(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 September 2009 (24.09.2009)

PCT

(10) International Publication Number
WO 2009/116077 A2

(51) International Patent Classification:
A61K 9/08 (2006.01) *A61K 31/44* (2006.01)

(21) International Application Number:
PCT/IN2009/000114

(22) International Filing Date:
19 February 2009 (19.02.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
356/MUM/2008 19 February 2008 (19.02.2008) IN

(71) Applicant: **M.J.BIOPHARM PVT. LTD.** [IN/IN]; 113, Jolly Maker Chambers No. 2, Nariman Point, Mumbai 400 021 (IN).

(72) Inventors: **VYAS, Ashok, Vasantray**; 26-27, Shakti Nagar Society, Gotri Road, Vadodara 390 021, Gujarat (IN). **JADHAV, Ravindra, Tukaram**; 304, Susheel Residency, Plot No. 67/1, Panvel 410 206, Maharashtra (IN). **PHAD, Subhash, Trimbak**; 202, Creative Arcade, Vijay Nagar Colony, Kukatpally, Hyderabad 500 072 (IN).

(74) Agent: **MOHAN, Dewan**; R.K. Dewan & Company, Trade Mark & Patent Attorneys, Podar Chambers, S.A. Brelvi Road, Fort, Mumbai 400 001 (IN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2009/116077 A2

(54) **Title**: INJECTABLE PREPARATIONS AND A PROCESS OF PREPARATION THEREOF

(57) **Abstract**: A pharmaceutically acceptable injection formulation comprising 3-hydroxy 2, 4,6 trimethylpyridine, pharmaceutically acceptable salts, esters, derivatives and polymorphs thereof for the treatment of ischemia, intraocular hemorrhage and macular degeneration is disclosed.

INJECTABLE PREPARATIONS AND A PROCESS OF PREPARATION THEREOF

FIELD OF THE INVENTION

The present invention relates to a pharmaceutically acceptable injectable formulation.

Particularly, the present invention relates to a pharmaceutically acceptable injectable formulation used in the treatment of ischemia, intraocular hemorrhage and macular degeneration.

BACKGROUND OF THE INVENTION

Ischemia is restriction in blood supply due to factors in the blood vessels, with resultant damage or dysfunction of tissue. Ischemia is absolute or relative shortage of the blood supply to an organ. Relative shortage means mismatch between blood supply (oxygen delivery) and blood requirement for adequate oxygenation of tissue. Ischemia results in tissue damage because of lack of oxygen and nutrients. Ultimately, this causes great damage because of a buildup of metabolic wastes. Ischemia can also be described as an inadequate flow of blood to a part of the body, caused by constriction or blockage of the blood vessels supplying it.
Depending on the type of organ that is affected, ischemia can be broadly classified in to the following categories:

1. Cardiac ischemia: Ischemia of heart muscle produces angina pectoris.
2. Bowel ischemia: An ischemia in the large bowel caused by an inflammation results in ischemic colitis and ischemia in the small bowel, caused by an inflammation results in mesenteric ischemia.
3. Cutaneous ischemia: Reduced blood flow to the skin layers may result in mottling or uneven, patchy discoloration of the skin.
4. Cerebral ischemia: is the localized reduction of blood flow to the brain or parts of the brain due to arterial obstruction or systematic hyperfusion.

Intraocular hemorrhage (hemophthalmos or hemophthalmia) is a condition in which bleeding occurs in the eyeball. It may be the result of physical trauma (direct injury to the eye) and/or medical illness. Severe hemorrhage, particularly when leading to rising pressure inside the eye, may lead to blindness.
There are different types of intraocular hemorrhage such as subconjunctival hemorrhage, hyphema, vitreous hemorrhage, subretinal hemorrhage and submacular hemorrhage. Different causes responsible for bleeding in different locations are terson's syndrome (as a result of subarachnoid hemorrhage), hemophilia (a severe bleeding disorder, usually hereditary), anticoagulants and thrombolysis (medication to reduce blood clotting tendency or to disperse blood clots, respectively).

It has been proved that hemophthalmia is always accompanied by activation of free radical oxidation processes and proceeded as chain reactions and involves accumulation of oxidation products of molecules in vitreous body and retina. Application of antioxidant preparations in early conservative therapy of intraocular hemorrhages essentially allows to speed up the resorption processes and thus reduces the risk of development of serious complications such as fibrosis of vitreous body and retinal detachment.

In the last decade researches of have established that inflammatory reaction is always accompanied by activation of processes of free radical oxidation, mutually aggravating each other and resulting in development of various post-inflammatory complications. Use of antioxidant in early conservative therapy of inflammatory pathology of eye allows to speed up recovery and to improve the disease prognosis.

3-Hydroxy-2,4,6-trimethylpyridine succinate and other such salts belong to a new biologically active compounds that exhibit important pharmacological activities such as anti oxidant for the vascular and inflammatory eye pathology. 3-Hydroxy-2,4,6-trimethylpyridine succinate and other such salts also posses geroprotective

action and can be used to treat conditions, diseases or disorders of the cornea, retina, lens, sclera and anterior and posterior segments of the eye.

Another critical indication where the aforementioned compound finds its use is macular degeneration (degenerative disease of the eye). In macular degeneration, lipofuscin accumulation is implicated as a major risk factor. Lipofuscin is finely granular yellow brown pigment granules composed of lipid-containing residues of lysosomal digestion. It is considered one of the aging or "wear and tear" pigments. It appears to be the product of the oxidation of unsaturated fatty acids and may be symptomatic of membrane damage or damage to mitochondria and lysosomes.

3-Hydroxy-2,4,6-trimethylpyridine has been found to be an active inhibitor of peroxide oxidation of lipids. Thus, it is capable of neutralizing toxic activity of lipofuscin granules to have geroprotective action.

EXISTING KNOWLEDGE

PCT/IB2005/003636 discloses a method of preparing 2,4,6-trimethyl-3-hydroxypyridine derivatives and salts thereof having antioxidant, geroprotective and anti-ischemic activity.

This patent application suggest the use of 2,4,6-trimethyl-3-hydroxypyridine derivatives as a geroprotective and anti ischemic. Disclosure of the said Pct application is limited to a method of synthesis of 2,4,6-trimethyl-3-hydroxypyridine derivative and salts. However, details of the formulations comprising said 2,4,6-trimethyl-3-hydroxypyridine derivative or salts have not been disclosed.

Accordingly, it is desirable to prepare a pharmaceutically acceptable dosage form containing 2,4,6-trimethyl-3-hydroxypiridine which will produce rapid onset of action .

OBJECTS OF THE INVENTION

It is an object of the present invention to provide a geroprotective and antioxidant formulation.

It is another object of the present invention to provide a pharmaceutically acceptable injection formulation that is useful for treatment of ischemia.

It is still another objective of the present invention to provide a stable pharmaceutically acceptable injection formulation.

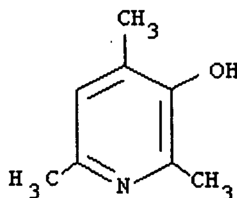
It is a further object of this invention to provide a pharmaceutically acceptable injection formulation which does not causes irritation at the site of application.

It is yet another object of the present invention to provide a pharmaceutically acceptable injection formulation with rapid onset of action.

It is another object of the present invention to provide a pharmaceutically acceptable injection formulation which is easy to prepare and is cost effective.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a pharmaceutically acceptable injection formulation comprising a compound of formula-I, pharmaceutically acceptable salts, esters, derivatives and polymorphs thereof in an amount of about 0.5 % to about 10 % of the mass of the formulation, preservative in an amount of about 0.001 % to about 0.5 % of the mass of the formulation and water for injection, wherein pH of said injection formulation is in the range of about 2.5 to 6.5.



Formula-I

Typically, the pharmaceutically acceptable injection formulation in accordance with this invention comprises a pharmaceutically acceptable salt of a compound of formula-I, selected from a group of pharmaceutically acceptable salts consisting of

succinate, maleate, tartrate, oxalate, fumarate, citrate, hydrochloride, salicylate, pamoate, hydrogen sulfate, sulfate methanesulphonate and benzenesulfonate.

Preferably, the concentration of 3-hydroxy-2,4,6-trimethylpyridine is in the range of about 0.5 % to about 5 % of the mass of the formulation.

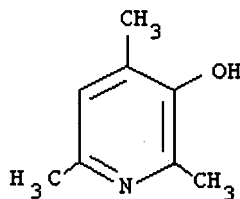
Typically, the preservative is at least one selected from a group consisting of benzalkonium chloride, benzyl alcohol, methyl paraben, propyl paraben, butyl paraben, chlorobutanol, metacresol, phenylmercuric nitrate, thiomersal, myristylgamma picolonium chloride and phenol.

Typically, the pharmaceutically acceptable injection formulation in accordance with this invention further comprises a tonicity agent.

Typically, the tonicity agent is at least one selected from a group consisting of glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate and sorbitol.

DETAILED DESCRIPTION

In accordance with the present invention, there is provided a pharmaceutically acceptable injection formulation comprising a compound of formula-I , pharmaceutically acceptable salts, esters, derivatives and polymorphs thereof in an amount of about 0.5 % to about 10 % of the mass of the formulation, preservative in an amount of about 0.001 % to about 0.5 % of the mass of the formulation and water for injection, wherein pH of said injection formulation is in the range of about 2.5 to about 6.5.



Formula-I

In accordance with the present invention, the pharmaceutically acceptable injection formulation comprises pharmaceutically acceptable salt of 3-hydroxy -2,4,6 trimethylpyridine, selected from a group of salts consisting of succinate, maleate, tartrate, oxalate, fumarate, citrate, hydrochloride, salicylate pamoate, hydrogen sulfate, sulfate methanesulphonate and benzenesulfonate.

The concentration of 3-hydroxy-2,4,6-trimethylpyridine used in the formulation prepared in accordance with the present invention is in the range of about 0.5 % to about 5 % of the mass of the formulation.

The preservative used in the formulation prepared in accordance with the present invention is at least one selected from a group consisting of benzalkonium chloride, benzyl alcohol, methyl paraben, propyl paraben, butyl paraben, chlorobutanol, metacresol, phenylmercuric nitrate, thiomersal, myristylgamma picolonium chloride and phenol.

According to one of the embodiment of the present invention, the pharmaceutically acceptable injection formulation further comprises a tonicity agent.

The tonicity agent used in the formulation is at least one selected from a group consisting of glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate and sorbitol.

Further, the pH of the injection formulation prepared in accordance with the present invention is adjusted in the range of about 2.5 to about 6.5 to prevent eye irritation. The pH adjusting agent is selected from a group consisting of sodium hydroxide, hydrochloric acid, triethanolamine, ammonia and mixtures thereof.

Following examples illustrate the invention, but are not intended to limit the scope of the present invention.
Injection formulations, in accordance with the present invention employing different salts of 3-hydroxy-2,4,6-trimethylpyridine were prepared.

Example 1

3-hydroxy-2,4,6-trimethylpyridine succinate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
Water for injection	upto 1 ml

3-hydroxy-2,4,6-trimethylpyridine succinate was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 2.9 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into ampoule.

Example 2

3-hydroxy-2,4,6-trimethylpyridine maleate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
Water for injection	upto 1 ml

The process of example 1 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 3

3-hydroxy-2,4,6-trimethylpyridine tartrate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 1 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine tartrate was employed as the active ingredient.

Example 4

3-hydroxy-2,4,6-trimethylpyridine oxalate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 1 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine oxalate was employed as the active ingredient.

Example 5

3-hydroxy-2,4,6-trimethylpyridine succinate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

3-hydroxy-2, 4, 6-trimethylpyridine succinate was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 2.9 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 50 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 6

3-hydroxy-2,4,6-trimethylpyridine maleate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 5 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 7

3-Hydroxy-2,4,6-trimethylpyridine tartarate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 5 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine tartarate was employed.

Example 8

3-Hydroxy-2,4,6-trimethylpyridine oxalate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 5 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine oxalate was employed as the active ingredient.

Example 9

3-hydroxy-2,4,6-trimethylpyridine succinate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

3-hydroxy-2,4,6-trimethylpyridine succinate was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.8 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 10

3-hydroxy-2,4,6-trimethylpyridine maleate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 9 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 11

3-hydroxy-2,4,6-trimethylpyridine tartarate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 9 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine tartarate was employed as the active ingredient.

Example 12

3-hydroxy-2,4,6-trimethylpyridine oxalate	10.0 mg/ml
10% aqueous solution of hydrochloric acid	QS

water for injection	upto 1 ml

The process of example 9 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine oxalate was employed.

Example 13

3-hydroxy-2,4,6-trimethylpyridine succinate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

3-hydroxy-2,4,6-trimethylpyridine succinate was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.7 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 50 mg per ml. Further the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 14

3-hydroxy-2,4,6-trimethylpyridine maleate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 13 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 15

3-Hydroxy-2,4,6-trimethylpyridine tartarate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS
water for injection	upto 1 ml

The process of example 13 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine tartarate was employed as the active ingredient.

Example 16

3-Hydroxy-2,4,6-trimethylpyridine oxalate	50.0 mg/ml
10% aqueous solution of hydrochloric acid	QS

water for injection	upto 1 ml

The process of example 13 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine oxalate was employed as the active ingredient.

Example 17

3-hydroxy-2,4,6-trimethylpyridine succinate	10.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of hydrochloric acid	QS
water for injection	up to 1 ml

3-hydroxy-2, 4, 6-trimethylpyridine succinate and benzalkonium chloride was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.7 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 18

3-hydroxy-2,4,6-trimethylpyridine maleate	10.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of hydrochloric acid	QS
water for injection	up to 1 ml

The process of example 17 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 19

3-hydroxy-2,4,6-trimethylpyridine tartarate	10.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of sodium hydroxide	QS
water for injection	up to 1 ml

3-hydroxy-2, 4, 6-trimethylpyridine tartarate and benzalkonium chloride was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.7 with 10% aqueous solution of sodium hydroxide. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 20

3-hydroxy-2,4,6-trimethylpyridine oxalate	10.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of sodium hydroxide	QS
water for injection	up to 1 ml

The process of example 19 was repeated except that 3-hydroxy-2, 4, 6-trimethylpyridine oxalate was employed as the active ingredient.

Example 21

3-hydroxy-2,4,6-trimethylpyridine succinate	50.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of hydrochloric acid	QS
water for injection	up to 1 ml

3-hydroxy-2,4,6-trimethylpyridine succinate and benzalkonium chloride was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.7 with 10% aqueous solution of hydrochloric acid. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 22

3-hydroxy-2,4,6-trimethylpyridine maleate	50.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of hydrochloric acid	QS
water for injection	up to 1 ml

The process of example 21 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine maleate was employed as the active ingredient.

Example 23

3-hydroxy-2,4,6-trimethylpyridine Tartarate	50.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of sodium hydroxide	QS
water for injection	up to 1 ml

3-hydroxy-2,4,6-trimethylpyridine tartarate and benzalkonium chloride was dissolved in water for injection with continuous stirring under inert gas. The pH of the solution was adjusted to 4.7 with 10% aqueous solution of sodium hydroxide. The solution was diluted with sterile water for injection to achieve required concentration of 10 mg per ml. Further, the resultant solution was sterilized by sterile filtration and autoclaving. The sterile formulation was aseptically filled into the ampoule.

Example 24

3-hydroxy-2,4,6-trimethylpyridine oxalate	50.0 mg/ml
benzalkonium chloride	0.05%
10% aqueous solution of Sodium Hydroxide	QS
Water for injection	up to 1 ml

The process of example 23 was repeated except that 3-hydroxy-2,4,6-trimethylpyridine oxalate was employed as the active ingredient.

Stability testing:

The formulations prepared in accordance with the present inventions were stored at temperatures ranging from 5^0 C to 60^0 C for 12 months. At various time intervals,

the samples were examined for colour change and development of insoluble material. The pH of the formulations was measured and chemical assays were performed to ascertain whether any significant chemical loss had occurred and to assure maintenance of potency.

The formulations of the invention demonstrated acceptable storage stability.

Preclinical trials:

i) The efficiency of injection formulation comprising 3-hydroxy-2,4,6-trimethylpyridine prepared in accordance with the present invention, in traumatic hemophthalmia, on the basis of the modern ultrasonic researches of eyeglobe (eyeball) and parameters of biochemical researches of blood serum, tear liquid, liquid of the anterior chamber and eye tissue was studied.

Material and methods:

Studies were carried out in 12 chinchilla rabbits having body weight 2.0-2.5 kg. A traumatic hemophthalmia was modeled by introduction of 0.5 to 0.7 ml of autoblood into a vitreous body through a puncture in a sclera on distance of 5 mm from a limbus, under a local anaesthesia. The basic group made with 6 rabbits (12 eyes), daily received a formulation prepared in accordance with the present invention parabulbarly (0.5 ml of 1 % solution). A control group presented by 6 animals (12 eyes), daily received parabulbar emoxipin (2-ethyl-6-methyl-3-hydroxypiridin) in the same dosage. Treatment was carried out within 14 days after a trauma.

Besides the traditional ophthalmologic examination such as biomicroscopy, direct and indirect ophthalmoscopy, all the animals passed through ultrasonic scanning of an eye by means of the device " Voluson 730 " ("Kretz"). The studies were carried out on the 1, 3, 7, 10 and 14th day of the experiment. For the estimation of efficiency of the treatment following tests were used: density, area, volume of a hemophthalmia and opportunity (possibility) of ophthalmoscopy of an eye ground. Density of a hemophthalmia was estimated in standard (conventional) units of density (SUD), 100 SUD were accepted for the maximal value, corresponding to

the ultrasonic sclera density. Depending on the degree of acoustic density of a hemophthalmia, a high (100 - 50 SUD), medium (50 - 25 SUD) and low (less than 25 SUD) densities were distinguished. To determine the hemophthalmia the area measurement in sm^2 was used. The volume of the intraocular hemorrhage was studied with the help of 3D modelling in the B-regimen of a grey scale and measurement in sm^3.

At the ultrasonic examination of the intact rabbit's eyes, the average volume of vitreous body (1.0±0.9 sm^3) and the average area of a vitreous body (1.04±0.8 sm^2) were fixed. Proceeding from the obtained data, the total hemophthalmia (from 100 up to 50 % of the vitreous body volume) was equated to the volume of 0.5-1.0 sm^3 and of 0.5-1.0 sm^2, a wide-spread hemophthalmia (50 - 25 % of volume of a vitreous) corresponded to the area of 0.5-0.25 sm^3 , both 0.5-0.25 sm^2, and the partial hemophthalmia occupied up to 0.25 sm^3 and 0.25 sm^2 (up to 25 % of the vitreous volume).

Capability of the eyeground ophthalmoscopy was estimated by a 3-mark(point) scale depending on the image sharpness (0 - ophthalmoscopy is impossible (not capable), 3 - details of an eyeground are clearly visible).

The materials used for biochemical examination were as follows: blood, liquid of the anterior chamber, tear liquid, tunics of eyeglobe (retina, a vitreous body). The blood was taken from the aural vein in an amount of 3 ml. Sampling of tear liquid was carried out by a microcapillary after instillation of distilled water into the conjunctival cavity. Liquid of the anterior chamber was obtained by paracentesis. The blood sampling as well as liquid of the anterior chamber and teat liquid were carried out on the 1st, 7th and 14th day of the experiment. On the 15th day of the studies, the animals were killed (pithed) by air embolism and both the eyes were enucleated. The eyes were prepared by tunic separation. Retina and vitreous body were obtained.

For estimation of activity of the preparations the concentration of products of the free radical oxidation, active in the reaction with thiobarbituric acid (TBA-active

products), protein concentration, and the antioxidant activity (AOA) were determined.

Results:

The data of preclinical and ultrasonic examinations at the experimental hemophthalmia in dynamics is shown in Table No. 1.

Table 1: Dynamics of parameters of preclinical and ultrasonic examination at the experimental hemophthalmia in the basic (A) and control (B) groups.

Day	Parameters							
	Volume of a hemophthalmia, sm^3		Area of hemophthalmia, sm^2		Density of hemophthalmia, SUD		Capability of ophthalmoscopy and eyeground, points	
	A	B	A	B	A	B	A	B
1st	0.53±0.4	0.54±0.8	0.49±0.7	0.48±0.5	84±2.4	82±1.4	1.5	1.5
3rd	0.78±0.2	0.81±0.8	0.74±0.9	0.76±0.1	72±3.1	76±2.6	0-0.5	0-0.5
7th	0.51±0.6	0.7±0.1	0.47±0.4	0.68±0.9	49±1.4	65±2.1	1.5	0.8
10th	0.24±0.1	0.53±0.4	0.2±0.1	0.5±0.2	23±1.1	48±1.5	2.2	1.6
14th	0.15±0.2	0.4±0.7	0.11±0.7	0.39±0.4	13±0.6	32±0.8	2.8	2.0

In the first day of experiment, the parameters of ultrasonic and clinical examination were practically identical in all groups: the density of hemophthalmia in average made from 81 up to 85 SUD, the area – 0.52-0.55 sm^2, the volume 0.47-0.5 sm^3, the capability of ophthalmoscopy of an eyeground was corresponded to 1-2 points. Thus, in all animals the intraocular hemorrhage which complicated the capability of ophthalmoscopy of an eyeground was marked rather small in volume, but essential in density.

By the 3rd day of studies the blood was regularly distributed in a vitreous body, thus the density of hemophthalmia was decreased a little. The area and the volume of a hemorrhage were corresponded to criteria of the total hemophthalmia (about 80 %

of a vitreous body), the density remained high (more than 70 SUD), the eye ground practically was not looked through (seen) (0 – 0.5 points). Since from the 3rd day of experiment the tendency to the faster resorption of hemorrhage of rabbits of the basic group was outlined, however, no statistically significant difference of ultrasonic examination was observed. To characterize clinical current of the given period it is necessary to refer the more expressed reaction of an eye to a trauma in animals of the control group in comparison with the experienced group (lacrimation, photophobia, injection of an eyeglobe, liquid opalescence of the anterior chamber).

By the 7th day, the difference of preclinical and ultrasonic parameters of a hemophthalmia became more noticeable. In the basic group, the area of a hemophthalmia was decreased up to 0.51 ±0.6 sm^2, volume up to 0.47±0.4 sm^3, density up to 49±1.4 SUD; capability of the eyeground ophthalmoscopy was equal to 1.5 points. In the basic group the wide-spread hemophthalmia of average density was observed. In the control group, the tendency to resorption of a hemorrhage was less expressed: the area of a hemophthalmia was 0.7±0.1 sm^2, volume 0.68±0.9 sm^3, density 65±2.1 SUD, capability of the eyeground ophthalmoscopy was 1 point. Thus, in animals of the control group the clinical picture practically did not changed.

By the 10th day, in the basic group the hemophthalmia was completely resolved in two animals, in other cases marked a noticeable decrease of the area (0.24±0,1sm^2), volume (0.2±0.1 sm^3) and density (23±1.1 SUD) of hemorrhage, the eyeground (2.2 points) was well seen. The intraocular hemorrhage corresponded to a partial hemophthalmia with a low density. In the control group the effect of treatment was less expressed: the area of a hemophthalmia averaged to 0.53±0.4 sm^2, volume 0.5±0.2 sm^3, density 48±1.5 SUD, capability of the eyeground ophthalmoscopy was equal to 1.8 points. On the 10th day of the studies, some rabbits receiving emoxipin had the tendency of sheet-anchor formation in a vitreous body.

On the 14th day the hemophthalmia was completely resolved in 50 % of animals in the basic group, the area, volume and density (0.15±0.2 sm^2, 0.11±0.7 sm^3 and 13±0.6 SUD, accordingly) of hemorrhage were considerably decreased, the

eyeground (2.6 points) was well seen in the other animals. In the control group, the result of the treatment appeared less expressed: the area of a hemophthalmia was 0.47±0.7 sm^2, volume 0.39±0.4 sm^3, density 32±0.8 SUD, the ophthalmoscopy picture of an eyeground was not clear (2 points). In the control group, no noticeable changes were obtained in comparison with the 10th day; the phenomena of fibrosis and sheet-anchor formation in a vitreous body were marked in 30 % of animals.

Table No.2 shows biochemical parameters in blood serum (BS), tear liquid (TL) and the anterior chamber liquid (ACL) at an experimental hemophthalmia in the basic (A) and the control (B) groups.

Table 2: Biochemical parameters

	Protein concentration (mg / ml)		Concentration of TBA-active products (nmol/ml)		AOA (relative unit)	
	A	B	A	B	A	B
1st day						
BS	90.0 ± 6.0	85.2 ± 0.9	1.29 ±0.13	2.53 ± 0.28	1.7 ±0.3	1.5±0.2
TL	0.32 ± 0.2	0.35 ±0.1	0.14 ±0.01	0.16 ±0.02	0.23± 0.02	0.13 ± 0.03
ACL	3.1 ± 1.2	3.9 ±1.0	0.97±0.18	1.6 ± 0.3	0.4± 0.01	0.33± 0.02
7th day						
BS	82.4 ± 1.3	103.0 ± 5.0	1.17 ±0.05	2.4 ± 0.09	2.4± 0.02	1.8 ±0.07
TL	0.7 ± 0.2	0.91±0.25	0.13 ±0.05	0.46± 0.07	0.24 ± 0.02	0.18 ±0.05
ACL	4.4 ±1.2	10.0 ± 3.0	0.8 ± 0.5	2.2 ± 0.4	0.8± 0.07	0.55 ± 0.04
14th day						
BS	47.0 ± 3.0	73.0 ±2.0	0.9±0.15	1.6 ±0.2	1.9 ±0.7	1.3 ±0.3
TL	0.6 ± 0.2	0.74 ±0.14	0.04± 0.07	0.3 ± 0.2	0.08± 0.02	0.06± 0.03
ACL	4.1 ±1.1	5.5 ±1.0	0.6 ±0.2	2.2 ± 0.3	0.5± 0.05	0.33± 0.06
Retina					0.4 ± 0.07	0.23 ± 0.08
Vitreous body					3.5 ±0.9	1.6 ±0.7

From the mentioned data it is evident that the protein concentration in the 1st day of experiment, and AOA in all biological liquids practically did not varied in the groups. Concentration of TBA-active products in the blood serum had no essential differences either, however, in the tear and anterior chamber liquid of the rabbits of the basic group, the concentration of TBA-active products was lower than that in the rabbits of the control group.

The greatest difference in biochemical parameters observed on the 7-th day of experiment and coincided with the beginning of the active hemorrhage resorption in rabbits of the basic group. In animals of the basic group AOA was in the average 1.5 times higher than in the control group in all measured substrata: in the blood serum 2.4±0.02 - 1.8±0.07/100 mcl, tear liquid 0.24±0.02-0.18±0.05/200 mcl and in the anterior chamber liquid 0.8±0.07 - 0.55±0.04/100 mcl. On the contrary, concentration of TBA-active products was grown in the control group (2.4±0.09 nmol/ml in the blood serum, 0.46±0.07 nmol/ml in the tear liquid, 2.2±0.4 nmol/ml in the anterior chamber liquid), and was reduced in the basic group (1.17±0.05 nmol/ml in the blood serum, 0.13±0.05 nmol/ml in the tear liquid, 0.8±0.5 nmol/ml in the anterior chamber liquid). Similar changes were observed at examination of protein concentration in the blood serum in rabbits of the control (103.0 ±5.0 mg / ml) and the basic (82.4±1.3 mg / ml) groups. In all animals the protein concentration was grown in the tear and the anterior chamber liquid on the 7th day.

By the 14th day of experiment, statistically significant differences in the biochemical parameters were marked. In both groups, AOA was insignificantly reduced, however the higher parameters were in the animals receiving formulation prepared in accordance with the present invention (1.9±0.7/100 mcl in the blood serum, 0.08±0.02/200 mcl in the tear liquid, 0.5±0.05/100 mcl in the anterior chamber liquid), and the lower parameters were in the animals receiving emoxipin (1.3±0.03/100 mcl in the blood serum, 0.06±0.03/200 mcl in the tear liquid, 0.33±0.06/100 mcl in the anterior chamber liquid). Concentration of TBA-active products was also decreased in all rabbits, thus in the basic group their values were lower, than in the control group (0.9±0.15 - 1.6±0.2 nmol/ml in the blood serum,

0.04±0.07 - 0.3±0.2 nmol/ml in the tear liquid, 0.6±0.2 - 2.2±0.3 nmol/ml in the anterior chamber liquid, accordingly). Similar changes were marked at the examination of parameters of protein concentration in both the groups of animals.
In AOA studies the augmentation of the given parameter was observed in 2 times in the eye tunics, a retina and a vitreous body, in rabbits of the basic group in comparison with the control group.

Conclusions:

1. The injection formulation prepared in accordance with the present invention renders a positive effect on the resorptive processes in a vitreous body at the experimental traumatic hemophthalmia.
2. On the basis of parameters of clinical and ultrasonic examination of eyes of the experimental animals at the traumatic hemophthalmia in dynamics, it is established that efficiency of the composition prepared in accordance with the present invention is much higher in comparison with emoxipin.
3. Results of biochemical studies of the blood serum, tear liquid, the anterior chamber liquid and eye tissues (*in vivo*) shows that the composition prepared in accordance with the present invention has higher antioxidant activity than emoxipin.

ii) Anti -ischemic activity:

The efficiency of injection formulation comprising 3-hydroxy-2,4,6-trimethylpyridine succinate prepared in accordance with the present invention, in acute myocardial ischemia was studied by evaluating effect of the formulation on the sizes of necrosis ischemia zones.
The experiment was done on non-linear male rats weighing about 250-300 g. The animals were anesthetized with sodium ethaminale (40 mg/kg intraperitoneally). A myocardial infarction was modeled for the animals. The animals were then transferred to controlled breathing by ligation of a descending branch of the left-hand coronary artery at a level of the lower edge of an auricula atrii.

The sizes of a necrosis zone and ischemia zones were detected in 4 hours after occlusion of a coronary artery by a differential indicator method, which is founded on separate quantitative determination of Evans' blue (indicator of a ischemia zone) and red phormazane (indicator of a necrosis zone).

The results of anti-ischemic activity of 3-hydroxy-2,4,6-trimethylpyridine succinate and other 3-oxypyridine derivatives (in 4 hours after occlusion of a coronary artery of rats) are shown in table No. 3.

Table 3: Anti-ischemic activity

Conditions of experience	Dose mg/kg	Number of animal	Ratio of a necrosis zone to total mass of the myocardium (%)	Ratio of a necrosis zone to an ischemia zone (%)
Control	-	17	22 ± 2.0	68 ± 4.3
3-hydroxy-2, 4, 6-trimethylpyridine succinate	16	7	4 ± 2.3	11 ± 3.3
Emoxypine	26	8	9 ± 2.4	32 ± 4.6
Mexydole	26	8	8 ± 1.4	46 ± 5.6
Nicorandile	12	8	10 ± 1.6	42± 5.4

The results as shown in table No. 3 clearly indicate that the injection formulation comprising 3-hydroxy-2, 4, 6-trimethylpyridine succinate prepared in accordance with the present invention displays considerably higher anti-ischemic activity than other 3-oxypyridines.

ANECDOTAL TRIALS:

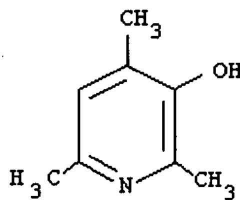
Dramatic and significant results have been obtained in individual anecdotal cases of patients presenting with ischemia, intraocular hemorrhage and macular degeneration in which the condition is either reversed or ameliorated.

The applicant craves leave to submit formal data to establish significant enhancement of efficacy by way of explanation to support these preliminary findings.

While considerable emphasis has been placed herein on the specific ingredients of the preferred formulation, it will be appreciated that many additional ingredients can be added and that many changes can be made in the preferred formulation without departing from the principles of the invention. These and other changes in the preferred formulation of the invention will be apparent to those skilled in the art from the disclosure herein, whereby it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the invention and not as a limitation.

Claims:

1. A pharmaceutically acceptable injection formulation comprising a compound of formula-I, pharmaceutically acceptable salts, esters, derivatives and polymorphs thereof in an amount of about 0.5 % to about 10 % of the mass of the formulation; preservative in an amount of about 0.001 % to about 0.5 % of the mass of the formulation and water for injection, wherein pH of said injection formulation is in the range of about 2.5 to about 6.5.



Formula-I

2. The pharmaceutically acceptable injection formulation as claimed in claim 1, comprises pharmaceutically acceptable salt of a compound of formula-I, selected from a group of salts consisting of succinate, maleate, tartrate, oxalate, fumarate, citrate, hydrochloride, salicylate pamoate, hydrogen sulfate, sulfate methanesulphonate, and benzenesulfonate.

3. The pharmaceutically acceptable injection formulation as claimed in claim 1, wherein the concentration of 3-hydroxy-2,4,6- trimethylpyridine is in the range of about 0.5 % to about 5 % of the mass of the formulation.

4. The pharmaceutically acceptable injection formulation as claimed in claim 1, wherein the preservative is at least one selected from a group consisting of benzalkonium chloride, benzyl alcohol, methyl paraben, propyl paraben, butyl paraben, chlorobutanol, metacresol, phenylmercuric nitrate, thiomersal, myristylgamma picolonium chloride and phenol.

5. The pharmaceutically acceptable injection formulation as claimed in claim 1, further comprises a tonicity agent.

6. The pharmaceutically acceptable injection formulation as claimed in claim 4, wherein the tonicity agent is at least one selected from a group consisting of glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate and sorbitol.