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SCANNING ELECTRON MICROSCOPY OF GLUCOSE-INDUCED CATARACT TREATED WITH *Garcinia mangostana Linn* PERICARP EXTRACT

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Abstract:

Garcinia Mangostana Linn (GML) plant is one of the biodiversity of wetland area in South Kalimantan. GML pericarp contain various secondary metabolites, one of which is xanthones. Xanthones have antioxidant, antiinflammatory, reductase aldose inhibitor activity. This study aims to prove the potential of GML pericarp in the prevention of cataractogenesis by incubating the clear goat eye lens into a growth medium 199 for 120 hours in 5 groups. The positive control group which only given 30 mM glucose into the medium and the treatment groups that were given 30 mM glucose together with the mangosteen pericarp ethanol extract (EEKM) at a dose of 100, 500 and 1000 µg/ml. The results of scanning electron microscope (SEM) on the lens of the positive control group show irregularities of the cell arrangement of the lens fibers were evident. The lens fiber cells in the group given 100 µg/ml EEKM are still regularly arranged. In the group given 500 µg/ml EEKM, the lens fiber cells are still arranged regularly. In the group given 1000 µg/ml EEKM, the lens fiber cells are arranged regularly like the arrangement of fiber cells in the negative control group. This study proves that EEKM is able to prevent cataracts at least through the maintain the regularity of lens fibre cells and the clarity of the lenses. The drawback of this study is the use of crude extracts from EEKM so that it cannot be known specifically which active compounds are involved in the prevention of cataractogenesis. It can be concluded that the administration of EEKM at doses of 100, 500 and 1000 µg/ml can prevent cataracts and damage to the lens structure of the eye.

Keywords: Cataract; *Garcinia Mangostana* Linn; Lens fibre cell; Scanning electron microscope

Introduction

Cataracts are the leading cause of blindness in the world, and no medicine has been demonstrated to be beneficial in the prevention and management of cataracts other than cataract surgery and removing secondary causes or trauma. There are 2 main risk factors for cataracts, namely age and diabetes mellitus (DM).[1] Administration of antioxidant vitamin supplements such as vitamin C, vitamin E and beta-carotene has not been shown to be effective in preventing or slowing the progression of age-related cataracts.[2,3] Cataracts can only be treated with surgery, however, surgery carries a higher risk of intraoperative and postoperative complications in diabetic cataracts.[4] Poor postoperative visual acuity in patients with diabetes may result from the formation of posterior capsule opacification (PCO) and postoperative cystoid macular edema (CME). The postoperative course may be further complicated by the appearance of diabetic macular edema (DME) or by the worsening of existing diabetic retinopathy.[5]

The number of blindness due to cataracts in Indonesia according to the basic health research data (Riskesdas) of the Ministry of Health of the Republic of Indonesia in 2013 was 1.8% of the total population and every year around 240,000 new cataract sufferers appear. Indonesia is a country with the highest number of cataract sufferers in ASEAN.[6] Cataract is one of the main causes of visual impairment in DM patients with a tendency to suffer from cataracts 5 times greater than those without DM, especially at a young age. The 5-year Beaver Dam Eye study consisting of 3684 participants aged 43 years and over also reported an association between DM and nuclear and cortical cataract formation.[7]

In hyperglycemic states, the crystalline lens is exposed to hyperosmotic aqueous humor and the glucose level is high and progressively increases. Excess intracellular glucose is converted to sorbitol by the enzyme of aldose reductase. Excessive accumulation of sorbitol in the cortex and nucleus of the eye causes a high osmotic gradient in the lens.[8] Accumulated sorbitol causes osmotic stress that can cause changes in lens epithelial cell permeability, decreasing ATPase activity, decreasing crystalline protein synthesis, decreasing amino acid uptake and changes in redox homeostasis.[9] Osmotic stress will cause stress on the endoplasmic reticulum (ER stress) of lens epithelial cells which induces unfolded protein response (UPR). UPR can also cause oxidative stress through the formation of reactive oxygen species (ROS) and can cause lens epithelial cell apoptosis.[10] The activity of SOD and catalase enzymes in cataract lenses with diabetes mellitus patients decreased significantly compared to non-diabetes mellitus patients. This shows that diabetes mellitus causes an increase in oxidative stress which has an impact on the oxidation of biomolecules in the eye lens, resulting in cataracts.[11]

Increased levels of glucose in the cortex and nucleus of the eye also induce non-enzymatic glycation of crystalline proteins in the lens of the eye, resulting in the formation of superoxide radicals and the formation of advanced glycation end products.[12] AGE-crystalline protein binding causes irreversible changes in the structure of the lens crystalline protein that can lead to protein aggregation and the formation of high molecular weight aggregates that scatter light and obstruct vision.[13]

Epidemiological studies and clinical studies of plant-derived compounds such as curcumin, lutein, zeaxanthin, dashen and ginseng on eye diseases in recent years have been widely carried out and have shown beneficial effects.[14] Recent studies have attempted to develop natural flavonoids that have potential as preventive drugs against diabetic cataracts through antioxidant activity, inhibitors of aldose reductase enzyme and inhibition of AGE formation as well as modulation of activity of α -crystalline protein chaperones to prevent lens epithelial cell apoptosis.[15,16]

Garcinia mangostana L. (Guttiferae) or commonly known as the mangosteen plant is

one of the biodiversity in Indonesia. Mangosteen plants are known to contain various secondary metabolites, one of which is a family of tricyclic isoprenylated polyphenols called xanthones.[17] Mangosteen pericarp contains active compounds with high antioxidant activity comparable to quercetin and kaempferol and greater than Trolox; this plant extract also shows protein glycation inhibitory activity comparable to aminoguanidine, anti-inflammatory activity and as inhibitor of aldose reductase.[18,19,20] Garcinia mangostana methanol extract produced polar and non-polar fractions, which were xanthone-rich fractions with anti-AGE formation activity. The polar fraction inhibited the formation of AGEs at the Amadori product stage and the formation of protein aggregation through protein thiol maintenance.[21]

Research on the effect of α mangostin on diabetic rat models showed a decreased effect of AGE-RAGE accumulation in the retinal tissue of the eye.[22] Faisal (2020) insilico study found the 1-isomangostin, 3-isomangostin, γ-mangostin, mangostanol, D-garcinone, and gartanin have potentially could inhibit the interaction and activity of imidazole in RAGE through a competitive binding mechanism. The inhibition of imidazole-RAGE activity by the mangosteen active components may inhibit the pathobiology of AGEs-RAGE axis.[23] With the ability as an antioxidant, the protein glycation inhibitory activity, anti-inflammatory and aldose reductase enzyme inhibitor from the active compound of mangosteen pericarp is thought to prevent cataractogenesis induced by high concentrations of glucose such as cataracts in DM patients.

Problem Formulation

The formulation of the problem in this study is: can the administration of EEKM prevent cataract formation in the lens of the eye exposed to high concentrations of glucose (30 mM)?

Sub problem

1. Can the administration of high concentrations of glucose (30 mM) damage the microscopic structure of the lens fiber cells? Can the administration of EEKM doses of 100, 500 and 1000 µg/ml prevent the microscopic structure of lens fiber cells exposed to high concentrations of glucose?

2. Can the administration of EEKM doses of 100, 500 and 1000 µg/ml prevent clouding (cataract) of the eye lens exposed to high concentrations of glucose?

Research purpose

General purpose

Proving the effect of giving ethanol extract of mangosteen pericarp (EEKM) doses of 100, 500 and 1000 µg/ml in preventing cataractogenesis in the lens of the eye exposed to high concentrations of glucose.

Special purpose

1. Observing changes in the histological structure of the eye lens and assessing the effect of the administration of EEKM doses of 100, 500 and 1000 µg/ml on the damage of the histological structure of the eye lens exposed to high concentrations of glucose (30 mM) by scanning electron microscope (SEM) examination.

2. Assessing the effect of the administration of EEKM doses of 100, 500 and 1000 µg/ml on the changes of the eye lens clarity exposed to high concentrations of glucose (30 mM) for 120 hours (5x24 hours).

Benefits of research

Practical Benefits

This study aimed to empirically prove the effect of giving ethanol extract of mangosteen pericarp (EEKM) at doses of 100 µg/ml, 500 µg/ml and 1000 µg/ml as a drug in preventing cataractogenesis in the lens of the eye due to the exposure to high concentrations of glucose.

Theoretical Benefits

This study is beneficial to determine the pathomechanism of the effect of administering ethanol extract of mangosteen pericarp (Garcinia mangostana linn.) at the doses of 100 µg/ml, 500 µg/ml and 1000 µg/ml in preventing damage to microscopic structures and maintaining the clarity of the eye lens exposed to high glucose concentrations (30 mM).

Research Method

This research was a laboratory experimental study. The research design used was a post test only control group design with in vitro incubation of goat's eye lens into 199 medium from Gibco Grand Island, New York, United States which resembled aqueous humor in the eyeball. This study consisted of 5 groups with the number of samples in the study calculated based on the number of groups (5 groups) with the Federer formula in 1963: (n-1) (t-1) ≥15. Information: t = number of groups per treatment, n = number of samples per group.

The minimum number of samples used for each treatment group was 5 eyepieces for each group. The division of experimental units to treatment groups was carried out by simple random sampling by drawing lots and numbering. The group division was as follows:

K- = eye lens, medium, glucose of 5 mM, as a negative control
K+ = eye lens, medium, glucose of 30 mM, as a positive control
P1 = eye lens, medium, glucose of 30 mM, mangosteen pericarp extract of 100 µg/ml
P2 = eye lens, medium, glucose of 30 mM, mangosteen pericarp extract of 500 µg/ml
P3 = eye lens, medium, glucose of 30 mM, mangosteen pericarp extract of 1000 µg/ml

Goat eye lenses were incubated for 120 hours (5 days) and put in an incubator at 37 degrees Celsius. After 120 hours of incubation, the lens was carefully removed with a spoon to assess its clarity and observed with a scanning electron microscope (SEM) on the lens cortex.

Making of Mangosteen Pericarp Ethanol Extract (EEKM)

The ethanol extract of mangosteen pericarp was a dry extract obtained by hot extraction with the Reflux method. Mangosteen pericarp comes from the mangosteen fruit (Garcinia mangostana Linn.) purchased from Kelayan market in Banjarmasin. The mangosteen fruit were from the forest in the Loksado sub-district, South Kalimantan. The mangosteen fruit has been verified and the mangosteen pericarp was extracted using the Reflux method at the Chemical Analysis Laboratory, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru City, South Kalimantan.

Assessment of eye lens clarity

After the incubation for 120 hours in an incubator at 37 degrees Celsius, all eye lenses were removed. The lens was washed with phosphate buffer solution and placed in a clean glass dish under which a checkered lined paper was placed as a background. The lens was then photographed with the iPhone X's handphpne digital camera.

The degree of opacity of the lens of the eye was determined based on 4 levels. Grade zero (no lens opacities), grade 1 (a slight cloudiness of the lens), grade 2 (uniform opacities) and grade 3 (thick and broad lens opacities). The determination of the degree of lens opacity refered to Jyoti's research.[24]

Scanning electron microscope (SEM) examination

After the incubation for 120 hours in an incubator at 37 degrees Celsius, all eye lenses were removed. The lens was washed with phosphate buffer solution and histopathological preparations were made. The histopathological preparation process was carried out using the paraffin block method. The lens was cut perpendicular to the center of the lens along the midsagittal axis with a microtome knife. SEM examination was performed using a Hitachi TM3000 brand electron microscope at the Biosciences

laboratory, Brawijaya University, Malang to see the arrangement of lens fiber cells.[25]

Results

Clarity of eye lens

In this study, the clarity of the lens was assessed by examining the transverse lines of the strimin paper placed on the lower surface of the lens by dividing the degree of opacity into 4 levels, namely degree 0, degree 1, degree 2 and degree 3.

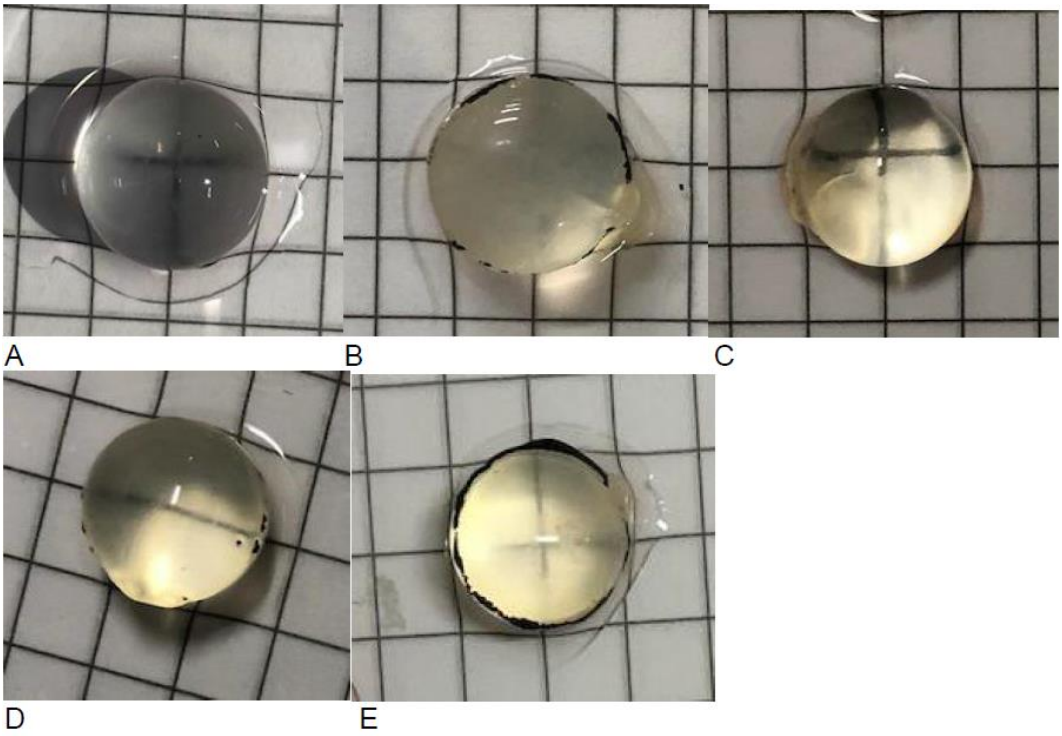

Figure 1. Clarity of the eye lens.
Information: (A) Group I: negative control group lens, (B) group II: positive control group lens, (C) group III: lens with 100 µg/ml ethanol extract of mangosteen pericarp (EEKM) (D) lens with administration of 500 µg/ml ethanol extract of mangosteen pericarp, (E) lens with administration of 1000 µg/ml of ethanolic extract of mangosteen pericarp.

Figure 1 (A) shows that the eye lens in the negative control group had mild cloudiness (grade 1 cataract). In the positive control group (B) it was seen that the lens had cloudiness in all parts of the lens (degree 3 cataract). In the group given EEKM doses of 100 and 500 µg/ml, the lens did not appear cloudy (cataract grade 0). In the group given EEKM at a dose of 1000 µg/ml, the lens appeared to have mild cloudiness (grade 1 cataract).

Microscopic structure of the eye lens by SEM

In this study, lens samples from all groups were examined by scanning electron microscope (SEM) to see the regularity of the arrangement of lens fiber cells in the cortex and lens nucleus area.

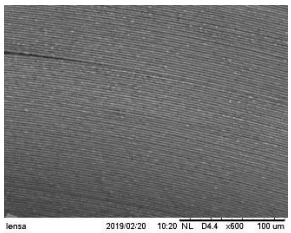

Figure 2. Negative control group at 600x . magnification

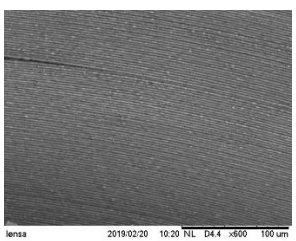

Figure 2. Negative control group at 600x . magnification

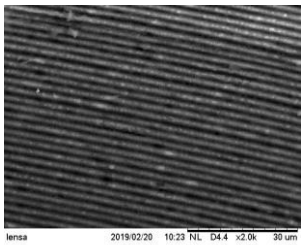

Figure 3. Negative control group at 2000x . magnification

In the negative control group, the lens fiber cells were arranged in an orderly fashion and there was no visible damage to the fiber cells at either 600x or 200x magnification.

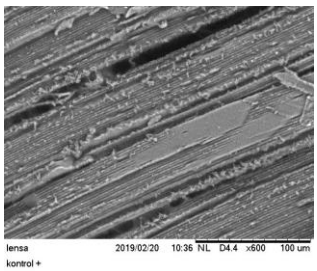

Figure 4. Positive control group at 600x . magnification

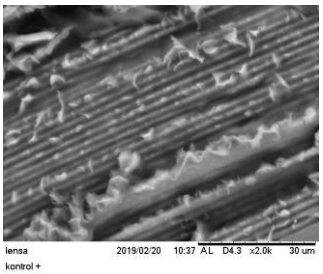

Figure 5. Positive control group at 2000x . magnification

In the positive control group, the irregularity in the arrangement of the lens fiber cells was vividly seen. There may have been severe damage to the microscopic structure of the lens fiber cells that make up the lens cortex. Structural irregularities result in reduced clarity or clouding of the eye lens.

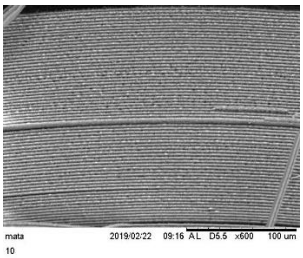

Figure 6. EEKM group of 100 µg/ml at 600x magnification

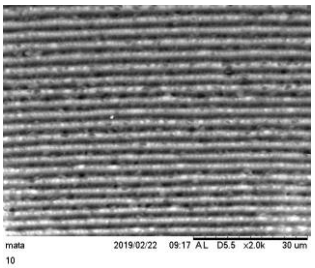

Figure 7. EEKM group 100 µg/ml at 2000x

In the group given ethanol extract of mangosteen pericarp (EEKM) at a dose of 100 µg/ml the lens fiber cells were still arranged regularly. At 2000x magnification, it was seen that the surface of the fiber cells was slightly rough, unlike the surface of the fiber cells of the negative control group.

In the group given the ethanol extract of mangosteen pericarp (EEKM) at a dose of 500 µg/ml the lens fiber cells were still arranged regularly. At 600x magnification, the cell surface was uneven which was more clearly seen at 2000x magnification. It seemed that the surface of the rough fiber cells was not like that of the fiber cell surface of the negative control group.

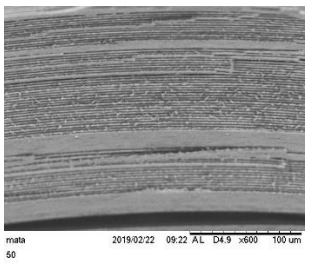

Figure 8. EEKM group of 500 µg/ml at 600x magnification

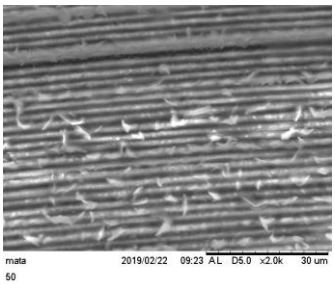

Figure 9. EEKM group of 500 µg/ml at 2000x magnification

In the group given the ethanol extract of mangosteen pericarp (EEKM) at a dose of 1000 µg/ml (figure 5.4, the lens fiber cells were arranged in a regular manner like the arrangement of the fiber cells in the negative control group. At 600x and 2000x magnification, the cell surface was flat as in the fiber cell surface of the negative control group.

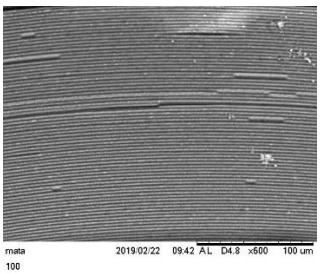

Figure 10. 1000 µg/ml EEKM group at 600x magnification

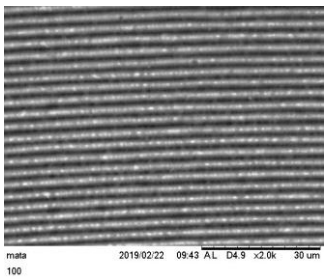

Figure 11. EEKM group 1000 µg/ml at 2000x magnification

The administration of ethanol extract of mangosteen pericarp (EEKM) doses of 100, 500 and 1000 µg/ml can maintain the regularity of the arrangement of lens fiber cells due to exposure to glucose concentrations of 30 mM. At EEKM doses of 100 and 500 µg/ml the regularity of the arrangement of the fibrous cells was still good, but the surface of the fibrous cells did not appear smooth. The administration of EEKM at a dose of 1000 µg/ml can maintain the regularity of the arrangement of the fibrous cells and the surface of the fibrous cells as in the negative control group.

Discussion

Administration of high concentrations of glucose (30 mM) causes opacity of the eye lens

In this study, the opacity of the eye lens in the positive control group began to appear on day 3 of the incubation period. In contrast to the study of Sivashanmugam (2012), the cloudiness of the eye lens began to occur from the edge of the lens, and at the end of the 8th hour on the posterior surface of the lens. This difference may be related to the 30 mM glucose dose used in this study. According to Chowdhury (2014) the lens culture in basic media alone (negative control) did not induce cataracts.[26, 27] When the lens medium was given 55 mM glucose (positive control) cataracts occurred in the eye lens.[26] Lens opacity increased progressively towards the lens nucleus and the total opacity occured at the end of 72 hours.[27] Lens opacity begins to appear after 8 hours on the posterior peripheral surface of the lens which increased progressively towards the center and complete opacities occured at 72 hours.[28]

Oxidative stress increases cell injury through protein oxidation, DNA damage, membrane lipid peroxidation, ER stress, UPR activation and imbalance in calcium homeostasis and others that contribute to cataractogenesis.[29] Glycation of the crystalline lens can cause conformational changes that lead to exposure to thiol group oxidation and the formation of protein cross-links. In addition, the eye lens has almost no turnover so that it accumulates AGEs which in turn causes lens crystal aggregation which produces high molecular weight materials which are responsible for opacities as shown by studies on animals.[30]

The negative control group experienced mild opacity (grade 1 cataract). In the positive

control group, the lens experienced cloudiness in all parts of the lens (degree 3 cataract). In the group given EEKM doses of 100 and 500 µg/ml, the lens did not have opacity (cataract grade 0), whereas in the group with 1000 µg/ml EEKM the lens seemed to have mild opacities (cataract grade 1).

This study proved that the administration of EEKM doses of 100 and 500 µg/ml was able to prevent cataractogenesis in lenses exposed to high concentrations of glucose (30mM).

Lens opacities of degree 1 in the negative control group occurred possibly due to the influence of oxidative stress that occurred before the lens incubation, namely oxidative stress due to ultraviolet B radiation from sunlight when these animals bask for food during the day. The incubation environment of the eye lens and the incubation medium which was not exactly the same as the intraocular environment with natural aqueous humor also caused oxidative stress during the incubation of the lens in medium 199 for 120 hours. In physiological conditions in the aqueous humor of the eye lens there are ascorbate levels as high as 20-70 times compared to other tissues that function to protect the eye lens from oxidative stress.[31]

Administration of glucose concentration of 30 mM damages the microscopic structure of the eye lens

In Figure 8, the negative control group lens SEM results show that the lens fiber cells are arranged in an orderly fashion and there is no visible damage to the fiber cells at either 600x or 2000x magnification. The lens of the eye is a transparent tissue composed of dense fibrous epithelial cells. To maintain homeostasis and lens transparency, it is necessary to circulate water, ions and metabolites. Junctional microdomains connect lens cells and ensure tight cell-to-cell attachment and intercellular flow of fluid through the microcirculation system.[32]

In the positive control group shown in Figure 5.9, the lens fiber cells are arranged in an irregular manner. There may have been severe damage to the microscopic structure of the lens fiber cells that make up the lens cortex. Structural irregularities result in reduced clarity or clouding or opacity of the eye lens. Torres-Bernal's (2014) study which performed scanning electron microscopy (SEM) on the lens of a normal human eye showed the characteristics of the fiber organization with a regular arrangement and interdigitation between fibers. An SEM scan of the lens of an eye with senile cataract or diabetic cataract shows an irregular and disorganized pattern of lens fibers.[33]

Buzhynskyy's (2011) study demonstrated reduced connexon at the edge of the junctional micro domain in senile cataract and type-II diabetic cataract. The absence of connexons was confirmed using gel electrophoresis and mass spectrometry techniques. Individual transmembrane Aquaporin 0 (AQP0) channels appear to remain unaffected, whereas connexons are degraded during cataract formation. As a consequence of the absence of a connexon, AQP0 deforms and becomes indistinct like downy thing. They branch into rows of several AQP0 molecules.[32]

Administration of EEKM doses of 500 and 1000 µg/ml was proven to prevent damage to the microscopic structure of the eye lens exposed to 30 mM glucose.

In the group given ethanol extract of mangosteen pericarp (EEKM) at a dose of 100 µg/ml the lens fiber cells were still arranged regularly. At 2000x magnification, the surface of the fiber cells was slightly rough, unlike the surface of the fiber cells of the negative control group. In the group given the ethanol extract of mangosteen pericarp (EEKM) at a dose of 500 µg/ml, the lens fiber cells were still arranged regularly. At 600x magnification, the cell surface was uneven which was more clearly seen at 2000x magnification. It seemed that the surface of the rough fiber cells was not like that of the fiber cell surface of the negative control group. In the group given the ethanol extract of mangosteen pericarp

(EEKM) at a dose of 1000 µg/ml (figure 5.4 the lens fiber cells were arranged in a regular manner like the arrangement of the fiber cells in the negative control group. At 600x and 2000x magnification, the cell surface was flat as in the fiber cell surface of the negative control group.

The administration of glucose 30 mM increased MDA, CML, DDIT3, and GRP78, and active caspase 3 levels. Groups which treated with GML pericarp extract at the doses of 100, 500 and 1000 µg/ml decreased MDA, CML, GRP78, and DDIT3, and active caspase 3 levels inside the lens. On lens capsule, the CML and GRP78 levels increased. Ethanol extract of GML pericarp potentially showed antioxidant, anti-glycation, DDIT3 inhibitor, and anti-apoptotic activity in Goat cultured lenses.[35]

The administration of ethanol extract of mangosteen pericarp (EEKM) at the doses of 100, 500 and 1000 µg/ml was proven to maintain the regularity of the arrangement of the lens fiber cells due to exposure to glucose concentrations of 30 mM. In the administration of EEKM doses of 100 and 500 µg/ml, the regularity of the arrangement of the fibrous cells was still good, but the surface of the fibrous cells did not appear smooth. Administration of EEKM at a dose of 1000 µg/ml can maintain the regularity of the arrangement of the fibrous cells and the surface of the fibrous cells as in the negative control group. The administration of EEKM is proven to be able to prevent damage to the microscopic structure of the epithelial cell arrangement of the lens fibers of the eye.

Conclusions

The administration of 30 mM glucose concentration has been shown to cause lens opacities and changes in the microscopic structure of the lens fiber cells. The administration of EEKM doses of 500 and 1000 µg/ml was proven to prevent damage to the microscopic structure of the eye lens exposed to 30 mM glucose.

It is necessary to conduct further researchers using in vivo methods on diabetic animal models.

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