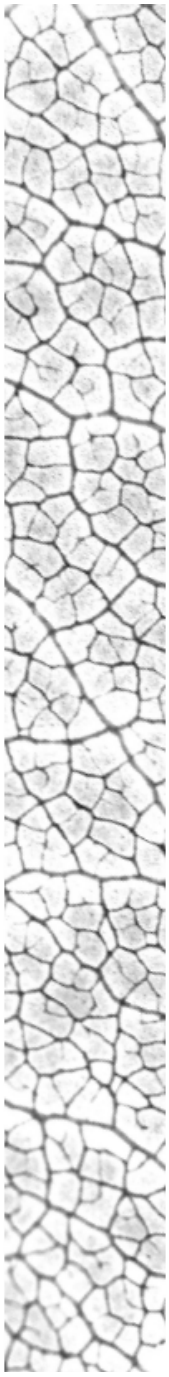

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Protective effect of methanol extract of Kelor (*Moringa oleifera*) leaves on Glutathione Peroxidase (GPx) levels in trabecular meshwork cell culture of primary congenital glaucoma patients

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Abstract

Background: The aim of this study was to determine differences in glutathione peroxidase (GPx) activity after multiple doses of methanol extract of *Moringa oleifera* leaves in a trabecular meshwork cell culture from primary congenital glaucoma patients.

Material and Methods: This was an experimental laboratory study; cell line cultures were divided into four groups (negative control and three treatment groups). The treatment groups were exposed to methanol extract of *Moringa oleifera* leaves at dosages of 15 µg/mL, 20 µg/mL, and 25 µg/mL. After four hours incubation, GPx activity was measured in all groups using a colorimetric microplate reader.

Results: ANOVA analysis found that there was a difference in GPx activity between treatment groups. Each *Moringa oleifera* extract dosage (15 µg/mL, 20 µg/mL, and 25 µg/mL) significantly differed from each other, with dosage 25 µg/mL having the highest GPx activity. The correlation analysis showed there was a significant association between exposure to *Moringa oleifera* extract and GPx activity with a positive correlation coefficient ($r = 0.962$, $p < 0.005$), which means higher dosages of *Moringa oleifera* extract will increase GPx activity. Linear regression analysis found thet every 1 mg/mL dosage of *Moringa oleifera* extract will increase GPx activity by 1.217 mU/mL.

Conclusion: It can be concluded that there were differences in GPx activity between treatment groups and there was an influence of dosage of methanol extract of *Moringa oleifera* leaves on GPx activity, such that increasing doses of methanol extract of *Moringa oleifera* leaves will further increase GPx activity.

Keywords: Gluthathione Peroxidase (GPx), methanol extract, *Moringa oleifera* leaves, primary congenital glaucoma

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BACKGROUND

Primary congenital glaucoma (PCG) is a specific form of glaucoma in children characterized by a developmental disorder of the trabecular meshwork that is unrelated to other developmental disorders and may be followed by increased intraocular pressure. PCG is an eye disorder that contributes to 0.01-0.04% of total blindness. About 60% were diagnosed with PCG at the age of six months and 80% were diagnosed within the first year of life (Krishnadas and Ramakrishnan 2008).

Increased intraocular pressure (IOP) in PCG, due to developmental abnormalities from the anterior chamber angle, cause a decrease in aqueous humor outflow. In some studies, it is said to be due to obstruction of the aqueous outflow in congenital glaucoma. Barkan assumed that imperfect resorption of mesodermal tissue causes the formation of membranes that cross the anterior chamber angle, called the Barkan membrane. Maumenee demonstrated there is a disorder in anterior ciliary muscle insertion that attaches directly to the trabecular meshwork (TM) compared to scleral spur and iris root attachment to trabecular meshwork (Saccà et al. 2007).

Oxidative damage is an important pathogenesis for triggering trabecular meshwork degeneration, which results in high ocular pressure. Oxidative stress can affect the trabecular meshwork and retinal ganglion cells (RGC) and may involve the death of neuron cells that

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affect the optic nerve in glaucoma patients. An increase in oxidative damage was seen in TM from patients with glaucoma. Antioxidants, such as resveratrol, ascorbate, p-coumarate and antioxidant enzyme cofactors (rGSH), protect TM cells from H_2O_2 that can cause cellular damage. Glutathione peroxidase (GPX) is an antioxidant enzyme that has selenocysteine at its active site and depends on selenium for its activity. Glutathione peroxidase is found in the cytosol and mitochondria of a number of tissues (Repetto et al. 2012).

The methanol extract of *Moringa oleifera* leaves contains nutrients that inhibit the occurrence of oxidative stress. Methanol antioxidant levels (quercetin and kaempferol) of *Moringa oleifera* leaves are higher than other plants. Water, methanol and ethanol extracts of *Moringa oleifera* leaves have been studied for a role in blocking free radicals and having antioxidant activity. Among the three extracts, the methanol extract (80%) and ethanol extract (70%) of *Moringa oleifera* leaves showed higher antioxidant activity and were the best solvents for extraction of antioxidant components of *Moringa oleifera* leaves (Jung 2014).

This was an interventional study about methanol extract of *Moringa oleifera* leaves against increased glutathione peroxidase levels in trabecular tissue culture from PCG patients. From the results of this study, it is expected that methanol extract of *Moringa oleifera* leaves can be an alternative treatment for trabecular tissue damage in PCG.

MATERIALS AND METHODS

This study was an experimental laboratory study conducted in vitro on a culture of the trabecular meshwork. The study was conducted in Physiology Laboratory of Molecular Physiology Division, Faculty of Medicine Brawijaya University Malang, from May 2016 until July 2016.

The samples for this experiment were trabecular tissue cultures exposed to methanol extract of *Moringa oleifera* leaves. The trabecular tissue cultures were divided into four groups, as follows: Control group: a group that did not get any exposure; Treatment group I: group exposed to 15 mg/mL methanol extract of *Moringa oleifera* leaves; Treatment group II: group exposed to 20 mg/mL methanol extract of *Moringa oleifera* leaves; and Treatment group III: group exposed to 25 mg/mL methanol extract of *Moringa oleifera* leaves dosages.

In this study, the number of samples was calculated using the formula (t-1) (r-1) ≥ 15, with t = number of experiment groups

r = number of replications

In this study, there were four experimental groups, with six replicates in each group. Thus, the number of samples required in this study was 24. Exclusion criteria in this study were if trabecular meshwork cell culture of PCG patients was contaminated by fungus or there was a mistake in treatment (wrong dosage, incorrect incubation time).

Trabecular meshwork tissue culture was obtained from the American Type Culture Collection (ATCC), Rockville, MD. The methanol extract of *Moringa oleifera* leaves was the result of *Moringa oleifera* leaves extracted with 80% methanol made at Pharmacology Laboratory of FKUB (Faculty of Medicine Brawijaya University).

GPx activity in this study was measured by a colorimetric microplate reader. The microplate reader also called a plate reader or microplate photometer is a tool to detect biological, chemical or physical content in microtiter plates. This tool can detect GPx activity in the 40-800 U/L range. The samples used were 10 µL of supernatant to which was added 40 µL of reaction mix reagent consisting of assay buffer, NADPH, GSH solution and glutathione reductase (GR). They were incubated for 15 minutes; the optical density of the samples was measured at 340 nm. Then, 5 µL of hydroperoxide was added to the samples and optical density was measured again at 340 nm using kinetic mode. The examination was performed three times and the average was calculated. The data for differences between optical density in samples and controls were recorded and processed to obtain GPx activity with units of nmol/min/mL or mU/mL (Avissar et al. 1996).

Obtained data analyzed using SPSS 16 for Windows. Kolmogorov-Smirnov test was performed to determine the normality of glutathione peroxidase data, and Levene's test was performed to determine the homogeneity of control and treatment samples.

One-way ANOVA test was performed to determine whether there was a difference in glutathione peroxidase levels between groups. If the ANOVA test result showed a significant difference in glutathione peroxidase levels, it was followed by Post Hoc Tukey test to determine the differences between each group. To determine the effect of methanol extract of Moringa oleifera leaves on glutathione peroxidase levels, regression test and correlation were performed.

RESULTS

Cell culture in this study used trabecular cell culture of PCG patients obtained from Shandong Kexing Bioproducts Co., Ltd. China. The description of the trabecular cell culture results is shown in the **Fig. 1**. Dosages of 15 µg/mL, 20 µg/mL, and 25 µg/mL, as well as positive control, were used to determine the effect of methanol extract of *Moringa oleifera* leaves on GPx levels in trabecular cell culture of PCG. Then the results of the study are as listed in **Table 1**.

Based on the examination of data normality using Kolmogorov-Smirnov test, data of GPx levels in trabecular cell culture of PCG has significance value of 0.556 ($p>0.05$) (**Table 2**). Therefore, it can be concluded

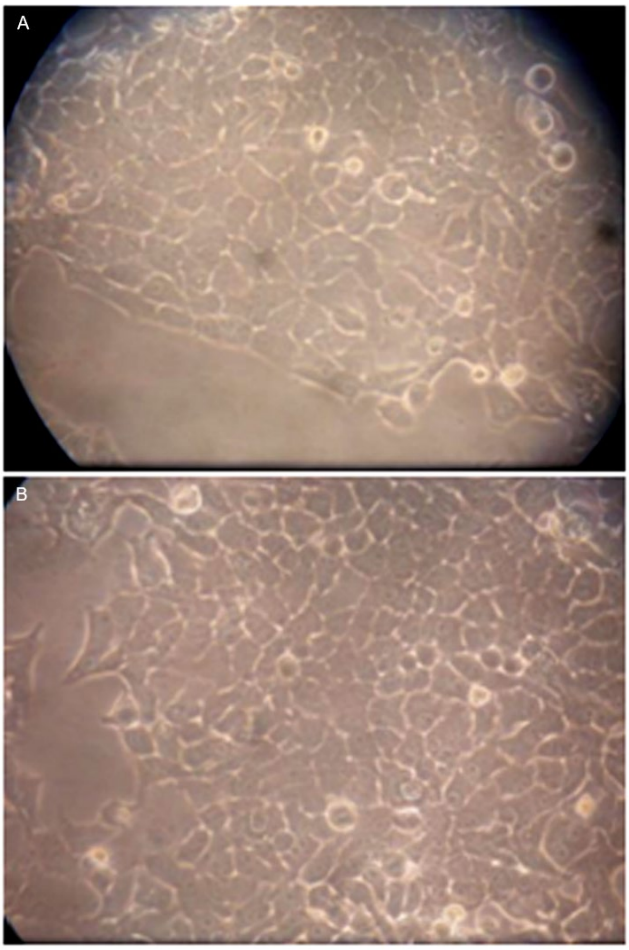

Fig. 1. Trabecular cell culture of primary congenital glaucoma observed using light microscope, without staining at 400x magnification. (A) Cell culture on the third day after incubation, cell shape is polygonal, thin, with a flat surface. Cells that are still around and floating are cells that have just divided, the cells are young and undifferentiated (arrows). (B) Cell culture after 13 days incubation, cells are confluent (arrow)

Table 1. The Research Results of Gpx Levels in Trabecular Cell Culture of Primary Congenital Glaucoma

Group	Sample Replicate (mU/mL)						Mean ± Std. dev.
	1	2	3	4	5	6	
Control	7.46	8.16	11.78	8.09	9.56	12.00	9.51 ± 1.97
MO 15 µg/mL	19.49	21.95	22.71	20.90	21.89	26.87	22.30 ± 2.50
MO 20 µg/mL	35.80	31.37	31.35	31.37	33.13	35.87	33.15 ± 2.19
MO 25 µg/mL	42.62	43.19	36.28	40.69	36.84	42.03	40.27 ± 3.00

Source: Data of Research Results

that the data is spread following a normal distribution. The examination showed that the Levene's test value for data of glutathione peroxidase in trabecular meshwork cell culture had a significance value (p) of 0.613 (p>0.05) (**Table 3**). It can be concluded that the data range of glutathione peroxidase on trabecular meshwork cell culture is homogeneous.

Table 2. Table of Normality Test

Variable	K-S Statistic	Value of Significance	Conclusion
GPx levels in trabecular cell culture of PCG	0.793	0.556	Data has normal distribution

Source: Processed Primary Data
Description: K-S = *Kolmogorov-Smirnov test Z*

Table 3. Homogeneity Test using Levene's Test

Information	Levene statistic	the	Decision
Glutathione peroxidase levels	0.616	0.613	Homogeneous

Source: Results of Data Analysis

Table 4. Table of ANOVA Test Result

Information	p-value
GPx levels in trabecular cell culture of primary congenital glaucoma	0.000

Source: Results of Data Analysis

Table 5. Table of Tukey Double Comparison Test for GPx Levels

Comparison between groups		Difference of mean	Sig.	Decision
Control	Dosage of 15 µg/mL	-12.794	0.000	Significantly different
	Dosage of 20 µg/mL	-23.639	0.000	Significantly different
	Dosage of 25 µg/mL	-30.766	0.000	Significantly different
Dosage of 15 µg/mL	Dosage of 20 µg/mL	-10.845	0.000	Significantly different
	Dosage of 25 µg/mL	-17.972	0.000	Significantly different
Dosage of 20 µg/mL	Dosage of 25 µg/mL	-7.162	0.000	Significantly different

Information: If the value of significance (p) < alpha 0.05 = there is a significant difference.
If the value of significance (p) > alpha 0.05 = there is no significant difference

Based on the results of the variant analysis with ANOVA (**Table 4**) for GPx levels in trabecular cell culture of PCG data, there was a significance value of 0.000 ($p < 0.05$), therefore Ho is rejected, and it can be concluded that there is a difference in GPx levels in trabecular cell culture of PCG in the treatment groups of methanol extract of *Moringa oleifera* leaves.

After the ANOVA test, a double comparison test (Tukey's Test) was performed on each treatment (**Table 5**), indicating that the mean GPx levels in the control group were significantly different (meaningful) from GPx levels in the groups given methanol extract of *Moringa oleifera* leaves at 15 µg/mL, 20 µg/mL and 25 µg/mL dosages ($p<0.05$).

To determine the amount of correlation and effect of giving methanol extract of *Moringa oleifera* leaves on mean GPx levels in trabecular cell culture of PCG, correlation test, and linear regression test were performed.

Based on the results of the analysis in the above table, it can be seen that the administration of methanol extract of *Moringa oleifera* leaves affects glutathione peroxidase levels in trabecular meshwork cell culture of PCG ($r=0.962$, $p=0.000$) with significant positive correlation ($p<0.05$) (because the correlation coefficient

Table 6. Correlation Test

Information	r	p-value	Conclusion
Administration of methanol extract of *Moringa oleifera* leaves on glutathione peroxidase levels in trabecular meshwork cell culture of primary congenital glaucoma	0.962	0.000	a significant correlation

Source: Processed Primary Data

Table 7. The Results of Regression Equations at Each Dosage

Regression equation	R *Square*
Y = 8.057 + 1.217X (Dosage of methanol extract of *Moringa oleifera* leaves)	92.5%

Information: Y = glutathione peroxidase levels in trabecular meshwork cell culture of PCG,
X = methanol extract of *Moringa oleifera* leaves

is positive). It means that higher dosage of methanol extract of *Moringa oleifera* leaves will tend to increase glutathione peroxidase levels in trabecular meshwork cell culture of PCG greater than the lower dosage of methanol extract of *Moringa oleifera* leaves, and otherwise.

How big the effect of methanol extract of *Moringa oleifera* leaves was on glutathione peroxidase levels in trabecular meshwork cell culture of PCG can be determined using relationship form analysis (regression), because the correlation test cannot explain it yet. Based on the test results using linear regression analysis, with the results of the regression equation at each dosage as shown in **Table 7**.

The regression equation for the effect of methanol extract of *Moringa oleifera* leaves on glutathione peroxidase levels in trabecular meshwork cell culture of PCG is Y = 8.057 + 1.217X (dosage of methanol extract of *Moringa oleifera* leaves), where Y is the glutathione peroxidase levels in trabecular meshwork cell culture of PCG; whereas, X is the treatment dosage of methanol extract of *Moringa oleifera* leaves.

This can be interpreted that, without considering the effect from the methanol extract of *Moringa oleifera* leaves, the glutathione peroxidase levels on trabecular meshwork cell culture of primary congenital glaucoma will tend to remain constantly high at 8.057 mU/mL (because the coefficient of the constant is positive).

DISCUSSION

From the results of the examination in this study, the average GPx levels obtained in the control group was 9.51 ± 1.97 mU/mL. The control group was trabecular cell culture of primary congenital glaucoma group that received no exposure. There has never been any research for measurement of GPx levels in trabeculae of PCG. However, GPx levels when compared to normal human lung epithelial lining fluid were slightly higher at 6.7 ± 0.64 mU/mL (Avissar et al. 1996, Gunjal et al. 2010).

As for the trabecular cell culture of PCG that received treatment, exposure to methanol extract of *Moringa oleifera* leaves of dosages of 15 µg/mL, 20 µg/mL and 25 µg/mL, generally, showed an increase in GPx levels. On microscopic observation, trabecular cell cultures that received treatment did not show any cell death cell; whereas, if death cell was found the dosage treatment could be said to be toxic.

The result of analysis using ANOVA test showed $p<0.05$; therefore, Ho is rejected, and it can be concluded that there is a difference between dosage groups of methanol extract of *Moringa oleifera* leaves of glutathione peroxidase levels in trabecular meshwork cell culture of primary congenital glaucoma. Post Hoc Test (Tukey Test) analysis, which is a further test of ANOVA, showed that there is a significant difference between GPx levels at each dosage.

In the correlation test, there was a significant correlation between administration of methanol extract of *Moringa oleifera* leaves and glutathione peroxidase levels in trabecular meshwork cell cultures. This correlation has a strong positive correlation coefficient ($r=0.962$, $p=0.000$), which signifies that the greater the dosage of ethanol extract, the greater the GPx levels.

In the regression test, the regression equation obtained from the effect of methanol extract of *Moringa oleifera* leaves on glutathione peroxidase levels in trabecular meshwork cell culture of primary congenital glaucoma was Y = 8.057 + 1.217X (where Y is GPx levels and X is the dosage of methanol extract of *Moringa oleifera* leaves). This test yields R2 = 92.5%, so it can be said that giving methanol extract of *Moringa oleifera* leaves are very influential on trabecular meshwork cell culture of primary congenital glaucoma up to 92.5%.

There have been several previous studies on *Moringa oleifera* leaves extract on GPx activity in mouse with several models, such as alcohol-induced hepatotoxic model, hydroxyurea-induced testicular toxicity model and cardiotoxicity model induced by isoproterenol (Alía et al. 2006, Gunjal et al. 2010, Saalu et al. 2012).

In alcohol-induced hepatotoxic model mice, the administration of *Moringa oleifera* leaves extract (at a dosage of 300 mg/kgBW/day oral for 56 days) alone did not result in a significant increase of GPx in serum. Giving only alcohol (5 mg/kgBW/day oral for 56 days) will decrease GPx significantly. Giving *Moringa oleifera* leaves extract after administration of alcohol (alcohol as much as 5 mg/kgBW/day oral for 56 days followed by *Moringa oleifera* leaves extract at dosage of 300 mg/kgBW/day oral for 56 days) showed an increase in GPX activity compared to the alcohol-only group ($p<0.05$). The conclusion of the study done by Saalu et al. was that *Moringa oleifera* leaves extract provides protection against oxidative damage due to its ability as an antidote to free radicals (Saalu et al. 2012).

In another study using a cardiotoxic mouse model induced by isoproterenol (ISO), it was found from serum biochemical assay tests that in the negative control

group (treated only with ISO 85 mg/day for 23 days) there was a significant decrease of GPx antioxidant activity. The group given a treatment of *Moringa oleifera* leaves extract, 500 mg/day, along with administration of ISO, 85 mg/day, for 23 days was shown to successfully prevent the decrease in this enzymatic activity ($p<0.05$). However, there were no similarly significant results in the dosage treatment group of 250 mg/day. These results suggest *Moringa oleifera* leaves extract provides a prophylactic cardioprotective effect (Gunjal et al. 2010).

In a mouse model of testicular toxicity, it was found that administration of *Moringa oleifera* leaves extracts increased GPx levels in mice given hydroxyurea compared with positive controls (hydroxyurea-induced only). However, these results were not statistically significant (Saalu et al. 2011).

Previously, there were two studies about *Moringa oleifera* leaves extract in trabecular cell culture of primary congenital glaucoma. The first study was conducted by Suryani et al. (2016), who observed changes in malondialdehyde (MDA) levels in the trabecular cell culture of PCG given *Moringa oleifera* leaves extract (Suryani et al. 2016). MDA is a metabolite of lipid peroxidation caused by free radicals. The results of the research showed that methanol extract of *Moringa oleifera* leaves at 5 µg/mL, 15 µg/mL and 25 µg/mL dosages had an effect or influence on MDA levels in trabecular cell culture of PCG, where a higher dosage is given will further decrease MDA levels in trabecular cell culture. There was a significant difference between MDA levels in trabecular cell culture of the PCG control group (group without any treatment) and trabecular cell cultures of treatment groups in the form of exposure to methanol extract of *Moringa oleifera* leaves at dosages of 5 µg/mL, 15 µg/mL, and 25 µg/mL. Among the three dosages, the most effective dosage to decrease MDA levels was 15 µg/mL (Suryani et al. 2016).

A second study conducted by Rijal et al (2016) observed changes in caspase-3 expression (apoptosis) in trabecular cell culture of PCG given *Moringa oleifera* leaves extract. The results showed a significant change in caspase-3 expression (apoptosis) after administration of methanol extract of *Moringa oleifera* leaves at dosages of 20 µg/mL, 30 µg/mL and 40µg/mL in trabecular meshwork cell cultures of primary congenital glaucoma. There was a significant effect between dosages of methanol extract of *Moringa oleifera* leaves on caspase-3 expression (apoptosis), where giving higher dosages will decrease apoptosis in trabecular meshwork cell culture (Rijal et al. 2016).

Limitation of this study includes that this study is relatively new so the relationship between increasing GPx levels and pathogenesis of PCG through trabecular tissue damage still requires more research. Another weakness of this study is that more treatment groups and more varied incubation times are needed to determine the effective dosage of methanol extract of Moringa oleifera leaves. In addition, the toxicity of methanol extract of Moringa oleifera leaves on the cell line also still requires further research.

CONCLUSION

The methanol extract of *Moringa oleifera* leaves of dosages 15 µg/mL, 20 µg/mL and 25 µg/mL showed an effect or influence on GPx levels on trabecular cell culture of PCG, where higher dosages will increase GPx levels in trabecular cell culture.

There was a significant difference in GPx levels in trabecular cell culture of PCG in the control group (group without any treatment) compared with trabecular cell culture of treatment groups in the form of exposure to methanol extract of *Moringa oleifera* leaves at dosages of 15 µg/mL, 20 µg/mL and 25 µg/mL. Of the three dosages, the most effective dosage to increase the GPx levels was the dosage of 25 µg/mL.

The suggestion from this study is that further studies are needed to determine dosage effectiveness and toxicity dosage of methanol extract of *Moringa oleifera* leaves in trabecular cell culture of PCG. In addition, to determine the subsequent benefits from increased GPx levels in trabecular of PCG, further research is needed so that methanol extract of *Moringa oleifera* leaves can be used clinically.

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REFERENCES

Alía M, Mateos R, Ramos S (2006) Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). European J Nutri, 45(1): 19-28. https://doi.org/10.1007/s00394-005-0558-7

Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW, Watkins RH, Khullar P, Xu YL, Cohen HJ (1996) Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. Am J Physiol, 270(2 Pt 1): L173-182. https://doi.org/10.1152/ajplung.1996.270.2.L173

Gunjal MA, Shah AS, Wakade AS, Juvekar AR (2010) Protective effect of aqueous extract of Moringa oleifera Lam. stem bark on serum lipids, marker enzymes, and heart antioxidants parameters in isoproterenol-induced cardiotoxicity in Wistar rats. IJNPR, 1(4): 1-5.

Jung IL (2014) Soluble Extract from Moringa oleifera Leaves with a New Anticancer Activity. PloS One, 9(4): e95492. https://doi.org/10.1371/journal.pone.0095492

Krishnadas R, Ramakrishnan R (2008) Congenital Glaucoma-a brief review. J Curr Glaucoma Pract, 2(2):17-25. https://doi.org/10.5005/jp-journals-10008-1028

Repetto M, Semprine J, Boveris A (2012) Lipid peroxidase: chemical mechanisms, biological implication, and analytical determination. Biochem Genet Mol Biol. Lipid Peroxidation, Dr. Angel Catala (Ed.), InTech. Retrieved on 10 December 2017 from https://www.intechopen.com/books/lipid-peroxidation/lipid-peroxidation-chemical-mechanism-biological-implications-and-analytical-determination

Rijal S, Wulandari LR, Sujuti H (2016) Pengaruh Ekstrak Methanol Daun Kelor (Moringa Oloifera) terhadap Ekspresi Caspase-3 pada Kultur Sel Trabekular Meshwork Penderita Glaukoma Kongenital Primer. Indonesia. Bagian Ilmu Kesehatan Mata Fakultas Kedokteran Universitas Brawijaya – Rumah Sakit dr. Saiful Anwar Malang.

Saalu LC, Ogunlade B, Ajayi GO, Oyewopo AO, Akunna GG, Ogunmodede OS (2012) The hepato-protective potentials of Moringa oleifera leaf extract on alcohol-induced hepato-toxicity in Wistar rat. Am J Biotechnol Mol Sci, 2(1): 6-14. https://doi.org/10.5251/ajbms.2012.2.1.6.14

Saalu LC, Osinubi AA, Akinbami AA (2011) Moringa oleifera Lamarck (drumstick) leaf extract modulates the evidence of hydroxyurea-induced testicular derangement. Int J Applied Res Nat Prod, 4(2): 32-45.

Saccà SC, Izzotti A, Rossi P, Traverso C (2007) Glaucomatous outflow pathway and oxidative stress. Exp Eye Res, 84(3):389-399. https://doi.org/10.1016/j.exer.2006.10.008

Suryani I, Wulandari LR, Sujuti H (2016) Pengaruh Ekstrak Methanol Daun Kelor (Moringa Oloifera) terhadap Kadar MDA pada Kultur Sel Trabekular Meshwork Penderita Glaukoma Kongenital Primer. Indonesia. Bagian Ilmu Kesehatan Mata Fakultas Kedokteran Universitas Brawijaya – Rumah Sakit dr. Saiful Anwar Malang.