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Ethanol Extract Activity of Yellow Pumpkin Flesh (*Cucurbita Moschata Duch*) on the Cataract Formation

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Abstract*—Objectives Oxidative stress is suspected as a major factor triggering cataracts. Yellow pumpkin contains beta-carotene compounds can reducing oxidative stress, and it is suspected to be able to reduce the occurrence of cataracts. This study aims at determining and proving the benefits of yellow pumpkin (Cucurbita moschata Duch) as a deterrent in cataract formation in male albino rats (Wistar strain) induced by Sodium Selenite. Macroscopic and microscopic features of cataract formation on negative controls included in mature cataracts (stadium 3), at the dose I and dose III including incipient cataracts (stadium 2) and dose II including immature cataracts (stadium 1). The average parameter diameter of the lens (µm) of group dose II (1738 ± 583) and dose III (1825 ± 106) experiences a widening of the lens diameter compared to the negative control (1492 ± 52). Whereas, at the dose I (1392 ± 38), there was a narrowing compared to the negative control. The parameters of lens epithelial cell thickness (µm) at dose I (15 ± 3.46), dose II (15.67 ± 5.03), and dose III (9.67 ± 4.62) of ethanol extract of yellow pumpkin showed an increase in thickness lens epithelial cells of mouse compared to negative controls (7 ± 1). The ethanol extract of the flesh of yellow pumpkin showed the improvement of eye health of albino rats (Wistar strain) experienced cataract, although it has not been able to improve it on the whole.

Keywords: cataract,* Cucurbita moschata Duch*, sodium selenit

I. INTRODUCTION

A cataract is a clouding of the lens in the eye that can reduces the amount of light entering the eye that affects vision. a cataract remaining in the eye for a very long time become cause of visual impairment and blindness around the world making at least 50% of blindness in most developing countries. Blindness is estimated to reach 75 million in 2020. Of these, cataracts are estimated at 35 million. This number is equivalent to the current combined total population of Australia, New Zealand, Sweden, and Denmark including Indonesia. Thus, the burden of cataracts increases [1].

Yellow pumpkin (Cucurbita moschata Duch) is a vegetable plant type, but it also can be used for various types of food, such as: bread, dodol , chips, kolak , manisan and so on which have a fairly complete nutritional content. Those nutritional content of foods are carbohydrates, proteins, fats, glycosides, sitosterol, and carotenoids [2]. In addition, those foods also contain some minerals, such as calcium, phosphorus, iron, and vitamins, vitamins B and C, as well as fiber. flesh of the fruit which is yellow or orange is a sign that it has a very high carotenoid content [3].

Indonesian society has long used flesh of yellow pumpkin as everyday dishes, and it is also used as a traditional medicine to treat various diseases. It has triggered many researchers conducting research on yellow pumpkin plants ranging from the chemical content within it to the advantage that can be obtained from it. Research on the use of yellow pumpkin has been investigated as antidiabetic, hepatoprotector, anti-cancer, anti-obesity [4]. In addition, yellow pumpkin is useful as anti-HIV, anti-diarrhea, anti-bacterial, laxative, carminative, anti-pyretic, anti-oxidant, anthelmintic, and anti-tuberculosis [5]. According to Valenuela et al. (2011), yellow pumpkin is used for the treatment of cataracts.

This study aims at determining the effect of ethanol extract of yellow pumpkin fruit in improving the health of eye experiencing cataracts from a laboratory rat having been induced of sodium selenite. Observations was macroscopically and microscopically done on the rat eyes.

II. MATERIAL AND METHOD

A. Procedure

Tools and materials

The tools used are rotary evaporator, Olympus binocular microscope, paraffin block molding place, tissue processor, embedding tools, microtome. The materials used were 2-3 months old yellow pumpkin fruit obtained from the Cimaragas, Ciamis, West Java, sodium selenit, BNF 10%, a laboratory rat used is male albino rats (Wistar strain) aged 9 days.

B. Research procedure

This research was conducted with the following stages:
Material Collection

The material used is Yellow Pumpkin (Cucurbita moschata Durch) obtained from plantations in Cimaragas, Ciamis, West Java.

Yellow Pumpkin Plant Determination

Determination of Yellow Pumpkin Plant (Cucurbita moschata Durch) at Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.

Simplicia Preparation

The flesh of yellow pumpkin as much as 11.6 kilograms is wet sorted by means of the flesh of yellow pumpkin being cleaned and washed, then drained. After being drained, the flesh of yellow pumpkin is cut into small sizes and then dried by placing it in an open place with good air circulation and not exposed to direct sunlight with a black flannel cloth covered. Simplicia is mashed using a blender to make the process of withdrawal of secondary metabolites by solvents easier.

Extraction

The flesh of yellow pumpkin which has become powder extracted by maceration, by soaking the powder of flesh of yellow pumpkin in 70% ethanol for 3x24 hours, every 24 hours replaced by a new solvent and stirring occasionally. After 3x24 hours, it is filtered with a filter cloth until it is perfectly filtered which is marked with the ethanol color becoming clear again. After that, the remaining ethanol solvent was evaporated with a rotary evaporator so that a thick extract of flesh of yellow pumpkin was obtained.

Phytochemical Test

Phytochemical test conducted includes alkaloid compounds, flavonoids, polyphenols, tannins, monoterpenoids and sesquiterpenoids, saponins, quinones, terpenoids and steroids.

Animal Preparation

In this study, the animals used male albino rats (Wistar strain) aged 9 days, and it was placed in a cage at room temperature (25 ± 100C) and relative humidity (55 ± 5%). Then, the animals are grouped into 6 groups, in which in each group consisted of 5 animals, and they were given treatment as shown in table 1:

Table 1. Animal Preparation

Test Group	Treatment
Normal	PGA 1% (2 mL/200 g of body weight rats)
Negative	Na. Selenite + PGA 1% (2 mL/200 g of body weight rats)
Positive	Na. Selenite + Vitamin C (0,0009 g/200 g of body weight rats)
Dose I	Na. Selenite + Ethanol Extract Flesh of Yellow Pumpkin (0,0227 g/200 g of body weight rats)
Dose II	Na. Selenite + Ethanol Extract Flesh of Yellow Pumpkin (0,0454 g/200 g of body weight rats)
Dose III	Na. Selenite + Ethanol Extract of Flesh of Yellow Pumpkin (0,0908 g/200 g of body weight rats)

Test Material Preparation

Dose Determination of Sodium Selenite

Based on previous research, sodium selenite dose of 25 µmol/Kg of body weight rats can induce cataracts. Sodium selenite dose is 4,32345 x 10-3 grams/Kg of body weight rats. Conversion dose = 0.25 x 10-3 g/200 g of body weight rats = 0.25 mg / 200 g of body weight rats rats. The concentration of solvent made is 0.25 mg/mL by dissolving 25 mg in aquabides to 100 mL.

Dose Determination Flesh of Yellow Pumpkin

Empirical dose flesh of yellow pumpkin for humans is 100 g of fresh fruit flesh. Dose conversion of yellow pumpkin powder = 100 g/8000 gx 477.6 = 5.97 grams. The dose conversion of extract = 5.97 g / 200 g x 84.63 g = 2.5262 g. By using a conversion factor of 0.018, a dose of 0.0454 gram/200 gram of weight of rats was obtained, 3 variations of dose were made, namely 0.0227 g / 200 gram of weight of rats, 0.0454 g / 200 gram of body weight rats, and 0.0908 g / 200 gram of body weight rats.

Dose Determination of Ascorbic Acids

The empirical dose of ascorbic acid of humans is 50 mg. The dose in rat is = 0.05 g x 0.018 g = 0.0009 g / 200 g of body weight rats. The concentration of solvent made is 0.9 mg/mL by dissolving 90 mg in aquadest to 100 mL.

Process of Cataract Testing

The procedure for cataract testing can be seen in the chart below:

A laboratory rat aged 9 days were grouped into 6 groups. Each group was induced (except the normal group) with subcutaneous sodium selenite. 24 hours after being induced, then it was given the test compound. The dose was given orally to each laboratory rat until it opened its eyes for the first time (± 18 days). Macroscopic observations of all laboratory rats were performed. All laboratory rats were sacrificed at the end of the study, and the eye organs were taken to make the lens histopathological preparations. The histopathological features of the lens experiencing cataracts in each eye of the laboratory rats.

Setup the histopathology preparations [6,7].

Soaking the tissue in 10% of buffer neutral formalin (BNF)

Soaking the tissue in 10% of buffer neutral formalin (BNF) functioned as a preservative to prevent tissue digestion by enzymes or bacteria and to protect the physical structure of cells. The process of tissue fixation by using 10% of BNF soaked for 3 days.

Process of Histopathology Preparation

It Included organ washing, dehydration process, purification process, vacuum, printing paraffin blocks, cutting tissue blocks, tissue coloring

Observation of Cataract Test Results

The parameters observed were macroscopically comparing normal eyes to the eye experienced cataracts, which was a clouding of the lens of the eyes of the laboratory rat. Microscopic observation by observing the changes of the parts/layers constructing the lens test of laboratory lab as well as the diameter and the thickness of the lens epithelium.

C. Data analysis

The data obtained were qualitative data in the form of lens histopathology features which was analyzed descriptively by comparing the comparison group with the test group and quantitative data in the form of diameter and thickness of the lens epithelium that were processed statistically using SPSS 16.

III. RESULTS

Results of Plant Determination

Plant determination was carried out at the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung. Determination is done with the purpose to ensure the type and family of plants. Determination results obtained that the sample used is a yellow pumpkin of *Cucurbita moschata* Duch type from Cucurbitaceae family.

Result of Simplicia and Yellow Pumpkin Powder

Simplicia or yellow pumpkin powder is obtained from fresh yellow pumpkin (*Cucurbita moschata* Duch) obtained from Cimaragas, Ciamis. yellow Pumpkin was harvested, and wet sorting was done on it. The flesh of yellow pumpkin is thinly sliced, and soaking is done with Sodium metabisulfite 0.3% of the total material for 20 minutes aimed at increasing the solubility of flour or yellow pumpkin powder. Sodium metabisulfite is an additional ingredient often used in food processing that functions as a preservative, and it is used to prevent damage due to enzymatic browning reactions and works as an antioxidant, to removes odors and bitter taste, especially on cassava, and to maintain the color to remain attractive [8]. Drying is carried out at 60oC using a cabinet dryer aiming at reducing the damage of β-carotene [9]. Production of simplicia powder was done by smoothing dry simplicia using a blender, and powder was sieved using mesh 60 obtained same size of flour or powder. Yellow pumpkin powder was obtained from fresh yellow pumpkin which has been sliced and cleaned as much as 8 kg, and it was dried and made to be powder so that yellow pumpkin powder as much as 477.6 grams was obtained, then yields of yellow pumpkin powder as much as 5.96% was obtained.

Extraction Results

The flesh of yellow pumpkin which has become 200 grams of powder was extracted by maceration, by soaking dried flesh of yellow pumpkin in 70% ethanol for 3x24 hours, every 24 hours replaced by new solvents, and stirring was done occasional. After 3x24 hours, it was filtered with a filter cloth until it appeared perfectly marked with the ethanol color becoming clear again. A liquid extract of 3.5 liters was obtained. After that, the remaining ethanol solvent was evaporated with a rotary evaporator so that a thick extract of flesh of yellow pumpkin was obtained as much as 84.63 grams and yields as much as 42.3%.

Results of Phytochemical Test

Phytochemical test is a preliminary examination to determine the content of secondary metabolites of a simplicia. The results of test conducted on pumpkin powder and extract of the yellow pumpkin containing secondary metabolites, namely flavonoids, triterpenoids, monoterpenoids, and sesquiterpenoids.

Metabolite compounds suspected to have activity as a deterrent to cataract formation in extracts of yellow pumpkin are flavonoids, triterpenoids, monoterpenoids and sesquiterpenoids. The secondary metabolite compounds are able to correct the imbalance of the antioxidant system in the body acting as a compound can delaying, slowing down and preventing free radical oxidation reactions in lipid oxidation. In addition, pumpkin (Cucurbita moschata) contains β-carotene (carotenoid) which is a precursor of vitamin A (retinol), which is useful in maintaining vision, growth, and differentiation of good tissues, as well as functioning as an oxygen singlet damper and free radicals [10].

Effects of Cataract Formation

Testing on cataract formation was carried out with the purpose to determine the potential of the extract of yellow pumpkin as a prevention of cataract formation. This test used three variations of test dose of the extract of yellow pumpkin, namely 0.0227g/200 gram of body weight rats (dose I), 0.0454g 200 gram of body weight rats (dose II), and 0.0908g/200 grams of body weight rats (dose III). The three comparison groups are normal control, negative control and positive control. The results obtained from the three variations of the test dose can be compared with the three control groups.

Ascorbic acid is used as a positive control. Ascorbic acid works as a coenzyme, and it is a reducing agent and antioxidant in certain circumstances. The ascorbic acid can directly or indirectly provide electrons to enzymes requiring reduced metal ions. The ascorbic acid is easily absorbed through the digestive tract. When normal conditions, it appear the increase levels of ascorbic acid in the blood after being adopted. Its distribution is extensive throughout the body with the highest levels in the gland and the lowest in muscle and fat tissue. Excretion through urine in the form of intact form and sulfate salts occur if blood levels exceed the threshold of excitatory kidney [10].

Sodium selenite was used as an inductor in testing because giving a single dose of sodium selenit given to young rats can cause cataract genesis that is morphologically and biochemically similar to senile cataracts that commonly occur in older people. Sodium selenite can cause oxidative defenses, and it can damage cells, so triggering cataract formation [11].

The metabolic processes of body, especially the reaction with oxygen, are formed molecules with loss of electrons. These substances are called free radicals which are very reactive and tend to attack molecules that can give the electrons. However, the body has a protective tissue from natural antioxidants that are easily oxidized si that it can be neutralized by most of these free radicals. Among them are vitamins A, C and E and natural

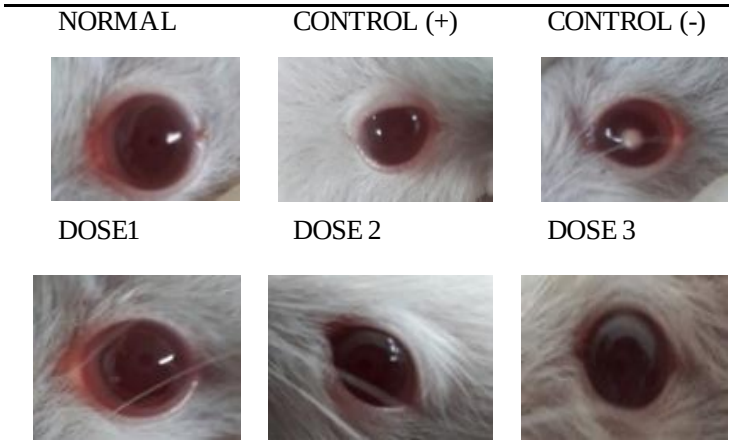
enzymes glutathione peroxydase (GPx), superoxidedismutase (SOD) and catalase. If the body lacks natural antioxidants, the cell membrane and/or nucleus of the cell can be damaged by free radicals. As a result, the process of tissue aging is accelerated, and defects occur in DNA. If it is not repaired or destroyed by the immune system, cells can multiply into malignant cells. In addition, free radicals are the cause of a number of other disorders such as clouding of the lens of the eye (cataracts) and deposition of oxy-LDL cholesterol in the walls of vessels with atherosclerosis [12].

Cataract Testing

Observations were made macroscopically and microscopically on the lens diameter and epithelial cells of the eyepiece. The results of microscopic testing showed that lens diameter and epithelial cells of the eyepiece were carried out to clarify macroscopic observations.

Macroscopic Observation

Macroscopic observations are observations with the naked eye, macroscopic results are shown in Figure 1.



Information:

Normal Control = The rat gets no treatment
Control (+) = induced by sodium selenite + Vitamin C
Control (-) = induced by sodium selenite only
Dose I = induced by sodium selenite + yellow pumpkin powder (0,0227g/200 gram of body weight rats)
Dose II = induced by sodium selenite + yellow pumpkin powder (0,0454 g/200 gram of body weight rats)
Dose III = induced by sodium selenite + yellow pumpkin powder (0,0908 g/200 gram of body weight rats).

Figure 1. Macroscopic features of Rats' Lenses

Macroscopic results of administration of ethanol extract of yellow pumpkin at all doses showed differences in the ability to prevent cataract formation. This depends on the dose given by pumpkin extract given to the rats. Macroscopic results can be seen in Figure 1.

In the positive control group with ascorbic acid dose of 0.9 mg/200 g of weight of rats, microscopic results showed that there was an influence on cataract formation. Cataracts occurred included in the category of stadium I (Insipient Cataract) which is a damage starting from the edge of the equator in the form of jellies to the anterior cortex. The damage begins to appear in the anterior subcapsular epithelium, a gap is formed between the lens fibers and the cortex containing degenerative tissue. This damage can cause polyopia because the refractive index is not the same in all parts of the lens [11].

Diameter of Rat Eyepiece

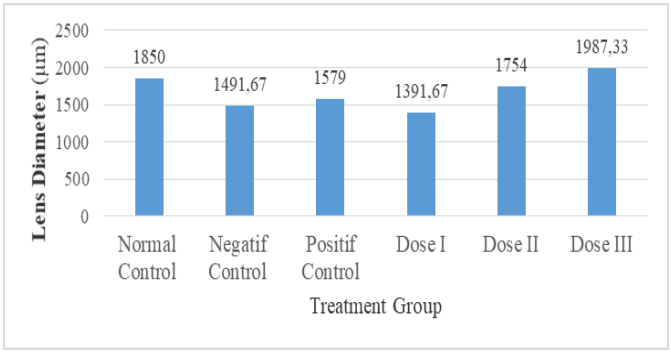


Figure 2. Diameter of Rat Eyepiece

The observations in Figure 2 showed that the lens diameter (µm) of rat of normal group (1850 ± 288), positive control (1563 ± 265), dose II (1738 ± 583) and dose III (1825 ± 106) widened compared with lens diameter of negative control (1492 ± 52). Whereas, at dose I (1392 ± 38), lens diameter was narrowed when compared to negative control.

Statistical analysis of the normality test was obtained significance value of 0.875> 0.05 meaning that all data are normally distributed. Homogeneity test of variance was obtained significance value of > 0.05, then H0 is accepted, meaning all data used are homogeneous. Because the sample is normally distributed and all variants are normal, showing a significance value of 0.150, the ANOVA test is then performed and a sig value of 0.095 < 0.05 is obtained, meaning that there is no difference among treatment groups, meaning that the provision of test compounds does not affect the widening of diameter rat eyepiece experienced cataract.

Thickness of Epithelial Cells of Rat Eyepiece

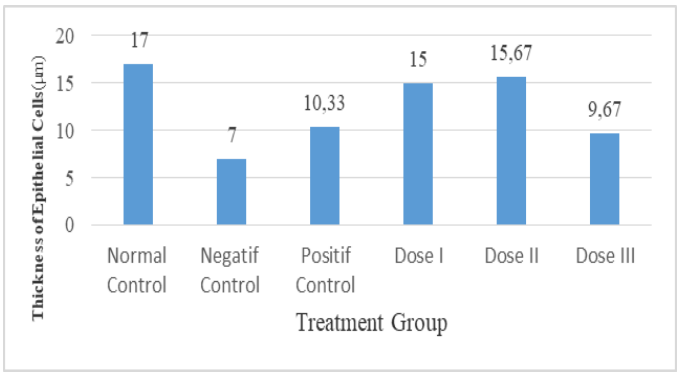


Figure 3. Thickness of Epithelial Cells of Rat Eyepiece

The observation results in Figure 3 show that the thickness of the epithelial cells (µm) of the rat eyepiece, the process of thickening on epithelial cell occurred compared to the negative

control meaning that the administration of ethanol extract of yellow pumpkin with a dose variant can increase the number of epithelial cell thickness of the rat eyepiece given a test compound to prevent cataracts. Thickness of epithelial cells in the normal group (17 ± 3.61), positive control (10.33 ± 2.31), dose I (15 ± 3.46), dose II (15.67 ± 5.03), and dose III (9.67 ± 4.62) of ethanol extract of yellow pumpkin showed an increase in the thickness of the epithelial cells of the rat eyepiece compared with the negative control (7 ± 1).

Statistical analysis of the normality test was obtained significance value of 0.802> 0.05 meaning that all data were normally distributed. Homogeneity test of variance was obtained significance value of > 0.05 then H0 is accepted, meaning all data used are homogeneous. Because the sample is normally distributed and all variants are normal, showing a significance value of 0.217, The ANOVA test is then performed and a sig value of 0.030 < 0.05 is obtained, meaning that there are differences among treatment groups, meaning that the provision of the test compounds does not affect the widening of the diameter of rat eyepiece experience cataract. Furthermore, the follow-up test of LSD was obtained a sig value of < 0.05, that is, the sig value of dose I (0.019*) and the sig value of dose II (0.012*) compared to negative control, there was a significant difference. This means that by administering ethanol extracts to dose I and II can increase the thickness of the epithelial cells of the rat eyepiece experienced cataract.

IV. DISCUSSION

The lens is one of the tissues can experiencing oxidative stress and damage on the eyepiece is irreversible. Chemical modification of lens components mediated by oxidative agents is believed to be the cause of cataract formation due to disruption of electrolyte homeostasis, protein aggregation, and loss of enzymatic function of the eye [13]. Cataract lenses have the characteristics of lens edema, protein changes, and damage to the continuity of the lens fibers. In general, lens edema varies according to the stadium of cataract development. Insipient cataract, the formed damage is still mild, and the lens fluid remains in a normal state. Immature cataracts are characterized by a partially damaged lens. Mature cataracts are characterized by clouding that has formed on the entire mass of the lens, and it has a slight edema. Edema occurs when the maximum water content, and lens capsules stretch. In hyper mature cataract, it is relatively dehydrated, and the lens capsules wrinkle due to water coming out of the lens and leaving clouding [14].

Based on the test results, it was obtained that sodium selenite can induce the occurrence of cataracts. The administration of sodium selenite at a dose of 0.25 mg/200 g of weight of rat can cause cataracts that morphologically and biochemically are similar to humans. The rats induced using sodium selenite can be said to be quite reproducible. Sodium selenite can cause damage to oxidative defenses, and it can damage cell membranes, so triggering cataract formation. Epithelial membrane oxidation and formation of insoluble protein aggregates are the initial occurrence of clouding in the lens [11].

Free radicals of superoxide and hydroxyl cause a damage to lipids and proteins of cell membrane that are stored on surface of the lens, cause damage to lens, cause a decrease of plasma levels of ascorbate, β-carotene, and cause an increase of the occurrence of cataract formation [15]. Chemically, cataract formation is characterized by reduced oxygen intake and increased water content which is then followed by dehydration. Increased sodium and calcium content, while the content of potassium, ascorbic acid, and protein is reduced [16].

Cataracts can be classified by various methods. Based on their location, Senile cataracts is classified in three lens zones, namely the lens capsule, cortex, and nucleus. The mechanism of cataract formation is very multifactorial because it is difficult to study. Oxidation of membrane lipids, structural or enzymatic of proteins, and DNA by peroxides caused by free radicals is the initial event that results in a loss of transparency both in the nucleus and in the cortical tissue in the lens. In cortical cataracts, electrolytes cause lens overhydration which causes melting from the lens. Nuclear damage on cataracts usually occurs due to protein denaturation as a result of oxidation, proteolytic and glycation processes. Protein aggregates cause higher protein molecular weights. This increase in optical density can cause a shift in myopia index resulting in vision errors. Besides, the lens area becomes turbid, yellowish appearance, and visible on the optical part with a microscope [17].

Based on the results of microscopic observations, the test group of the three doses and positive control of ascorbic acid showed a cataract formation prevention activity. Ascorbic acid can donate electrons to intracellular and extracellular biochemical reactions. Ascorbic acid can eliminate reactive oxygen compounds in neutrophil cells, lens proteins, and the retina. Ascorbic acid reduces superoxide radicals, hydroxyl, and hypo-chloric acid. Because ascorbic acid is able to react with free radicals and then convert it to radical ascorbate and dehydroascorbate, it minimizes the damage occurring in the eyepiece. Indirectly, ascorbic acid can reduce the activity of free radicals arising from sodium selenite by changing ascorbic acid into a reduced form. Reaction of ascorbic acid to superoxide as follows:

$$2O2^- + 2H^+ + \text{ascorbate} \rightarrow 2H2O2 + \text{dehydroascorbate}$$

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

$$H2O2 + 2\ \text{ascorbate} \rightarrow 2H2O + 2\ \text{monodehydroascorbate}$$

Based on microscopic results with staining HE (Hematoxylin and Eosin) 40x magnification, the results were obtained that each test group compared with the negative control group showed a preventive activity. The stadium of microscopic cataract can be categorized into 4 parts, namely stadium I (Insipient Cataracts), stadium II (Immature Cataract), stadium III (Mature Cataract) and stadium IV (Hyper-mature Cataract) [11].

V. CONCLUSION

Macroscopic features of cataract formation after being induced by sodium selenite that occurs in negative controls included mature cataracts. Whereas, at dose I and dose III include insipient cataracts, and at dose II included immature cataracts. Microscopic features of cataract formation of rats that occurs in negative controls included stadium 3. Whereas, at dose I and dose III include stadium 2, and at dose II included stadium 1. The average parameter diameter of the lens (µm) of group dose II (1738 ± 583) and dose III (1825 ± 106) experiences a widening of the lens diameter compared to the negative control (1492 ± 52). Whereas, at dose I (1392 ± 38), there was a narrowing compared to the negative control. The parameters of lens epithelial cell thickness (µm) at dose I (15 ± 3.46), dose II (15.67 ± 5.03), and dose III (9.67 ± 4.62) of ethanol extract of yellow pumpkin showed an increase in thickness lens epithelial cells of mouse compared to negative controls (7 ± 1). The ethanol extract of flesh of yellow pumpkin showed the improvement of eye health of albino rats (wistar strain) experienced cataract although it has not been able to improve it on the whole.

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