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Ginkgo Biloba Extract Effect on Oxidative Stress Marker Malonildialdehyde, Redox Enzyme Gluthation Peroxidase, Visual Field Damage, and Retinal Nerve Fiber Layer Thickness in Primary Open Angle Glaucoma

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Abstract: Objective: To investigate ginkgo biloba extract effect on oxidative stress marker malonildialdehyde, redox enzyme gluthation peroxidase, visual field damage and retinal nerve fiber layer thicknessin primary open angle glaucoma.
Methods : An experimental study, prospective, double blind was conducted at the Adam Malik Hospital from Agustus 2012 to Agustus 2013 after approved by the Ethics Committee for Health Research University of North Sumatera School of Medicine. Diagnosis of Primary Open Glaucoma was based on presence of an open iridocorneal angle, the characteristic appearance of glaucomatous optic neuropathy such as enlargement of optic cup ratio, focal thinning of neuroretinal rim, bayoneting and corresponding visual field defects and elevated intraocular pressure. Subjects underwent assessment visual field defects with Octopus 301, retinal nerve fiber layer thickness with Cirrus HD-OCT and venous blood was taken to measure plasma levels of oxidative stress marker malonildialdeyde(MDA) and redox enzyme gluthathion peroxidase (GPx).Then subjects were divided into 2 groups. The first group was given 40 mg GBE 2 times daily and 20 patients POAG as a control given placebo (identical capsules filled with 40 mg fructose) for 6 months. We evaluated MDA and GPx, visual field for the change of progression rate using Mean Deviation (MD) and Pattern Standart Deviation (PSD), retinal nerve fiber layer thickness (RNFL) both of groups before and after treatment.
Results : After GBE treatment, a signicant improvement in oxidative stress marker and redox enzyme indices at the sixth month was recorded: MDA level (p=0.001) and GPx level (p=0,001), visual fields MD (p=0,011), PSD (p=0,020), and retinal nerve fiber layer superior (p=0,001), inferior (p =0,035). No significant changes were found in intraocular pressure, retinal nerve fiber layer nasal, temporal, mean and optic nerve head after GBE extract or placebo.
Conclusion: GBE administration as a neuroprotective and antioxidant slowed of visual field and retinal nerve fiber layer damage in primary open angle glaucoma.
Keywords : malonyldialdehyde (MDA), gluthathion peroxidase (GPx), ginkgo biloba, primary open angle glaucoma.

Introduction

Glaucoma refers to a group of diseases that have common a characteristic progressive optic neuropathy with associated with visual function loss and higher of intraocular pressure as a risk factor[(1,2)]. Glaucoma

usually called “the silent thief of sight” because the onset usually suddenly without symptom beforely. Glaucoma can caused irreversible blindness worldwide, including in Indonesia[3].

The pathogenesis of glaucoma is multifactorial and until now still investigate.Retinal ganglion cell (RGC) death due to apoptosis and loss of RGC axons leads to glaucomatous optic neuropathy. Many factors play role in pathogenesis glaucoma included genetic, glutamate excitotoxicity, NMDA, nitric oxide and oxidative stress[4]. Oxidative stress appears play a role in progressive neuronal death that is characteristic of glaucomatous optic nerve damage[4,5]. Oxidative stress generally is induced through formation of multiple reactive oxigen species including hydrogen peroxide and superoxide that can initiate and propagate free radicals.The oxidative burden between prooxidant and antioxidant is oxidative stress that damages cellular and tissue macromolecules such as lipids, proteins and results in cellular and tissue dysfunction and cellular death. After identification of the circulating autoantibodies against antioxidant stress enzymes and gluthathion S-transferase.

Many biologic substances growth as an oxidative stress marker especially malonyldialdehyde (MDA). Beside MDA, catalase and sodium dismutase also as oxidative stress marker[6]. Increased marker of oxidative stress that have been reported in glaucoma included oxidized DNA bases, lipid oxidation products and total oxidative stress marker. A number of studies in vitro and in vivo suggested that there was a role of oxidative stress marker in glaucoma patient[5,7]. Haefiger et al reported destroyed oxidized DNA in trabecular meshwork change of matrix extracellular regulate and cytokine in glaucoma patient[8]. Flammer reported the destroyed of DNA in trabecular meshwork was higher in glaucoma patients compared to normal subjects[9].

The correlation between oxidative stress marker with glaucoma has already been reported. Flammer reported the concentration of oxidative nucleotide modification (8-OH-dG) in trabecular meshwork with glaucoma patients were correlated with the higher of intraocular pressure and decreased of visual field. Faschinger et al reported there was a positive correlation between malonyldialdehyde (MDA) in aquous humor glaucoma patients compared to normal subjects[9].

The goal treatment in glaucoma to preserve visual function by lowering IOP below a level that is likely to produce futher damage of the nerve[2]. Beside of lowering IOP, until now still investigate neuroprotective to repair the the optic nerve head damage dbecause glaucoma leads to the loss of retinal ganglion cells and their axons but also to tissue remodeling which involved both of the optic nerve head and the retina. The optic nerve gets thinner and the cells of the lateral geniculate ganglion disappear partially. Nevertheless, there is little doubt that other risk factors besides IOP are involved so that even an ideal IOP does not stop progression in all patients[10].

Ginkgo biloba is one of neuroprotective and also as an antioxidant. The extract of ginkgo biloba leaves have been used to treat various disorders such as asthma, vertigo, fatique and circulatory problems.These extracts consist mainly of flavonoids and terpenoids. One of the main extract is EGb 761. Most pharmacological, toxicological and clinical studies have focused on the neuroprotective value of the main extract[11]. Winter reported intake ginkgo biloba extract 100 mg/kg in mice for 4-8 weeks can increased memorized[11]. Kriglestein and Cowokers reported given ginkgo biloba can decreased infarc area in brain surface mice before occlusion happened and ginkgo biloba injection after global forebrain ischemia can increased cerebral outflow[12].

In eyes, Evan reported ginkgo biloba have been tried in age related macular degeneration and founded restore of retinal ganglion cell[13]. Hiroka et all reported in mice wiith increased intraocular pressure have been tried with ginkgo biloba treatment for 5 months and founded restore the retinal ganglion cell compared to control[14].

From these studies, the objective of this research is to provide a scientific opinion on the indications for Ginkgo Biloba as a neuroprotective and antioxidant in primary open angle glaucoma.

Subjects and Methods

Subjects

This was a prospective, noninvasive, experimental study comprising fourty patients primary open angle glaucoma divided into two groups. The first group 20 patients POAG was given 40 mg GBE 2 times daily and 20 patients POAG as a control given placebo (identical capsules filled with 40 mg fructose) with the same age range and sexwere included in this study for the six months. All the patients given standart therapy with combination latanoprost 0,005% and timololmaleat 0,5%. The patients measured the first day came to hospital before treatment, third months and sixth months after treatment.These subjects were recruited consecutively at Haji Adam Malik Hospital North Sumatera, Indonesia. The current study adhered to the tenets of the Declaration of Helsinki.The institusional review boards of Hospital reviewed and approved the research. All subjects provided written informed consent and all subjects underwent ophthalmologic examination included measured of best corrected visual acuity (BCVA), intraocular pressure by Goldman applanation tonometry and slitlamp examination, gonioscopic(Carl Zeiss Meditec AG, Jenna, Germany), visual field with Octopus 301 and reliable SAP, retinal nerve fiber layer and optic disc with Cirrus HD-OCT(Carl Zeiss, Meditec, Dublin, CA). POAG was diagnosed based on presence of an open iridocorneal angle, the characteristic appearance of glaucomatous optic neuropathy such as enlargement of optic cup disc ratio, focal thinning of neuroretinal rim, bayoneting and corresponding visual field defects with Octopus 301(Haag-Streit, InterzeagInternational AG, Schlieren, Switzerland)and elevated intraocular pressure The subjects had best corrected visual acuity >6/60.The exception criteria included patient with cataract, ocular infection and retinopathy. Blood pressure systolic and diastolyc was measured for all patient before the blood samples were collected.Venous blood specimens were collected from the antecubital vein into evacuated tubes 5 cc. Plasma samples obtained by centrifugation 3500 rpm. The collected venous blood were stored at 4^0C, supernatants keep in -20^0C and determination of the samples occurred with 6 months.

Oxidative Stress Marker Measurement

All blood analyses were performed by using a free radical analyzer system included spectrophotometry device reader with length waves 586 nm and measurement kits for MDA with MDA Oxis catalogue number 21044 and measurement kits for GPx with GPxRandox using spectrophotometry with length waves 340 nm. All analyses were performed within 48 hours of blood collection to avoid falsely high or low result. Malonildialdehyde (MDA) was measured as thiobarbituric acid reacting substance (TBARS) production. Spectrophotometri can result specific MDA concentration with coefficient variation 1,2-3,4%. The specificity of MDA-586 method was determined by measuring the absorbance ratios recovery 100% and for the sensitivityMDA-586 average 0,0102. Concentration of plasma MDA normally 1.04±0,43nmol/l. The sensitivity of GPx was 7,3pg/ml.

Retinal Nerve Fiber Layer and Optic Disc Analyses

All patients had their RNFL measured by Cirrus HD- OCT (Carl Zeiss, Meditec, Dublin, CA). Cirrus HD-OCT (Carl ZeisssMeditec) improves on time-domain systems, allowing performance of up 27000 axial scans per second. Cirrus HD-OCT imaging, the Macular Cube 200 x 200 Combo protocol was used. The protocol consists of two perpendicular line scans centered at the fovea followed by a cube scan also centered at the fovea. The line scans were 6 mm in the transverse direction, had a 2 mm acial depth, and was composed of 200 x 200 axial scans. The Cirrus RNFL map represents a 6x6 mm cube of A-scan data centered over the optic nerve in which a 3.4 mm diameter circle RNFL data is extracted to create what is refered It to as the TSNIT map (temporal, superior, nasal, inferior) is displayed as a false color scale with the thickness values by quadrants and clock hours, and the RNFL peaks give a sense of the anatomic distribution of nerve fiber axons represented by the superior and inferior bundles that emanate from optic neve. SD OCT had a sensitivity of 83% and a specificity of 88%[15,16]

Cirrus HD-OCT also automatically outlines the optic nerve head, optic cup, and disc borders similar to mental estimations by clinicians, but then also calculates more objective measurements such as optic disc area and neuroretinal rim area in addition to the classic clinician subjective average. And vertical cup to disc ratio. This allows the 3,4 mm RNFL circle to always be centered in the same spot within the cube. ONH parameters have also been found to have excellent ability to discriminate between normal eyes and eyes with even mild

glaucoma. The parameters found to have the greatest diagnostic capability are vertical rim thickness, rim area, and vertical cup to disc ratio. These ONH parameters were found to be as good as RNFL thickness parameters in diagnosing glaucoma[17].

Standart automated perimetry

SAP was performed with Normal strategy on OCTOPUS 301 (Haag-Streit, Interzeag International AG, Schlieren, Switzerland). A reliable VF defect was defined as one with less than 33% fixation loss and less than 20% positive and negative catch trial. Glaucomatous VF defect was defined as MD >2.0 dB (equivalent to being triggered at the 5% level on the Humprey Field Analyzer) or both in at least two reliable examinations.[18]

Statistical Analysis

The collected data write in the research publication and keep in the computer. The collected data kept in computer analysed by using the statical software. For the clinical characteristic between the two groups using t-test. To compare quantitative variables serial measurement between the two groups in the beginning, third month and sixth months using Anova test.

Results

The research was examined twenty patients (20 eyes) with ginkgo biloba extract treatment and twenty patients (20 eyes) as a control subjects. The patients measured the first day came to hospital before treatment, third months and sixth months.

Table1.The demographic parameter from 20 patients with GBE and 20 patients as a control presented.

Characteristic	**GBE group x ± SD**	**Control group x ±SD**	**P**
Sex			
- Men	6(30%)	8(40%)	
- Women	14 (70%)	12(60%)	
Age	54,63±4,34	54,92±4,26	0,869
IOP	25,30±2,92	25,95±3,13	0,865
MDA (nmol/l)	2,50±0,56	2,23±0,62	0,110
GPx (µ/gHb)	25,78±3,24	24,50±3,26	0,359
Visual Field			
- MD (db)	3,25±0,02	3,32±0,06	0,942
- PSD (db)	0,97±0,26	0,94±0,23	0,788
Retinal Nerve fiber layer			
- Superior	89,00±8,60	92,96±8,37	0,201
- Nasal	47,74±5,58	50,92±7,96	0,075
- Inferior	92,04±4,58	94,58±7,59	0,069
- Temp	47,52±3,03	49,65±7,42	0,451
- Mean	77,00±8,10	73,73±7,00	0,122
Optic Nerve Head			
- Disc ratio	0,72±0,14	0,70±0,14	0,656
- Disc ratio vertical	0,72±0,21	0,75±0,15	0,501

Based the clinical characteristic there was no significant differences between the two groups (p>0,05).

Table 2. Comparison IOP before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	25,31±2,92	25,95±3,13	0,865
Third month	19,00±1,96	19,77±2,51	0,712
Sixth month	15,48± 1,90	17,69±1,32	0,367

Based on the above tablethere was no significant between the two groups (p>0,05).

Table 3. MDA level before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	2,50±0,66	2,23±0,62	0,110
Third month	1,87±0,47	2,11±0,59	0,176
Sixth month	1,12±0,35	2,09±0,51	0,001*

Based on above table appear significant differences MDA level in the sixth month after treatment in GBE group

Table 4. Comparison GPx level before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	25,78±3,24	24,50±3,26	0,359
Third month	26,21±4,02	25,00±3,21	0,288
Sixth month	34,12±4,63	26,68±3,16	0,001*

Based on above table there was significant differences GPx level in sixth month after treatment in GBE group (p<0,05)

Table 5.Mean Deviation (MD) level before and after treatment between the two groups.

	GBE group	Control group	P
Before treatment	3,25±0,02	3,32±0,06	0,942
Third month	2,75±0,21	3,06±0,03	0,365
Six Month	2,39±0,07	2,91±0,02	0,011*

Based on above table appear there was significant differences in the sixth month after treatment in GBE group

Table 6. Pattern Standart Deviation (PSD) level before and aftertreatment between the two groups.

	GBE group	Control group	P
Before treatment	0,97±0,26	0.94±0,23	0,786
Third month	0,91±0,24	1,01±0,31	0.226
Sixth month	0,64±0,18	1,21±0,37	0,020*

Based on above table appear significant differences PSD level in the sixth month after treatment in GBEgroup(p<0,05).

Table 7. Superior RNFL before and after treatment between the two groups.

	GBE group	Control group	P
Before treatment	89,00±8,60	92,96±8,37	0,201
Third month	94,26±7,96	91,35±8,37,	0,065
Sixth month	101,65±7,99	86,58±5,87	0,001*

Based on above table appear significant differences superior retinal nerve fiber layer level in the sixth month after treatmentin GBE group(p<0,05).

Table 8. Nasal RNFLbefore and after treatment between the two groups.

	GBE group	Control group	P
Before treatment	47,74±5,58	50,92±7,96	0,252
Third month	51,15±5,23	48,65±7,05	0,167
Six month	53,85±4,83	50,12±5,40	0,081

Based on above table there was no significantly differences between the two groups(p>0,05).

Table 9. Inferior RNFL before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	92,04±4,58	94,58±7,59	0,069
Third month	96.00±5,01	94,81±6,11	0,057
Six month	99,41±5,63	94,05±4,86	0,035*

Based on above table there was significantly differences inferior RNFL in the sixth month after treatment in GBE group(p<0,05).

Table 10. Temporal RNFLbefore and after treatment between the two groups

	GBE group	Control group	P
Before treatment	47,52±3,03	49,65±7,42	0,451
Third month	49,57±3,56	48,02±6,99	0.426
Sixth month	50,56±4,04	46,73±6,18	0,067

Based on above table appear there was no significant differences in temporal RNFL between the two groups (p>0,05)

Table 11. Mean/ average RNFLbefore and after treatment between the two groups

	GBE group	Control group	P
Before treatment	77,00±8,10	73,73±7,00	0,122
Third month	78,78±8,33	74,38±6,34	0.136
Sixth month	79,85±8,48	75,11±5,78	0,118

Based on above table appear there was no significant differences in mean/average RNFL between the two groups (p>0,05).

Table 12 Disc ratio before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	0,72±0,14	0,70±0,15	0,656
Third month	0,68±0,13	0,70±0,03	0.336
Sixth month	0,65±0,15	0,71±5,78	0,057

Based on above table there was no significant differences optic disc between the two groups (p>0,05)

Table 13. Disc ratio vertical before and after treatment between the two groups

	GBE group	Control group	P
Before treatment	0,73±0,21	0,75±0,15	0,505
Third month	0,70±0,15	0,76±0,03	0.136
Sixth month	0,68 ±0,16	0,74±0,12	0,103

Based on above table there was no significant differences optic disc vertical between the two groups(p>0,05).

Discussion

Glaucoma is the second leading caused blindness after cataract in the world. Glaucoma referes to a group of diseases with optic neuropathy and decreased of visual field and higher intraocular pressure as a risk factor. Glaucoma can developed after as early as birth until older age, but the most in older people. The recent study reported 60,5 million people had primary open angle glaucoma and 8,4 million people result bilateral blindness[19].

The pathogenesis of glaucoma is multifactorialand until now still investigate. From some literature reported glaucoma not only caused destroyed of retinal ganglion cell and its axon, but involvement optic nerve head and retina which caused blood supply decreased[20]. Numerous scientific investigations have confirmed the presence of oxidative stress in ocular diseases. From some study also reported involvement free radical is one of pathogenesis of glaucoma, so reactive oxygen species play a role of pathogenesis of glaucoma.In ophthalmology, oxidative stress has been reported to induced the progression of cataract and diabetic retinopathy. In glaucoma, antioxidant levels decrease in aquous humor compared with normal subject. It's suggested that peroxidation involved in development of glaucoma[21].

Recent datas indicate that oxidative stress plays an important role of pathogenesis of glaucoma, but until now the mechanism is unclear. The possible caused of increased oxidative stress might be include increased of free radical or impaired antioxidant defencesystem. Free radical can reacts with macromolecules as membrane lipid, protein, DNA which can changed the structure, functional and neuronal death endly[22]. From one study reported that there was increased of lipid peroxidation concentrate in aquous humor, trabecular meshwork and canalissclemmi primary open angle glaucoma patients compared to controls dan the study reported lipid peroxidation caused destruction of trabecular meshwork and canalissclemmi. From one study animal trial with higher intraocular pressure reported that there was an increased of MDA levels in vitreous humor[23].

Malonyldialdehyde and Gluthathion Peroxidase are well known markers in the pathologic molecular process in oxidative stress. In this study, we selected two biomarkers that are widely used, sensitive, and appropriate for use in large study. MDA is a decomposition product of peroxidized polyunsaturated fatty acids[24]. Hydrogen peroxide is removed by two enzyme: catalase and gluthathion peroxidase. Gluthathion peroxidase removes H2O using gluthathion as a cofactor.

Production of ROS and lipid peroxidation are increased in glaucoma patients. Bunin reported increased levels of lipid peroxides in the aquous humor, trabecular meshwork, and SChlemm's canal in POAG compared with control eyes, and suggested that lipid peroxidation was responsible for destruction of trabecular meshwork and Schlemm'scanal.Increased vitreous and retina MDA levels were also detected in rats with elevated intraocular pressure[25]. We found that POAG had significantly higher MDA and GPx.

Ginkgo biloba (GBE) is one of neuroprotectiveand also can used as an antioxidant. In this reseach found that GBE administration as a neuroprotective and antioxidant slowed of visual field and retinal nerve fiber layer damage in primary open angle glaucoma.

Based on result examination, we found that oxidative stress marker MDA and GPx play a role in primary open angle glaucoma (Table 1). This result correlated with several studies have reported lower systemic levels of antioxidants or antioxidative stress capacity in glaucoma, that is a reduced form of gluthathione level was lower in red blood cells of glaucoma patients[26]. Faschinger et al reported there was a positive correlation between malonyldialdehyde (MDA) in aquous humor glaucoma patients compared to normal subjects. The systemic antioxidant capacity could reflect the local ocular redox status. In experimental studies, free radical scavengers effectively prevented glaucomatous tissue such as glutamate and IOP induced RGC death, tumor necrosis factor induced axonal injury[27]. Collectively decreased levels of systemic antioxidant capacity might be in the glaucomatous TM and neuronal damage as a result of local inadequate defense against oxidative stress[26]. After GBE treatment, oxidative marker MDA level was decrease and redox enzyme GPxlevel was increased after the six month treatment (Table 3,4). This showed the benefit of ginkgo biloba extract as an antioxidant.

There was elevated of visual field (table 5,6) and retinal nerve fiber layer (table 7,9) in patient with ginkgo bilobaextract treatment. This showed that ginkgo biloba extract as a neuroprotective. Recent study found that flavonoids can increased collagen fibers and extracellular fibrovascular in vitro and protect apoptosis from ischemia and exicito toxicity glutamate[28,29]. Another study found that flavonoids restore the blood vessel and metabolic toxin when ischemic[29,30].

In conclusion, we found that oxidative stress and redox enzyme plays a role in pathogenesis of primary open angle glaucoma and GBE can be used in primary open angle glaucoma as an adjuvantfor neuroprotective and an antioxidant. GBE slowed visual field and retinal nerve fiber layer damage. Therefore, assessment of oxidative stress and redox enzyme in POAG may be important for the therapy and prevention of primary open angle glaucoma and GBE as an adjuvant therapy in primary open angle glaucoma. Prospective longitudinal clinical trials on larger populations and longer times are therefore needed for another types of glaucoma.

Disclosure

Patients have been approved prior to the studyconducted. Costs borne by researchers.

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