ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

New herbal products for treatment of chronic prostatitis. I. Some pharmacological properties of Prostanorm - liquid extract from a mix of herbs (*Glycyrrhiza glabra, Hypericum perforatum, Solidago canadensis, Echinaceae purpurea*).

Inna V Voskoboynikova[1], Alexandra K Voskoboynikova[2], and Vladimir K Kolkhir[1]*.

[1]Department of Experimental and Clinical Pharmacology, National Institute of Therapeutic and Aromatic Plants (VILAR), 7 Grina Str., Moscow 117216, Russia
[2]Medical analytical department, Pharmaceutical & Industrial Company PharmVILAR, 7 Grina Str., Moscow 117216, Russia

ABSTRACT

Animal models were used to study pharmacological activity of a multi-component Russian-made herbal product (prostanorm) designed for treatment of chronic prostatitis, containing ethanol-aqueous extract of a mixture of St. John's wort (*Hypericum perforatum* L.), golden rod (*Solidago Canadensis* L.), licorice root (*Glycyrrhiza glabra* L.), *Echinacea purpurea (*L*.)* and *Moench* rhizome and roots taken in equal quantities. Prostanorm showed pronounced antiinflammatory activity, presented as significant decrease of peritoneal exudate volume in rat peritonitis model by 15-50% and by alleviation of formalin- and histamine-iduced hindlimb edema in mice as compared to control animals. The product administration dose-dependently increased pain sensitivity threshold in the hot plate model by 12-70% for at least 3 hours after drug administration as compared to initial level. It significantly and dose-dependently enhanced diuresis induced by water load in rats. It also has gonadotrophic effect. Intragastric administration to rats before or concomitantly with ulcer-inducing agents provided protection of gastric mucosa. The beneficial effect on rat liver microsomal monoxygenase system supports detoxifying and hepatoprotective properties of the product. Prostanorm displays a number of pharmacological effects, including antiinflammatory, angioprotective, analgesic, diuretic and androgenic ones. This profile of the product's pharmacological activity suggested a possibly favorable Prostanorm application for treatment of chronic non-specific prostatitis.

Keywords: prostanorm; prostatitis; anti-inflammatory, analgesic, angioprotective, diuretic, gastro- and hepatoprotective activity

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chronic prostatitis is one of the most prevalent diseases of men. Inflammatory diseases of male genital tract may arise from its infection by pathogenic or opportunistic microorganisms, from disturbance of prostate hemodynamics, alteration of immune status, etc. [1, 2]. The present inadequate efficacy of chronic prostatitis treatment and the significant rate of complications (e.g. allergic reactions, toxic effect on spermatogenesis) caused by pharmaceutical therapies strongly support the need for new approaches to the disease treatment. One of the promising fields of research covers complex therapy of chronic prostatitis, which would include herbal products [3-5]. Many herbal products are known to be used for treatment of chronic prostatitits in different countries [6, 7].

Here we describe the results of experimental pharmacological study of a new herbal product designed for treatment of chronic prostatitis - prostanorm, which is a liquid water-ethanol extract of herbal raw material mixture, including root *Glycyrrhiza glabra L*. (Leguminosae), grasses *Hypericum perforatum L.* (Hypericaceae), grasses *Solidago canadensis L.*(Asteraceae), rhizomes with roots *Echinaceae purpurea Moench* (Asteraceae).

EXPERIMENTAL

General

Prostanorm used in the present study was produced according to regulatory standards developed at VILAR.

Appearance. Brown-reddish liquid with aromatic odor. Its basic characteristics:
Active compounds - substances extracted by 50% ethyl alcohol from a mixture of plants, including triterpene glycosides, flavonoids, oxycinnamic acids and other biologically active substances [8].

Identity: Determination of saponins - stable foam upon shaking; determination of phenols - green coloration upon addition of ferrioxide chloride solution. TLC - chromatogram must contain a blue spot with R_f about 0.73 (*G. glabra*), a red spot with R_f about 0.65 (*H. perforatum*), a dark-red spot with R_f about 0.15 (*S. canadensis*) and a dark-blue spot developing to yellow with R_f about 0.06 (E. purpurea). Spectrophotometric assay of total flavonoids (as rutin) - not less than 1.3%. Dry residue content not less than 21%. Ethyl alcohol content not less than 37%.

Before the study prostanorm was made free of alcohol. Control animals received an adequate amount of solvent (water) by the same route of administration. Each animal group (experimental, control and intact) consisted of not less than 10 animals.

Plant material

All plants have been prepared in the European part of the Russian Federation and meet the requirements of the Russian Pharmacopoeia.

Animals

White not purebred mice in weight 17-20 g and white not purebred rats in weight 180-220 g have been used. All animals were housed in standard environmental condition and fed under specific pathogen-free condition.

Anti-inflammatory properties

These were studied in two ways: (i) by measuring an acute inflammatory swelling produced in mice after an injection into the subaponeurosis of the hind legs of either 50 µL of a 1% solution of formalin or a 0,1% solution of histamine and (ii) by inducing peritonitis in rats after intraperitoneal injection of 1mL/100g wt of a 0,2% solution of silver nitrate (1mL/100g wt) [9]. The effect of anti-inflammatory action of prostanorm was measured by noting the decrease of the swelling in comparison to a control after an intragastric

administration of prostanorm in a dose of 2-10 ml/kg in the first case 1 h prior to the administration of the irritant, and in the second case 1 h after administration of silver nitrate.

Angioprotective action

This was studied [10] using mice. prostanorm was introduced at a dose of 2, 5 and 10 ml/kg 1 h before intraperitoneal injection of 250 µL of a 1% solution of trypan blue. The criteria used for intracapillary penetration was the time of penetration by trypan blue into the centre of inflammation caused by application of 50 µL of xylol on the depilated skin surface.

Analgesic activity

This was studied by measuring pain sensitivity threshold (PST) in hot plate test [11], 1944). Measurements were carried out using mice on a standard device (Ugo Basil, Italy) with a temperature of 54.5^{o}C. Time to discomfort reaction (limb withdrawal, vertical position, "washing") was registered 30, 60 and 120 min after a single drug intragastric administration at doses 2 or 10 ml/kg.

Diuretic action

This was studied in white male rats according to the method of [12] with increased water load after intragastric administration of prostanorm in a dose 2 and 10 ml/kg.

Gonadotrophic and androgenic properties

Prostanorm gonadotrophic activity was studied by chronic drug intragastric administration to juvenile male rats (aged 21-23 days) at a dose of 10 ml/kg. Prostanorm *androgenic activity* was studied using castrated juvenile male rats. Castration was carried out at the age of 23-25 days by method of [13]. Prostanorm was administered by gastric tube for 7 days starting from the day after castration with concomitant administration of testosterone propionate. The latter was diluted by olive oil to the final concentration 0.5 mg/kg and injected subcutaneously to both experimental and control animals. The weight of gonads, musculus levator ani and hypophysis was measured at day 8 or 11 after castration.

Gastro- and hepatoprotective action.

This was studied in white rats of both sexes weighing 200-220 g after intragastric administration of Prostanorm in a dose of 10 ml/kg. The antiulcerative activity of prostanorm was studied using different models of experimental gastric ulcers elicited by ulcerproducing agents with different mechanisms of action: ethanol ulcers [14]; ulcers produced by pyloric stricture [15]; and by induction of caffeine-arsenic ulcers [16]. To check the protective action of prostanorm of the gastric mucosa the total number of ulcers, the percentage of rats with ulcers, and the average rate of the appearance of ulceration were measured. Using these indicators Paulus index was calculated, being the integral parameter of antiulcerative activity [17]. Hepatoprotective properties were assessed by measurement of microsomal monooxygenase (cytochrome P450) activity in rats with hepatic lesions caused by caffeine-arsenic mixture.

Statistical analyses

The data obtained were subjected to the Student's *t*-test.

RESULTS AND DISCUSSION

Prostanorm showed pronounced antiinflammatory activity, presented as significant decrease of peritoneal exudate volume in rat peritonitis model by 15-50% and by alleviation of formalin- and histamine-iduced hindlimb edema in mice as compared to control animals (Table 1).

Besides, prostanorm displayed capillary protective activity by elongation of time to Trypan blue penetration into inflammation locus by 32-50% vs. control (Table 1).

ISSN: 0975-8585

Table 1: Effects of prostanorm on inflammatory hindlimb edema in mice, experimental peritonitis in rats and capillary permeability in mice skin

Dose, ml/kg	Formalin swelling, mg (% vs. control)	Histamine swelling, mg (% vs. control)	Peritoneal exudate, ml (% vs. control)	Time of penetration of trypan blue, s(% vs. control)
Control	60,6±3,15	37,3±2,96	1,93±0,29	68,3±5,9
2	60,6±2,75	29,6±1,76 (-21)	1,64±0,29 (-15)	91,6±5,4* (+34)
5	49,0±3,97 (-19)	26,8±2,95 (-25)	1,54±0,19 (-20)	90,8±2,4* (+32)
10	46,4±3,95* (-24)	27,3±2,36* (-27)	0,97±0,24* (-50)	100,0±5,0** (+46)

* or ** - significant difference from control with p<0,05 or p<0,01 resp.

Mechanism of pain development during thermal stimulation includes emotional reaction to pain; reaction in the hot plate model is primarily regulated by central mechanisms. Prostanorm administration dose-dependently increased PST in the hot plate model by 12-70% for at least 3 hours after drug administration as compared to initial level (Table 2).

Table 2: Effect of prostanormon pain sensitivity threshold in the hot plate model

Dose, ml/kg	Pain sensitivity threshold (% of initial) at specified time points after Prostanorm administration			
	30 min	60 min	120 min	180 min
Control	94±7	106±8	88±5	93±5
2	113±7	121±7	119±6*	124±6*
10	153±8**	169±7**	171±7**	161±6**

* or ** - significant difference from control with p<0,05 or p<0,01 resp.

Prostanorm significantly and dose-dependently enhanced diuresis induced by water load in rats. Compared to control level, total diuresis (within 5 hours after drug administration) increased by 42.4% at a dose of 2 ml/kg and by 83.1% at a dose of 10 ml/kg (Table 3).

Table 3: Effect of prostanormon diuresis in rats

Parameter	Control	Prostanorm, 2 ml/kg	Prostanorm. 10ml/kg
Diuresis, ml/100 g, % of control	2,95±0,27	4,2±0,32* +42,4	5,4±0,32** +83,1

* or ** - significant difference from control with p<0,05 or p<0,01 resp.

Prostanorm administration to juvenile rats at doses 2 or 10 ml/kg significantly increased the weight of seminal vesicles by 25% or 28% respectively and the weight of ventral prostate by 30% and 34% respectively (Table 4). These data demonstrate the gonadotrophic effect of prostanorm. Small increase of the weight of musculus levator ani suggests some anabolic activity of the drug. Similar results were obtained in the study of Prostanorm androgenic activity: the weight of seminal vesicles increased by 60% vs. control (Table 4), suggesting the androgenic activity of prostanorm.

Table 4: Effects of prostanorm (10 ml/kg) on the weight of gonads and sex organs of juvenile and castrated rats

Weight, % of control				
Seminal vesicles	Ventral prostate	Testes	Hypophysis	Musculuslevatorani
Juvenile rats				
+28*	+34*	+14	+12	+20*
Castrated rats				
+60**	+20	-	-	+19*

* or ** - significant difference from control with p<0,05 or p<0,01 resp.

ISSN: 0975-8585

Prostanorm intragastric administration to rats before or concomitantly with ulcer-inducing agents provided protection of gastric mucosa (Table 5). This protective effect was most pronounced in the models of acute ulcers and was seen as the decrease of the number of ulcer-bearing animals by 70% (ethanol-induced ulcers) or by 57% (pylorus ligation-induced ulcers), decrease of the number and area of ulcer lesions by 76-97%, and decrease of Pauls index 10 times vs. control.

Table 5: Effects of prostanorm (10 ml/kg) on experimental ulcers in rats

Group	Rats with ulcers,%	Number of ulcers,%	Pauls index	Therapeu-tic effect	Ulcer area, %	Pauls index	Therapeu-tic effect
Ethanol-induced ulcers							
Control	100	100±8	2,9	-	100±4	34,1	-
Experim.	28,8	24±14**	0,3	14,5	15±6**	1,4	24,4
Pylorus ligation-induced ulcers							
Control	100	100±27	19,2	-	100±25	14,4	-
Experim.	42,8	7±15*	0,6	3,2	3±10*	0,2	72,0
Ulcers induced by caffeine-arsenic mixtrure							
Control	100	100±27	7,4	-	100±21	22,7	-
Experim.	88,9	55±21	3,6	2,0	56±17	11,4	2,0

* or ** - significant difference from control with p<0,05 or p<0,01 resp.

Table 6: Effects of prostanorm (10 ml/kg) on microsomal monoxygenase system in rats with hepatic lesions caused by caffeine-arsenic mixture

Group	***P_{450} content***		***Reaction rate***			
	nmoles/ml	nmoles/mg protein	Aniline hydroxilation		Dimethylalanine N-demethylation	
			nmoles/min per mg protein	nmoles/min per mg P_{450}	nmoles/min per mg protein	nmoles/min per mg P_{450}
Control	16,25±0,02	0,65±0,02	0,8±0,02	1,23±0,03	1,39±0,06	2,14±0,08
Experim.	15,3±0,44	0,5±0,01**	0,59±0,02**	1,61±0,01**	1,12±0,03*	3,06±0,01**
Exp/contr, %	94	77	74	131	81	143

*or ** - significant difference from control with p<0,05 or p<0,01 resp.

Study of prostanorm hepatoprotective properties showed that the activity of microsomal hydroxylating and demethylating enzymes, calculated per 1 mole of cytochrome P450, increased vs. control by 31% and 43% respectively (Table 6). The beneficial prostanorm effect on rat liver microsomal monoxygenase system supports detoxifying and hepatoprotective properties of the product.

The wide spectrum of prostanorm pharmacological activity arises from the diversity of plant extracts comprising the product. These extracts possess prostatetrophic properties, improve prostate microcirculation, normalize urination, have antiinflammatory, capillary protective, analgesic and antimicrobial properties [18].

Prostanorm specific pharmacological activity is primarily determined by triterpene and flavonoid glycosides of G.glabra (liquorice). Decoct of liquorice root shows significant gonadotrophic, androgenic and diuretic activity, which exceeds that of the well-known antiprostatitic product Cernilton. Liquorice preparation has been recommended for prevention and treatment of prostatitis [19]. Besides, liquorice triterpenoids show antiallergic, detoxifying, lipid-lowering and oxygen radical scavenging properties, and flavonoids show antimicrobial and oxygen radical scavenging properties [20, 21]. Extract of goldenrod is an essential component of products like Inconturin, Prostaforton, Antiprostin, Prostamed, Cefasable (Germany), which are used to treat prostatitis, prostate adenoma, nycturia, cystitis [19]. S. canadensis (goldenrod) flavonoids show diuretic, antiinflammatory and stone-dissolving properties [22]. H.perforatum wort preparations are used as astringents and antidepressants [23]. E. purpurea preparations are used as immune stimulators; besides, they show androgenic and potency-improving properties [24].

CONCLUSION

Herbal product (Prostanorm) is a multi-component herbal drug containing extract of a mixture of St. John's wort (Hypericum perforatum L.), golden rod (Solidago Canadensis L.), licorice root (Glycyrrhiza glabra L.), Echinacea purpurea (L.) Moench rhizome and roots in equal quantitites. Prostanorm displays a number of pharmacological effects, including antiinflammatory, angioprotective, analgesic, diuretic and androgenic ones. This profile of the product's pharmacological activity suggested a possibly favorable Prostanorm application for treatment of chronic non-specific prostatitis. The positive prostanorm characteristic is further supported by its pronounced gastric protective properties.

Next publications will describe pharmacological properties of Prostanorm as dry extract and safety data.

REFERENCES

[1] Karpuchin IV, Li AA, Kolkhir VK Fitoterapia 2003; 1: 37-40 (in Russian)
[2] Holt JD, Garrett WA, McCurry TK, Teichman JM. Am Fam Physician. 2016; 15; 93(4): 290-296
[3] Snow DC1, Shoskes DA. Expert Opin Pharmacother. 2010;11(14): 2319-2330
[4] Lara KS, Franklin CL Curr Urol Rep. 2011;12(4): 284–287
[5] Morán E, Budía A, Broseta E, Boronat F. Actas Urol Esp. 2013; 37(3): 174-180
[6] Galeone G, Spadavecchia R, Balducci MT, Pagliarulo V. Minerva Urol Nefrol. 2012; 64(2): 135-141.
[7] Iwamura H, Koie T, Soma O, Matsumoto T, Imai A, Hatakeyama S, Yoneyama T, Hashimoto Y, Ohyama C. BMC Urol. 2015; 7; 15:120
[8] Beloborodov V, Voskoboynikova I, Kolkhir V, Savvateev A, Zakharova N. RJPBCS. 2016; 7(2): 423-429
[9] Liberman SS, Kutchak SN, Rudzit EA Shakhnazarova NG Pharmacol Toxicol 1972; 35: 333-335 (in Russian).
[10] Oivin IA, Monakova KN Pharmacol Toxicol 1953; 16: 50-51 (in Russian)
[11] Gomaa AA Acta Pharmacol et Toxicol 1985; 57: 18
[12] Berhin EB, Ivanov YuI The Experimental Methods of Investigation of Kidneys and Water Exchange. Barnaul 1972, p. 199(in Russian).
[13] Kabak YaM Practical work on Endocrinology. Moscow 1968, p.126 (in Russian).
[14] Robert A, Nezamis JE, Lancaster C, Davis JP et al Am J Physiol 1983; 245: 113-121.
[15] Shay H, Komarov SA, Fels SS, Meranze D et al Gastroenterology 1945; 5: 43-61.
[16] Mescherskaya KA Pharmacol Toxicol 1954; 17: 26-28
[17] Pauls F, Wick AN, MacKay EM Gastroenterology 1947; 8: 774-782.
[18] Kolkhir VK, Stihin VA, Ephremov AP et al. The Patent №2098119, 1997 (in Russian).
[19] Ephremov AP, Shreter AI Fitoterapia for men. 1996; p. 321 (in Russian)
[20] Vichkanova SA Ingibitory of microorganisms among substances from the supreme plants. The dissertation. Moscow, 1981 (in Russian).
[21] Shibata S. Yakugaku Zasshi 2000; 120:849.
[22] Batuk VS, Vasilchenko EA, Kovaleva CN. Vegetative resources 1988; 24: 92-99(in Russian).
[23] Gorkov VA, Raushkin VA, Olejchik IV, Churilin UU, Karamisheva EI. Psychiatry and psychopharm 2000; 2: 1-7 (in Russian).
[24] Kolkhir VK, Sokolskaya TA. in: Actual problems of creation of new medicinal preparations of natural origin. St-Petersburg-Pushkin 2003, p.540-545 (in Russian).