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ORIGINAL ARTICLE

Evaluation of the Possible Anti-inflammatory Effects of Loratadine in Acute Gouty Arthritis in Rats

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ABSTRACT

Keywords: Acute Gouty Arthritis, Anti-inflammatory, Loratadine, Rats

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Background: Gouty arthritis (GA) affects joints due to the body losing its ability to metabolize uric acid. Loratadine is an antihistamine drug that shows promise as an anti-inflammatory drug. **Purpose:** Evaluation of the possible anti-inflammatory activity of loratadine in acute gouty arthritis in rats. **Methodology:** 40 rats randomly divided into 4 equal groups: Group 1 received intra-articular injection (IA) of phosphate buffer saline (PBS). Group 2 received IA of Mono urate crystals (MUC) 0.25 mg/ml. Group 3 received single dose of ibuprofen (20 mg/kg) orally 2 hours before induction of acute GA. Group 4 received a single dose of loratadine orally (10mg/ kg), 2 hours before induction of GA. Ankle diameter was assessed at 0 time, 6h, 12 h, 24 h, 48h and 72 h (after injection). Blood samples and serum were used for C- reactive protein (CRP) level assessment. **Results:** A significant decrease in ankle diameter in the group treated with loratadine compared to other groups. Repeated measures of ankle diameter after IA MUC; shows highly significant increase. CRP level was significantly decreased among the group treated by ibuprofen and loratadine versus MUC group. **Conclusions:** loratadine has a significant anti-inflammatory effect compared to ibuprofen in MUC-induced acute gouty arthritis in rats.

INTRODUCTION

Gouty arthritis ranks among the prevailing rheumatic conditions. The clinical implications of gouty arthritis have long been acknowledged in the medical field. However, it is common for gout to be inaccurately diagnosed and improperly treated. The occurrence of gout is increasing and can be attributed to various factors such as a higher occurrence of comorbidities, lifestyle choices, and increased utilization of medications that can cause gout [1].

Gouty arthritis is a prevalent manifestation of arthritis among the male population. The deposition of uric acid crystals in the intra-articular and peri-articular spaces and subsequent activation of the innate immune system are observed as a result of persistent hyperuricaemia.

The sudden appearance of arthritis affecting a single joint in the lower limbs, with notable clinical characteristics, strongly indicates the occurrence of a gout attack [2].

Presently, the primary therapeutic interventions for acute gouty arthritis encompass the administration of anti-inflammatory agents, such as colchicine, non-steroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids. Nevertheless, the utilization of these medications in clinical settings is restricted due to the presence of unfavorable side effects, including but not limited to severe gastrointestinal reactions, hepatic injury, cardiovascular complications, and drug interactions. This is particularly evident in elderly patients and individuals with comorbidities such as chronic kidney disease. Hence, in the context of acute gouty arthritis, it becomes imperative to pursue a treatment modality that offers enhanced efficacy [3].

Loratadine, an antihistamine medication, exhibits potential as an agent with anti-inflammatory properties. Loratadine exerts a molecular-level effect by diminishing the concentrations of nitric oxide, interleukin-1 beta, tumor necrosis “factor alpha”, interleukin-6, and cyclooxygenase-2. Loratadine exhibited a specific inhibitory effect on the NF-kB pathway by selectively targeting the Syk and Src proteins [4].

Loratadine exhibits binding affinity towards H1-receptors located in various cellular contexts, thereby eliciting a reduction in vascular permeability, which in turn mitigates the occurrence of edema and flushing. Additionally, loratadine induces a decline in smooth muscle tone, leading to bronchodilation. Furthermore, it diminishes the activation of peripheral nociceptive receptors, thereby resulting in a reduction in both pain and pruritus, According to Sidhu and Akhondi [5], Loratadine has exhibited efficacy in managing asthma symptoms, enhancing pulmonary function, and exhibiting prolonged duration of action in individuals diagnosed with allergic bronchial asthma. The aim of our study was to evaluate the possible effects and anti-inflammatory activity of loratadine in a model of acute gouty arthritis in rats.

MATERIALS AND METHODS

The Laboratory of the Pharmacology Department, located in the Faculty of Medicine at Assiut University, conducted procedures involving the administration of drugs through a stomach tube, intra-articular injections in the hind paws, and subsequent follow-up assessments.

2.1. Ethical considerations:

The experiment conducted adhered to the Ethical Guidelines set forth by the Ethical Committee of the Faculty of Medicine at Aswan University.

2.2. Drugs and chemicals:

- The following chemicals were procured from El Nasr company for chemical in Cairo, Egypt: phosphate buffer, uric acid, NaOH, N HCl, and saline.
- Loratadine was procured from the pharmaceutical industry S.A.E, located in Egypt, while Ibuprofen was acquired from KAHIRA Company, situated in Cairo, Egypt.
- Monourate crystals will be prepared according to the method reported by Reber et al. [6].

2.3. Experimental Procedures

The experiment utilized adult male wistar rats weighing between 200 and 250 grams. The rats were divided into four groups, with each group consisting of ten rats. The animals were accommodated in appropriate enclosures with provisions of food and water. The experimental

animals were housed in controlled laboratory conditions at ambient temperature, following a 12-hour light-dark cycle.

- Group 1, 10 rats: were administered 10 ml of phosphate buffer saline (PBS) through intra-articular injection in the right hind paw of each rat after undergoing sterilization. This group served as the negative control in the study .
- 10 rats (Group 2): were used to induce acute gouty arthritis. The rats in the positive control group were subjected to intra-articular injection of 0.25 mg/ml monosodium urate crystals (MUC) into the right ankle (tibiotarsal) joint.
- Ten rats (Group 3): were administered ibuprofens orally at a dosage of 20 mg per kilogram of body weight. This administration took place two hours prior to the induction of acute gouty arthritis by intra-articular injection of 0.25 mg/ml MUC into the right ankle (tibiotarsal) joint [7].
- Ten rats, Group 4: were administered a single oral dose of loratadine at a dosage of 10mg per kilogram of body weight. This dosage was given two hours prior to the induction of acute gouty arthritis by intra-articular injection of 0.25 mg/ml MUC into the right ankle (tibiotarsal) joint [7].

Upon the conclusion of our experiment, blood samples were collected after induction of gouty arthritis from each rat. The serum samples were stored at a temperature of -80°C, using freezing as the preservation method.

2.4. Measurements:

- The alterations in the diameter of the ankle in the right hind paws, which were injected under sterilized conditions, will be evaluated at various time intervals: 0 hours (immediately following injection), 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours post-injection. The measurements were obtained using a digital caliper .
- Serum C-reactive protein (CRP)

2.5. Statistical analysis:

Data for ankle joint diameter and biochemical measurement were represented as mean ± SD and SE and then were analyzed using a one-way analysis of variance (ANOVA), or a Kruskal–Wallis non-parametric test. Statistical analysis and figure preparation were performed on untransformed data using Graph Pad Prism (version 5; San Diego, CA). Significance for all tests was set at $p < 0.05$. P values of Sidak's multiple comparisons are shown among different groups.

RESULTS

In table (1): The intra-articular injection of MUC led to increase in the diameter of the right ankle joint compared to control group (injected by PBS) at all measuring times (6, 12, 24, 48, and 72 h)

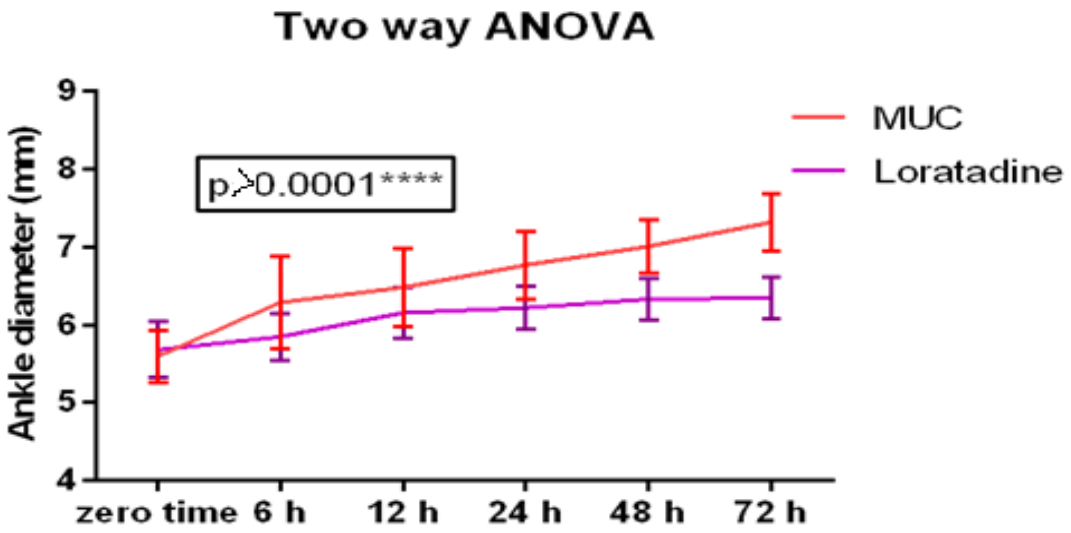
Table (1): Effects of all pretreatments on diameter of ankle joints

		0 time	6 h	12h	24 h	48 h	72 h
	Ankle joint diameter						
Control group (PBS intra-articular injection)	Mean	5.752	6.043	6.042	5.825	5.878	5.992
	SD	0.3435	0.1901	0.1998	0.4516	0.4098	0.3068
	SE	0.1402	0.07762	0.08158	0.1844	0.1673	0.1253
Gouty arthritis group Saline before MUC (intra-articular injection)	Mean	5.602	6.293	6.475	6.765	7.01	7.318
	SD	0.3446	0.6	0.4981	0.4376	0.3366	0.3733
	SE	0.1407	0.2449	0.2034	0.1786	0.1374	0.1524
Ibuprofen before MUC (intra-articular injection)	Mean	5.503	5.977	6.153	6.307	6.3	6.15
	SD	0.2803	0.4412	0.5274	0.3594	0.3721	0.3528
	SE	0.1144	0.1801	0.2153	0.1467	0.1519	0.144
Loratadine before MUC (intra-articular injection)	Mean	5.682	5.848	6.155	6.22	6.33	6.347
	SD	0.3592	0.3056	0.3306	0.2806	0.2769	0.2677
	SE	0.1467	0.1248	0.135	0.1145	0.113	0.1093

PBS; phosphate Buffer saline, SD; standard deviation, SE; standard error, MUC; Monourate crystals

In figure (1): The Intra-articular injection of MUC (after single dose loratadine) showed significant progressive decrease in the diameter of ankle joint when compared to the group received intra-articular injection of MUC, with high significant differences (p>0.0001***)

Figure (1): changes in ankle joint diameter at different times in loratadine and MUC groups.



In table (2): changes in the diameter of ankle joints among different treated groups at different times of measurements (zero, 6h, 12h, 24h, 28h, and 72 h), show that both time factor and treatment factor led to significant changes in the diameter of ankle joints and highly significant differences (p>0.0001***)**.**

Table (2): results of two-way ANOVA for effects of Treatment group, and time factors on changes in the ankle diameters

ANOVA table	F (DFn, DFd)	*P* value	"*P* value summary"
Interaction	F (20, 150) = 2.402	P=0.0015	**
Time Factor	F (5, 150) = 15.77	P<0.0001	****
Group Factor	F (4, 150) = 22.97	P<0.0001	****

In table (3): serum levels of C-reactive protein (CRP) were significantly elevated in the gouty arthritis group (MUC) versus control group (PBS); the levels of CRP were significantly decreased among groups treated by ibuprofen, and loratadine versus the gouty arthritis group (MUC); (p>0.0001***)

Table (3): Sidak's multiple comparisons in C-reactive protein (CRP) among different groups

Sidaks multiple comparisons test for CRP	Summary	Adjusted P Value
Control vs. MUC	****	<0.0001
Control vs. MUC Plus ibupofen	*	0.0437
Control vs. MUC plus loratadine	*	0.0447
MUC vs. MUC Plus ibupofen	****	<0.0001
MUC vs. MUC plus loratadine	****	<0.0001
MUC Plus ibupofen vs. MUC plus loratadine	ns	>0.9999

DISCUSSION

Gouty arthritis is a chronic condition characterized by the presence of hyperuricemia, which leads to joint inflammation. This condition arises from the body's impaired ability to metabolize uric acid, as described by Harre et al. [8].

According to Molloy and McCarthy [9], monosodium urate (MSU) crystals possess significant pro-inflammatory properties and play a crucial role in the development of gouty arthritis through the activation of the innate immune system and subsequent inflammatory response.

The activation of cells by microcrystals derived from monosodium urate (MSU) is a key characteristic of acute gouty arthritis. These proinflammatory microcrystals have the ability to interact with various types of synovial cells, such as neutrophils, monocytes/macrophages, and fibroblast-like synoviocytes of type B. This phenomenon has been extensively studied and documented by Terkeltaub et al. [10] and Pouliot et al. [11].

The primary approach to managing gout in a clinical setting involves the administration of drugs that inhibit the synthesis of uric acid (such as allopurinol), drugs that promote its excretion (such as probenecid), and non-steroidal anti-inflammatory drugs (such as

indomethacin). Nevertheless, the prolonged utilization of this substance results in numerous detrimental responses, including hepatotoxicity, nephrotoxicity, myelosuppression, and gastrointestinal irritation [12]. Therefore, the pursuit of novel pharmaceuticals that are both safe and possess potent anti-hyperuricemic and anti-gout properties holds considerable importance .

The findings of our study indicate that prior to the intra-articular MUC injection, there were no notable variations in the intra-articular diameter among the groups that received a single dose of loratadine, ibuprofen, or were in the control group (treated with phosphate buffer saline, PBS). The repeated measurements of ankle diameter following intra-articular injection of Mono-urate crystals (MUC) demonstrate a statistically significant and substantial increase. However, a significant reduction in ankle diameter was observed in the group treated with loratadine compared to the group treated with a single dose of ibuprofen.

Jiang et al. [13] conducted a study to examine the therapeutic effects of the ethanol extract from Mangifera indica (EMI) in rats with monosodium urate (MSU) crystals-induced gouty arthritis, as per the obtained results. The present study aimed to evaluate the impact of EMI administration at varying doses (50, 100, and 200 mg/kg, p.o.) over a period of 9 days on ankle swelling, synovial tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β) levels in a rat model of MSU crystal-induced inflammation. According to Jiang et al. [13], the data indicated that rats with gouty arthritis induced by MSU crystal exhibited increased levels of ankle swelling in comparison to the control group at various time intervals, namely 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, and 48 hours.

In a study conducted by Han et al. [14], an experimental model using MSU crystals in rats was established to examine the potential impact of Zisheng Shenqi decoction (ZSD), a herbal preparation, on gouty arthritis. The present study also investigated the potential underlying mechanisms responsible for the anti-inflammatory and anti-oxidative effects observed in rats with gouty arthritis treated with ZSD. The findings from these investigations revealed that the injection of MSU crystals into the ankle joint cavity resulted in notable increases in ankle swelling.

Based on the findings reported by Tavares et al. [15], the experimental model employed to induce gouty arthritis through the use of urate crystals successfully replicated symptoms comparable to those observed during an acute episode of the disease. Furthermore, these symptoms were measured through functional assessments and analysis of synovial fluid. Both groups of injured individuals exhibited an augmented joint diameter six hours following the occurrence of the lesion .

The presence of inflammatory cells infiltrating the synovium could potentially contribute to the observed increase in ankle swelling. Gouty arthritis is a persistent inflammatory condition that is distinguished by intense pain and swelling in one or more synovial joints. This condition arises from abnormalities in nucleic acid metabolism, leading to the accumulation of monosodium urate (MSU) crystals within the affected joints. During episodes of gouty arthritis, the presence of MSU crystals triggers a significant influx of leukocytes into the joint cavity. These crystals are then engulfed by monocytes/macrophages, leading to the breakdown of cell membranes, the generation of reactive oxygen species (ROS), and the release of lysosomal enzymes. This phenomenon has been documented in studies conducted by Han et al. [14].

According to Khanhare et al. [16], loratadine demonstrates anti-histamine effects and functions as an anti-inflammatory agent in the context of immune-mediated disorders.

In our study, we observed that the administration of intra-articular MUC injections, following a saline solution, resulted in noteworthy improvements in the diameter of ankle joints. Notably, both the time factor and treatment factor were found to have a significant positive impact on these changes, particularly when pretreatment with loratadine was employed.

In a study conducted by Reber et al. [6], the researchers examined the impact of intraarticular (IA) injection of MSU crystals on different strains of mice with mast cell deficiency, either constitutive or inducible, as well as mice lacking interleukin-1β (IL-1β) or other components of innate immunity. The researchers also evaluated the reaction to intra-articular administration of MSU crystals in mice that lacked mast cells genetically, following the introduction of wild-type or IL-1β bone marrow-derived cultured mast cells into the joint. Histamine was found to be present in synovial fluid samples obtained from patients diagnosed with gout at comparable levels to those observed in specimens collected from patients with Rheumatoid Arthritis. The findings of this study provide evidence that mast cells undergo local activation in the context of acute gout attacks in human subjects. Furthermore, it was observed that synovial fluid samples obtained from individuals diagnosed with gout exhibited markedly elevated concentrations of IL-1β in comparison to samples obtained from patients diagnosed with Rheumatoid arthritis.

In the conducted experiment, it was observed that the serum levels of C-reactive protein (CRP) were notably higher in the group with gouty arthritis (MUC) compared to the control group (PBS). Additionally, the levels of CRP beta were significantly lower in the groups treated with ibuprofen and loratadine in comparison to the gouty arthritis group (MUC).

In a study conducted by Riaz et al. [17] aimed to examine the impact of carvacrol on the oxidative and inflammatory pathways in hyperuricemic rats. The researchers assessed the therapeutic potential of carvacrol and observed a dose-dependent relationship in the groups treated with carvacrol. The outcomes of these groups were found to be similar to those of rats treated with allopurinol. To induce hyperuricemia, the researchers validated the condition by measuring elevated levels of serum uric acid and C-reactive protein (CRP) in the rats. The researchers discovered that the administration of carvacrol resulted in a reduction of serum C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) levels in the joint tissues of rats with hyperuricemia.

In order to identify inflammatory processes, blood tests such as CRP/ESR and CBC with differential can be employed, as suggested by Termeer et al. [18]. Loratadine has been demonstrated to possess anti-inflammatory properties, which are believed to be attributed to its potent inverse agonism of the histamine receptor H1, thereby inhibiting even the basal signaling of this receptor [16].

CONCLUSION

The results of our study indicate that Loratadine exhibits promising therapeutic potential. Loratadine has a significant anti-inflammatory effect compared to ibuprofen in MUC-induced acute gouty arthritis in rats.

Conflict of interest

None

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