Description

This invention relates to pharmaceutical compositions for the treatment of ophthalmic diseases, and specifically to the use of selected anthocyanidins to elicit a specific ophthalmic pharmacological effect.

Anthocyanidins are known to have valuable pharmacological properties in treating peripheral vascular diseases. Thus our British Patent Nos. 1 589 294 and 1 595 531 describe and claim anthocyanidins which have cicatrizing, epithelium-regenerating, anti-inflammatory, vaso-protective, hypolipaemic, hypocholesterolaemic and hypoglycaemic activity. Owing to their low toxicity, these compounds are particularly useful for the prolonged treatment of patients with impaired peripheral circulation.

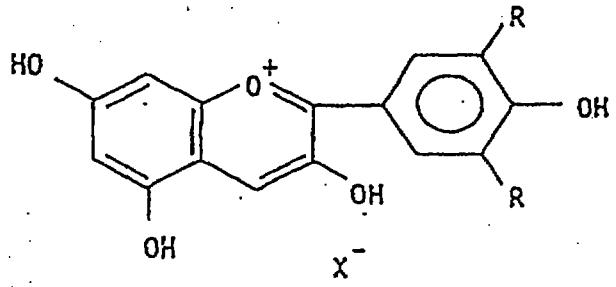
The glycosides of anthocyanidins (anthocyanosides) which are present as mixtures in extracts of certain fruits, have been described as being active in improving the visual function, particularly night vision (French Patent No. 1369 M, 25 June 1962, Chibret). These compounds apparently accelerate the regeneration of rhodopsin, and thus facilitate adaptation to darkness.

We have now found that two of the anthocyanidins described in GB 1 589 294, namely pelargonidin and delphidin, have a novel and unexpected activity in the ophthalmic field, specifically that they are active in reducing the permeability of ciliary body vessels.

These blood vessels differ in their anatomic structure from the other ocular blood vessels and from the blood vessels of the general circulation and are involved in the regulation of endo-ocular pressure and of the blood-water barrier, and in the production of aqueous humor.

This newly-discovered activity of reducing the permeability of ciliary body vessels could not have been forecast from the known properties of anthocyanins or anthocyanosides.

The structure of pelargonidin and delphidin is as follows:



wherein

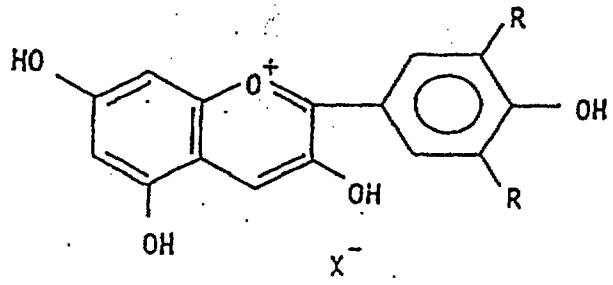
in pelargonidin (Compound I) each R represents hydrogen,

in delphinidin (Compound II) each R represents hydroxyl,

and X^- is a pharmaceutically acceptable anion, e.g. Cl^-.

The novel ophthalmic activity of these compounds has been established by various *in vivo* experiments in animal models.

Thus according to one aspect of the present invention there is provided the use of an anthocyanidin of formula



wherein

(I) each R represents hydrogen, or

(II) each R represents hydroxy, and

X^- is a pharmaceutically acceptable anion, e.g. Cl^- in the manufacture of a pharmaceutical composition for

reducing the hyperpermeability of the ciliary body ocular vessels in a subject.

The invention is of particular utility in the manufacture of compositions for reducing the hyperpermeability of the ciliary body ocular vessels induced by injury or by harmful agents.

Various routes of administration may be used in carrying out the invention. For example, the anthocyanidins may be administered orally or they may be administered locally to the eye.

The invention further provides topical pharmaceutical preparations adapted for administration locally to the eye of a subject for reducing the hyperpermeability of the ciliary body ocular vessels comprising an anthocyanidin of formula (I) and (II) above and an ophthalmically acceptable diluent.

The specific pharmacological effects obtained according to the invention will now be described in more detail.

A. EFFECT ON THE PERMEABILITY OF THE HAEMATO-OPHTHALMIC BARRIER

The effect on the permeability of the haemato-ophthalmic barrier was measured by inducing an increase in the permeability of the vessels of the ciliary body, either by paracentesis or by instillation of sodium hydroxide into the conjunctival sac. The reduction in permeability induced by oral administration of the products under examination was then measured.

1. The Paracentesis Method

(i) Acute Administration

The experiment was carried out on 80 New Zealand albino rabbits, average body weight 2.8 kg, divided into eight groups each of ten animals.

The vasal permeability was measured by the dye method: 1 ml of Evans Blue in a concentration of 0.5% was injected into the marginal vein of the auricle.

After 10 minutes the left eye was subjected to paracentesis and after 12 minutes a sample of aqueous humour was taken. The quantity of Evans Blue in the sampled aqueous humour was used to assess the permeability of the haemato-ophthalmic barrier. Immediately afterwards, compounds I and II were administered in doses of 100 or 200 mg/kg by stomach probe.

Two or four hours after administration, paracentesis was carried out on the right eye and after 12 minutes a sample of aqueous humour was taken.

The quantity of Evans Blue in the aqueous humour was evaluated by spectrophotometer reading at 623 nm.

(ii) Repeated Administration

The experiment was carried out on 20 New Zealand rabbits, average body weight 2.5 kg, using the same experimental technique as for acute administration.

The substances were administered in doses of 100 mg/kg os/day for seven days, and the effect on the permeability of the barrier was measured on the eighth day, 24 hours after the last administration.

Table 1 gives the results obtained with compounds I and II administered in a single dose of 100 or 200 mg/kg. The amount of dye coming out of the vessels of the ciliary body, two hours or four hours after administration, was reduced in a statistically significant manner. When the substances are repeatedly administered their effectiveness increases. The inhibition of the amount of dye emerging rose from 15% to 21-22% and was still statistically significant 24 hours after the last administration.

TABLE 1

Effect On The Permeability Of The Haemato-Ophthalmic Barrier Induced By Paracentesis In The Rabbit

Substance	Dose mg/kg os	No. of Administrations	No. of Animals	Evans Blue (mcg/ml of aqueous humour) m ± s.d. Time 0	2 hours	4 hours	24 hours
Compound I	100	1	10	41.2±10.6	34.9±8.5** (-15.4%)		
	100	1	10	55.5±20.6		46.9±17.5** (-15.5%)	
	200	1	10	50.5±17.9	42.2±15.0** (-16.4%)		
	200	1	10	53.1±23.7		44.8±20.2** (-15.6%)	
	100	7	10	56.9±14.8			44.2±11.7** (-22.4%)
Compound II	100	1	10	48.3±7.2	41.5±7.9** (-14.1%)		
	100	1	10	53.6±12.3		45.6±9.7** (-14.9%)	
	200	1	10	53.1±12.2	45.1±10.3** (-15.1%)		
	200	1	10	54.1±11.5		45.9±12.2** (-15.1%)	
	100	7	10	53.7±12.8			42.5±13.3** (-20.8%)

The brackets give the percentage variations relative to time 0
** $p < 0.01$ Student's "t" for paired data

2. Sodium Hydroxide Method

The experiment was carried out on 50 New Zealand rabbits, average body weight 2.2 kg. The substance was administered once a day for seven days in doses of 50 or 100 mg/kg. A control group was

treated with tap water.

Thirty minutes after the last administration, 1 ml of a 0.5% solution of Evans Blue was administered in the marginal vein of the ear. After 30 minutes, inflammation of the eye tissue was produced by instilling two drops of 0.25 N sodium hydroxide in the conjunctival sac. Two hours later the animal was anesthesized and a sample of aqueous humour was taken in order to measure the proportion of dye.

As Table 2 shows, compounds II and I both induced a dose-dependent reduction in the permeability of the vessels of the ciliary body caused by the irritating agent. The reduction following administration of 100 mg/kg was statistically significant compared with the controls.

TABLE 2

Effects Of Compounds I And II On The Permeability Of The Haemato-Ophthalmic Barrier Induced By NaOH In The Rabbit				
Substance	No. of Animals	Dose mg/kg/day os	Evans Blue mcg/ml m± s.e.	Percentage Variation
Controls	10	-	5.9±0.6	
Compound II	10	50	5.1±0.7	-14
	10	100	3.2±0.4*	-45
Compound I	10	50	5.8±0.8	- 2
	10	100	3.5±0.6*	-40

* $p < 0.05$ according to Student's "t" test

3. Corneal Lens Method

The experiment was carried out on 40 New Zealand rabbits, average body weight 2.5 kg, divided into three groups of ten animals each.

The vasal permeability was measured by the dye method: 1 ml of Evans Blue in a concentration of 0.5% was injected into the marginal vein of the ear. After 10 min a soft corneal lens embedded in a 2% solution of histamine hydrochloride was applied for 30 min to the left eye.

The left eye was subjected to paracentesis and a sample of aqueous humour was taken.

Immediately after, the substances were given by gavage at the dose of 100 mg/kg. Two or four hours after administration a lens embedded with histamine as described before was applied for 30 min on the right eye. Paracentesis was carried out and aqueous humor was taken.

The quantity of Evans Blue in the aqueous humor was evaluated as described in 1(i).

As Table 3 shows, compounds II and I both reduced permeability of the vessels of the ciliary body caused by histamine. The effects after 2 and 4 hours were statistically significant compared with the basal values.

TABLE 3

Effect on the Permeability of the Haemato-Ophthalmic Barrier Induced by Histamine					
Substance	Dose mg/kg/os	No. of animals	Evans Blue (mcg/ml of aqueous humour) m ± sd		
			Time 0	2 hours	4 hours
Compound II	100	10	110.9±32.7	82.0±7.0*** (-25.3)	
	100	10	100.6±38.9		68.8±8.7*** (-31.7)
Compound I	100	10	106.4±29.5	86.2±6.1** (-19.0)	
	100	10	108.2±16.4		75.9±10.3*** (-29.9)

*** P < 0.001

** P < 0.01 Student's "t" for paired data

In brackets the percentage variations relative to time 0.

B. ACUTE TOXICITY

This was tested on mice and rats after administration by stomach probe. Doses up to 6 g/kg of compounds I and II did not cause toxic symptoms or death. The LD 50 must therefore be above 6 g/kg.

C. OCULAR TOLERABILITY

The ocular tolerability of compounds I and II was measured in New Zealand rabbits on the anterior segment (conjunctiva, cornea, iris and crystalline lens) and on the posterior segment (vitreous humour and retina) after oral administration, local application and intra-ocular injection.

1. Oral Administration

Both products were administered by stomach probe, either in a single dose of 200 mg/kg or in repeated doses of 100 mg/kg for 7 days. Surveys were made before and 1, 2 and 4 hours after acute administration, on the third and seventh days after repeated administration and 5 days after suspension of treatment.

2. Local Application

The substances were instilled into the conjunctival sac in a quantity of 0.5 ml day of 1% aqueous suspensions for 30 days.

3. Intra-Ocular Injection

The substances were injected under anaesthesia into the front chamber at a concentration of 0.1%, after evacuation of the chamber. The ocular structures were examined by slit lamp, 1, 24 and 48 hours after injection.

Single or repeated oral administration of compounds I and II did not induce modifications in the conjunctiva, cornea, iris, crystalline lens, vitrous humour or retina.

Local application was also perfectly tolerated.

After intracamerular injection, a blue coloration of the intracamerular structures was observed, together with hyperaemia of the conjunctiva and an inflammatory reaction which, in most cases, disappeared in 48 hours.

The intensity of the reactions was similar for both products.

On the basis of the test results for pharmacological and clinical activity, compounds I and II can be used in the treatment of conditions of increased capillary permeability following a noxious stimulus resulting in opening of intercellular junctions, break of blood aqueous barrier and leakage of seric proteins in the anterior chamber.

Such conditions of capillary fragility are frequent complications of diabetes. Therefore a direct action of compounds I and II on vasal permeability can improve ophthalmic affections in diabetic patients.

Various routes of administration may be used in carrying out the invention. For example, the anthocyanidins may be administered orally or they may be administered locally to the eye.

The products according to the invention can be used in oral pharmaceutical forms such as tablets, capsules and sachets of granulate.

The following examples illustrate the invention without being limitative.

EXAMPLE 1 (Compound I, 100 mg, capsules)	
Each capsule, size 2, contains:	
Compound I	100 mg
Lactose	138 mg
Methylcellulose	4 mg
Sodium carboxymethylcellulose	4 mg
Magnesium stearate	2 mg
Talc	2 mg

EXAMPLE 2 (Compound II, 300 mg, capsules)	
Each capsule, size 1, contains:	
Compound II	300 mg
Methylcellulose	6 mg
Sodium carboxymethylcellulose	6 mg
Talc	3 mg
Magnesium stearate	3 mg

EXAMPLE 3) (Compound II, 100 mg, tablets)	
Each 160 mg tablet contains:	
Compound II	100 mg
Methylcellulose	2 mg
Sodium carboxymethylcellulose	10 mg
Microgranular cellulose	30 mg
Lactose	12 mg
Talc	3 mg
Magnesium stearate	3 mg

EXAMPLE 4) (Compound I, 300 mg, tablets)	
Each 500 mg tablet contains:	
Compound I	300 mg
Methylcellulose	6 mg
Microgranular cellulose	100 mg
Lactose	70 mg
Sodium carboxymethylcellulose	18 mg
Magnesium stearate	3 mg
Talc	3 mg

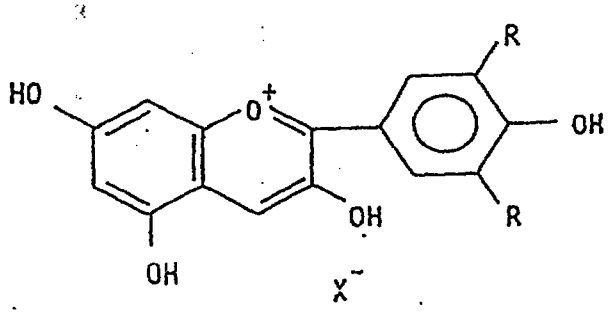
EXAMPLE 5 (Compound 1, 100 mg, granulate sachets)	
Each 1 g sachet contains:	
Compound I	100 mg
Mannitol	400 mg
Ammonium glycyrrhizinate	10 mg
Flavouring powder	10 mg
Methylcellulose	5 mg
Lactose	475 mg

EXAMPLE 6 (Compound II, 300 mg, granulate sachets)	
Each 1 g sachet contains:	
Compound II	300 mg
Mannitol	300 mg
Ammonium glycyrrhizinate	10 mg
Flavouring powder	10 mg
Methylcellulose	5 mg
Lactose	375 mg

Example 7 (Compound II, 100 mg lyophilized collyrium)	
Each "lyophilized" ampoule contains	
Compound II	100 mg
Mannitol	300 mg
Each "Solvent" ampoule contains:	
Hydroxypropylmethylcellulose	40 mg
Benzalkonium chloride	1 mg
Monobasic sodium phosphate	82.8 mg
Dibasic sodium phosphate	23.9 mg
Water for injection q.s. to	10 ml

Claims

1. The use of an anthocyanidin of formula



wherein

(I) each R represents hydrogen, or

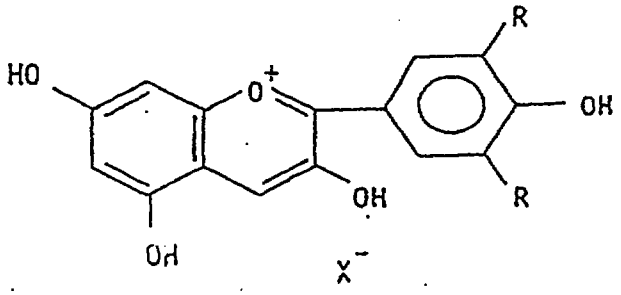
EXAMPLE 5 (Compound 1, 100 mg, granulate sachets)	
Each 1 g sachet contains:	
Compound I	100 mg
Mannitol	400 mg
Ammonium glycyrrhizinate	10 mg
Flavouring powder	10 mg
Methylcellulose	5 mg
Lactose	475 mg

EXAMPLE 6 (Compound II, 300 mg, granulate sachets)	
Each 1 g sachet contains:	
Compound II	300 mg
Mannitol	300 mg
Ammonium glycyrrhizinate	10 mg
Flavouring powder	10 mg
Methylcellulose	5 mg
Lactose	375 mg

Example 7 (Compound II, 100 mg lyophilized collyrium)	
Each "lyophilized" ampoule contains	
Compound II	100 mg
Mannitol	300 mg
Each "Solvent" ampoule contains:	
Hydroxypropylmethylcellulose	40 mg
Benzalkonium chloride	1 mg
Monobasic sodium phosphate	82.8 mg
Dibasic sodium phosphate	23.9 mg
Water for injection q.s. to	10 ml

Claims

1. The use of an anthocyanidin of formula



wherein

(I) each R represents hydrogen, or

(II) each R represents hydroxy, and
X^- represents one equivalent of a pharmaceutically acceptable anion in the manufacture of a pharmaceutical composition for reducing the hyperpermeability of the ciliary body ocular vessels in a subject.

2. The use claimed in Claim 1 wherein the composition is formulated for administering the anthocyanidin systemically.

3. The use claimed in Claim 2 wherein the composition is formulated for administering the anthocyanidin orally.

4. The use claimed in Claim 1 wherein the composition is formulated for administering the anthocyanidin locally to the eye.

5. The use claimed in any of Claim 1 to 4 wherein the composition is for reducing the permeability of the haemato-ophthalmic barrier.

6. The use claimed in any of Claims 1 to 4 wherein the composition is for use in the treatment of degenerative retinopathy, myopia and ophthalmic complications of diabetes.

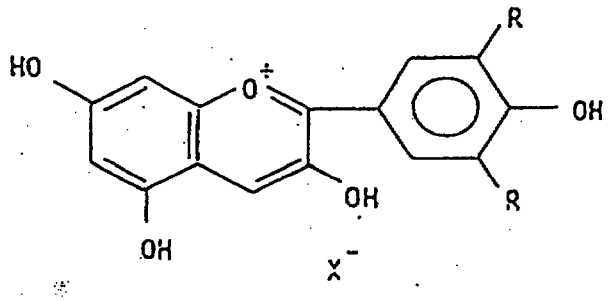
7. The use claimed in any of Claim 1 to 4 wherein the composition is for use in the treatment of secondary ophthalmic disorders in vascular and metabolic diseases or in increasing the retinal sensitivity.

8. A topical pharmaceutical preparation adapted for administration locally to the eye of a subject for reducing the hyperpermeability of the ocular vessels comprising an anthocyanidin of formula (I) or (II) as defined in Claim 1 and an ophthalmically acceptable diluent.

9. A composition according to Claim 8 in the form of an eye drop, opthalmic ointment or collyrium.

Patentansprüche

1. Verwendung eines Anthocyanidins der Formel



in der
(I) jedes R Wasserstoff bedeutet oder
(II) jedes R Hydroxy bedeutet und
X^- ein Äquivalent eines pharmazeutisch verträglichen Anions bedeutet, bei der Herstellung einer pharmazeutischen Zubereitung zur Verringerung der Hyperpermeabilität der Ziliarkörperaugengefäße bei einem Patienten.

2. Verwendung nach Anspruch 1, bei der die Zubereitung so formuliert ist, daß das Anthocyanidin systemisch verabreicht wird.

3. Verwendung nach Anspruch 1, bei der die Zubereitung so formuliert ist, daß das Anthocyanidin oral verabreicht wird.

4. Verwendung nach Anspruch 1, bei der die Zubereitung so formuliert ist, daß das Anthocyanidin lokal am Auge verabreicht wird.

5. Verwendung nach einem der Ansprüche 1 bis 4, bei der die Zubereitung die Permeabilität der hämato-ophthalmischen Barriere herabsetzen soll.

6. Verwendung nach einem der Ansprüche 1 bis 4, bei der die Zubereitung zur Behandlung degenerativer Retinopathie, von Kurzsichtigkeit sowie ophthalmischer Komplikationen von Diabetes dient.

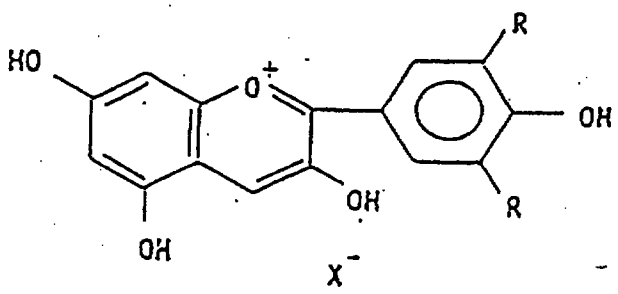
7. Verwendung nach einem der Ansprüche 1 bis 4, bei der die Zusammensetzung zur Behandlung sekundärer ophthalmischer Störungen bei Gefäß- und Stoffwechselkrankheiten sowie zur Steigerung der retinalen Sensibilität dient.

8. Topische pharmazeutische Zubereitung, die für die lokale Verabreichung am Auge des Patienten adaptiert ist, um die Hyperpermeabilität der Augengefäße zu verringern, und ein Anthocyanidin der Formel (I) oder (II) wie in Anspruch 1 definiert sowie einen ophthalmisch verträglichen Verdünner enthält.

9. Zusammensetzung nach Anspruch 8 in Form von Augentropfen, einer Augensalbe oder eines Augenwassers.

Revendications

1. Utilisation d' une anthocyanidine de formule :



dans laquelle
(I) chaque R représente un atome d' hydrogène, ou
(II) chaque R représente un groupe hydroxy, et
X^- représente un équivalent d' un anion acceptable du point de vue pharmaceutique, pour la fabrication d' une composition pharmaceutique destinée à réduire l' hyperperméabilité des vaisseaux oculaires du corps ciliaire chez un sujet.

2. Utilisation suivant la revendication 1, dans laquelle la composition est formulée pour l' administration systémique de l' anthocyanidine.

3. Utilisation suivant la revendication 2, dans laquelle la composition est formulée pour l' administration orale de l' anthocyanidine.

4. Utilisation suivant la revendication 1, dans laquelle la composition est formulée pour l' administration locale, dans l' oeil, de l' anthocyanidine.

5. Utilisation suivant l' une quelconque des revendications 1 à 4, dans laquelle la composition est utilisée pour réduire la perméabilité de la barrière hémato-ophtalmique.

6. Utilisation suivant l' une quelconque des revendications 1 à 4, dans laquelle la composition est utilisée pour le traitement de la rétinopathie dégénérative, de la myopie, et des complications ophtalmiques du diabète.

* C L M S *

7. Utilisation suivant l' une quelconque des revendications 1 à 4, dans laquelle la composition est utilisée pour le traitement des troubles ophtalmiques secondaires dans les maladies vasculaires et métaboliques, ou pour augmenter la sensibilité rétinienne.

8. Préparation pharmaceutique topique adaptée à l' administration locale dans l' oeil d' un sujet, en vue de la réduction de l' hyperperméabilité des vaisseaux oculaires, comprenant une anthocyanidine de formule (I) ou (II) suivant la revendication 1, et un diluant acceptable du point de vue ophtalmique.

9. Composition suivant la revendication 8, sous forme de gouttes oculaires, de pommade ophtalmique, ou de collyre.