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The Development and Standardization of a Proctological Agent Based on Black Cumin (Nigella Sativa L.) Extract

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Abstract

Annotation. Nigella sativa L. (Ranunculaceae) is one of the promising plants that has a wide spectrum of pharmacological activity. The black cumin is known to have been used for the treatment of cataracts, bronchial asthma, cholelithiasis and urolithiasis, helminthic invasions, diabetes, hemorrhoids, and many other diseases.

The aim of this study is to develop and standardize a drug based on a dry extract of the black seed– black cumin (Nigella sativa L.).

The technology of preparing suppositories of N. sativa dry extract by fusion molding was proposed, and its antiulcer activity was studied. The quality of suppositories was assessed according to the following indicators: characteristics, average weight of suppositories, melting point, identification, quantification. The study of the antiulcer activity of the drug was carried out on the model of phenolic proctitis. It was found that the tested drug significantly reduces the area of necrosis, hyperemia, and also significantly reduces the edema and the length of the damaged section of the intestine relative to the entire length of the rectum, on the model of phenolic proctitis. The data obtained indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases with concomitant inflammation, edema, hyperemia and ulceration.

Key words: dry extract of Nigella sativa, suppositories, thymoquinone, anti-inflammatory activity.

Introduction

Black cumin (*Nigella sativa L*) is a well-known annual herb of the buttercup family (Ranunculaceae). It is cultivated in the Mediterranean, North Africa, Central Asia, India, the Middle East and Russia[1]. It has a wide therapeutic effect[2], and information about its medicinal properties can be found in the works of Abu Ali ibn Sina (Avicenna)[3].

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According to literary sources, *N. sativa* seeds are used as an antitussive[4], gastroprotective[5], sedative[6], antiulcer[7], anti-inflammatory, immunomodulatory and antitumor drug [8-11], hepatoprotective agent[12], healing gastric ulcer[13], and as an agent for treating male infertility[14]. There is evidence of their use in cardiovascular disorders[15], improving memory[16], and as an antioxidant[17].

The component composition of *N. sativa* seeds is represented by the content of fatty acids, essential oils, vitamins, phenolic compounds, alkaloids, saponins, sterols, minerals, amino acids, proteins and carbohydrates[18]. Thymoquinone (TQ) is one of the most active biological compounds of *N. sativa*[19]. Thymoquinone also has a wide pharmaco-biological spectrum, in particular, antioxidant, antimicrobial, anti-inflammatory, antiparasitic, antitumor, hypoglycemic, hypotensive, hepatoprotective and anti-asthmatic, as well as neuroprotective effects[20,21,22].

To date, the pure TQ is not used independently. However, *N. sativa* seeds and oil, being the raw materials for its extracting, are mainly used in France, Germany, Italy, Great Britain, and the United States to prepare pharmaceutical products. A dry extract is also obtained from it.

Based on the above, the development of anadvantageous drug in the form of suppositories based on dry extract of *N. sativa* for introduction into medical practice in areas such as genitourinary, proctology, oncology, and endocrinology is promising and seems to be reasonable.

Aim of the research: The development and standardization of medicinal product based on dry extract of *N. sativa*.

Materials and Methods

The study object was dry extract of black cumin, suppository bases widely used in factory production: Witepsol H 15 (IOI Oleo GmbH, Germany), Witepsol W 35 (IOI Oleo GmbH, Germany) andlocally-made solid confectionery fat, as well as cocoa butter as a classic suppository base for comparison.

The quality of theprepared suppositories was assessed by the appearance, average weight of the suppositories, aqueous solution PH, the TQ identification and quantitative content, the time of complete deformation and the melting point in accordance with the procedures developed by us and the standard oness given in the State Pharmacopoeia of the Republic of Uzbekistan (SP RUz) 1st ed., Vol. 1 (2020).

To determine the rate and completeness of the TQ release from suppositories, we conducted the "Dissolution" test using the "rotating basket"apparatus. 500 ml purified water served as the dissolution medium at 37±10°C. The basket rotation speed was 100 rpm. At regular intervals, samples were taken from the dissolution medium with its replenishment. The samples were filtered and the TQ quantitative content was determined therein by the spectrophotometric method in the UV region.

The identification and quantitative analysis were conducted using a Shimadzu UV-1800 spectrophotometer.

Results and Discussion

To conduct a study aimed at choosing the best suppository base, the following lipophilic bases widely used in factory production were used: Witepsol H 15, Witepsol W 35, and locally-madesolid confectionery fat, as well as cocoa butter, as a classic suppository base for comparison. For the listed bases, the physicochemical and structural-mechanical indicators recommended by the State Pharmacopoeia of the Republic of Uzbekistan, 1st edition, Vol. 1, 2020 ("melting point" 2.2.16, p.38; "freezing point" 2.2.18, p.40) were determined.

One of the important requirements for fatty suppository bases is stability under conditions of long-term storage. The main indicators reflecting the intensity of oxidative processes in fats during storage are acid, iodine, peroxide indices, which were determined on the samples.

The dosing accuracy in case of fusion molding depends on the size of the mold deepening, the density of the base and the APIs included in the suppositories, on the uniformity of mixing the base with the active agent.

The substitution factor is variable and varies with the base viscosity, drug particle size, API density and

amount, and air volume included. The substitution factor for the dry extract of *Nigella sativa* (NSDE) was determined as follows: 30 suppositories were prepared from the suppository base by fusion molding without NSDE and weighed on an analytical balance. 80% of the base by weight of 30 suppositories was melted in a porcelain dish in water bath. The same procedure was used to prepare NSDE containing suppositories. In doing so, a weighed amount of NSDE was added to the molten base, dispersed and evenly poured into the same mold, after which the cavities were filled with the remaining base from the weight of 30 suppositories. The molds were cooled at –4°C for 15 minutes.

The substitution factor F was calculated by the formula:

P is the weight of 30 suppositories without NSDE, g; O is the weight of 30 suppositories with NSDE, g;

A is the total weight of the NSDE in 30 suppositories, g; *F* is the substitution factor. The amount of the base, taking into account the substitution factor, was calculated by the formula:

$$J = (P\text{-}F*A)*n,$$

where, *P* is the average weight of the suppository without NSDE, g; *A* is the amount of NSDE in grams spent on the preparation of 1 suppository; *F* is the substitution factor; *n* is the number of suppositories for which the calculation is performed. The loading quantities of various bases with NSDE, taking into account the calculated substitution factors, are shown in Table 1.

Table 1: The substitution factors and the number of bases required for the preparation of NSDE-containing suppositories

Type of base	Weight of supp. without NSDE(placebo), g (P)	Weight of supp. with NSDE, g (O)	Substitution factor (F)	Number of supp. for which calculation is performed (n)	Calculation of bases taking into account the displacement factor
Cocoa butter	21.5954	22.9432	0.6533	20	19.056
Witepsol W 35	21.5970	22.9452	0.6713	20	19.085
Witepsol H 15	21.5969	22.9451	0.6712	20	19.084
Solid confectionery fat	21.686	23.7106	0.6696	20	18.918

Type of base	Average weight of supp. withoutNSDE (P),g	Amount of NSDE (g) spent on the preparation of 1 suppository (A), g
Cocoa butter	1.1472	0.2133
Witepsol W 35	1.0918	0.2050
Witepsol H 15	1.0918	0.2050
Solid confectionery fat	1.0843	0.2067

As the results show, the NSDE substitution factors for the bases used are approximately the same, and their values are in the range of 0.6712 – 0.6696.

Suppositories were prepared by fusion molding with the presence of a certain amount of NSDE, which was injected directly into suppository bases. To make NSDE-containing suppositories by fusion molding, plastic and metal molds were used, which made it possible to obtain suppositories weighing 0.95-1.05 g. The mold cavities were preliminarily lubricated with a hydrophilic lubricant in order to facilitate the removal of congealed suppositories. To prepare a certain number of suppositories, the amount of base and NSDE was calculated. Witepsol-based suppositories were prepared without the use of an emulsifier, since the bases themselves have emulsifying properties. When molding suppositories based on cocoa butter and solid confectionery fat, distilled monoglycerides were used as an emulsifier, i.e. emulsifier No.1 and emulsifier No.2.

The suppository base was placed in an evaporation dish and melted in water bath. The NSDE was added to the molten base in parts, with constant stirring. The resulting suppository mass was stirred until the temperature of the mixture became close to the freezing point of the suppositories and quickly poured into pre-cooled molds. The resulting suppositories were inspected for quality. Suppositories had a smooth surface, the same torpedo shape, a homogeneous mass without inclusions. The uniformity of suppositories was determined visually by the absence of spangles, inclusions and pieces of the base on a longitudinal section. The average weight was determined according to the procedure given in the SPRUz, 1st ed., Vol. 1, 2.9.5. "Uniformity of the mass of dosed drugs" (p. 412), weighing 20 suppositories with an accuracy of 0.001 g.

The results obtained indicate the compliance of the prepared suppositories with the requirements of regulatory documents in terms of the average weight variation. The average weight variations do not exceed ±5%.

Further, for NSDE-containing suppositories, the iodine index, peroxide index and acid index were determined according to the above-procedures. The study results have shown that the addition of NSDE does not affect the indices of lipophilic bases.

The adding of a dry extract does not significantly affect the physicochemical and structural and mechanical properties of suppository bases.

The rate and completeness of the TQ release from the suppositories was determined according to the above procedure ("Dissolution" test).

Sampling in a volume of 5 ml was performed after 15, 30, 60, 120, 180, 240 minutes with replenishment of the dialysis medium. 5 ml of dialysate was placed in a 25 ml volumetric flask, adjusted to the mark with solvent. The samples were filtered through filter paper and the optical density of the resulting dialysate was measured on a spectrophotometer at a wavelength of 255 nm in a cuvette with a layer thickness of 1.0 cm relative to the solvent.

The percentage of TQ release o(X, mg) from suppositories in dialysates was calculated by the formula:

$$D_1 \times m_0 \times 200 \times 100 \times 1 \times b \times W \times 1000\ X = \frac{D_1 \times m_0 \times b \times W \times 20}{D_o \times m_1 \times V \times 100 \times 100 \times 100}$$

$$D_o \times m_1 \times V$$

where, D_1 and D_o the optical density of the test solution and that of the TQ reference sample solution (RSS), respectively;

m_1 and m_0 is a weighed amount of the suppository taken for analysis and that ofTQused to prepare the TQ RSS, g, respectively;

P is the average weight of suppositories, g;

V the volume of samples used foranalysis, ml;

W is the TQ content in its reference sample(98%).

Preparation of the TQreference sample solution (RSS). About 0.0195 g (accurately weighed amount) of TQ (Sigma-Aldrich) should be placed in a 50 ml volumetric flask, dissolved in 30 ml of 70% ethyl alcohol, the volume of the solution is brought to the mark with the same solvent and stirred. 0.5 ml of the resulting solution is placed in a 50 ml volumetric flask, the volume of the solution is brought to the mark with 70% ethyl alcohol. The solution is used freshly prepared.The results are shown in Table 2.

Table 2: Results of the TQ release from suppositories

Type of suppository bases	Thermostating time, min	NSDE content in the sample	
		X, g	X,%
Cocoabutter	15	0.000069	2.3
	30	0.000156	5.2
	60	0.000351	11.7
	120	0.000618	20.6

Type of suppository bases	Thermostating time, min	NSDE content in the sample	
		X, g	X,%
	180	0.000984	32.8
	240	0.001344	44.8
Witepsol W 35	15	0.000093	3.3
	30	0.000351	11.9
	60	0.000525	17.5
	120	0.000819	27.3
	180	0.001323	44.1
	240	0.001758	68.6
Witepsol H 15	15	0.000096	3.2
	30	0.000267	8,9
	60	0.000543	18.1
	120	0.000825	27.5
	180	0.001326	44.2
	240	0.001836	61.2
Solidconfectionaryfat	15	0.000099	3.1
	30	0.000357	9.3
	60	0.000525	12.5
	120	0.000702	20.4
	180	0.001041	31.7
	240	0.001374	38.8

The results obtained indicate that the nature of the base affects the TQ release from suppositories. The most complete and fastest release of TQ occurs from Witepsol H 15 and Witepsol W 35.

Based on the studies carried out, the following composition of NSDE-containing suppositories was proposed.

Composition: NSDE– 0.15g

Witepsol H 15 or W 35, a sufficient amount to prepare suppositories weighing 1.03 - 0.999.

The evaluation of the quality of suppositories was carried out in accordance with the requirements for this dosage form.

Appearance: suppositories of dark cinnamon color, torpedo-shaped, of the same size, with a smooth surface, of sufficient hardness to ensure ease of administration. The absence of inclusions, determined visually on the section, indicates the homogeneity of the suppositories. In appearance, the suppositories met the requirements of the SP RUZ, p 5.11, p.1099.

The average weight of the NSDE-based suppositories was 1.03±0.05 g, and the weight variation was within ±5%.

The pH value of 10% water extraction.

The melting point for the NSDE-based suppositories was 36.4±0.5°C, which meets the requirements of regulatory documents.

For the identification and quantification analysis of suppositories, we used a previously developed technique using a spectrophotometric method of analysis in the UV spectrum by TQ, which is the main active ingredient of the dry extract of *Nigella sativa*. Previously, it was found that the suppository base does not absorb in this region of the spectrum and does not affect the results of the quantitative determination of active substances.

1.0g (accurately weighed amount) of crushed suppositories was placed in a 50 ml flask, 5 ml of 70%

ethyl alcohol was added and heated in water bath until completely melted. The flask with the contents was stirred for 3 minutes, cooled and filtered. The extraction was repeated with another 5 ml portion of the solvent and combined. TQ was identified by the position of the maximum at 255±2 nm in the 220–350 nm region in the UV absorption spectrum in an alcohol solution prepared for quantitative determination (Fig. 1).

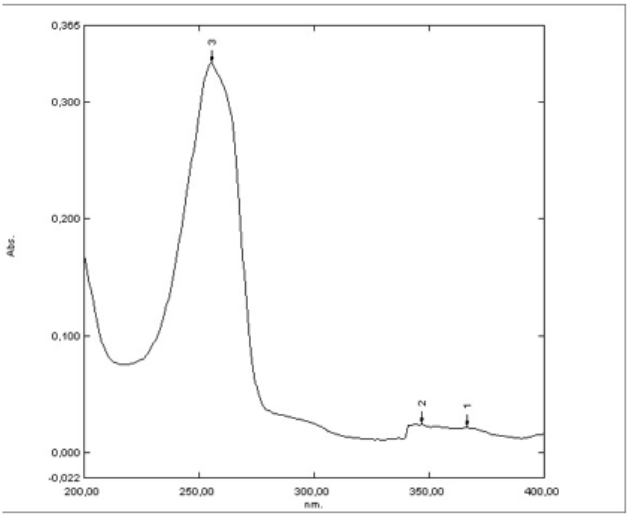

Figure 1: UV spectrum of an alcoholsolution of suppositories

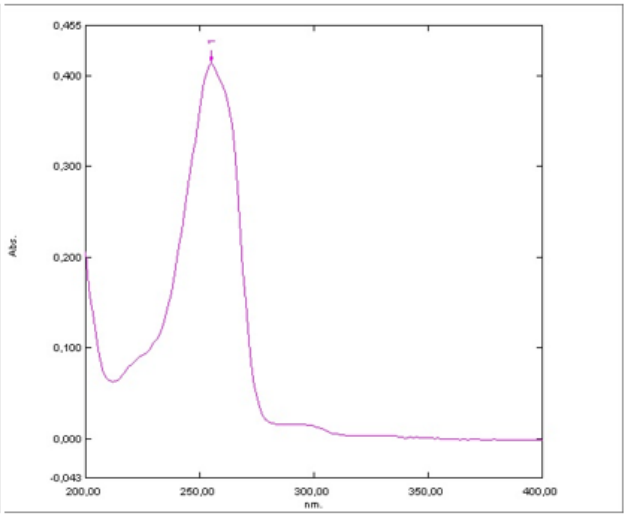

Figure 2: UV spectrum of an alcohol solution of the reference sample solution (RSS) of thymoquinone

To confirm the assignment of these maxima to TQ, the absorption spectrum of reliable TQ RSS was recorded under the same conditions. It was revealed that both spectral curves have the same character (Fig. 2).

In addition, it was proposed to identify TQ in suppositories by TLC on TCL Plates, Silica gel on Aluminum, 20 X 25x25 cm (Sigma-Aldrich) plates, followed by visualization of light brown adsorption zones by irradiation in UV light with a wavelength of 365 nm. For this, an alcohol extract of suppositories, prepared for quantitative analysis, and a 0.01% solution of the TQ RSS in 95% ethyl alcohol were applied by 0.005 ml (5 µg) per chromatographic plate. The plate with the applied samples was dried in air for 1–2 min, then placed in a chromatographic chamber saturated with a solvent system (o-xylene: cyclohexane in a ratio of 8: 2). Chromatography was performed in an ascending manner. When the front of solvents reached the end of the plate (finish), it was taken out and dried in air for 2-3 minutes. Then it was heated for 5 minutes in a drying chamber at a temperature of 100ºC and viewed under a UV lamp. TQ appeared as brown spots on a white background. The chromatogram showed 2 spots with Rf 0.37 and at the start, respectively.

In a quantitative analysis, the TQ content in one suppository in milligrams (X, mg) in terms of the average weight of the suppository was calculated by the formula:

$$X = \frac{D_1 \times m_0 \times b \times W \times 20}{D_o \times m_1 \times V}$$

where, D_1 and D_o are the optical density of thetest solution and the TQ RSS, respectively;

m_1 and m_0 is a weighed amount of the suppository taken for analysisand that ofTQ used to preparethe TQ RSS, g, respectively;

Pis the average weight of suppositories, g;

Vis the volume of samples used for analysis, ml;

Wis the TQ content in its standard sample (98%).

The content of TQ ($C_8H_9NO_2$) in one suppository should be from 14.4 mg to 17.6 mg.

The results of the quantitative determination of TQ in suppositories and the metrological characteristics of the analysis method are shown in Table 3.

Table 3: Results of quantitative determination of TQ in suppositories by spectrophotometry

TQ found (X, mg)	Metrological characteristics
16.99	X av.=16.62
16.34	S^2 = 0.11905
16.74	S = 0.345
16.85	ΔX = 0.9591
16.18	ε= 5.77%

As can be seen from the table, the TQ content is 16.62 mg, and the error of the analysis method does not exceed ± 5.77%.

Microbiological purity and pharmacological tests were conducted on the basis of the laboratory of microbiology and pharmacology of the Testing Center at "Med Standard" LLC Scientific Center.

Microbiological control was carried out in accordance with the SP RUz 1st ed., Vol. 1, 2.6.11. "Microbiological purity"[p.245]. The tests were carried out immediately after the manufacture of the suppositories, after a year and finally after two years. To test suppositories for microbiological purity, a 10 g sample of suppositories under aseptic conditions was emulsified in 100 ml of phosphate buffer solution pH 7.0 (PBS) using glass beads and a minimum amount of emulsifier Tween-80; in this case, mechanical shaking was used, and heating to a temperature not exceeding 45°C. After obtaining a homogeneous emulsion, the sample was diluted in 10 ml of sterile PBS to 1:100 and 1:1000, then 1 ml of each dilution was introduced into sterile Petri dishes, followed by pouring onto medium No. 1 (nutritious meat-peptone agar or soybean-casein agar) and No. 2 (Sabouraud medium with glucose). The Petri dishes with the solidified medium were inverted and incubated at 32.4°C and 22.5°C. After 48 hours and finally after 5 days, the number of grown colonies of aerobic bacteria and fungi was counted. The final number of bacterial colonies was counted from two dishes, the average value was found and multiplied by the corresponding dilution rate, and the CFU number was calculated in grams or ml.

When testing for Escherichia coli, 10 ml or an amount corresponding to 1 g or 1 ml, for inoculation in a suitable amount of casein soybean broth, should be mixed and incubate at a temperature from 300C to 350C for 18-24 hours.

The container is shaken, transferring 1 ml of the inoculated casein soybean broth to 100 ml of MacConkey broth, incubated at a temperature of 42ºC to 44ºC for 24-18 hours. Then, it is subcultures on plates with MacConkey agar at a temperature of 30ºC to 35ºC for 18-24 hours.

As a result of the studies, no colonies were found, which indicated the compliance of the drug with the requirements for microbiological purity of the State Pharmacopoeia RUz,1sted. (2020).

One of the most important criteria for assessing the quality of any dosage form is its stability during storage. The stability study was carried out on 5 series of suppositories during storage in a refrigerator (4±1°C). The suppositories were stored in a polyvinyl chloride (PVC) cell strip. The quality of the suppositories was checked on the day of preparation, as well as after 3, 6, 12, 18, 24 months. Quality control of the developed dosage form during storage was carried out using standard indicators in accordance with the SP RUz: in appearance, determination of the average weight, melting temperature, pH of the aqueous medium, time of complete deformation, identification and quantitative determination. When determining the stability indicators of the Witepsol-based NSDE-containing suppositories, it was found that in the process of natural storage, the appearance, physicochemical indicators of identification and the quantitative content of the medicinal substance met the requirements of the regulatory documentation for this dosage form (Table 4).

Table 4: Quality indicators of the NSDE-containing suppositories during natural storage at a temperature of 4±1°C

Quality indicators	Standardized requirements	Shelf life, months					
		0	6	12	18	24	30
Appearance	Opaque brown torpedo-shaped suppositories	Opaque brown torpedo-shaped suppositories	same	same	same	same	same
Average weight, g	should be in the range of 0.95 g to 1.05 g.	in the range of 0.95 g to 1.05 g	conforms	conforms	conforms	conforms	conforms
pH of the aqueous extract	6.1-6.7	6.3±0.2	conforms	conforms	conforms	conforms	conforms
Melting point, °C	37^{0}C max.	37^{0}C	conforms	conforms	conforms	conforms	conforms
Time of complete deformation, min.	15 min max.	10.0±1.0	conforms	conforms	conforms	conforms	conforms
Identification -maximum absorption in the region of 220-350 nm of alcohol solution -value in UV light at a wavelength of 365 nm	255±2 nm 2 spots with Rf, respectively 0.37 and at the start	255±2 nm conforms	conforms	conforms	conforms	conforms	conforms
Content, mg/g	14.4-17.6	16.8	conforms	conforms	conforms	conforms	conforms

As follows from the data presented in Table 4, the prototypes of NSDE-containing suppositories had quality indicators that meet the regulatory requirements during the observed period of 2 years, therefore, the shelf life is 2 years.

The antiulcer activity of the suppository was preliminary studied. The experiments were carried out on a phenolic proctitis model. The criterion for assessing the pharmacological activity of the drug was: a decrease in the area of necrosis (in mm2) and the intensity of inflammation of the mucous membrane (in points), as well as the length of the damaged section of the intestine relative to the entire length of the rectum (in percent), compared with the control. The results obtained were processed by the method of variation statistics according to the Student's criterion at p = 0.05. As a result of studying the antiulcer activity of the suppository, it was found that the test drug at a dose of 90.3 mg/kg has the highest effect. The data obtained indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases concomitant with inflammation, edema, hyperemia, and ulceration.

Conclusions

1. The effect of suppository bases on the physicochemical and structural-mechanical properties of suppositories has been studied. It was found that the selected bases provide the necessary quality parameters for the developed suppositories.
2. The indicator of the substitution factor for the NSDE has been established, its value is in the range of 0.6712 to 0.6696.
3. The best results were obtained based on Witepsol H-15 and W-35. The proposed composition of NSDE-containing suppositories: NSDE – 0.345 g; Witepsol H-15 and W-35 are sufficient to prepare suppositories weighing 1.03 - 0.999. Based on the results of the studies, a technological scheme for the preparation of NSDE-containing suppositories was proposed.
4. The quality of suppositories containing a dry extract of N. sativa was evaluated according to indicators: appearance, average weight, pH of the aqueous extract, melting point, time of complete deformation, identification, quantitative content of thymoquinone, microbiological purity. The stability of suppositories has been studied and the shelf life has been established when stored in a refrigerator at 8°C, namely 2 years.
5. The obtained preliminary data on the study of the pharmacological activity of suppositories indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases concomitant with inflammation, edema, hyperemia, and ulceration.

Ethical Approval: The manuscript was checked by the staff of the ethical approval committee under the Ministry of health of the Republic of Uzbekistan.

Conflict of Interest: The author declare that they have no competing interests.

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