PharmacologyOnLine

Newsletter • 2021 • vol. 3 • 151-156

STUDY OF ANTI-INFLAMMATORY ACTIVITY OF HOMEOPATHIC GRANULES LILIUM IN THE THERAPY OF FEMALE GENITAL SYSTEM DISEASES

Yuryeva G. B.*, Herasymova I. V., Konovalenko I. S.
National University of Pharmacy, 53 Pushkinska str., 61002, Kharkiv, Ukraine
*yurieva.anyuta@gmail.com

Abstract

This article represents the results of pharmacological study of homeopathic medicines under the conditions of experimental carrageenan-induced paw edema in rats.

Materials and methods. Edema was caused by subplantar injection of 0.1 ml of 1 % carrageenan solutionl into the hind paw. The measurements were performed before edema induction and 1, 2, 4, and 5 hours afterwards. The main task was to study the effect of homeopathic granules Lilium of two dilutions D3 and C3 in comparison with a reference drug - Diclofenac.

Results. The most pronounced antiexudative effect at the level of the reference drug (AA = 59.39%) was shown by the homeopathic medicine Lilium D3, for which the average anti-inflammatory activity for 5 hours was 64.38%. In the case of homeopathic granules C3, it was found that high activity in the first 2 hours (73.30% and 63.57%, respectively) decreased by 5 hours and amounted to 37.14%. The data obtained indicate the effectiveness of D3 granules in the treatment of chronic inflammation and granules in a higher dilution (C3) for acute conditions.

Conclusions. The mechanism of the anti-inflammatory activity of the homeopathic granules Lilium D3 is mediated by the inhibition of all inflammatory mediators, which indicates its analgesic and antiallergic effect. Homeopathic medicine Lilium C3 has a pronounced anti-inflammatory activity in the first 2 hours after introduction, which indicates its ability to inhibit serotonin, histamine and kinins, and also suggests that this medicine also has antiallergic and analgesic properties.

Keywords: *homeopathy, anti-inflammatory activity, carrageenan-induced inflammation, female genital system.*

Introduction

Medico-biological significance of inflammatory diseases of the female reproductive system is determined by the high level of morbidity, progressive course of the disease, significant complications. One of the pathogenetic links in the development and progression of inflammatory diseases of the female genital organs is inflammation. Acute inflammation is characterized by rapid development of local inflammatory reaction in response to phlogogen and systemic reaction in the acute-phase response (Enikeev et al. 2017).

Inflammation is a complex reaction of living tissues to local damage. It consists of gradual changes in the microcirculatory system, blood system and connective tissue, which are aimed at isolating and eliminating the damaging agent and repairing damaged tissues. It is cyclical: the hemo- and lymphatic vascular response to alteration provides an increase in vascular permeability for both plasma and blood cells (exudation), which leads to the appearance of cellular inflammatory infiltrate, which is subject to phagocytosis, and to secondary tissue destruction (purification). Throughout the process in the inflammatory focus there is a change in cell cooperation, the transformation of cells and vessels, which are aimed at the formation of regenerative proliferate and ends with cell differentiation, repair and recovery [1].

Violation of the barrier properties of the lipid layer of biological membranes (BM) plays an important role in the development of inflammation. It has been established that one of the triggers of BM damage is the activation of lipid peroxidation (LPO) processes, which affect membrane phospholipids and thus significantly disrupt membrane transport [2]. Lipid peroxidation in cells and tissues is controlled by the physiological antioxidant system (FAS), which contains several levels of protection against BPO lipids:

1. Superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GPO) promote enzymatic removal of formed oxygen radicals (O2-) and hydroperoxides (H2O2).

2. Glutathione transferase (GTP) performs enzymatic reduction of hydroperoxides of polyunsaturated fatty acids (PUFAs).

3. Antioxidants - traps for free radicals. All levels of the antioxidant system act in the body as synergists. Weakening of any link of protection (SOD, GPO, KT, antioxidants) inevitably leads to activation of POL, and if the remained links do not compensate weakening, pathological process develops. It is known that in the process of inflammation development the formation of free radicals is enhanced due to the enzymatic oxidation of PUFA - arachidonic acid, resulting in the formation of prostaglandins (PGs) and leukotrienes (LTs). Free radicals in turn enter into the LPO reaction, the substrate of which is also polyunsaturated arachidonic acid [3-5].

Thus, there is a vicious circle that helps maintain an unquenchable inflammatory process, which can be broken using antioxidants (α-tocopherol and ascorbic acid, other natural and synthetic antioxidants).

The major classes of drugs to suppress inflammation are nonsteroidal anti-inflammatory agents (NSAIDS) and corticosteroids, but their toxic adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome have limited their use [6, 7].

Homeopathic medicines Lilium are used in the treatment of inflammatory diseases of the female reproductive system in the form of oral single drugs such as: granules, triturations, dilutions, drops which are prepared according to the requirements of the State Pharmacopoeia of Ukraine using the basic medicine Lilium Θ.

The basic medicine was prepared from fresh flowering (July) raw materials - *Tiger Lily herb* (Lilium lancifolium, Synonym: L. tigrinum). It is known that the raw material contains a complex of biologically active substances (BAS), which includes flavonoids, saponins, a small amount of alkaloids [8, 9]. Preliminary research of literature sources and folk medicine has predicted a number of pharmacological effects inherent in the studied homeopathic medicines Lilium D3 and C3 according to its chemical composition, which are aimed at reducing inflammation: anti-inflammatory, analgesic, membrane stabilizing, antioxidant, etc. [10-12].

Thus, the development of herbal medicines for female genital system therapy is a topical issue for the current pharmacy and medicine.

Based on the above, the aim of our work was to study the effect of homeopathic granules Lilium D3 and C3 on the course of experimental inflammation with a predominant exudative mechanism.

Methods

The objects of the study were homeopathic granules diluted according to the decimal (D3) and centesimal (C3) scales, which were prepared from the basic medicine Lilium Θ (method 1a, SPhU, 1 ed., suppl. 3. P. 142-143) at the Drug Technology Department of the National University of Pharmacy (Kharkiv, Ukraine).

Animals

The experiment was carried out on 40 white outbred female rats of the same age with the body weight of 180-200 g. The experimental animals were kept in the vivarium according to standard sanitary standards and recommended conditions on the necessary diet [13]. All studies were carried out in accordance with the EU Council Directive 86/609 of the EEC dated November, 24, 1986 on the compliance with the laws, regulations and administrative provisions of the EU Member States on the protection of animals used for experimental and other scientific purposes [14].

The effect of homeopathic granules Lilium D3 and C3 on the exudative phase of inflammation was studied in a model of carrageenan edema of the foot in rats.

Carrageenan-Induced Paw Edema in the Rat

The Carrageenan-induced hind paw edema model has extensively been used as a model of acute inflammation [15-19]. Female rats of experimental groups were injected with 0.1 ml of 1 % carrageenan solution. The needle was inserted into the pad region of the glabrous skin and moved 6 to 8 mm proximal towards the tarsal region. The contralateral paws were injected with 0.1 ml of purified water as control.

The comparison drug is Diclofenac (PhC Darnitsa, Ukraine) in dose 8.0 mg/kg (LD50 antiexudative effect for data of Sigidin Ya. A., Schwartz G. Ya. et al.) [20].

Experimental Design

The animals were weighed and divided into groups. Each group contained 10 animals. All groups were coded:

Group 1 – animals with the model control pathology;
Group 2 – animals with the experimental pathology treated by Lilium D3 granules;
Group 3 – animals with the experimental pathology treated by Lilium C3 granules;
Group 4 – animals with the experimental pathology, treated by sodium diclofenac.

The dose of homeopathic medicines for female rats was calculated from the human dose using the coefficient of species sensitivity (CSS) by Yu. Rybolovlev. The daily human dose for an adult with an average body weight of 70 kg is 18 granules (6 granules 3 times a day). The amount it per 1 kg of human body weight: $D_h = 18 / 70 = 0.26$ granules / kg. The value of CSS for rats is 1.89, for humans - 0.45 [21].

The formula for calculating the dose for rats from the dose for humans:

$$D_h / CSS_h = D_{rats} / CSS_{rats}$$

Therefore, the daily dose for rats is calculated:

$$0.26 \text{ granules/kg} / 0.45 = D_{rats}/1.89 = 1 \text{ granule/kg}.$$

It was decided to use this dose (1 granule / kg) for the objective evaluation of the effectiveness of the test samples and the expediency of their further studies; the relevant interpretation of the results obtained.

The development of edema was judged by the increase in foot volume, which was measured in the dynamics of 1; 2; 3; 4 and 5 hours after the introduction of carrageenan solution using a mechanical oncometer according to A.S. Zakharevsky.

The anti-inflammatory activity (AA, %) of the drugs was evaluated by a decrease in the paw volume increment, expressed as a percentage of the initial volume, compared to the control group. The volume of paws of the animal before the injection of carrageenan was taken as the initial volume (100%). A share (%) of paw volume increment = [(volume after injection of carrageenan at a certain moment – initial volume) / initial volume] × 100.

Statistical analysis

All obtained experimental data were processed statistically. The results obtained were statistically processed using the one-way ANOVA

test with the standard software package STATISTICA 7.0 and the 4PL statistical and logistic method using the My Assays Internet service for free use [22].

Results and Discussion

While creation a new anti-inflammatory drugs, the classic approach is to study their effect on the exudative phase of inflammation in appropriate model, which allows to determine the mechanism of pharmacological activity of APIs.

By experimental researches an acute carrageenan paw inflammation in rats has been studied as a most widely used model for antiexudative properties of new potential API.

An experimental carrageenan edema in rats reproduces the main signs of inflammatory reactions: impaired microcirculation, hyperplasia, pain, cellular chemotaxis, and fever. This is due to nutrients such as biogenic amines, kinins, prostaglandins and leukotrienes.

In the model of carrageenan-induced rat paw edema model, which is characterized by the ability to study the process in dynamics; it was found that at the different stages of carrageenan inflammation involved the following phlogotropic agents: kinin system, biogenic amines and prostaglandins, which indirectly suggest mechanisms of anti-inflammatory action (Table 1).

Thus, in the first 30-90 min in the pathogenesis of carrageenan inflammation in rats involved serotonin and histamine, in the interval between 1.5 - 2.5 hours - kinins, and between 2.5-5.5 hours - prostaglandins.

A direct relationship between the effectiveness of the drug in the model of carrageenan edema in rats and its effectiveness in the clinic has been proven.

The analysis of the obtained results showed (Table 1) that the reference drug Diclofenac in dose 8 mg / kg has a pronounced antiexudative effect (more than 50 %), which indicates the adequacy of the reproducibility of the carrageenan edema model and the reliability of the results obtained.

A pronounced antiexudative effect at the level of the reference drug Diclofenac in dose 8 mg / kg (average over 5 hours AA = 59.39%) was shown by the homeopathic medicine Lilium D3. An average anti-inflammatory activity within 5 hours is 64.38 % , and the homeopathic medicine Lilium C3 is inferior to them in terms of the severity of anti-inflammatory action by almost 2 times: its average anti-inflammatory activity within 5 hours is 37.14 %.

Thus, considering the data that serotonin and histamine are involved in the pathogenesis of carrageenan inflammation in rats in the first 30-90 minutes in this model, kinins are involved in the interval between 1.5-2.5 hours, and between 2.5-5.5 hours - prostaglandins, the analysis of the dynamics of the anti-inflammatory activity of the homeopathic medicine Lilium D3 and C3 (Table 1) made it possible to draw the following conclusions:

- the mechanism of anti-inflammatory activity of the homeopathic medicine Lilium D3 is mediated by the inhibition of all inflammatory mediators: serotonin, histamine, kinins and prostaglandins. In view of the fact that serotonin and histamine are also mediators of pain and allergies, and kinins and prostaglandins are pain mediators, it can be assumed that medicine Lilium D3 may have anesthetic and anti-allergic effects;

- the homeopathic medicine Lilium C3 has a pronounced anti-inflammatory activity in the first 2 hours after introduction, which indicates its ability to inhibit serotonin, histamine and kinins, and also suggests that this medicine also has antiallergic and analgesic properties.

The data obtained indicate the effectiveness of D3 granules in the treatment of chronic inflammation and granules in a higher dilution (C3) for acute conditions.

Therefore, the above indicates that homeopathic granules of Lilium D3 are a promising medicine of natural origin with anti-inflammatory activity for further study in order to substantiate the use in the treatment of inflammatory diseases of the female genital organs.

References

1. Rogers RG. (2006). Pelvic Inflammatory Disease. N Engl J Med. 2006 August 17; 355:743-744.
2. Yin H, Xu L, Porter NA. (2011). Free radical lipid peroxidation: mechanisms and analysis. Chemical Reviews. 2011; 111(10): 5944–5972.
3. Calixto JB, Otuki MF, Santos ARS. (2003). Anti-Inflammatory Compounds of Plant Origin. Part I. Action on Arachidonic Acid

Pathway, Nitric Oxide and Nuclear Factor κ B (NF-κB) Planta Med. 2003:69:973–983.
4. Kulkarni RG, Achaiah G, Sastry GN. (2006). Novel targets for antiinflammatory and antiarthritic agents. Curr Pharm Des. 2006; 12(19):2437-54.
5. Larsen GL, Henson PM. (1983). Mediators of inflammation. Annu. Rev. Immunol. 1983;1:335–359.
6. Qandil AM. (2012). Prodrugs of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), More Than Meets the Eye: A Critical Review. Int. J. Mol. Sci. 2012;13:17244–17274.
7. Um SY, Chung MW, Kim K-B, Kim SH, Oh JS, Oh HY, Lee HJ, Choi KH. (2009). Pattern Recognition Analysis for the Prediction of Adverse Effects by Nonsteroidal Anti-Inflammatory Drugs Using1H nmr-Based Metabolomics in Rats. Anal. Chem. 2009;81:4734–4741. doi: 10.1021/ac9000282.
8. Gautam R, Jachak SM. (2009). Recent developments in anti-inflammatory natural products. Med. Res. Rev. 2009;29:767–820.
9. Hahnemann CFS. (1994). In: Organon of Medicine. Joseph Reves from the 5th and 6th edition (1842), editor. Haifa: Homeopress Ltd; 1994.
10. Jin L, Zhang Y, Yan L, Guo Y, Niu L. (2002). Phenolic compounds and antioxidant activity of bulb extracts of six Lilium species native to China. Molecules. 2002;17:9361–9378.
11. Conforti A, Bertani S, Metelmann H, Chirumbolo S, Lussignoli S, Bellavite P. (1997). Experimental studies on the anti-inflammatory activity of a homeopathic preparation. Biomed Ther. 1997;15:28–31.
12. Bellik Y, Boukraa L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi SM, Iguer-Ouada M. (2012). Molecular Mechanism Underlying Anti-Inflammatory and Anti-Allergic Activities of Phytochemicals: An Update. Molecules. 2012;18:322–353.
13. Oukseub L, Ivancic D, Subhashini A, Shidfar A, Kenney K, Helenowski I, Sullivan ME, Muzzio M, Scholtens D, Chatterton RTJr, Bethke K P, Hansen NM, Khan SA. (2015). Local Transdermal Therapy to the Breast for Breast Cancer Prevention and DCIS Therapy: Preclinical and Clinical Evaluation: Cancer Chemother Pharmacol. 2015 Dec;76(6):1235–46.
14. Rozemond H, Vet Q. (1986). Laboratory animal protection: the European Convention and the Dutch Act.. 1986. PMID: 3798719.
15. Winter CA, Risley EA, Nuss GV. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for anti inflammatory drugs. Proc Soc Exp Biol Med. 1962, 111: 544-547.
16. Morris CJ. (2003). Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol. 2003;225:115–121.
17. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G. (2004). Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br. J. Pharmacol. 2004;142:331–338. doi: 10.1038/sj.bjp.0705650.
18. Whiteley PE, Dalrymple SA. (2015). Models of Inflammation: Carrageenan-Induced Paw Edema in the Rat. Curr. Protoc. Pharmacol. 2001:5–6. doi: 10.1002/04711417.ph0506s00.55.
19. Patel M, Murugananthan G, Gowda K. (2012). In vivo animal models in preclinical evaluation of anti-inflammatory activity - A review. Int. J. Pharm. Res. Allied Sci. 2012;1:1–5.
20. Sigidin YaA, Schwartz GYa, Arzamastsev AP, Liberman SS. (1998). Medicinal therapy of inflammatory process. Moscow, 1998.
21. Anroop BN, Shery J. (2016). A simple practice guide for dose conversion between animals and human: J Basic Clin Pharm. – 2016; 7(2): 27–31.
22. Rebrova OYu. (2006). Statistical analysis of medical data. Application of the STATISTICA application package. 3rd ed. – M .: MediaSfera, 2006.312 p.

Table 1. Study of the anti-inflammatory activity of homeopathic drug Lilium D3 and C3 on the course of acute exudative carrageenan inflammation of the rat's foot in dynamics

Dynamics of inflammation, hours	Anti-inflammatory activity (AA), %				
	Indicator	Control pathology	Lilium D3, 1 granule / kg	Lilium C3, 1 granule / kg	Diclofenac, 8 mg / kg
1	ΔV	10.00±1.69	3.83±1.19*	2.67±0.61*	4.33±0.94*
	AA, %	–	61.70	73.30	56.70
2	ΔV	7.33±1.05	1.17±0.17*	2.67±1.92*	2.50±1.01*
	AA, %	–	84.03	63.57	65.89
3	ΔV	13.00±1.41	4.50±1.23*	8.67±2.02	5.17±1.06*
	AA, %	–	65.38	33.31	60.23
4	ΔV	14.70±1.61	5.33±1.94*	11.83±1.30	5.83±1.03*
	AA, %	–	61.93	15.50	60.34
5	ΔV	10.83±1.56	5.50±1.61*	11.83±1.13	5.00±0.87
	AA, %	–	49.21	0	53.83
Average in 5 hours	AA, %	–	64.38±4.44	37.14±10.99	59.39±1.60

Note:
1) * – differences are likely in relation to animals of the control pathology (p≤0.05).