

Anti-inflammatory and antiarthritic activity of UNIM-301 (a polyherbal unani formulation) in Wistar rats

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ABSTRACT

Background: UNIM-301 is a polyherbal formulation used in the Unani system of medicine for the treatment of joint pain and rheumatoid arthritis (RA). **Objective:** The objective was to evaluate the anti-inflammatory and antiarthritic activity of UNIM-301 in carrageenan-induced paw edema and complete Freund's Adjuvant (CFA) induced arthritis. **Materials and Methods:** The anti-inflammatory and antiarthritic activity of UNIM-301 was evaluated using carrageenan-induced paw edema and CFA induced animal arthritis models, respectively, in doses of 250, 500, and 1000 mg/kg body weight. Anti-inflammatory activity of UNIM-301 was evaluated using carrageenan-induced paw edema model using a digital plethysmometer. Anti-arthritis activity was evaluated using CFA induced arthritis, and joint sizes were measured at regular intervals using a micrometer screw gauge. Serum was collected and subjected to estimation of pro-inflammatory cytokine. Indomethacin 3 mg/kg body weight) was used as a standard drug in both the models. The acute and chronic toxicity study was carried out to evaluate the safety of the test drug. **Results:** UNIM-301 treatment produced a dose-dependent reduction in paw edema and paw thickness in carrageenan-induced paw edema and CFA-induced arthritis, respectively, as compared to control. UNIM 301 also reduced the expression of pro-inflammatory mediator in a dose-dependent manner as compared to control. **Conclusion:** The result of the present study suggests that anti-inflammatory and anti-arthritis activity of UNIM-301, which might be accredited to inhibitory activity on pro-inflammatory cytokines to its various individual constituents.

Key words: Adjuvant arthritis, carrageenan-induced paw edema, UNIM-301

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, disabling, chronic multisystem disease which is characterized by pain, swelling, and stiffness of synovial joint which is due to unknown causes. An inflammatory reaction increased cellularity of synovial tissue, and joint damage are pathological hallmark of RA.^[1]

Current treatment used for RA are nonsteroidal anti-inflammatory drugs like etoricoxib, disease modifying anti-rheumatic drugs such as methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, cyclosporine, and corticosteroids. Due to their chronic use, these agents

have multiple side effects like immunosuppression, emergence of opportunistic infection which adds mortality and morbidity to the disease. Because of these reasons patient with RA often seek an alternative method for symptomatic relief.^[2] The use of herbal and complementary medicine in the management of inflammatory diseases is well-documented in various literature, but there is limited evidence for effectiveness and safety of these complementary therapies.^[3]

In Unani system of medicine, many polyherbal formulations are used for the treatment of arthritis and UNIM-301 is one such a polyherbal formulation composed of *Withania somnifera*, *Alpinia galanga*, *Operculina turpenthum*, *Colchicum luteum*, *Zingiber officinale*.^[4] Efficacy of UNIM-301 in RA has not been validated using modern scientific parameter. Therefore, the present study has been undertaken to evaluate the antiarthritic activity of the polyherbal formulation UNIM-301 using experimental models of arthritis.

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MATERIALS AND METHODS

Animals

Adult male Wistar albino rats (150–200 g) from the Central Animal Facility, All India Institute of Medical Sciences (AIIMS), were used in the study. Animals were housed under standard laboratory conditions at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in groups of three with free access to food and water ad libitum. They were acclimatized to the laboratory conditions for a period of 5 days before the study. The study was carried out in the Department of Pharmacology after approval of the Institutional Animal Ethics Committee, AIIMS, New Delhi (633/IAEC/11).

Drugs and chemicals

UNIM-301 was provided by Central Council for Research in Unani Medicine, Department of AYUSH, Government of India, New Delhi. Indomethacin was used as standard drug (Merck Chemicals, India). Both drugs were suspended in 1% gum acacia (vehicle) and administered by oral gavage. Indomethacin was administered in a dose of 3 mg/kg body weight, and UNIM-301 was administered in a dose of 250, 500 or 1000 mg/kg body weight, respectively. λ -Carrageenan was purchased from Sigma-Aldrich, USA and complete Freund's adjuvant (CFA) from Difco Laboratories, USA.

Carrageenan induced paw edema in rats

Five groups of male Wistar albino rats ($n = 6$) were used in this study. Animals were fasted overnight with free access to water before the experiment. On the day of the experiment, baseline paw volumes were recorded using a plethysmometer (Ugo Basile 7140). Group I received vehicle (1% gum acacia per orally) and served as the control; group II received indomethacin (3 mg/kg per orally); and groups III, IV, and V received UNIM-301 at a dose of 250, 500, and 1000 mg/kg, respectively, per orally. Sixty minutes after administration of the drug/vehicle, paw edema was induced by sub-plantar administration of 0.1 ml of 1% λ -carrageenan, constituted in normal saline into the left hind paw of the animal. Thereafter, the increase in paw volume was measured at 1, 3, and 6 h postcarrageenan administration.^[5-7]

Adjuvant induced arthritis

Five groups of male Wistar albino rats ($n = 6$) were used in this study. Baseline recording of the joint diameter was made using a micrometer screw gauge. Grouping of animals and drug treatments were same as for carrageenan-induced paw edema. Sixty minutes after administration of the vehicle/drug, arthritis was induced by subplantar administration of 0.1 ml of CFA (0.05% w/v *Mycobacterium butyricum* in mineral oil) into the left hind paw of all the rats^[8] as described by us earlier.^[9] This was

designated as day 0. Vehicle/drug treatment was continued for 20 more days. Joint size measurements were carried out on days 3, 7, 14, and 21.^[10]

Detection of circulating cytokines

Circulating tumor necrosis factor-alpha (TNF- α) level was estimated by ELISA (U-CyTech Biosciences, The Netherlands) and dot-blot analysis was used for the estimation of circulating interleukin (IL)-6 and IL-1 β protein in the serum of control, indomethacin, and highest dose UNIM 301 (1000 mg/kg) treated animals as described by us earlier.^[11]

Toxicity studies

Evaluation of oral acute toxicity of UNIM-301 was carried out according to the Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals-425.^[12] To reduce the number of animals, a limit test at 2000 mg/kg body weight was performed using five male Wistar albino rats (150–180 g).

Evaluation of oral 28-day toxicity of UNIM-301 was carried out according to the OECD guidelines for testing of chemicals-407.^[13] Twelve male Wistar rats (150–180 g) were divided into two groups ($n = 6$). Group I received the vehicle (2 ml/kg body weight, 1% gum acacia) and served as normal control and group II received UNIM-301 in a dose of 2000 mg/kg body weight. Drug/vehicle was administered daily for the duration of 28 days.

Statistical analysis

Difference between groups was compared using one-way ANOVA followed by Dunnett's Multiple Comparison. $P < 0.05$ was considered significant.

RESULTS

Anti-inflammatory activity of UNIM-301 in carrageenan-induced paw edema

Administration of carrageenan produced a time-dependent increase in paw edema in all the tested animals [Figure 1]. A dose-dependent decrease in paw edema was observed in the UNIM-301-treated groups. Reference drug, indomethacin, also significantly suppressed the paw edema at 3 and 6 h postcarrageenan administration. However, the maximum reduction in paw edema was produced by UNIM-301 (1000 mg/kg) at all-time points.

Effect of UNIM-301 on joint swelling in complete freund's induced arthritis

Immunization with CFA produced an increase in the ankle joint diameter of the injected limb in all the animals [Figure 2]. Maximum joint swelling in all the groups was observed on day 3. This was followed by a gradual

reduction in joint swelling, except in group I (control) and III (UNIM-301, 250 mg/kg) where there was a slight increase in joint diameter after day 14. The standard drug indomethacin produced a significant decrease in the joint diameter as compared to control on all observation days. UNIM-301 produced a significant and dose-dependent reduction of joint swelling throughout the study. At the highest dose tested, UNIM-301 (1000 mg/kg) produced anti-arthritic activity that was comparable to indomethacin (3 mg/kg).

Effect of UNIM-301 treatment on circulating cytokine

Serum TNF- α level in normal rats was not in the detectable range for the ELISA kit that was used in the study.

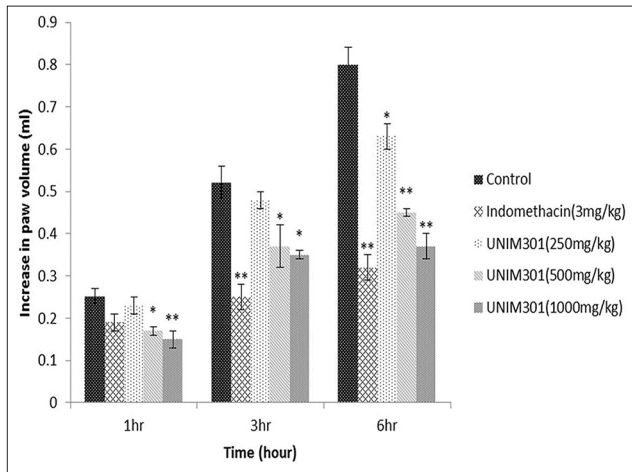


Figure 1: Effect of UNIM-301 treatment on carrageenan-induced paw edema in rats. Difference between initial paw volume and paw volume at observation points post-carrageenan administration was considered to be an increase in paw volume (depictive of paw edema). Each bar represents the mean \pm SE of 6 animals. Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison ($*P < 0.05$, $**P < 0.01$)

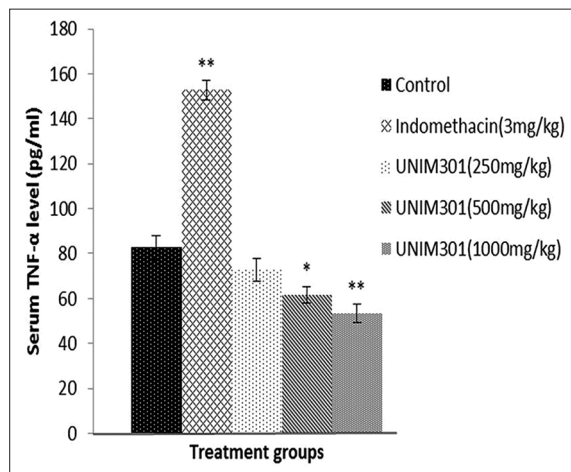


Figure 3: Effect of UNIM-301 treatment on serum tumor necrosis factor- α level in complete Freund's Adjuvant induced arthritis in rats. Each bar represents the mean \pm SE of six animals. Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparisons ($*P < 0.05$, $**P < 0.01$)

CFA administration produced an increase in the serum TNF- α level in both vehicle-treated and drug-treated groups [Figure 3]. Although UNIM-301 produced a dose-dependent decrease in the serum TNF- α level as compared with control, the decrease was significant only in the group treated at dose 500 and 1000 mg/kg. A significant reduction in serum IL-1 β and IL-6 expression was observed in UNIM-301 treated group as compared to control. However in the indomethacin treated group, there is a significant increase in serum IL-1 β level and IL-6 level as compared to control, this increase was not statistically significant [Figures 4 and 5].

Toxicity studies

Oral LD50 of UNIM-301 in rats was found to be >2000 mg/kg body weight as administration of

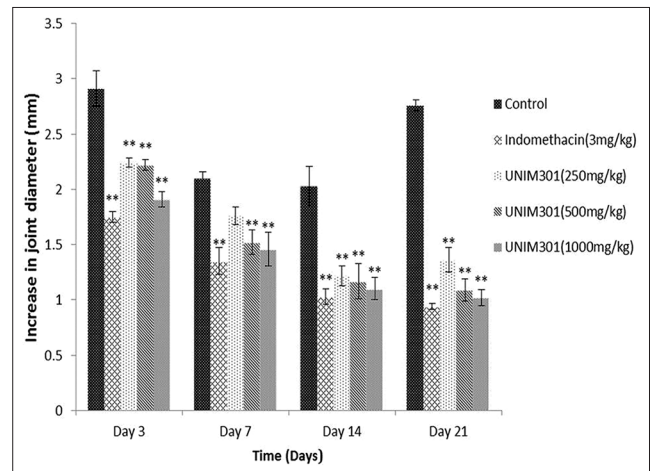


Figure 2: Effect of UNIM-301 treatment on joint swelling in complete Freund's Adjuvant induced arthritis in rats. Each bar represents the mean \pm SE of 6 animals. Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison ($*P < 0.05$, $**P < 0.01$)

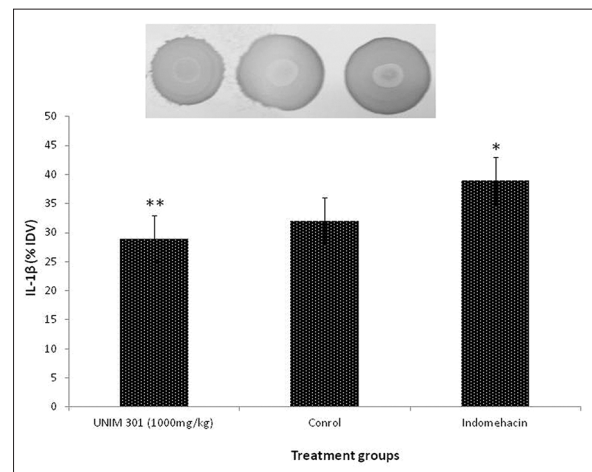


Figure 4: Effect of UNIM-301 treatment on serum IL-1 β level in complete Freund's Adjuvant induced arthritis in rats. Each bar represents the mean \pm SE of 6 animals. Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparisons ($*P < 0.05$, $**P < 0.01$)

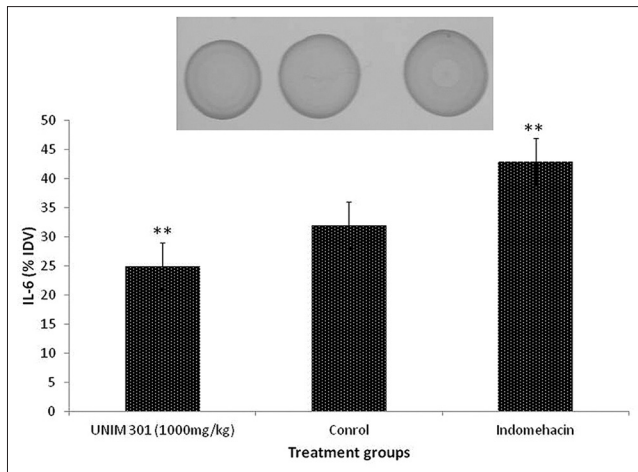


Figure 5: Effect of UNIM 301 treatment on serum IL-6 level in complete Freund's Adjuvant induced arthritis in rats. Each bar represents the mean \pm SE of 6 animals. Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparisons (* $P < 0.05$, ** $P < 0.01$)

UNIM-301 at a dose of 2000 mg/kg weight did not produce any mortality in the tested animals. In chronic administration of UNIM-301 at a dose of 2000 mg/kg body weight for 28 days did not produce any pathological changes as compared to normal animals (data not shown).

DISCUSSION

UNIM-301 is a polyherbal formulation that is used in the Unani formulation for the treatment of rheumatoid arthritis.^[4] It comprises of five medicinal plants which are *W. somnifera*, *A. galangal*, *O. turpenthum*, *C. luteum*, *Z. officinale*. Some of the plant constituents which are used in UNIM-301 have been evaluated for their anti-inflammatory and anti-arthritic activity. *W. somnifera* has been shown to be effective in reducing the paw diameter and lysosomal enzyme activity in CFA-induced arthritis.^[14] It has been reported that *Z. officinale* has decreased pain and swelling in the arthritic patient.^[15] *C. luteum* had shown to be effective in carrageenan-induced paw edema, CFA-induced arthritis, Cotton pellet-induced granuloma formation, and CFA-induced stimulation of peritoneal macrophage in rats and also found to reduced pro-inflammatory cytokine levels of TNF- α , IL-1 β and IL-6 in these rats.^[5,16] However, despite the presence of a large number of active principles its efficacy has not been validated in experimental models. Therefore, the present study was carried out to scientifically evaluate the anti-inflammatory and antiarthritic effect of UNIM 301 using two different animal models. Carrageenan-induced paw edema was used to evaluate the anti-inflammatory activity, and complete Freund's induced arthritis model was used to evaluate the anti-arthritic efficacy of the formulation.

To evaluate the effect of the drug on autacoid system, carrageenan-induced paw edema model is widely

used.^[6] This model has three distinct phases of autacoid involvement. During first phase (1–2 h after carrageenan administration), the primary involvement of histamine in inflammation, in second phase (2–3 h after carrageenan administration), serotonin and kinin are the primary mediator for inflammation and the last third phase (3 h onwards) the primary mediators are prostaglandin and leukotrienes.^[17] In our study, the standard drug indomethacin reduced paw edema significantly at third and 6th h postcarrageenan administration as compared to control. Thus, depicting its primary activity against prostaglandin synthesis. On the other hand, UNIM 301 decrease paw edema at all observation point thus demonstrating its activity against multiple mediators involved in carrageenan-induced paw edema.

The CFA-induced arthritis model is widely used for evaluating the antiarthritic activity of drugs. It shares a number of clinical and immunological features with human arthritis. Therefore, CFA model has a high degree of validity.^[8] In this model along with measurement of joint inflammation (joint diameter), serum TNF- α level was also measured for evaluating its disease modifying activity of the formulation.

In the group I (control) and group III (UNIM-301 at dose of 250 mg/kg), there was increased in paw thickness after day 14 which could be the result of heightened cell-mediated response.^[16] However, this trend was not observed in indomethacin and higher two doses of UNIM-301 treated group. Immunomodulatory activity was confirmed by reduced serum pro-inflammatory cytokine levels viz. TNF- α , IL-1 β and IL-6 level in UNIM-301 group. The effect of indomethacin on serum proinflammatory cytokine levels is similar to the results obtained in our previous reports.^[9,11]

The probable mechanism might be immunomodulatory activity of individual constituents present in UNIM-301 mainly by *C. luteum* and *W. somnifera*. As *C. luteum* have already shown disease modifying activity by inhibiting the pro-inflammatory cytokines.^[5,16] *W. somnifera* also showed potent inhibitory activity toward complement system, mitogen-induced lymphocyte proliferation, and delayed-type hypersensitivity reaction in *in vivo* and *in vitro* study.^[17] However, due to the presence of other constituents in UNIM 301, this immunomodulatory effect cannot be solely attributed to *C. luteum* and *W. somnifera*.

In our study, UNIM-301 was found to have an oral LD50 above 2000 mg/kg. Chronic administration of UNIM-301 also did not produce any pathological changes in tested animals, thus demonstrating its safety on long-term administration. Based on our results, we

conclude that UNIM-301 has the potential to be used as a disease-modifying agent in the treatment of RA and could be further explored for the safer alternative in the treatment of RA.

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