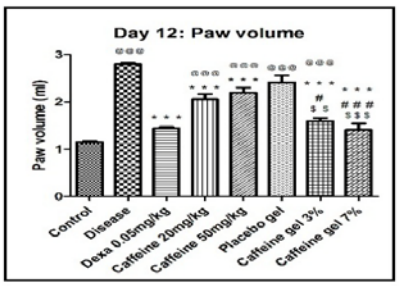

Fig. 2: It shows Paw volume at day 12

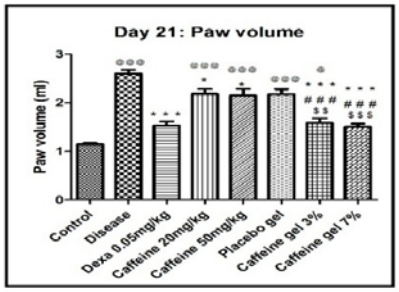

Fig. 3: It shows Paw volume at day 21

C - reactive protein

Fig. 4 and 5 shows significant ($p < 0.001$) increase in CRP values for all groups when compared with normal control animals on day 12 and day 21. All treatment Groups V, VII and VIII showed significantly ($p < 0.001$) lower level of serum CRP value when compared with Group II on day 12 and 21 Group IV showed significantly ($p < 0.001$, $p < 0.01$) lower level of serum CRP value on day 12 and 21 respectively when compared with Group II.

Statistically significant ($p < 0.01$, $p < 0.05$) reduction in circulating serum CRP value was observed in Group VII when compared with Group IV and V respectively on day 12 and day 21. Group VIII showed significantly ($p < 0.001$) lower level of serum CRP value compared with Group IV on day 12, 21 and with Group V on day 12, 21 at a significance of $p < 0.01$, $p < 0.001$.

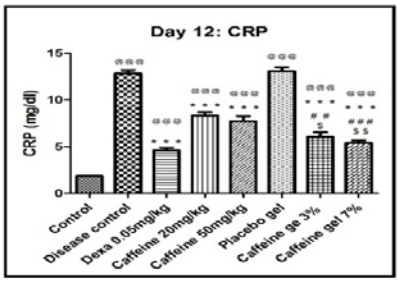

Fig. 4: It shows CRP value at day 12

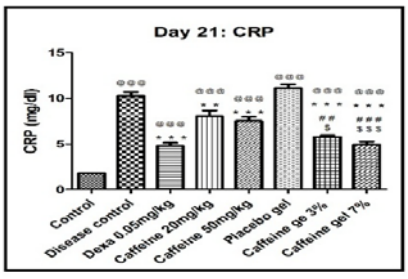

Fig. 5: It shows CRP value at day 21

Serum rheumatoid factor

Circulating serum RF value is referred as ongoing inflammatory state. Higher values of RF were found to be significant ($p < 0.001$) on day 12 and 21 for all groups when compared with Group I as shown in fig. 6 and 7. While Group IV, V, VII, VIII showed significantly ($p < 0.001$) lower level of serum RF when compared with Group II on day 12 and 21. Group VII showed significantly ($p < 0.001$, $p < 0.05$) lower level of serum CRP value with Group IV and V respectively on day 12 and 21. Group VIII showed significantly ($p < 0.001$) lower level of serum CRP value with Group IV and V on day 12 and 21.

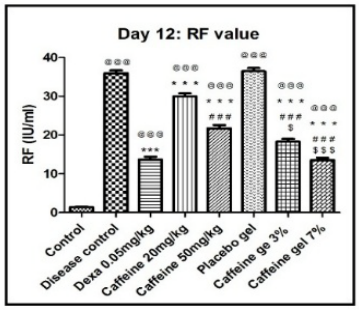

Fig. 6: It shows RF value at day 12

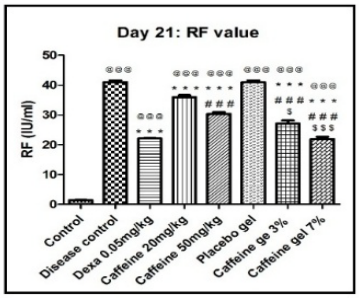

Fig. 7: It shows RF value at day 21

Percentage change in body weight

Fig. 8 shows no significant change in body weight on day 12. On day 21 there was a significant ($p < 0.05$, $p < 0.01$) change in body weight in Group V when compared with Group II and VI respectively as shown in fig. 9.

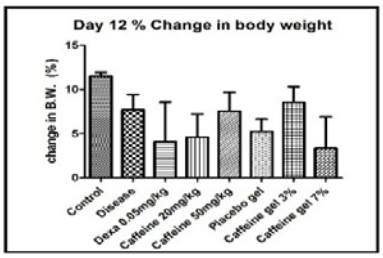

Fig. 8: It shows % change in body weight at day 12

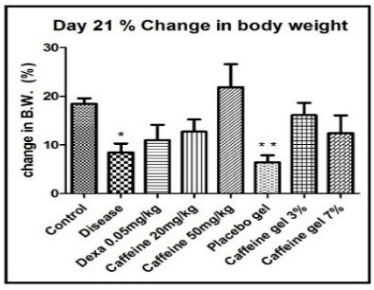

Fig. 9: It shows % change in body weight at day 21

Arthritic index

Fully developed arthritis including redness and swollen paws were observed after 12 days of onset of inflammation. The clinical score in the arthritic control group reached approximately 14 days after immunization with CFA. Rats in arthritic control group showed severe inflammation with marked lesions and deformity. Secondary lesions such as inflammation of non-injected sites and nodule formation in nose, ear, and tail, fore paws and hind paws region were also observed in arthritic group. The arthritic scoring were significantly ($p < 0.05$, $p < 0.001$) less in Group VII, VIII when compared with Group II as shown in fig. 10. Group VIII also showed significantly ($p < 0.05$) lower arthritic index than Group VI and VII.

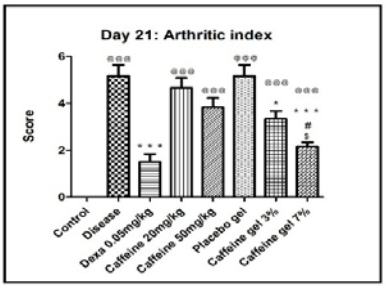

Fig. 10: It shows Arthritic index at day 21

DISCUSSION

Caffeine is xanthine derivative and xanthine derivatives inhibit Streptococcus pneumoniae-Stimulated production of tumor necrosis factor alpha, Interleukin-1b (IL-1b), and IL-10 by human leukocytes [14]. It is reported that caffeine suppresses TNF-α production via activation of the cyclic AMP/protein kinase a pathway (in Vitro). In vitro exposure of caffeine and its major metabolite paraxanthine on lipopolysaccharide (LPS)-stimulated cytokine production in human blood cells cause inhibition of pro inflammatory mediators [7]. Also elevation of cellular cAMP inhibits tumor necrosis factor alpha (TNF-α) production and increases the expression of interleukin (IL)-10 in mononuclear cells [12]. Caffeine also modulates TNF-α production by cord blood monocytes: the role of adenosine receptors [13]. So in support of all above researches we made an attempt to evaluate the anti-inflammatory effect of caffeine in CFA induced arthritis. The main aim of this study was to evaluate anti-inflammatory activity of caffeine and also to compare topical caffeine gel with oral caffeine solution. Complete Freund's adjuvant (CFA) administration is known to produce both primary and secondary lesions [15] with majority of consequent pathological changes similar to that observed in rheumatoid arthritis. Considering this advantage, we used Freund's adjuvant induced rheumatoid arthritis model in rats in the present study.

Following a single injection of CFA at the sub-plantar surface of left hind paw, rats developed pronounced arthritis in the paws, showing 100% incidence. Chronic inflammation in the CFA model is manifested as a progressive increase in the paw volume at the injected site. In the present study, injection of CFA in the left hind paw of rats resulted in a sustained increase in the paw volume which remained at elevated level even up to 21 days of study. After injection of 0.1 ml CFA in the sub-plantar region of left hind paw on day 0, the peak effect was observed within 24 hours as a significantly increase in paw volume of all groups when compared with control group. Treatment with caffeine 20 mg/kg, caffeine 50 mg/kg, caffeine gel 3% and caffeine gel 7% showed statistically significant reduction in paw volume, CRP value, and RF value of all treated groups on 12th and 21st day. CRP was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of any inflammation and tissue damage [16]. Production of CRP is mainly under transcriptional control by cytokine IL-6. In most diseases, the circulating value of CRP reflects ongoing inflammation and/or tissue damage much more accurately than any other laboratory parameters of the acute phase response, such as plasma viscosity and the erythrocyte sedimentation rate [17]. So in support of that our study of rheumatoid arthritis also showed significant reduction in serum CRP value when treated with topical caffeine gel and oral caffeine solutions. As a consequence of arthritis,

immunologic abnormalities are supported leading to expression of serum rheumatoid factor. Plasma cells produce antibodies (e. g. IgM) that contribute to these complexes. Serum RF measures the amount of antibody IgM titre present in the serum [18]. As inflammation increases there is significant reduction in circulating IgM which ultimately decreases the clearance of apoptotic cells. Moreover serum RF measures the quantity of circulating IgM and decreases as inflammation increases [19]. In our study topical caffeine gels also supported this. Topical caffeine gel reduced serum RF value more than oral caffeine solution. Arthritic index includes the combined index of inflammation, formation of nodules and extent of spread of the disease to other organs. Symmetric involvement of small hand joints (especially proximal interphalangeal and metatarsophalangeal), foot joints (metatarsophalangeal), wrists, elbows, and ankles is typical, but initial manifestations may occur in any joint. Inflammation and/or nodules were observed on ears, nose, and tail, fore paws and hind paws. Arthritic index is the average of the score given to severity of the lesions in these places CFA treated animals showed significantly higher arthritic index as compared with normal untreated animals [20]. When topical caffeine gels were compared with oral caffeine solutions, topical gels showed statistically significant reduction in arthritic index than caffeine solutions on 12th and 21st day.

Caffeine 20 mg/kg and caffeine 50 mg/kg also showed reduction in paw volume, CRP and RF value but no statistical significance on day 12 and day 21. Similar results were also observed in case of caffeine gel 3% and caffeine gel 7% where both showed no statistical significance in paw volume reduction on 12th and 21st day. Statistically significant difference was observed in between oral caffeine solutions (caffeine 20 mg/kg, caffeine 50 mg/kg) and caffeine gels (Caffeine gel 3%, caffeine gel 7%) on day 12th and day 21st. When topical caffeine gels (caffeine gel 3%, caffeine gel 7%) were compared with oral caffeine solutions (caffeine 20 mg/kg, caffeine 50 mg/kg) for scoring of arthritic index on day 21, topical caffeine gels (caffeine gel 3%, caffeine gel 7%) showed statistically significant reduction in arthritic index than caffeine solutions (caffeine 20 mg/kg, caffeine 50 mg/kg).

Body weight normally gets reduced in most of auto-immune disorders which may be due to development of cachexia. However in this study, no statistically significant changes were observed on 12th day and 21st day.

CONCLUSION

From the present study it can be concluded that topical caffeine gel shows more significant anti-inflammatory effect in CFA induced arthritic rats as compared to oral caffeine solution. As caffeine gel was applied topically so there was less chance of side effects as compared to oral solutions. Caffeine gel is more effective then caffeine oral solution as observed in results. Caffeine gel 7% has more anti-inflammatory activity then caffeine gel 3% but caffeine gel 7% did not show statistically significant anti-inflammatory activity as compared to caffeine gel 3%.

CONFLICT OF INTERESTS

Declared None

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