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Influence of Flavonoids Containing Extract from Medicinal Plants to the Course of Aseptic Inflammation

Z. Z. Khakimov, A. Kh. Rakhmanov*, Sh. R. Mavlanov, A. A. Abduvaliev, M. J. Allayeva, G. K. Botiralieva

Tashkent Medical Academy, Tashkent, Uzbekistan

Abstract The anti-inflammatory effect of multi-component dry extract consisting local medicinal plants such as *Hipericum scabrum L., Ziziphora pedicellata Pazij Vved., Mediazia macrophylla* and *Glycirhiza glabra L.* on dextran and histamine induced inflammation model was studied on laboratory animals. It was determined that dry extract of medicinal plants in doses 10, 25, 50 and 100 mg/kg distinctly suppresses the process of exudation in dextran and histamine induced inflammation in experimental animals. Dry extract of medicinal plants is superior to LIV-52 (100 mg/kg) with its anti-inflammatory activity and is not inferior to the reference non-steroidal anti-inflammatory drug- diclofenac sodium.

Keywords Dry extract, Inflammation, Diclofenac sodium, Edema, Histamine, Dextran

1. Introduction

Recently, much attention has been paid to the development of anti-inflammatory drugs based on substances of natural origin, due to their relative high biological activity and low toxicity to the human body.

The experimental substantiation of new properties of natural compounds is one of the most important directions of pharmacological research. Earlier, we identified choleretic and hepatoprotective properties of compounds representing the sum of dry extract of medicinal plants (DEMP) *Mediazia macrophylla, Glycyrrhizin glabra L., Hipericum scabrum L.* and *Ziziphora pedicellata Pazij Vved* [1-3]. The main mechanism of pharmacological effect is its antioxidant properties expressing by the content of flavonoids and other substances, which block free radical reactions [4].

This allowed us to assume the presence of a possible anti-inflammatory effect of the complex of dry extract compounds from the above-mentioned medicinal plants, which determined the purpose of this work.

2. Material and Methods

2.1. Plant Material and Preparation of Dried Extract

Aerial parts of *Hypericum perforatum L., Ziziphora pedicellata Pazij et Vved.* and *Mediazia macrophylla* as well as root and rhizome parts of *Glycirhiza glabra L.* were obtained in summer of 2017 from foothills to medium zones of mountains of Tashkent region, Fergana, Samarkand and Surkhandaryo regions of Uzbekistan. Plant material was dried under dark conditions at room temperature for 10 days. The dry material was milled, obtaining 4-6 mm particles and mixed in proportion 1,25:1,0:1,25:1,5 (productivity of dried extract was higher than other proportions) then extracted by water at 93-95єC temperature for 3 hours. The extract was then separated from the sample residue by filtration through filter paper. The resulting extracts were concentrated in vacuum until remaining a crude solid extract, which then was dried in dryer apparatus [1].

2.2. Experiments

The experiments were carried out on outbred white male rats with a body weight of 155-170 g. They kept in standard condition and on a standard diet that had been quarantined for at least 12-14 days. Each experimental group consisted of 6 animals. The antiexudative effect of the preparations was studied on the model of acute inflammatory edema of the animal's paw, induced by the introduction into the plantar aponeurosis of the right posterior limb of rats with 0.1 ml of a 6% dextran solution and 1% histamine solution [5]. Measurement of the volume of paw of animals was performed by plethysmometer [6] before and 30, 60, 120, 180 and 240 minutes after the introduction of the histamine and dextran. The value of the anti-inflammatory activity (VAA) of the drugs was calculated according to the formula VVA = Vcon-Vexp / Vcon x 100 =% [7]. DEMP was administered preventive per os at doses of 10, 25, 50 and 100

* Corresponding author:
dr.ali.fl@mail.ru (A. Kh. Rakhmanov)
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mg/kg [7]. A separate group of animals was administered diclofenac sodium orally at a dose of 10 mg/kg [8], and another LIV-52 at a dose of 100 mg/kg [9] before the administration of the dextran and histamine.

All experiments were performed in compliance with the requirements of the European Convention "On Protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg 1986) and in accordance with the Russian Federal Law "On protection of animals from cruel treatment".

2.3. Statistical Analysis

The results of the study were statistically processed using the Biostat 2009 software package. The data are presented as the mean (M) and standard error of the mean value (m). Statically significant changes were taken at a probability level of 95% or more ($p < 0.05$).

3. Results and Discussion

It is seen from the data given in table 1, the volume of the paws increased more than three times in intact animals under the influence of dextran. In this case, the greatest increase in the volume of the paws was noted 1 hour after the introduction of dextrane, which statically significantly remained for the next four hours. In contrast, in animals that received DEMP at a dose of 10 mg/kg, the maximum increase in the volume of the paw was 240%, while the it was statistically significantly less than 72%. Calculation of anti-inflammatory activity of the drug showed that in this dose it was 22.1%.

Increasing of the dose of DEMP to 25 mg/kg led to an increase of the effect. At the same time, the anti-inflammatory activity of the medicine was 27.4%. the administration of DEMP in a dose twice high then the previous one led to a more significant suppression of the process of exudation, in which the anti-inflammatory activity was 35.8%. It was shown from the obtained data that the increasing of the dose of the medicine led to an increase of the anti-exudative effect of DEMP. However, despite of increasing of doses five times, the increase of the anti-inflammatory activity of the medicine was only 13,7%. Further increasing of the dose of compound to 100 mg/kg showed a distinct anti-inflammatory effect, the degree of which was slightly less than doses of 25 and 50 mg/kg. The value of anti-inflammatory activity was 26.3%.

Consequently, the studied compound, which consisting sum of dry extracts from local medicinal plants, has a distinct anti-exudative effect indicating its anti-inflammatory activity. It is known that if the level of anti-inflammatory activity of the studying substance exceeds 30%, it is considered that it has a expressed anti-inflammatory action [10].

Table 1. Efficiency of DEMP, diclofenac sodium and LIV-52 in dextran induced paw edema

Groups	Dose, mg/kg	Volume of paw, ml				
		Initial	60 min	120 min	180 min	240 min
Control	-	0,45±0,01	1,41±0,06*	1,37±0,05*	1,25±0,07*	1,18±0,06*
Diclofenac	10	0,49±0,02	1,11±0,04*	1,02±0,07*	0,93±0,04*	0,84±0,05*
DEMP	10	0,53±0,02	1,27±0,08*	1,15±0,07*	1,17±0,06*	0,99±0,06*
DEMP	25	0,54±0,02	1,22±0,05*	1,12±0,04*#	1,06±0,04*	0,97±0,04*#
DEMP	50	0,51±0,02	1,11±0,06*#	1,08±0,04*#	0,99±0,03*#	0,91±0,04*#
DEMP	100	0,53±0,03	1,19±0,06*#	1,11±0,06*#	1,03±0,04*#	0,93±0,05*#
LIV-52	100	0,52±0,02	1,39±0,04*	1,23±0,03*	1,17±0,04*	1,05±0,04*

Note: *-in comparison with initial index (P<0,05);
#- in comparison with control group respectively to the same hours (P<0,05).

Table 2. Efficiency of DEMP and LIV-52 in histamine induced paw edema

Groups	Dose, mg/kg	Volume of paw, ml					
		Initial	30 min	60 min	120 min	180 min	240 min
Control	-	0,67±0,05	1,70±0,05*	1,63±0,05*	1,53±0,05*	1,45±0,06*	1,38±0.05*
DEMP	10	0,68±0,01	1,49±0,06*	1,41±0,06*#	1,32±0,05*#	1,21±0,06*#	1,13±0,07*#
DEMP	25	0,65±0,02	1,41±0,07*#	1,32±0,07*#	1,23±0,06*#	1,14±0,06*#	1,04±0,05*#
DEMP	50	0,68±0,03	1,36±0,06*#	1,29±0,05*#	1,19±0,05*#	1,10±0,04*#	0,97±0,04*#
DEMP	100	0,61±0,03	1,34±0,07*#	1,26±0,07*#	1,18±0,07*#	1,10±0,07*#	1,02±0,07*#
LIV-52	100	0,69±0,03	1,46±0,07*#	1,38±0,08*#	1,31±0,08*	1,23±0,07*	1,13±0,07*

Note: *-in comparison with initial index (P<0,05);
#- in comparison with control group respectively to the same hours (P<0,05).

For recommendation to practical application, it is important to establish an effective dose of new compounds. It is seen from the given data that the effective dose of the studied compound is 50 mg/kg in this model of experimental aseptic arthritis. As long as, the compound is the extract of several medicinal plants, phyto-preparation LIV-52 was chosen for comparison of the anti-inflammatory activity. It is important to note that a literature data about the specific studies on the anti-exudative activity of this medicine is not enough. Thereby, the study of the anti-exudative action of LIV-52 on the model of dextran inflammation was conducted in a separate series of experiments. LIV-52 was administered in a dose of 100 mg/kg, which is effective as a hepatoprotector by literature data [9]. The results of the conducted separate studies showed that LIV-52 has substantial anti-exudative action. Its degree of activity was equal to the effect of DEMP (10 mg/kg), where the anti-inflammatory activity of LIV-52 was 23%. Consequently, the investigated DEMP with respect to its anti-exudative activity exceeds the known drug - LIV-52 even in a ten-time small dose.

In the treatment of human diseases (in the pathogenesis of them lies inflammation process) are widely used nonsteroidal anti-inflammatory drug (NSAID) from various groups of chemical compounds not selectively blocking cyclooxygenase. Among these NSAID- diclofenac sodium is considered a reference drug [11]. Therefore, we studied the anti-inflammatory activity of diclofenac sodium using its effective dose in a separate group of animals [7]. The results of the experiments showed that diclofenac sodium strongly suppressed the development of exudation with a maximum manifestation of anti-inflammatory activity after 1 hour from the beginning of the experiment. At the same time, the calculation of anti-inflammatory activity showed that it was equal to 37.9%. It can be seen that the effect of diclofenac sodium is insignificant (by 3.4%) higher than the effect of DEMP in a dose of 50 mg/kg.

Thus, the results of this series of experiments showed that DEMP emerges a distinct anti-exudative effect, which in twice low dose exceeds LIV-52 at anti-inflammatory activity and it is not significantly inferior the reference NSAID-diclofenac sodium. It is believed that the development of aseptic inflammation induced by dextran is due to the release of histamine and serotonin from mast cells which are considered main inflammatory mediators [11]. According to this, we investigated the effect of DEMP on the course of histamine inflammation in a separate series of experiments.

Histamine has a multi-effect on the human body, in particular, by excitation H1-receptors located in the vessels, bronchi and in the stomach, causes an increase of vascular permeability, bronchospasm, lowering blood pressure as well as increase secretion of gastric juice [12, 13]. Histamine is detected simultaneously in the focus of inflammation with the occurrence of damage. It causes the expansion of the vessels of the microcirculatory path, increases their permeability, stimulates the marginal pain receptors. Thus, histamine "triggers" an acute inflammatory response. The emergence of histamine in the focus of inflammation is closely related to the degranulation of mast cells. The latter stimulates the synthesis of new mediators from the membrane lipids of activated mast cells and basophiles, such as proteases, proteoglycans, eosinophilchematoxys factors, kinins, complementases, ekazonoids, leukotrienes, platelet activating factor (PAF), etc. [12].

The results of the studies have shown that under the influence of histamine there was an expressed intensifying of the processes of exudation (table 2). Thus, in control rats, histamine led to an increase of the paw volume more than 2.5 times after 30 minutes from the start of its introduction. Subsequently, the effect gradually weakened, however, the paw volume exceeded by 2.1 times in comparison to initial indexes even after 4 hours of experiment. In contrast, in rats that had previously been administered DEMP at a dose of 10 mg/kg, the degree of increasing of paw volume was smaller and it was 2.2 times in comparison to initial indexes. When using the preparation at a dose of 25 mg/kg, anti-inflammatory effect increased and suppression of edema was 116.9%. The increasing the dose of preparation twice led to the rising of the effect, where the increasing of the volume of the paws were only 2 times in comparison with the previous dose. At the same time, the value of the anti-inflammatory activity of the preparation (VAA) in these doses was 21.4%, 26.2%, 33.9% and 29.1%, respectively. It is noteworthy that DEMP was superior to the known medicine LIV-52 in its anti-exudative activity and VAA of it was only 25.2% during the experiment.

Consequently, DEMP, which is a dry extract from the medicinal plants of *Mediazia macrophylla, Glycirhiza glabra L., Hipericum scabrum L.* and *Ziziphora pedicellata PazijVved.* has an expressed anti-inflammatory activity, especially at doses of 50 and 100 mg/kg.

Probably, the mechanism of such action of the test compound is to suppress the intensity of the processes of lipid peroxidation, as mentioned above, there are a number of substances in content of medicinal plants, which bind the free radicals, inhibit free radical oxidation of lipids and the activity of the lipooxygenaseenzyme. Moreover, in recent years, the ability of inhibition of cyclooxygenase, lipoxygenase, whose activity increases with inflammation, have been found in substances with the membrane-stabilizing effect, in particular, in silymarin [13]. Along with this, the results of this study point out the antihistamine effect of DEMP, which prevents the development of histamine- induced aseptic inflammation. It can be assumed that DEMP might be effective in treating not only aseptic inflammations, but also diseases accompanied by an allergic background.

It is possible to consider that DEMP has such properties which probably lead to the strengthening of membranes of mast cells and prevent the release of inflammatory mediators. It is known that such membrane-stabilizing effect is characteristic to compounds with antioxidant effect. Since, they suppress the intensity of free radical processes that

prevent damage of cells membrane and subcellular structures. Earlier, we showed that DEMP reduces the level of malonic dealdehyde in the blood, which is an intermediate product of lipid peroxidation [4]. In our opinion, this action of DEMP is due to a large number of flavonoids in content of it, which has an antioxidant activity [4, 14, 15]. Antioxidants interfere the release of arachidic acid- a precursor of prostaglandins from phospholipids by the suppressing of free radical oxidation of lipids. Probability of an inhibitory effect of DEMP on cyclooxygenases is very low. However, it cannot be excluded that glycyrrhizinic acid (triterpenoid glycoside) possesses anti-inflammatory properties, provides an expression of the pharmacodynamic effect of DEMP [15].

Thus, DEMP has a distinct anti-exudative effect, which is not inferior in its activity to diclofenac sodium and clearly superior to LIV-52 - dry extract from number of medicinal plants. So that DEMP is a non-toxic compound [16] and has sufficient anti-inflammatory activity and it can be recommended as an agent for the treatment of diseases, in the pathogenesis of which lies inflammation process.

4. Conclusions

1. DEMP-extracted from mediazia macrophylla, glycyrrhiza glabra l., hipericum scabrum l., and ziziphora pedicellata pazij vved., distinctly suppresses the process of exudation in dextran and histamine induced inflammation in experimental animals.
2. DEMP is superior to liv-52 with its anti-inflammatory activity and is not inferior to the reference non-steroidal anti-inflammatory drug- diclofenac sodium.
3. The mechanism of anti-inflammatory activity of demp is associated with its antihistamine and antioxidant property.
4. demp can be recommended as a pathogenetic agent in the treatment of inflammatory diseases.

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REFERENCES

[1] Mavlanov Sh.R., Khakimov Z.Z., Rakhmanov A.Kh., Khodzimatov O.K., 2015, The importance of dry extract from plants of Central Asia for eliminating alterations of functional state of liver in its alcoholic lesion, Amer. Journal of Medic. and Med. Scien., 5(5), p.260-263.

[2] Mavlanov Sh.R., Khakimov Z.Z., Rakhmanov A.Kh., Paizieva L.A., 2015, Comparative study of the influence of Lesbokhol and LIV-52 on excretory liver function in acute drug lesion. Monthly scientific journal of Novosibirsk., 9(16), p.64-67.

[3] Mavlanov Sh.R., Khakimov Z.Z., Rakhmanov A.Kh., 2016, The influence of Lesbokhol on some biochemical indixes of blood in acute intoxication with tetrachloromethane in rats. Infection, immunity and pharmacology., 6, p.180-183.

[4] Mavlanov Sh.R., Khakimov Z.Z., Rakhmanov A.Kh., 2017, Effect of Lesbokhol on glycogen content in the liver in acute toxic damage. Infection, immunity and pharmacology, 1, p.129-134.

[5] Coure C.O., Souza R.B., Rodrigues J. et al. Mechanisms Involved in the Anti-Inflammatory Action of a Polysulfated Fraction from Gracilaria cornea in Rats. PLoS ONE 10 (3): e0119319. doi: 10.1371 / journal.pone.0119319.

[6] Khakimov Z.Z., Azimov R.I., 2001, A device for the measurement of volume of the paw of small experimental animals. Rasmiy akhborotnomf, 1, p. 8.

[7] Rakhmanov A.Kh., Khakimov Z.Z., Mavlanov Sh.R. et al., 2018, Anti-inflammatory activity of dry extract of medicinal plants. European Journal of Pharmaceutical and Medical Reasearch, 5 (8), p.55-57.

[8] Hasani, A., Soljakova, M., Jakupi, M., & Ustalar-Ozgen, S., 2011, Preemptive analgesic effects of midazolam and diclofenac in rat model. Bosnian Journal of Basic Medical Sciences, 11(2), p.113-118.

[9] Kushwaha Sh., Semwal Bh., Upadhayay P.K., 2017, Hepatoprotective activity of ethanolic extract of Euphorbia royleana linn in carbon tetrachloride treated guinea pig. Innovations in Pharmaceuticals and Pharmacotherapy, 4 (1), p.22-32.

[10] Talaleva O.S., Miyenko N.P., Bryukhanov V.M. et al., 2012, Influence of histochrome on the exudative and proliferative phase of experimental inflammation.Bulletin of SB RAMS, 32(4), p.28-31.

[11] Khakimov Z.Z., Rakhmanov A.Kh., Tadjibaev A.B., 2017, Experimental studies of the influence of the precursor of dopamine on the course of aseptic inflammation. Reports of the Academy of Sciences of the Republic of Uzbekistan, 1, p. 81-83.

[12] Poryadin G.V. Inflammatory mediators // Russian State Medical University. Toolkit. Moscow, (2006), p.22.

[13] Tsirkin V.I., Khlybova S.V., 2006, The role of histamine in reproduction. Vyatsky medical bulletin, 2, p.1-12.

[14] Novikov V.E., Klimkina E.I., 2005, Pharmacology of hepatoprotectors.Reviews on clinical pharmacology and drug therapy, 4(1), p.2-20.

[15] Mavlanov Sh.R., Khakimov Z.Z., Khodzhimatov O.K. Patent of Uzbekistan. Choleretic substance. IAP 05334.

[16] Mavlanov Sh.R., Khakimov Z.Z., Rakhmanov A.H., Gildieva M.S., 2017, Preclinical toxicological research of "Lesbokhol" - a mixture of dry extracts from the local flora. Pharmaceutical Bulletin of Uzbekistan, 2, p.74-81.