Čes. slov. Farm. 2023; 72, 184–189

ORIGINAL ARTICLE

Study of the anti-inflammatory properties of a thick extract of *Tribulus terrestris* L.

Studium protizánětlivých vlastností hustého extraktu *Tribulus terrestris* L.

Saida Yunusova • Yaroslav Rozhkovskyi • Bohdan Prystupa • Svitlana Bohatu

Received February 26, 2023 / Accepted Juny 1, 2023

Summary

The most promising direction in the treatment of chronic prostatitis is the use of medicinal plants and preparations based on them, which contain natural compounds with a broad spectrum of pharmacological activity: anti-inflammatory, antimicrobial, reparative, immunomodulatory, hormone-regulating, antisclerotic, etc., and which can provide a complex therapeutic effect on the course of chronic prostatitis. A promising raw material in this direction is *Tribulus terrestris* L., a herbal preparation traditionally used to treat erectile dysfunction and atherosclerosis. This experimental work aims to study the anti-inflammatory activity of a thick extract of the *Tribulus terrestris* grass (freed from fruits) on the models of carrageenan and zymosan inflammation in rats.

In the models of carrageenan and zymosan edema in rats, a thick extract of *Tribulus terrestris* L. in doses from 50 mg/kg to 200 mg/kg shows anti-inflammatory activity, the efficacy of which in the dose range of 150–200 mg/g in the initial stages of carrageenan inflammation is not inferior to sodium diclofenac at a dose of 8.0 mg/kg, and in the initial stages of zymosan inflammation, respectively, before the reference drug corvitin at a dose of 10 mg/kg. It indicates the anticyclogenase and antilipoxygenase properties of this thick extract.
Key words: thick extract • *Tribulus terrestris* • diclofenac sodium • corvitin • anti-inflammatory effect

Souhrn

Nejslibnějším směrem v léčbě chronické prostatitidy je využití léčivých rostlin a přípravků na jejich bázi, které obsahují přírodní látky se širokým spektrem farmakologické aktivity: protizánětlivé, antimikrobiální, reparační, imunomodulační, hormonálně regulační, antisklerotické atd. a které mohou poskytnout komplexní terapeutický účinek na průběh chronické prostatitidy. Slibnou surovinou v tomto směru je *Tribulus terrestris* L., rostlinný přípravek tradičně používaný k léčbě erektilní dysfunkce a aterosklerózy. Cílem této experimentální práce je studium protizánětlivé aktivity hustého extraktu z trávy *Tribulus terrestris* (zbaveného plodů) na modelech zánětu vyvolaného karagenanem a zymosanem u potkanů.

Na modelech karagenanového a zymosanového edému u potkanů vykazuje hustý extrakt *Tribulus terrestris* L. v dávkách od 50 mg/kg do 200 mg/kg protizánětlivou aktivitu, jejíž účinnost v rozmezí dávek 150–200 mg/g není v počátečních stadiích karagenanového zánětu horší než u diklofenaku sodného v dávce 8,0 mg/kg, respektive v počátečních stadiích zymosanového zánětu, a není lepší než účinek referenčního léčiva korvitinu v dávce 10 mg/kg. To svědčí o anticyklogenázových a antilipoxygenázových vlastnostech tohoto hustého extraktu.
Klíčová slova: hustý extrakt • *Tribulus terrestris* • diklofenak sodný • korvitin • protizánětlivý účinek.

Introduction

One of the most common diseases of the male genital area is chronic prostatitis (CP), which leads not only to the development of erectile dysfunction but also to infertility. In the modern pharmaceutical market, choosing effective and safe drugs for treating CP is extremely limited, determining the urgency of developing new drugs. Given the lack of proven effectiveness of conventional treatments such as antibiotics, it is not surprising that patients are increasingly turning to herbal medicine and other alternative therapies. The most promising direction in the treatment of CP is the use of medicinal plant raw

Saida Yunusova, PhD student (✉) • Y. Rozhkovskyi • B. Prystupa • S. Bohatu
Department of Pharmacology and Pharmacognosy
Odesa National Medical University
Valikhovsky Lane 2, 65082 Odessa, Ukraine
e-mail: saida.yunusova28@gmail.com

materials (MPM) and phytoremedies, which contain biologically active substances with a broad spectrum of pharmacological activity: anti-inflammatory, antimicrobial, reparative, immunomodulatory, hormone-regulating, antisclerotic, etc., and which can provide a complex therapeutic effect on the course of CP[1–5]. Such a medicinal plant (MP) is *Tribulus terrestris* L., herbal preparation traditionally used in treating erectile dysfunction and atherosclerosis[6–8]. The MPM of *Tribulus terrestris* L. includes a significant amount of polyphenolic compounds, phytosterols, steroidal saponins, and a complex of macro- and microelements with potential anti-inflammatory, antioxidant, and antimicrobial properties[9, 10]. At the Department of Chemistry of Natural Compounds of the National Pharmaceutical University, Kharkiv (Ukraine) (head Prof. V.S.Kyslychenko), in scientific cooperation with the Department of Pharmacology and Pharmacognosy of the Odesa National Medical University, the "Thick extract of the *Tribulus terrestris* grass (freed from fruits)" (TETT) was obtained and standardized. Due to a unique combination of natural compounds (a complex of phenolic compounds, flavonoids, and saponins), it could have potential protective activity on the prostate gland. Based on the leading role of inflammation in the pathogenesis of CP, it was expedient to investigate the anti-inflammatory properties of TETT in an experiment. Therefore the aim of the work is to investigate the anti-inflammatory activity of TETT on models of carrageenan and zymosan inflammation in rats.

Materials and methods

Animal groups

Experiments were conducted on 96 purebred white rats weighing 220–240 g. All experiments were conducted following the general ethical principles of animal experiments, regulated by the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986, as amended, 1998) and Law of Ukraine No. 249 dated 01.03.2012 "The procedure for conducting research and experiments on animals by scientific institutions".

Plant raw material

Medicinal plant raw materials of *Tribulus terrestris* L. were collected and harvested in the Northern Black Sea region in September 2020. TETT was obtained by extracting medicinal plant raw materials with 50% ethanol followed by evaporation on a rotary vacuum evaporator and was standardized by the total content of phenolic compounds, steroidal saponins, and flavonoids[11].

Anti-inflammatory activity testing

Based on the available literature data focused on using phytoremedies with anti-inflammatory properties, screening studies to determine the most effective dose of TETT were conducted using doses of 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg.

Diclofenac sodium (DS) and corvitin (Quercetin) were used as comparative drugs. Diclofenac sodium was used at a dose of ED50 of 8.0 mg/kg and at a dose of 10 mg/kg as in classical tests[13].

Animals in each series of experiments were divided into the following groups: intact, a group of control pathology without treatment, and groups of animals on the background of treatment, respectively, TETT at a dose of 50 mg/kg; 100 mg/kg; 150 mg/kg; 200 mg/kg and DS 8 mg/kg. When investigating the effect of TETT on the zymosan edema model, a separate group of rats received corvitin at a dose of 10 mg/kg. The carrageenan inflammation model was reproduced by subplantar injection of 0.1 ml of 1% aqueous carrageenin solution (ICN, USA) into rats[14]. Zymosan edema in rats was caused by the subplantary introducing zymosan in a volume of 0.1 ml as a 2% suspension[15]. The drugs were administered orally 1 hour before the injection of the phlogogenic agent. The control group of animals received an equivalent amount of water. The development of edema was observed dynamically after 1, 2, 3, 6, and 24 hours, for which paw volume was measured using a mechanical oncometer. The drug activity was evaluated by the ability to reduce edema in comparison with the control pathology group (CPG) according to the formula:

$$AA = (R_{cp} - R_{eg})/R_{cp},$$

where: AA – anti-inflammatory activity, R_{cp} – the average difference between the swollen paw and its initial size in the control pathology group, mm, R_{eg} – the average difference between the swollen paw and its initial size in the experimental group, mm.

The obtained experimental data were processed statistically using the Student's t-test using the Statistica 7 for Windows software.

Results and discussion

The results of the experiments showed that against the background of reproduction of the carrageenan model of inflammation, the maximum swelling of the affected limb in rats was recorded at the 3rd hour of the experiment and amounted to 18.12 ± 0.34 units and remained until 6 hours at the level of 17.20 ± 0.41 units. After 24 hours, it decreased to 10.31 ± 0.46 units, not reaching the level of the intact control. The previous introduction of TETT in different doses and DS reduced the intensity of the inflammatory process. At the same time, the anti-inflammatory effectiveness of TETT in doses of 50 – 100 – 150 mg/kg was directly proportional to the dose of medicine of plant origin. One hour after the start of the experiment, the anti-inflammatory activity of TETT at a dose of 50 mg/kg was 23.6%, at a dose of 100 mg/kg – 49.9%, at a dose of 150 mg/kg – 54.2%, at a dose of 200 mg/kg – 53.1%. In contrast, the

anti-inflammatory effect of DS under similar conditions is 51.1%. It shows that at the initial stages of the inflammatory process, the anti-inflammatory effect of TETT in doses of 100–200 mg/kg is not inferior to the comparative drug DS (Table 1).

At the second, and especially the third hour of the experiment, which is known to be mediated, first of all, by the production of prostaglandins, TETT in all tested doses also showed quite clear anti-inflammatory properties, but the anti-inflammatory effect was inferior to DS. In particular, after 3 hours of the inflammatory process, which was characterized by the greatest edema of the affected limb in rats (18.12 ± 0.34 units), TETT at a dose of 50 mg/kg reduced it to 16.13 ± 0.44 units, at a dose of 100 mg/kg – up to 12.76 ± 0.33 units, at a dose of 150 mg/kg – up to 11.31 ± 0.24 units, at a dose of 200 mg/kg – up to 12.46 ± 0.23 units, compared to the effect of DS – 5.77 ± 0.28 units. Therefore, in the period of observation of the greatest manifestations of the inflammatory process, the anti-inflammatory activity of TETT gradually increased with increasing doses in the range of doses from 50 mg/kg to 150 mg/kg. At the same time, a further increase in the dose of TETT to 200 mg/kg did not reveal an additional increase in the anti-inflammatory activity of this phytonutrient. At the same time, the anti-inflammatory activity of DS in this period of observation was significantly higher and amounted to 68.2%, which confirms the known data on the exceptional ability of this anti-inflammatory medicine to suppress the synthesis of prostaglandins in the focus of inflammation[11, 13, 16]. At the late stages of monitoring the inflammatory process, after 24 hours, we also confirmed the presence of a sufficiently pronounced anti-inflammatory effect of TETT, which was 23.3% at a dose of 50 mg/kg, 47.0% at a dose of 100 mg/kg, and 47.0% at a dose of 150 mg/kg – 57.0%, and at a dose of 200 mg/kg – 59.2%, which was only slightly inferior to DS – 66.5% (Fig. 1).

So, on the carrageenan inflammation model in rats, TETT's rather pronounced anti-inflammatory effect

Table 1. Anti-inflammatory activity of TETT and diclofenac sodium on the model of carrageenan edema in rats (M ± m), n = 8

Observation period (hours)	Indicator (units)	Control pathology	TETT 50 mg/kg	TETT 100 mg/kg	TETT 150 mg/kg	TETT 200 mg/kg	DS 8 mg/kg
1 h	M ± м	11.05 ± 0.30	8.44 ± 0.26*,**	5.54 ± 0.25*	5.06 ± 0.15*	5.18 ± 0.20*	5.40 ± 0.30*
	AA, %		23,6	49.9	54.2	53.1	51.1
2 h	M ± м	16.96 ± 0.23	14.53 ± 0.30*,**	12.30 ± 0.31*,**	7.31 ± 0.31*,**	7.88 ± 0.33*,**	6.02 ± 0.24*
	AA, %		14.3	27.5	56.9	53.5	64.5
3 h	M ± м	18.12 ± 0.34	16.13 ± 0.44*,**	12.76 ± 0.33*,**	11.31 ± 0.24*,**	12.46 ± 0.23*,**	5.77 ± 0.28*
	AA, %		11.0	29.6	37.6	31.2	68.2
6 h	M ± м	17.20 ± 0.41	15.30 ± 0.39*,**	12.05 ± 0.36*,**	10.34 ± 0.20*,**	10.04 ± 0.29*,**	5.48 ± 0.33*
	AA, %		11.0	29.9	39.9	41.6	68.1
24 h	M ± м	10.31 ± 0.46	7.91 ± 0.20*,**	5.46 ± 0.40*,**	4.43 ± 0.27*,**	4.20 ± 0.32*,**	3.45 ± 0.32*
	AA, %		23.3	47.0	57.0	59.2	66.5

*P < 0.05 compared to the indicator of the control pathology group
**P < 0.05 compared to the group which received DS

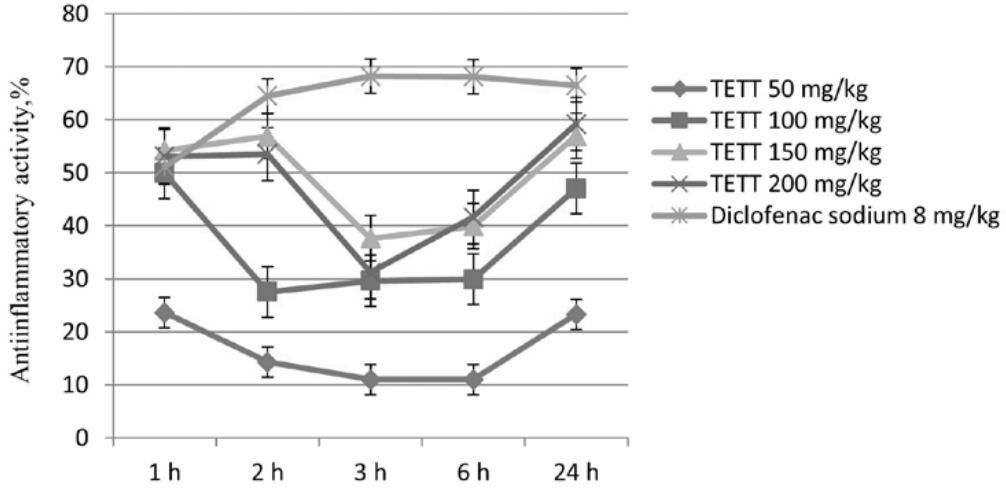

Fig. 1. Dynamics of anti-inflammatory activity (in %) of different doses of TETT and DS (8 mg/kg) on the model of carrageenan edema in rats (M ± m), n = 8

was found, which increases in the range of doses from 50 mg/kg to 150 mg/kg. A further increase in the dose of TETT up to 200 mg/kg does not significantly increase the anti-inflammatory properties of this phytonutrient.

At the initial stages of carrageenan inflammation, which is known to reflect the cyclooxygenase mechanisms of the inflammatory process, the antiphlogistic activity of TETT in doses from 100 mg/kg to 200 mg/kg was quite comparable to the similar effect of DS at a dose of 8.0 mg/kg, which indicates on the high ability of TETT in the specified doses to inhibit the activity of cyclooxygenase, primarily due to the negative effect on the release of early mediators of inflammation – biogenic amines, such as histamine and serotonin, and also to a lesser extent, due to the inhibition of the synthesis of pro-inflammatory prostaglandins at later stages of the experiment.

However, it is known that lipoxygenases play an essential role in the mechanisms of inflammation, the activation of which is accompanied by excess production of leukotrienes – pro-inflammatory protein mediators[18]. At the same time, special importance is attached to the enzyme 5-lipoxygenase (5-LO). The 5-lipoxygenase pathway of arachidonic acid metabolism is responsible for producing not only leukotrienes but also mono hydroxy eicosatetraenoic acids and lipoxins. Leukotrienes have pronounced vasoconstrictor, coronary constrictor, aggregant and arrhythmogenic activities. They contribute to forming free radicals and accumulating polymorphonuclear neutrophils – cells with significant pro-oxidant, lipoxygenase, and proteolytic potential[18–20]. Based on this, it is possible to predict that the anti-leukotriene properties of various compounds can be the basis for developing new approaches in treating inflammatory processes, including the male genital area.

We investigated the comparative anti-inflammatory activity of TETT in different doses with reference drugs (sodium diclofenac – 8 mg/kg and the classic 5-LO blocker corvitin – 10 mg/kg) on the model of zymosan

Table 2. Anti-inflammatory activity of TETT, DS, and corvitin on the zymosan inflammation model in rats (M ± m), n = 8

Observation period (hours)	Indicator (units)	Control pathology	TETT 50 mg/kg	TETT 100 mg/kg	TETT 150 mg/kg	TETT 200 mg/kg	DS 8 mg/kg	Corvitin 10 mg/kg
1 h	M ± м	20.10 ± 0.36	17.05 ± 0.26*,**	11.12 ± 0.30*,**	11.06 ± 0.25*,**	10.88 ± 0.28*,**	15.02 ± 0.33*	9.87 ± 0.29 *,**
	AA, %		15.2	44.8	45.0	45.9	25.3	50.9
2 h	M ± м	24.16 ± 0.20	19.10 ± 0.20*,**	12.39 ± 0.33*	12.00 ± 0.39*	11.68 ± 0.30*	12.08 ± 0.19*	12.34 ± 0.31*
	AA, %		20.9	48.7	50.3	51.7	50.0	48.9
3 h	M ± м	23.55 ± 0.39	19.27 ± 0.50*,**	14.06 ± 0.23*,**	14.31 ± 0.28*,**	14.06 ± 0.29*,**	11.70 ± 0.31*	12.55 ± 0.44*
	AA, %		18.2	40.3	39.2	40.3	50.3	46.7
6 h	M ± м	18.31 ± 0.40	16.06 ± 0.33*,**	12.88 ± 0.31*,**	11.34 ± 0.29*,**	11.00 ± 0.30*,**	9.50 ± 0.30*	11.02 ± 0.26*,**
	AA, %		12.3	29.7	38.1	39.9	48.1	39.8

*P < 0.05 compared to the indicator of the control pathology group
**P < 0.05 compared to the group which received DS

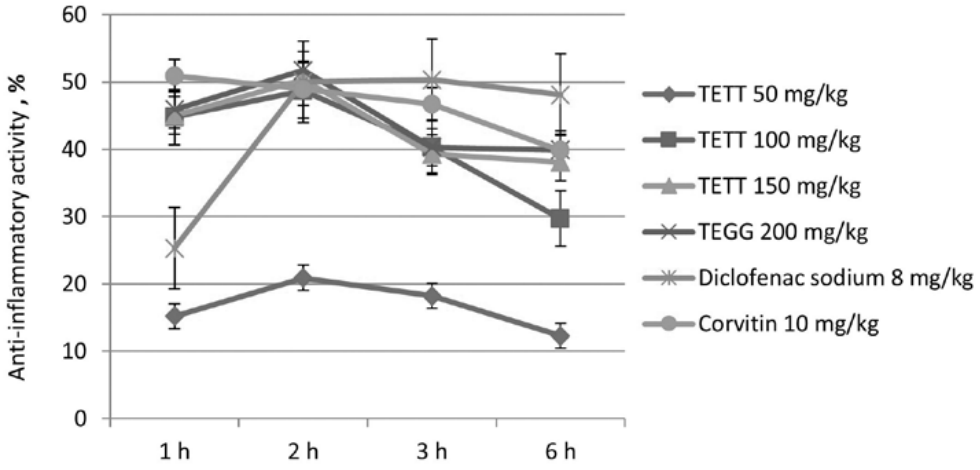

Fig. 2. The dynamics of anti-inflammatory activity (in %) of different doses of TETT, DS and corvitin on the zymosan edema model in rats (M ± m), n = 8

edema. This model, as is known, at the initial stages of reproduction (up to 1 hour) indirectly reflects the activity of 5-lipoxygenase, and at later stages – 2–3 hours after the introduction of phlogogen – the activity of lipoxygenase and cyclooxygenase[21].

The research results showed that when zymosan was administered, the greatest increase in foot edema in rats was observed in the period of 1–3 hours of observation. As early as 6 hours after the introduction of phlogogen, the amount of edema significantly decreased (Table 2).

As in previous experiments with the phlogogen carrageenin, the prophylactic administration of TETT in different doses, as well as sodium diclofenac and corvitin, significantly reduced the intensity of the inflammatory process. At the same time, the anti-inflammatory effectiveness of TETT was maximal in doses of 100–200 mg/kg. After 1 hour of the start of the experiment, the anti-inflammatory activity of TETT at a dose of 50 mg/kg was 15.2%, at a dose of 100 mg/kg – 44.8%, at a dose of 150 mg/kg – 45.0%, at a dose of 200 mg/kg – 45.9%, the selective 5-LO blocker corvitin at a dose of 10 mg/kg – 50.9%. In contrast, the anti-inflammatory effect of DS under similar conditions was only 25.3%.

It indicates that at the initial stages of the inflammatory process, the anti-inflammatory effect of TETT in doses of 100-200 mg/kg is almost not inferior to the 5-LO blocker corvitin. In terms of its activity, it is twice as effective as the comparison drug DS (Table 2).

Pronounced anti-inflammatory properties of TETT in doses of 100–200 mg/kg were also found at other times of the experiment. In particular, 2 hours after the introduction of zymosan, the anti-inflammatory activity of TETT in the specified doses was comparable to the effects of sodium diclofenac and corvitin.

After three hours of the experiment, the anti-inflammatory activity of TETT remained at a sufficient level, but in terms of expressiveness, it was somewhat inferior to both reference drugs. And after 6 hours of observation, TETT at a dose of 50 mg/kg reduced the inflammatory process by 12.3%, at a dose of 100 mg/kg by 29.7%, at a dose of 150 mg/kg by 38.1%, at a dose of 200 mg/kg – by 39.9%, while the anti-inflammatory activity of corvitin under similar conditions was 39.8%, and DS – 48.1% (Fig. 2).

Therefore, the ability of TETT to suppress zymosan inflammation was maintained during the entire duration of the experiment and was highest precisely at the initial stages (1 hour) after the introduction of this phlogogen, which indicates the ability of this medicine of plant origin also to inhibit the lipoxygenase pathway of arachidonic acid metabolism. In terms of the expressiveness of this inhibitory effect in the first hour of inflammation, the herbal remedy TETT is not inferior to the classic 5-LO blocker corvitin, and the similar impact of diclofenac prevails. At the later stages of the experiment (hours 3–6), which is known to reflect the additional effect of phytoremedies on cyclooxygenase activity, the effect of DS was expected to be higher, which fully corresponds to the known mechanisms of anti-inflammatory action of this drug, which are associated with a predominant effect on the synthesis of prostaglandins, which, under the conditions of zymosan inflammation, are maximally produced precisely in this period[21]. In this way, we have established that TETT has an inhibitory effect on both links of zymosan inflammation. At the same time, the anti-inflammatory activity of TETT in doses from 100 to 200 mg/kg for this inflammation model turned out to be commensurate.

The obtained results are fully consistent with the data of other studies. In particular, the ability to suppress the expression of inducible nitric oxide synthase and the expression of cyclooxygenase-2 was detected in the compound tribulusamide D isolated from the ethanol extract *Tribulus terrestris*[24]. Other studies on the inflammation model induced by xylene in mice confirmed the high anti-inflammatory properties of the flavonoid fraction obtained by extraction with 25% ethanol from the leaves of *Tribulus terrestris*[23]. Anti-inflammatory properties of saponin fraction from leaves *Tribulus terrestris* were installed on models of inflammation, induced lipopolysaccharide on RAW 264.7 cells, as well as on models with acute lung injury in mice[24].

Therefore, the pronounced anti-inflammatory activity of TETT on the models of carrageenan and zymosan inflammation, which we have established, indicates the ability of the extract to modulate cyclooxygenase and 5-lipoxygenase in the focus of inflammation, fully corresponds to the existing ideas about the anti-inflammatory properties of biologically active compounds, which are part of this phytomedicine[10, 11] and is probably implemented due to the combination of flavonoids, polyphenolic compounds, and *Tribulus terrestris* L.

Thus, based on the leading role of inflammation in the pathogenesis of CP, the established mechanisms of the anti-inflammatory action of TETT open prospects for further research into its potential protective effect on the prostate gland to create new medicine based on it.

Conclusions

In carrageenan and zymosan inflammation models in rats, the herbal medicine "Thick extract of the *Tribulus terrestris* grass (freed from fruits)" in doses from 50 mg/kg to 200 mg/kg shows anti-inflammatory activity. In the expressiveness of the anti-inflammatory effect of "Thick extract of the *Tribulus terrestris* grass (freed from fruits)" in doses of 150–200 mg/kg in the initial stages of carrageenan inflammation, it is not inferior to diclofenac sodium at a dose of 8.0 mg/kg, which indicates its ability in the indicated doses to suppress primarily the release of early mediators of inflammation – biogenic amines, as well as to a lesser extent pro-inflammatory

prostaglandins at later stages of the experiment. The effectiveness of "Thick extract of the *Tribulus terrestris* grass (freed from fruits)" at a dose of 100–200 mg/kg in the initial stages of zymosan inflammation they are not inferior to the classic 5-lipoxygenase blocker corvitin (10 mg/kg) and the similar effect of sodium diclofenac (8 mg/kg) prevails, which indicates the ability of this herbal medicine to modulate lipoxygenase in the center of inflammation and its antilipoxygenase activity. The established anti-inflammatory properties of «Thick extract of the *Tribulus terrestris* grass (freed from fruits)» open perspectives for further studies of its potential protective effect on the prostate gland.

Conflict of interests: none.

References

1. **Shoskes D.A.** Phytotherapy in chronic prostatitis. Urology 2002; 60, 35–37.
2. **Cai T., Verze P., La Rocca R., Anceschi U., De Nunzio C., Mirone V.** The role of flower pollen extract in managing patients affected by chronic prostatitis/chronic pelvic pain syndrome: a comprehensive analysis of all published clinical trials. BMC Urol. 2017; 17(1), 32.
3. **Zhang K., Guo R. Q., Chen S. W., et al.** The efficacy and safety of *Serenoa repens* extract for the treatment of patients with chronic prostatitis/chronic pelvic pain syndrome: a multicenter, randomized, double-blind, placebo-controlled trial. World J. Urol. 2021; 39, 3489–3495.
4. **Hu M., Wazir J., Ullah R., Wang W., Cui X., Tang M., Zhou X.** Phytotherapy and physical therapy in the management of chronic prostatitis-chronic pelvic pain syndrome. Int. Urol. Nephrol. 2019; 51(7), 1081–1088.
5. **Park M. J., Park H. J., Cheon W. H., Park J. H., Shin B. C., Park N. C.** Herbal Phytotherapy in Chronic Nonbacterial Prostatitis. World J. Mens Health 2019.
6. **Fernández-Lázaro D., Fernandez-Lazaro C. I., Seco-Calvo J., Garrosa E., Adams D. P., Mielgo-Ayuso J.** Effects of *Tribulus terrestris* L. on Sport and Health Biomarkers in Physically Active Adult Males: A Systematic Review. Int. J. Environ. Res. Public Health 2022; 19(15), 9533.
7. **Kamenov Z., Fileva S., Kalinov K., Jannini E. A.** Evaluation of the efficacy and safety of Tribulus terrestris in male sexual dysfunction-A prospective, randomized, double-blind, placebo-controlled clinical trial. Maturitas 2017; 99, 20–26.
8. **Nejati M., Dehghan P., Khani M., Sarbakhsh P.** The effect of Tribulus terrestris supplementation on inflammation, oxidative stress, and performance of recreational runners: study protocol for a randomized placebo-controlled trial. Trials 2022; 23(1), 689.
9. **Naseri L., Akbari bazm M., Khazaei M.** A Review on Therapeutic Effects of *Tribulus terrestris*. J. Med. Plants 2019; 18(72), 1–22.
10. **Semerdjieva I. B., Zheljazkov V. D.** Chemical Constituents, Biological Properties, and Uses of Tribulus terrestris: A Review. Nat. Prod. Commun. 2019; 14(8).
11. **Burda N. Ye., Dababneh M. F., Klivniak B. M., Zhuravel I. A., Rozhkovsky Ya. V.** The element composition study of thick extract from Tribulus terrestris L. herb. Res. J. Pharm. Biol. Chem. Sci. 2016; 7(6), 2200–2202.
12. **Franco J. V., Turk T., Jung J. H., Xiao Y. T., Iakhno S., Tirapegui F. I., Garrote V., Vietto V.** Pharmacological interventions for treating chronic prostatitis/chronic pelvic pain syndrome. Cochrane Database Syst. Rev. 2019;10(10), CD012552.
13. **Sroka Z., Sowa A., Dryś A.** Inhibition of Lipoxygenase and Peroxidase Reaction by Some Flavonols and Flavones: The Structure-Activity Relationship. Nat. Prod. Commun. 2017; 12(11).
14. **Fehrenbacher J. C., Vasko M. R., Duarte D. B.** Models of inflammation: Carrageenan- or complete Freund's Adjuvant (CFA)-induced edema and hypersensitivity in the rat. Curr. Protoc. Pharmacol. 2012; 5 (5–4).
15. **Cash J. L., White G. E., Greaves D. R.** Chapter 17. Zymosan-induced peritonitis as a simple experimental system for the study of inflammation. Methods Enzymol. 2009; 461, 379–396.
16. **Alfaro R. A., Davis D. D.** Diclofenac. StatPearls. Treasure Island (FL): StatPearls Publishing 2021.
17. **Gan T. J.** Diclofenac: an update on its mechanism of action and safety profile. Curr. Med. Res. Opin. 2010; 26(7), 1715–1731.
18. **Mashima R., Okuyama T.** The role of lipoxygenases in pathophysiology; new insights and future perspectives. Redox. Biol. 2015; 6, 297–310.
19. **Brain S. D., Williams T. J.** Leukotrienes and inflammation. Pharmacol. Ther. 1990; 46(1), 57–66.
20. **Jo-Watanabe A., Okuno T., Yokomizo T.** The Role of Leukotrienes as Potential Therapeutic Targets in Allergic Disorders. Int. J. Mol. Sci. 2019; 20(14), 3580.
21. **Volman T. J., Hendriks T., Goris R. J.** Zymosan-induced generalized inflammation: experimental studies into mechanisms leading to multiple organ dysfunction syndrome. Shock. 2005; 23(4), 291–297.
22. **Lee H. H., Ahn E. K., Hong S. S., Oh J. S.** Anti-inflammatory effect of tribulusamide D isolated from Tribulus terrestris in lipopolysaccharide-stimulated RAW264.7 macrophages. Mol. Med. Rep. 2017; 16(4), 4421–4428.
23. **Tian C., Chang Y., Zhang Z., Wang H., Xiao S., Cui C., Liu M.** Extraction technology, component analysis, antioxidant, antibacterial, analgesic and anti-inflammatory activities of flavonoids fraction from *Tribulus terrestris* L. leaves. Heliyon 2019; 5(8), e02234.
24. **Tian C., Chang Y., Wang R., Kang Z., Wang Q,. Tong Z., Zhou A., Cui C., Liu M.** Optimization of ultrasound extraction of Tribulus terrestris L. leaves saponins and their HPLC-DAD-ESI-MS ^{n} profiling, anti-inflammatory activity and mechanism in vitro and in vivo. J. Ethnopharmacol. 2021; 278, 114225.