The Effect of Pare (Momordica Charantia) L. Ethanol Extract on The Formation of Cataracts in Wistar White Rats

Nur Rahayuningsih¹ Desi Agni Mutia¹ Tita Nofianti¹

¹Program Studi S1 Farmasi, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada Corresponding author: nur.rahayuridwan@gmail.com

Abstract

Cataract is a clouding of the lens of the eye that reduces the amount of light entering and results in impaired vision. Cataracts remain the leading cause of visual impairment and blindness worldwide. Bitter melon fruit contain flavonoids and polyphenols which have antioxidant activities. Thirty of 9 days age wistar rats was divided into six group. Normal and negative group were given 2 ml/200 g BW rats 1% of PGA orally. Positive control was given 0,0009 g/200 g BW rat of vitamin C. Test group 1, 2, and 3 (were given 0,025 g/200 g BW rats; 0,051 g/200 g BW rats; and 0,1 g/200 g BW rats bitter melon fruit ethanol extract orally, respectively). All of the group except Normal were inducted with 0,25 mg/200 g BW rats sodium selenit subcutaneously. Treatment was given until the rat open the eyes for the first time (\pm 18 days). Eyes were observed macroscopic and microscopically in all group. The ethanol extract of bitter melon (Momordica charantia L.) has preventive activity against the formation of cataracts. The negative control group and dose I included in stage IV, positive control included in stage I, dose II included in stage III and dose III included in stage II. The most effective dose which prevent the formation of eye cataracts was 0,1 g/200 g BW rats bitter melon fruit (Momordica charantia L.) ethanol extract.

Keywords : Cataract, Bitter melon fruit, flavonoids

Introduction

Medicinal plants are found in various corners of the country, which are used as a form of traditional medicine. The use of traditional medicine by the community has always been an alternative to meet the basic needs of the community. Traditional medicine has been empirically proven to be relatively safe for human consumption. However, scientific evidence is still needed (Suharmiati, 2006). One of the plants that is widely known and widely used by the people of Indonesia is bitter melon (Momordica charantia L.). Cataracts are the leading cause of visual impairment and blindness worldwide, accounting for at least 50% of blindness in most developing countries. Based on the description above, this study was conducted to determine the

effect of bitter melon which contains antioxidant compounds on improving cataract eye health and can be used as information on the effect of prevention on the formation of cataracts from ethanol extract of bitter melon as one of the plants that is known and widely used. by society.

Method

Extraction

Pare which has become powder 500 g was extracted using 70% ethanol by maceration method 3x24 hours, stirring occasionally every 1x24 hours until the solvent becomes clear. The liquid extract was evaporated using a rotary evaporator to obtain a thick extract of bitter melon.

Phytochemical Screening

The examination included alkaloids, flavonoids, terpenoids/steroids, saponins, tannins, polyphenols, monosesquiterpenoids, sesquiterpenoids, and quinones.

Preparation of Animal

The animals used in this study were wistar rats aged 9 days and placed in cages at room temperature (25 \pm 10 oC) and relative humidity (55 \pm 5%). Then the animals were grouped into 6 groups, namely the normal control group; negative control group; positive control group; dose group I; dose group II and group III dose each as many as 5 individuals.

Preparation of Test Dossage

The dose of sodium selenite is 4.32345 x 10-3 g/KgBW. Conversion dose = 0.25 x 10-3 g/200 gBW rats = 0.25 mg/200 gBW rats. The concentration of the solution made was 0.25 mg/mL by dissolving 25 mg in aquabides to 100 mL.

The empirical dose of bitter melon in humans is 15 - 30 g. By using a conversion factor of 0.018 g and a DER of 10.5 g, a dose of 0.255 g/KgBW rats was obtained. Then made 3 dose variations, namely 0.125 g/KgBW rats, 0.255 g/KgBW rats and 0.5 g/KgBW rats.

The empirical dose of ascorbic acid in humans is 50 mg. The dose in rats is = 0.05 g x 0.018 g = 0.0009 g/200 gBB rats. The concentration of the solution made was 0.9 mg/mL by dissolving 90 mg in distilled water to 100 mL.

Cataract Testing

Animals aged 9 days were grouped into 6 groups. Each group was induced (except the normal group) with sodium selenite subcutaneously, 24 hours after being given induction, then each group was given a test preparation. The preparation was given orally to each animal until the rats opened their eyes for the first time (± 18 days of age). Macroscopic observations were made of all test animals, all animals were sacrificed at the end of the study and eye organs were taken to make lens histopathological preparations.

Histopathological Preparation

The eye lens that has been prepared is then viewed under a microscope using hematoxylin and eosin (HE) reagents.

Data analysis

The data obtained are qualitative data in the form of lens histopathology which were analyzed descriptively by comparing the comparison group with the test group.

Results And Discussion

The thick extract obtained from 447.27 g of Momordica charantia L. simplicia powder after maceration for 3 days was 42.563 g with a DER value of 10.5%.

Table 1. Phytochemical Screening Results

Metabolit	Simplicia	Extract
Secunder		
Alcaloid	+	_
Flavonoid	+	+
Terpenoid/Steroid	+	—
Saponin	+	+
Polifenol	+	+
Tanin	+	_
Monoterpenoid and	+	-
seskuiterpenoid		
quinon	+	_

Description: + Detected; - Not detected

Cataract Test

Observations were made macroscopically and microscopically. Microscopic results to further clarify macroscopic observations.



Figure 1. Macroscopic Observation Normal Eyes (A) And Cataracts (B)

From macroscopic results, administration of bitter melon ethanol extract for all doses did not show any ability to prevent the formation of cataracts. This could be due to the fact that the content of compounds in bitter melon extract was not proven to prevent the formation of cataracts caused by sodium selenite. In contrast to the positive control which was able to prevent the formation of cataracts.

The lens is one of the tissues that can experience oxidative stress and damage to the eye lens is irreversible. Chemical modification of lens components mediated by oxidative agents is believed to be the cause of cataract formation due to disturbances in electrolyte homeostasis, protein aggregation and loss of eye enzymatic function (Shambhu et al, 1984).

From the test results obtained that sodium selenite can induce cataracts. Administration of sodium selenite at a dose of 0.25 mg/200 g BW rats can cause cataracts that are morphologically and biochemically similar in humans. Animals induced using sodium selenite are said to be quite reproducible. Sodium selenite can cause damage to oxidative defenses and damage cell membranes thereby triggering the formation of cataracts. Oxidation of the epithelial membrane and the formation of insoluble protein aggregates is the initial occurrence of cloudiness in the eye lens (Gupta et al, 2003).

Na₂SeO₃ + H₂O \longrightarrow SeO₂ + 2NaOH SeO₂ + H₂O \longrightarrow SeO₃²⁻ + 2H⁺ SeO₃²⁻ \longrightarrow SeO₂ + O²⁻

Figure 2. Reaction for the formation of free radicals from sodium selenite

Superoxide and hydroxyl free radicals cause damage to lipids and cell membrane proteins deposited on the lens surface. Decrease in plasma levels of ascorbate and -carotene which causes cloudiness of the eye lens resulting in the formation of cataracts (Selim et al, 2001).

Chemically, cataract formation is characterized by reduced oxygen intake and increased water content followed by dehydration. The content of sodium and calcium increases, while the content of potassium, ascorbic acid and protein decreases (Tamsuri, 2002).

Microscopic Observation



Figure 2. Microscopically Normal Lens Parts Information :

- 1: Lens capsule
- 2: Subcapsular epithelium
- 3: Cornea
- 4: Iris
- 5: Cortex
- 6: Nucleus

Based on the results of microscopic observations, the test group of the three doses showed activity to prevent cataract formation but was not better than the positive control with ascorbic acid administration. Ascorbic acid can donate electrons in intracellular and extracellular biochemical reactions. Ascorbic acid is able to remove reactive oxygen compounds in neutrophil cells, lens proteins and retina. Ascorbic acid is able to reduce superoxide, hydroxyl and hypochloric acid radicals. Because ascorbic acid is able to react with free radicals and then convert them into ascorbyl radicals and dehydroascorbate, thus minimizing the damage that occurs to the lens of the eye. Indirectly, ascorbic acid can reduce the activity of free radicals caused by sodium selenite by converting ascorbic acid into a reduced form.

The reaction of ascorbic acid with superoxide is as follows:

 $2O_2 + 2H^+ + ascorbic \rightarrow 2H_2O_2 + dehydroascorbic$

The reaction with hydrogen peroxide is catalyzed by the enzyme ascorbic acid peroxidase:

$$H_2O_2$$
 + 2ascorbic \rightarrow 2 H_2O + 2monodehidroascorbic

Based on microscopic results with HE staining (hematoxylin and eosin) at 200x magnification, it was found that each test group compared to the negative control group showed preventive activity, but not good enough compared to the positive control group. Microscopically, cataract stage can be categorized into 4 parts, namely Stage I (Incipient Cataract), Stage II (Immature Cataract), Stage III (Mature Cataract) and Stage IV (Hypermature Cataract) (Gupta et al, 2003).

Conclusion

Based on macroscopic observations, it was found that the ethanolic extract of bitter melon (Momordica charantia L.) was not proven to prevent cataract formation. Based on microscopic observations, it was found that the ethanolic extract of bitter melon (Momordica charantia L.) had preventive activity against cataract formation, namely the negative control group and dose I included in stage IV, positive control included in stage I, dose II included in stage III and dose I included in stage IV. III is included in stage II. So that the ethanol extract of bitter melon (Momordica charantia L.) was not strong enough to prevent cataract formation in the lens of the eye compared to the positive control group.

Reference

Budiyono, Setiadi. 2011. *Anatomi Tubuh Manusia*, cetakan ke I. Laskar Aksara : Bekasi, hal 29-30.

Departemen Farmakologi Dan Terapeutik. Mardjono, Mahar. 2009. Farmakologi Dan Terapi. Edisi 5. Penerbit Fakultas Kedokteran Universitas Indonesia : Jakarta.

Departemen Kesehatan Republik Indonesia. Farmakope Indonesia. 1995. Edisi IV. Jakarta.

- Ilyas S. 2007. *Ilmu Penyakit Mata.* Tajam penglihatan, kelainan refraksi dan penglihatan warna. Edisi 3. Balai Penerbit Fakultas Kedokteran Universitas Indonesia : Jakarta.
- Green, J.H. 2013. *Pengantar Fisiologi Tubuh Manusia*. Widjajakusumah, Djauhari, penerjemah. Bina Putra Aksara : Tanggerang, hal 428.
- Gupta, S.K., Trivedi, D., Srivastava, S., Joshi, S., Halder, N., and Verma, S.D, 2003. Lycopene

attenuates oxidative stress induced experimental cataract development an in vitro and in vivo study. Basic Naturally Investigation. University of Maryland. Collage Park. Maryland, USA.

Nair, Kavitha Nair, Kirti Patel, Tejal Gandhi. 2010. Effect Antioxidant of Embelica in selenite induced Cataract. Spring. 9 (2): 147-152.

Rukmana, Rahmat. 1997. Budidaya Pare. Penerbit Kanisius : Yogjakarta.

- Selim, Doganay., Yusuf, Turkoz., Cem, Evereklioglu., Hamdi Er, Mehmet., Bozaran., Elif, Ozerol. 2001. Use of Caffeic Acid Phenthyl Ester To Prevent Sodium Selenit Induced Cataract and Refractive Sugery. Vol. 28. Page : 1457 – 1462.
- Shambhu D. varma, Diwan Chand., Yog R. Sharma., Jhon. F., Kuck andRichard D. Richards., 1984. Oxidative stress on lens and cataract formation : role of light and oxygen. Vol. 3. No. 1. Page : 35 – 58.
- Tamsuri, Anas. 2011. *Klien Gangguan Mata dan Penglihatan.* Penerbit Buku Kedokteran EGC : Jakarta, hal 9-12.
- Tjay, Hoan Tan, Rahardja, Kirana. 2006. *Obat Obat Penting*. Edisi 6. Penerbit PT. Alex Media Komputindo : Jakarta.
- Vaugan G. D, Asbury T, Eva R.P. 2000. Oftalmologi Umum Lensa. Bab. 20 Edisi 14. Penerbit Widya Medika : Jakarta. hal 401 – 406.
- Winarsi, Hery. 2007. Antioksidan Alami Dan Radikal Bebas. Penerbit Kanisus : Yogjakarta.
- Yuliani, Sapto. 2012. Efek Protektif Ekstrak Etanol Herba Pegagan (Centella asitica (L.) Urban) Terbadap Pembentukan Katrak Tikus Wistar Yang Diinduksi Sodium Selenit, Skripsi, Fakultas Farmasi, UAD.