RESEARCH PAPER

Evaluation Of The Anti-Inflammatory Activity Of Metformin In The Carrageenan-Induced Paw Edema Model In Rats

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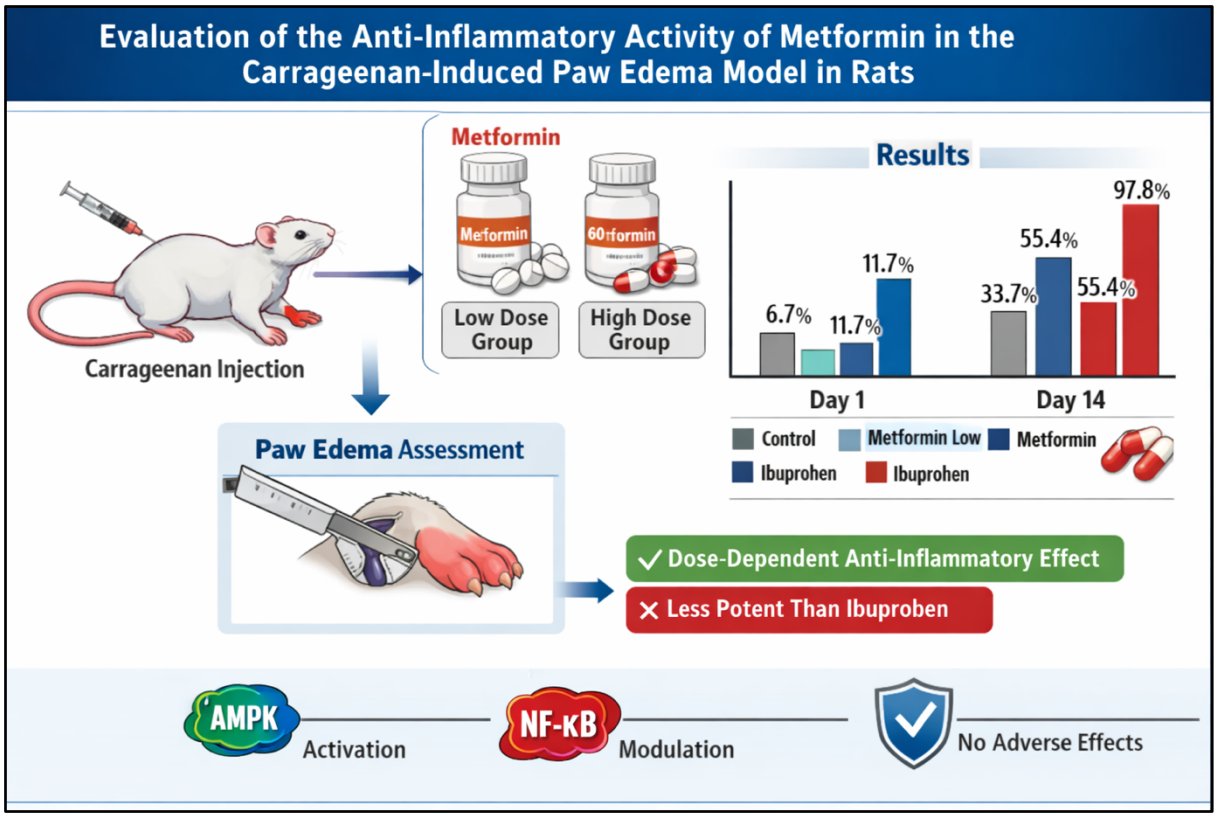
Graphical Abstract:



Graphical representation of the experimental design and key findings demonstrating the anti-inflammatory activity of Metformin in the carrageenan-induced paw edema model in rats. Oral administration of Metformin (30 mg/kg and 60 mg/kg) resulted in dose-dependent inhibition of paw edema compared with control, while Ibuprofen showed greater potency at all time points. Sustained anti-inflammatory effects were observed up to Day 14. The proposed mechanism may involve modulation of inflammatory signalling pathways through AMPK activation and NF-κB inhibition. No observable adverse effects were noted during the study period.

Abstract:

Background: Inflammation is a complicated process of defence, but unregulated inflammation is the cause of chronic illnesses, including diabetes, atherosclerosis, and arthritis. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is a first line treatment, which has side effects limiting its use. Metformin, a biguanide antidiabetic medication has been reported to have pleiotropic anti-inflammatory effects by activating AMP-activated protein kinase (AMPK) and inhibiting nuclear factor kappa B (NF-kB).

Objective: The following study was conducted to compare and assess the anti-inflammatory activity of Metformin at two oral doses with the conventional use of Ibuprofen on the carrageenan-induced paw edema in rats.

Methodology: Twenty-four male albino rats (180 +- 20 g) were separated into four groups (n = 6): control (saline), Metformin (30 mg/kg), Metformin (60mg/kg) and Ibuprofen (30mg/kg). The drugs were injected orally (30 minutes before the carrageenan injection) (0.05 mL, 1% w/v) into the subplantar area of the left hind paw. Paw volume was also assessed at baseline, 30 min, 1 h, 3 h and 5 h during Day 1 and Day 5 and 14. Edema was

inhibited as a percentage. The statistical analysis was performed using one-way ANOVA with the Tukey post hoc test. **Results:** Metformin demonstrated dose-effect anti-inflammatory activity. Inhibition on Day 1 was 6.7 and 11.7 with 30 mg /kg and 60mg/kg respectively, versus 41.2 with Ibuprofen ($p < 0.05$). On Day 5, Metformin inhibition had risen to 15.7% and 25.9% whilst Ibuprofen had an inhibition of 85.2%. At Day 14 sustained inhibition rates were met with 33.7 and 55.4 per cent with Metformin and Ibuprofen respectively. Metformin treatment of animals showed no negative side effects.

Conclusion: Metformin in the carrageenan-induced paw edema model has a dose-dependent and long-lasting anti-inflammatory effect which is not as potent as Ibuprofen. These results imply the possibility of Metformin as a supplementary anti-inflammatory drug in inflammatory states that are chronic.

Keywords: Metformin; Anti-inflammatory activity; Carrageenan; Paw edema; AMP-activated protein kinase; NF-κB; Ibuprofen; Rat model

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Introduction

Inflammation is a complicated, extremely controlled biological reaction of vascularized tissue to dangerous agents like infections, bodily harm, or chemicals. It is one of the defence mechanisms and it is intended to remove the harmful cause and begin tissue repair[1]. Celsus was the first to describe the classical hallmarks of inflammation, which include redness, heat, swelling, pain, and loss of function, and was later extended by Virchow who considered inflammation an essential part of tissue pathology[2].

Despite the fact that acute inflammation is simply beneficial and self-limiting, chronic or uncontrolled inflammation is also a pathogenesis of a variety of disorders, such as diabetes mellitus, atherosclerosis, rheumatoid arthritis, and neurodegenerative diseases[3,4]. The conditions are defined by the chronic release of cytokines, oxidative stress, and endothelial dysfunction, which also worsen tissue damage and metabolic imbalance[5].

Traditional inflammatory management is mostly based on nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen which inhibits the production of cyclooxygenase (COX) enzymes which in turn inhibit the formation of prostaglandins[6]. Nevertheless, long-term NSAID administration is commonly constrained by gastrointestinal, cardiovascular, and renal adverse effects, which led to the identification of less toxic anti-inflammatory drugs capable of regulating the underlying pathophysiological processes instead of suppressing the symptoms[7].

The initial pharmacotherapy of type 2 diabetes mellitus is biguanide in the form of Metformin. Metformin, traditionally known to have the antihyperglycemic effect, reduces hepatic gluconeogenesis and enhances peripheral glucose uptake by activating AMP-activated protein kinase (AMPK), one of the major metabolic regulators[8]. Recent works have also found out the pleiotropic effects of Metformin, such as antioxidative, anti-aging, and anti-inflammatory effects[9-11].

Metformin-induced activation of AMPK prevents nuclear translocation of nuclear factor kappa B (NF-κB), which is a transcription factor that regulates a variety of pro-inflammatory genes including tumor necrosis factor-alpha (TNF- 6), interleukin-1 beta (IL-1 6) and interleukin-6 (IL-6)[10, 12]. Metformin is also found to decrease the synthesis of nitric oxide (NO) and prostaglandin E2 (PGE2), thus regulating the release of macrophage inflammatory mediators[11,13]. These mechanisms indicate that Metformin has anti-inflammatory effects that do not depend on its glycemic effect.

The carrageenan-induced rat paw edema model is the standard of the gold standard in the assessment of acute anti-inflammatory agents[14]. The model shows a biphasic inflammatory reaction with an early phase (02 h) with the release of histamine, serotonin, and bradykinin, and the late phase (35 h) with the action of the prostaglandins and leukotrienes[14, 15]. The agents that have the potential to decrease the edema formation during either of the two stages are said to have great anti-inflammatory qualities.

Since the emerging evidence shows that Metformin inhibits the inflammatory pathways in the AMPK-dependent manner and has been reported to exert the endothelial-protective effect, this study aimed to compare the anti-inflammatory action of Metformin at two oral doses (30 mg/kg and 60 mg/kg) to a standard NSAID, Ibuprofen (30 mg/kg) using the carrageenan-induced paw edema model in albino rats.

Materials and Methods

Animals

Male albino rats weighing 180 ± 20 g were used for this study. Animals were procured from the Central Animal House of Bharati Vidyapeeth (Deemed to be

University), Medical College and Hospital, Sangli. They were housed under standard laboratory conditions-temperature 25 ± 2 °C, relative humidity 60 ± 5 %, and a 12 h light/dark cycle-with free access to standard pellet diet and water ad libitum. Prior to experimentation, animals were acclimatized for one week. All experimental procedures were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) under approval number BVDUMC /Sangli/IAEC/2017/03 (Reg. No. 972/PO/Re/Bi/S/2006/CPCSEA).

Drugs and Chemicals

Metformin hydrochloride tablets (250 mg; commercially available formulation) were powdered and freshly suspended in normal saline before each use. Ibuprofen (30 mg/kg) was used as the standard reference NSAID for comparison (6). Carrageenan (Sigma-Aldrich, USA) was used to induce inflammation as a 1 % w/v suspension in normal saline. All other reagents were of analytical grade and prepared freshly. The selection of Metformin doses (30 mg/kg and 60 mg/kg) was based on prior pharmacological studies demonstrating its systemic anti-inflammatory effects and extrapolated to rats using the body surface area conversion method[16-19].

Experimental Design

Twenty-four healthy rats were randomly divided into four groups of six animals each:

Group	Treatment	Dose and Route
I	Control	0.2 mL normal saline, p.o.
II	Metformin	30 mg/kg, p.o.
III	Metformin	60 mg/kg, p.o.
IV	Ibuprofen (standard)	30 mg/kg, p.o.

Drugs were administered orally 30 minutes prior to inflammation induction. Animals were fasted overnight before dosing but had free access to water. This design ensured uniform exposure and minimized inter-animal variability in drug absorption and pharmacodynamic response[18, 19].

Induction of Inflammation

Acute inflammation was induced by subplantar injection of 0.05 mL of 1 % carrageenan suspension in sterile normal saline into the left hind paw of each rat using a fine 26-gauge needle[17, 18]. The carrageenan-induced paw edema model is a well-validated and sensitive method for evaluating both early and late phases of acute inflammation. The early phase (0-2 h) is mediated by histamine, serotonin, and bradykinin, while the late phase (3-5 h) predominantly involves prostaglandins and leukotrienes[17].

Measurement of Paw Edema

Paw volume was measured using a mercury displacement plethysmograph (Ugo Basile, Italy) before carrageenan injection (baseline, 0 h) and subsequently at 30 min, 1 h, 3 h, and 5 h after carrageenan administration on Day 1 (acute phase). The same parameters were assessed again on Days 5 and 14 to evaluate the persistence of anti-inflammatory activity[17].

The percentage inhibition of paw edema was calculated using the formula[18]:

$$\text{Percentage inhibition} = [(Vc - Vt) / Vc] \times 100$$

where Vc represents the mean paw volume of the control group and Vt represents the mean paw volume of the treated group.

Observation Period and Clinical Monitoring

During the experimental period, animals were monitored regarding any abnormality of behavior or physiological aspect. There were no toxicity, hypersensitivity, and mortality observed. The animals treated with Metformin were normal in grooming and feeding behaviour, which is in line with other studies on the safety of the drug in rodent models[16,18].

Statistical Analysis

All quantitative data were expressed as mean ± standard error of mean (SEM). Statistical evaluation was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test (Graph Pad Prism 9.0, San Diego, USA). A value of $p < 0.05$ was considered statistically significant (18). Statistical methods followed established recommendations for preclinical pharmacological screening (18).

Data expressed as mean ± SEM (n = 6). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control.

Results

Effect of Metformin on Carrageenan-Induced Paw Edema

Carrageenan injection caused a marked and sustained increase in paw volume in the control group, confirming successful induction of acute inflammation. Treatment with Metformin (30 mg/kg and 60 mg/kg) significantly reduced paw edema compared to the control group ($p < 0.05$), though the response was less potent than that of Ibuprofen (30 mg/kg, $p < 0.001$).

Metformin produced a dose-dependent inhibition of paw edema. On Day 1, inhibition was 6.72 ± 0.45 % and 11.70 ± 0.53 % for 30 mg/kg and 60 mg/kg, respectively, compared to 41.17 ± 2.14 % for Ibuprofen ($p = 0.042$ vs control, $p = 0.038$ vs control, $p < 0.001$ vs Metformin groups).

Table 1. Carrageenan-induced paw edema (Day 1)

Group	Treatment	Mean Paw Volume (mL) ± SEM	% Inhibition ± SEM	p-value (vs Control)
A	Control (0.2 mL NS)	1.98 ± 0.04	-	-
B	Metformin 30 mg/kg	1.85 ± 0.05	6.72 ± 0.45	0.042 *
C	Metformin 60 mg/kg	1.75 ± 0.05	11.70 ± 0.53	0.038 *
D	Ibuprofen 30 mg/kg	1.16 ± 0.15	41.17 ± 2.14	< 0.001 ***

* $p < 0.05$ vs control; *** $p < 0.001$ vs control and Metformin groups.

By Day 5, inhibition rose to 15.74 ± 0.87 % (30 mg/kg) and 25.90 ± 0.92 % (60 mg/kg), while Ibuprofen showed 85.18 ± 2.33 % inhibition ($p = 0.031$ and $p = 0.028$ vs control, $p < 0.001$ vs Metformin groups).

Table 2. Carrageenan-induced paw edema (Day 5)

Group	Treatment	Mean Paw Volume (mL) ± SEM	% Inhibition ± SEM	p-value (vs Control)
A	Control	1.80 ± 0.06	-	-
B	Metformin 30 mg/kg	1.51 ± 0.07	15.74 ± 0.87	0.031 *
C	Metformin 60 mg/kg	1.33 ± 0.12	25.90 ± 0.92	0.028 *
D	Ibuprofen 30 mg/kg	0.26 ± 0.10	85.18 ± 2.33	< 0.001 ***

On Day 14, Metformin maintained 33.69 ± 1.24 % and 55.43 ± 1.11 % inhibition for 30 mg/kg and 60 mg/kg, respectively, whereas Ibuprofen achieved 97.82 ± 0.87 % ($p = 0.018$ and $p = 0.011$ vs control; $p < 0.001$ vs Metformin).

These results demonstrate that Metformin exerts a sustained, dose-dependent anti-inflammatory effect but remains significantly less potent than the standard NSAID Ibuprofen (one-way ANOVA, $F(3,20) = 14.57$, $p < 0.001$).

(Table 3, Figure 1).

Table 3. Carrageenan-induced paw edema (Day 14)

Group	Treatment	Mean Paw Volume (mL) ± SEM	% Inhibition ± SEM	p-value (vs Control)
A	Control	1.53 ± 0.05	-	-
B	Metformin 30 mg/kg	1.01 ± 0.27	33.69 ± 1.24	0.018 *
C	Metformin 60 mg/kg	0.68 ± 0.21	55.43 ± 1.11	0.011 *
D	Ibuprofen 30 mg/kg	0.03 ± 0.05	97.82 ± 0.87	< 0.001 ***

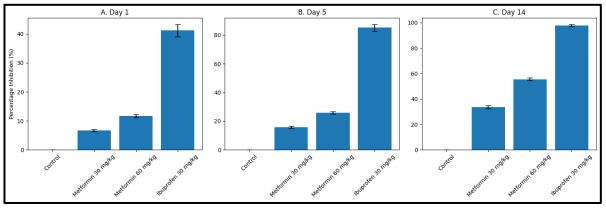

Figure 1. Anti-inflammatory effect of Metformin in carrageenan-induced paw edema model.

Metformin (30 mg/kg and 60 mg/kg, p.o.) produced dose-dependent inhibition of carrageenan-induced paw edema compared with control, whereas Ibuprofen (30 mg/kg, p.o.) showed marked suppression of inflammation at all-time points. A progressive increase in anti-inflammatory activity of Metformin was observed from Day 1 to Day 14, indicating sustained effects. Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ vs control.

Dose-Dependent Response

The anti-inflammatory effect of Metformin demonstrated a dose-dependent trend. The 60 mg/kg group consistently showed greater inhibition than the 30 mg/kg group, particularly evident on Days 5 and 14. This suggests that Metformin's efficacy is related to systemic exposure and its AMPK-mediated modulation of cytokine pathways[16,19].

Safety Observations

Throughout the study, no adverse behavioural changes, hypersensitivity reactions, or mortality were observed in any group. Body weight and feeding behaviour remained stable across treatment groups, confirming the safety profile of Metformin in this experimental setting[16].

Discussion

The current paper assessed Metformin anti-inflammatory effect in the carrageenan-induced paw edema model, which is one of the well-established assays of screening new anti-inflammatory drugs (17,18). Metformin was found to play a significant role in reducing paw edema in dose-dependent manner where 60mg/kg inhibited more than 30mg/kg. No more effective than Ibuprofen, the standard reference NSAID, the results show Metformin has anti-inflammatory actions that can be measured.

Carrageenan induces inflammation which is biphasic in nature. The initial (02h) stage is related to the emissions of histamine, serotonin, and bradykinin, and the second (35h) stage is the work of prostaglandins, cytokines, and neutrophil invasion[17]. Metformin in our study also had a modest effect on edema during the initial phase however, its effects were more pronounced on Days 5 and 14 indicating that its main effect could be inhibition of late phase mediators like prostaglandins and cytokines. The findings are in line with the previous literature that Metformin has slow but persistent anti-inflammatory effects[20,21].

Mechanistically, Metformin stimulates the action of the metabolic regulator, the AMPK, which modulates the action of inflammatory signalling. AMPK activation prevents nuclear factor kappa B (NF- κB) translocation and decreases pro-inflammatory gene transcription such as TNF- 2, IL-1 2, and IL-6[21,22]. Past literature has shown that Metformin reduces the production of nitric oxide (NO), production of prostaglandin E 2, and release of cytokines in stimulated macrophages, and thus inhibits inflammatory events[22]. These molecular impacts probably justify the slow, yet continuous edema of the paw reduction in our study, especially on Day 14.

Conversely, Ibuprofen caused fast and deep inhibition of paw edema, which is in line with its direct inhibition of cyclooxygenase (COX) enzymes and production of prostaglandins[06]. The mechanistic divergence is illustrated by the difference in potency: whereas NSAIDs inhibit the synthesis of prostaglandins, Metformin affects the upstream mechanisms which mediate the oxidative stress and the production of cytokines. This implies that the anti-inflammatory effect of Metformin might be more applicable in chronic and low degree inflammation conditions but not acute ones.

This is consistent with our findings and the results obtained by Isoda et al. who indicated that Metformin suppressed proinflammatory reaction in vascular wall cells through AMPK activation[21]. Likewise, Cameron et al. indicated that Metformin decreased systemic inflammation regardless of its antihyperglycemic effect, which confirmed its immunomodulatory effects[20]. Saisho also highlighted the fact that anti-inflammatory effects of Metformin can be involved in cardiovascular protective effects of this medication on patients with metabolic syndrome[22].

Notably, animals treated with Metformin did not show any side effects in the study. This helps to justify the safety established history of Metformin in diabetic and non-diabetic models[16]. Metformin has a lower risk of long-term gastrointestinal, renal, and cardiovascular toxicity compared to NSAIDs, and is a safer alternative or adjunct in the management of inflammatory disorders[7,23-25].

When combined, the findings indicate that Metformin may exert anti-inflammatory effects through AMPK activation and subsequent modulation of NF-κB signaling pathways. The results suggest the possible re-use of Metformin as an adjunct anti-inflammatory therapy, especially in persistent metabolic or vascular inflammatory diseases.

Conclusion

The current research has shown that Metformin has a strong anti-inflammatory effect in rats as indicated by dose-dependent decrease in paw edema when induced by carrageenan. Even though the effect of Metformin was not as potent as Ibuprofen, the anti-inflammatory effect persisted during the study. It is possible that the observed effects are due to the regulation of the inflammatory signalling pathways, possibly, through the activation of AMPK and the inhibition of NF-kB. Its safety profile in this model of experiment is also supported by the fact there were no observed adverse effects. It requires future research that involves the use of molecular biomarkers and histopathological assessment to learn more about its mechanisms and determine whether it can be used as an adjunct treatment of chronic inflammatory disease.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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