DISSERTATION PROTOCOL

Effects of Green Tea Consumption on Retinal Nerve Fibre Layer Thickness and Intraocular Pressure in Patients with Primary Glaucoma

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1. INTRODUCTION

Glaucoma is a progressive optic neuropathy characterised by excavated appearance of optic disc and loss of retinal ganglion cells (RGCs) with corresponding visual field loss (AAO, 2023). It affects more than 70 million people worldwide, making it one of the leading causes of irreversible blindness. Patients suffer from a centripetal loss of visual field associated with an increasing optic disc cupping while remaining asymptomatic until reaching advanced stages. Due to the lack of symptoms in the early stages, detection and treatment may be delayed until advanced stages of disease (Cohen et al, 2014). It is estimated that half of patients with glaucoma are unaware of their diagnosis (Vajarant et al, 2016).

The current established treatment of glaucoma is by reducing IOP, as one of neuroprotectant factor to prevent further loss RGCs. However, it is not always sufficient to fully prevent disease progression. Oxidative stress is one of biological insult contribute to etiology and progression of glaucoma (Tezel et al, 2006). Antioxidants represent the first line of defence against oxidative stress and are obtained through the diet and produced internally has shown to be protective towards glaucoma (Braakhuis et al, 2017).

Several studies reported glaucoma patients with younger age and more highly educated tend to use complementary and alternative medication in belief that can control their disease (Wan et al, 2012). Study by wan et al reported 34% of patients consumed herbal medication and 22% had dietary modification. There is no adequate clinical evidence to encourage patients to have certain dietary modification to support glaucoma management. Thus, we would like to study the effect of high antioxidant drink which is green tea on the glaucoma. High antioxidant drink can be more cost effective and safer to be promising potential as primary prevention for glaucoma.

2. LITERATURE REVIEW

2.1. Management of glaucoma

The current treatment of glaucoma is by reducing intraocular pressure (IOP) toward a target pressure through medication, laser, or surgery (AAO) to preserve visual function by lowering intraocular pressure (IOP) (AAO, 2023). In The Advanced Glaucoma Intervention Study (AGIS), reported lower IOP was associated with less visual field progression. In Collaborative Normal- Tension Glaucoma Study (CNTGS), IOP lowering by at least 30% reduced the 5- year risk of visual field progression from 35% to 12%.

Target pressure is a therapeutic IOP upper range in which visual field loss is unlikely to significantly reduce a patient's quality of life over his or her lifetime. In general, the initial target aims for about 20% to 50% reduction in pressure (AAO, 2023). Target pressure should be individualized for each eye initially based on the IOP level and the severity of glaucoma damage. Other several factors to consider including the observed rate of progression; life expectancy of the patient; history of disc hemorrhages, corneal thickness, or a family history of severe vision loss in the setting of glaucoma (AAO, 2023).

The clinician must decide whether to achieve target pressure goal medically or surgically after discussing with patient the benefits and justify the risks of the opted treatment modalities. Laser trabeculoplasty is relatively safe outpatient procedure and effective in reducing IOP by increasing trabecular outflow. However, it does not replace medical as primary treatment in view of gradual diminishes effect over time (Juzych et al, 2004).

Surgical trabeculectomy is indicated when maximally tolerated medical therapy and laser treatments fail to prevent progressive damage in view of its known complication including hypotony, malignant glaucoma and bleb fibrosis. In Collaborative Initial Glaucoma Treatment Study (CIGTS), reported patient with newly diagnosed open angle glaucoma showed greater IOP reduction in immediate trabeculectomy group compared to initial medical therapy. However, it does not show greater visual field preservation in the trabeculectomy group.

Other surgical options of glaucoma treatment are plate- based tube implants to shunt aqueous from the anterior chamber to subconjunctival space (AAO, 2023). These implants usually reserved in eyes that had failed multiple prior trabeculectomies, active inflammation or neovascularization, or severe conjunctival scarring. In landmark study of The Tube Versus Trabeculectomy Study reported high success rates with tube shunt surgery in eyes with previous cataract surgery or trabeculectomy. Common complications are corneal endothelial loss and cornea decompensation.

2.2. Role of oxidative stress in glaucoma

Current evidence suggests that oxidative stress has been implicated in a wide variety of neurodegenerative diseases (Juzych et al, 2004), including glaucoma (Tezel et al, 2006). Reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide (H2O2), hydroxyl radical, and singlet oxygen, are generated intracellularly through many processes. For examples as by-products of aerobic metabolism and as second messengers in various signal transduction pathway. ROS can also be derived from exogenous sources including by exposure to various natural and synthetic toxicants and ultraviolet/ionizing radiation (Chen et al, 2003).

An imbalance between ROS and antioxidants can result in excessive generation of ROS. Studies have observed diminished total antioxidant capacity in plasma or serum of glaucoma (Sorkhabi et al, 2011) (Mousa et al, 2015). These excessive ROS may cause damage to cellular nucleic acids, structural proteins, and lipids, and organelles including mitochondria, and also may promote cell death via apoptosis (Pinazo et al, 2015).

Following acute elevation of IOP, oxidative stress may exert damage to retinal ganglion cells (Quan et al, 2007). An animal study has shown that chronic IOP elevation induced by weekly injections of hyaluronic acid into the anterior chambers of rat eyes reduced anti-oxidant levels of superoxide dismutase (SOD), catalase, and glutathione associated with significant increased retinal lipid peroxidation (Moreno et al, 2004).

Oxidative stress may also cause degeneration of the trabecular meshwork leading to interference with normal drainage of aqueous humour, and an increase in IOP thus priming the glaucoma pathogenetic cascade. There is evidence that cells within the trabecular meshwork of glaucoma cases have higher levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative damage to the DNA and its level correlate with visual field damage and IOP (Sacca et al, 2005).

Unstable ocular perfusion especially when IOP or blood pressure fluctuates leads to a repeated reperfusion and thereby ROS formation contributing to glaucomatous damage (Mozaffarieh et al, 2008). Vascular diseases, including stroke, cardiovascular disease, migraine, and hypertension, are associated with increased risk for glaucoma, strongly suggesting that vascular abnormalities and impaired blood flow to the retina may play a role in development of the disease.

2.3.Nutrients and antioxidant content of green tea

Tea is one of the most popular beverages consumed worldwide (Neves et al, 2011), which is made up from leaf of the plant Camellia sinensis. Depending on the manufacturing process, teas are classified into three major types which are 'non-fermented' green tea, 'semi-fermented' oolong tea and 'fermented' black and red. Non fermented green tea is produced by drying and steaming the fresh leaves to impede oxidation by polyphenol oxidase. These processes preserve natural polyphenols contributing to the health-promoting properties (Naghma et al, 2007). For this reason, it is one of the most antioxidant rich drink due to its high content of flavonoids commonly known as catechins (flavan-3-ols).

The most abundant and the most active catechins are epigallocatechin-3-gallate (EGCG), that represents approximately 59% of the total of catechins followed by approximately 19% of epigallocatechin (EGC); approximately 13.6% of epicatechin-3-gallate (ECG); and approximately 6.4% of epicatechin (EC) (Henning et al, 2003). Henning et al also reported Lipton Green Tea has the 2nd highest catechin level of EGCG followed by Cellestial Seasoning Green Tea.

The calorie in green tea without added sugar is insignificant about 1-2kcal. The caffeine content is lower than in coffee, black tea, or cola soft-drinks. In addition to phytochemical substances, it also contains vitamins such as vitamin C, vitamin B, and minerals such as Mn, Cr, Se, Zn (Nutrient Data Laboratory, 2013). In this study, it is safe for subject to consume green tea with amount of 2 cup per day in 5 days per week. According to 'National Center for Complementary and Intergrative Health (NCCIH),' it is safe to consume green tea up to 8 cups per day.

In vitro study has shown that catechins found in green tea can penetrate the tissue of the rat eye after oral administration (Chu et al, 2010). Another study by Yang et al evidenced green tea extract acts as a neuroprotectant to rat RGCs after exposed to an oxidative stress induced by ischemia-reperfusion injury (Yang et al, 2019). Thus it can be speculated that green tea may have potential to act as antioxidant to human RGCs specifically for patients with glaucoma.

In the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), which included 116,484 participants ≥40 years of age, reported tea has been be possibly protective against developing glaucoma or its progression. Consuming 2 cups daily or median of 13.1 cups/week was associated with a significant 18% lower glaucoma risk (Kang et al, 2018). In clinical study of 43 healthy subjects demonstrates that green tea extracts can also decrease IOP, however its clinically significant in patient with glaucoma is not known (Gasiunas et al, 2022). Another clinical study reported significant effect on inner retinal function as evaluated by PERG after taking EGCG supplement in patient with early to moderate open angle glaucoma (Falsini et al, 2009).

3. RATIONAL OF STUDY

The current established treatment of glaucoma is by lowering of intraocular pressure (IOP) using medications, laser treatment, or surgery. However, these interventions are not always sufficient. Some patients continue to show disease progression causing severe vision loss despite achieving therapeutic IOP level. The identification of other options of modifiable risk factors can give significant implications for glaucoma prevention and perhaps can include in future treatment modalities. Many evidences showed an association between pathophysiology of oxidative stress and risk for glaucoma which suggesting that consumption of dietary antioxidants may be a factor capable of modifying disease risk.

Hence in this study, green tea is chosen as it is among the highest antioxidant drink attribute to its flavonoid based catechin content. Green tea is considerable cheap, safe drink, easily prepared and easily available worldwide. Although green tea extract has higher content of polyphenol especially EGCG compared to green tea, green tea is chosen as it is more beneficial and safer. Green tea extract form in large bolus dose has been linked to liver problems, but not when consumed as brewed tea (Hu et al, 2018).

In this study we would like to evaluate the effect of green tea consumption in patient with primary glaucoma. In view of its high antioxidant property, it may have potential as a complementary treatment to prevent or slow down progression of glaucoma. The simple modification of dietary drink is cheap, easy, and safe option to be taken as complementary management.

4. OBJECTIVE

4.1. General Objective

To evaluate the effect of 6 months consumption of green tea on intraocular pressure (IOP) and retinal nerve fibre layer thickness (RNFL) in patients with primary glaucoma.

4.2. Specific Objective

4.2.1. To compare the IOP measurement at baseline, after 1 month, 3 months and 6 months, between patients with primary glaucoma that consume green tea and not consume green tea.

4.2.2. To compare the retinal nerve fibre layer thickness at baseline, after 1 month, 3 months and 6 months, between patients with primary glaucoma that consuming green tea and not consuming green tea.

4.3. Research Questions

4.3.1. Is there any difference of IOP in patient with primary glaucoma after 1 month, 3 months and 6 months who is consuming green tea compared to patient not consuming green tea?

4.3.2. Is there any difference of retina nerve fibre layer in patient with primary glaucoma after 1 month, 3months and 6 months who is consuming green tea compared to patient not consuming green tea?

5. METHODOLOGY

5.1. Research Design

This is a randomized controlled trial study.

5.2. Study Location

1.1.1. Department of Ophthalmology, Hospital Sultanah Bahiyah (HSB), Kedah

1.1.2. Department of Ophthalmology, Hospital Universiti Sains Malaysia (HUSM), Kelantan

1.1.3. Department of Hospital Raja Perempuan Zainab (HRPZ), Kelantan

5.3. Study Duration

June 2023- June 2025

5.4. Study Reference

Population of primary glaucoma patients.

5.5. Study Source Population

Primary glaucoma patients attending eye clinic in Hospital Sultanah Bahiyah, Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab, Kelantan

5.6. Sampling Frame

All primary glaucoma patients attending ophthalmology clinic HSB, HUSM and HRPZ during study duration and fulfil selective criteria

5.7. Sampling Method

Block sample randomisation will be applied.

5.8. Study Participants

Primary glaucoma patients who attended eye clinic HSB, HUSM and HRPZ selected according to inclusion and exclusion criteria

5.9. Selection Criteria

5.9.1. Inclusion Criteria

5.9.1.1. Patient with confirmed diagnosis of primary glaucoma (POAG/PACG/NTG) that achieve target IOP with medical therapy at least for 6 months.

5.9.1.2. Never consume green tea as daily drinks.

5.9.2. Exclusion Criteria

5.9.2.1. Dense cataract with Lens Opacity Classification Sytem (LOCS II) of more than grade 2

5.9.2.2. Any other ocular media opacity that may interfere with OCT imaging (eg: cornea scar/vitreous haemorrhage)

5.9.2.3. Any history of optic neuropathy

5.9.2.4. History of glaucoma or retinal surgery

5.9.2.5. Macular degeneration and retinal disorder

5.9.2.6. Patient with caffein sensitive that causes unpleasant reaction (eg: palpitation, insomnia, nausea, constipation)

5.9.2.7. Allergic to green tea

5.10. Randomisation

Block randomization method using ‘Sealed Envelope’ website.

5.11. Blinding

Single blinded study where OCT operator and personnel taking IOP measurement will be blinded

5.12. Sample Size

Sample size determination for IOP and RNFL thickness are done using sample size calculator by Wan Nor Ariffin.

Standard Deviation pooled :

$$= \sqrt{\frac{\sigma_1^2 + \sigma_2^2}{2}}$$

where σ_1 = standard deviation in Group 1, and
σ_2 = standard deviation in Group 2.

$$= \sqrt{\frac{(\ 3.45\ )^2 + (\ 3.90\ )^2}{2}}$$

$$= 3.69$$

Sample size (n) :

$$= \frac{2\sigma^2\left[Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right]^2}{(\mu_1 - \mu_2)^2}$$

where n = sample size,
Z = level of confidence,
σ = standard deviation,
α = alpha,
β = beta,
μ_1 = mean in Group 1, and
μ_2 = mean in Group 2.

$$= \frac{2(\ 3.69\ )^2\left[Z_{1-\frac{0.05}{2}} + Z_{1\ -\ 0.20}\right]^2}{(\quad 18.6\ -\ 16.2\quad)^2}$$

$$= \frac{27.2322[\ 1.96 + 0.85\ ]^2}{5.62}$$

$$\approx \mathbf{39\ samples}$$

The final total sample size is 100 patients including 30% dropout with 50 patients in group A (interventional) and 50 patients in group B (non-interventional)

5.13. Definition of Terms

5.13.1. Primary Glaucoma

Progressive optic neuropathy characterised by excavated appearance of optic disc and loss of retinal ganglion cells with corresponding visual field loss with no identifiable cause (AAO 2022-2023).

5.13.2. Intraocular Pressure

The IOP of the eye is determined by the balance between the amount of aqueous humor production and aqueous humor outflow. The parameters that contribute to intraocular pressure (IOP) are modeled by the modified Goldmann equation include outflow facility, aqueous humor production rate, episcleral venous pressure, and uveoscleral outflow rate. (AAO, 2022-2023)

5.13.3. RNFL Thickness

The RNFL is formed by the expansion of the fibers of the optic nerve. RNFL thickness can be measured by optical coherence tomography (OCT) which can discriminate glaucomatous eyes from healthy eyes and to monitor the progress of disease. OCT RNFL thickness measurements are usually acquired in the peripapillary 3.45mm around the ONH. OCT reports global average peripapillary RNFL thickness and average RNFL thickness in quadrants or in small clock- hour sectors. RNFL thickness measurements are generally lower in glaucomatous eyes compared with those in non-glaucomatous eyes. (AAO, 2022-2023).

5.14. Withdrawal Criteria

Withdrawal of subjects from study are considered when:

i) Subject is unable to comply with the protocol.
ii) If the subject in group A consumes green tea more than 2 cups or less per day
iii) Subject in group A and B consumes antioxidant supplement during study period.
iv) If the study subject developed gastrointestinal discomfort upon consumption of green tea.

Subjects can choose to withdraw at any time. Subjects may be withdrawn if the investigator deems that it is detrimental or risky for the subject to continue. Withdrawn subjects will not be replaced.

5.15. Research Tools

5.15.1. Instruments

i) Snellen Visual Acuity Chart for distance (Reichert, NY, USA)
ii) Pinhole occluder
iii) Slit lamp biomicroscopy (HAAG-STREIT International, UK)
iv) Goldmann applanation tonometer (GAT) AT-900 (HAAG-STREIT International, UK)
v) Condensing lens of 90D and 78D lenses (VOLK, USA)
vi) Optical Coherence Tomography (Heidelberg Spectralis OCT, USA)
vii) Dietary Diary

5.15.2. Medication/Reagent

5.15.2.1. Tropicamide 1% eye drop (mydriatic agent)

5.15.2.2. Proparacaine Hydrochloride (Alcaine) 0.5% eye drop (topical local anesthesia)

5.15.2.3. Phenylephrine Hydrochloride (Mydfrin) 2.5% eye drop (mydriatic agent)

5.16. Data Collection

This study will be conducted after obtaining approval from the Universiti Sains Malaysia Ethical Committee (JPeM) and Medical Research and Ethics Committee, Ministry of Health, Malaysia and will be conducted in accordance to World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects.

5.16.1. Patient Recruitment

Primary glaucoma patients who attending ophthalmology clinic at HSB, HUSM and HRPZ during study duration June 2023–June 2025. Evaluation of study sample including systemic and ocular history, baseline ocular examination, IOP and OCT RNFL. Subjects who fulfil the inclusion and exclusion criteria for the study will be selected. An informed and written consent will be taken and information form given to patients. The selected patients will be randomized into 2 groups (A and B) using block sample randomisation. Group A patient will be consuming green tea drink and group B patient will not consume green tea drink. A data collection sheet will be used to gather all the data from the subjects.

5.16.2. Informed Consent Process

Patients shall be informed of the study during their usual clinic visits. They will be requested to contact investigators if they are interested. An appointment will be made where the patient information sheet will be provided and explained to them. If they are willing to participate, the consent forms will be signed and dated. If they need to, they are allowed to take the information sheet home to consult with their family members, and another day for getting consent arranged.

5.16.3. Interventional Protocol

50 patients in Group A will have to consume two cups (250ml for each cup) of hot green tea, five days per week for 6 months. A tea bag is brewed in 250ml hot water for 3 minutes. They are only allowed to consume as hot drink, not for iced tea. Any sweetener or sugar is not allowed to mix in the drink. The green tea bag will be distributed to patient at beginning of research and at every visit. Patient will be reminded weekly using text messages or phone call by a research assistant for green tea consumption.

50 patients in Group B will not be consuming green tea during 6 months of study period.

Patients in group A and group B will be given a dietary diary throughout the study period, where the patient will mark the intake of other drinks. The purpose of the diary is also to monitor patient intake of green tea and other drinks as well.

5.16.4. IOP measurement

IOP measurement will be done using Goldmann Applanation Tonometer (GAT) in sitting position by the primary investigator who will be blinded. GAT will be calibrated daily before used to measure the IOP. In order to prevent diurnal variation of IOP, measurement done in morning around 8am – 10am. Only one eye will be measured. If both eyes are eligible, only right eye will be selected regardless of severity of glaucoma. Three readings of IOP will be measured and the mean reading will be taken as result. IOP measurement will be taken at baseline, first, and 6th-month post recruitment for group A and B.

5.16.5. OCT evaluation on RNFL thickness

Spectral domain Heidelberg Spectralis OCT will be used to measure RNFL thickness by trained personnel who is blinded. Patient is instructed to sit in front of machine, place his/her chin on the chinrest, lean forehead against the forehead rest, and then to look at target pointer inside the machine. The OCT will then scan patient's eye without any direct eye contact. Good quality image included well centred optic nerve head and homogenous signal of overall image will be choosen. Poor image with artifacts, or with segmentation errors will be repeated. If there is unsatisfactory measurement after 3 attempts, the patient will be excluded from the study. In patient without media opacities but with poor signal strength the eye will be dilated first and if after dilation still unable to get satisfactory reading after 3 attempts, the patient will be excluded from the study. OCT will be conducted at baseline, 1 month, 3 month and 6 month post recruitment for group A and B.

5.17. Data Entry and Statistical Analysis

Data entry and analysis will be performed by using the IBM Statistical Package for the Social Sciences (SPSS) licensed to USM. Paired t-test will be used to compare numerical data and chi-square test for categorical data between the two groups.

5.18. Plans for Minimizing Study Errors

These steps will be taken to minimize the errors while conducting the study:

i. The same instruments and equipment will be used for repeated measurement in this study.
ii. The measurements of IOP will be done by same person.

iii. The measurement of IOP will be done at the same time (8am-10am) in a day for all patients to prevent diurnal variation.
iv. OCT measurements will be performed by qualified and trained personnel only.

6. ETHICAL CONSIDERATION

This study will be submitted to the Universiti Sains Malaysia Ethical Committee (JPeM) and Medical Research Ethics Committee (MREC), Ministry of Health of Malaysia and will be conducted in accordance to World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. The information form will be given to all participants and an informed and written consent will be taken prior to data collection.

6.1. Handling Privacy and Data Confidentiality

6.1.1. Confidentiality

All the information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. The confidentiality of the data will be strictly maintained, whereby only the author, supervisor and cosupervisor could access the data. Personal information and data will not be disclosed. The data collection sheets will have serial numbers instead of the names of the subjects to prevent recognition. Subjects are not given access to the personal information and study date. Subjects can write to the investigators to request access to study findings.

6.1.2. Data Storage

Research records will be stored securely in a locked cabinet and study data will be stored in a password-protected thumb drive. Medical information will be held and processed on a computer and will be entered using unique numbers into Microsoft Excel before analysis process using SPSS. Duration of storage and archival of medical records and study data will take about 3 years after completion of study. All digital data will be deleted permanently and all data collection sheets which contain the data will be disposed after the period of storage. The participants' name will not appear on the materials published and the medical information of each subject will keep confidential unless disclosure is required by law. Participants will be offered to read the manuscripts and to see all publish materials in which they are included.

6.2. Potential Risk to Subjects

It is safe for subject to consume green tea with amount of 2 cup per day in 5 days per week. According to 'National Center for Complementary and Intergrative Health (NCCIH),' it is safe to consume green tea up to 8 cups per day. There are no serious side effects known to be caused by the investigational product.

The study procedures are all routine procedures for the disease/condition studied. However, the participants will be monitored by the researcher until confirmed not to have any side effect of the investigational product. If any side effects medical treatment will be given as necessary. However, if there are any important new information found during this study that may affect the decision in being part of the study, participants will be told about it right away.

6.3. Direct and Indirect Benefits to Subjects

Study drug and study procedures will be provided at no cost to all participants. Participants may receive information about their health from any physical examination and investigation tests to be done in this study. This intervention would increase the participant's knowledge regarding dietary modification specifically high antioxidant drinks that may help towards successful treatment outcome in primary glaucoma.

6.4. Incentive, Compensation and/or Reimbursement

There is no any incentive, compensation and/or reimbursement given to all participants.

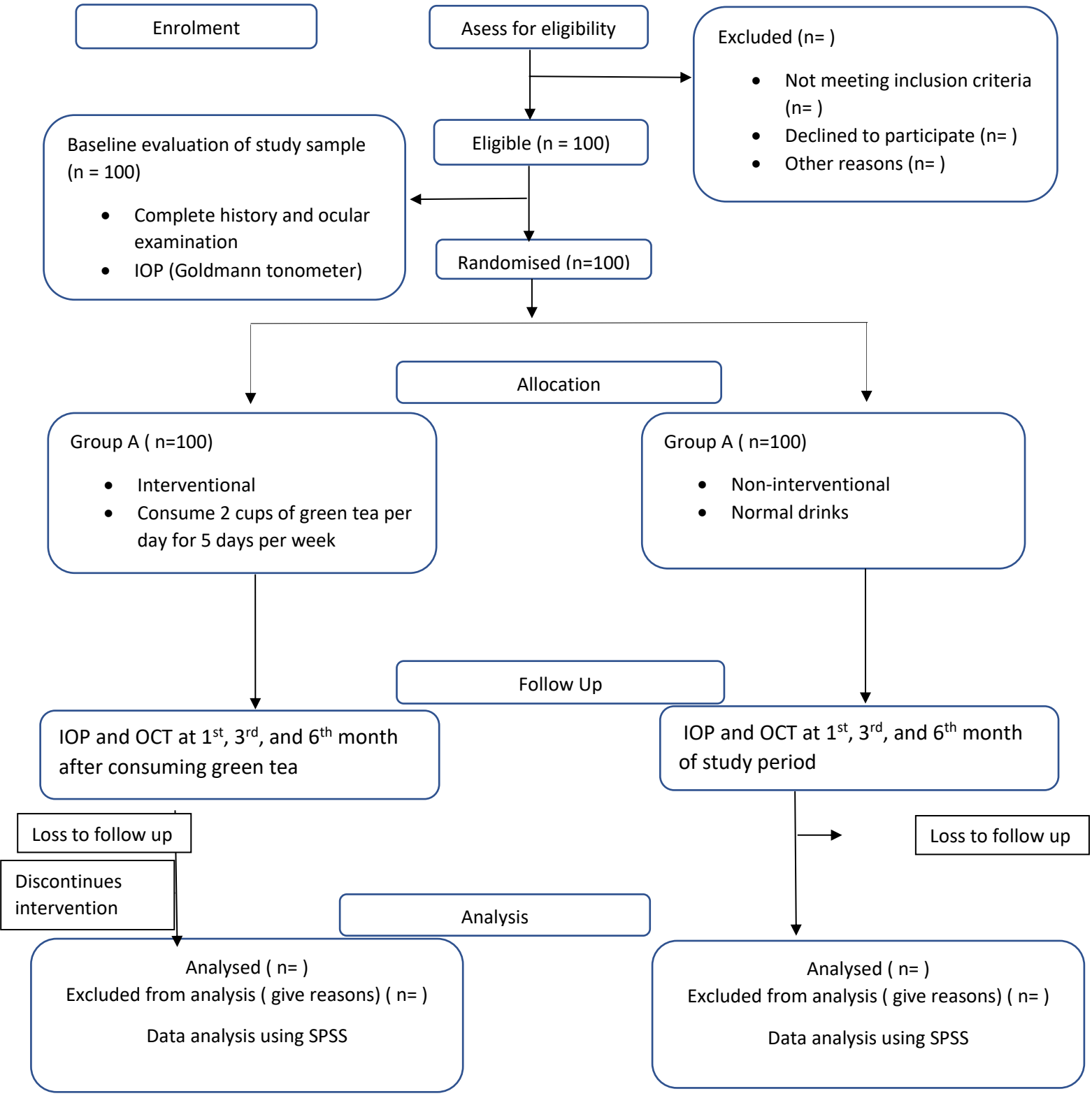
6.5. Declaration of Conflict of Interest

No conflict of interest is present.

6.6. Collaborative Study Term of Reference

There is no collaboration between researchers and other institute or ministry in this study.

7. FLOW CHART OF STUDY



8. EXPECTED RESULT

8.1. Objective 1:

Table 1: Mean of IOP in green tea group and control group for 6 months

IOP (mmHg)	Time (month)	Group A	Group B	P value
Mean, SD	Baseline			
	1			
	3			
	6			

Statistical analyses will be performed with the Paired T test. $P < 0.05$ is significant.

Table 2: Changes of IOP at every visit from the baseline IOP in green tea group and control group

Duration of study (Month)	Group A				Group B			
	Mean of IOP differences	95% CI of the difference		P-value	Mean of IOP differences	95% CI of the difference		P-value
		Upper border	Lower border			Upper border	Lower border	
V_0-V_1								
V_0-V_3								
V_0-V_6								

V_0: baseline reading, V_1: visit at 1 month, V_3: visit at 3month, V_6: visit at 6 month

Statistical analyses will be performed with one-way repeated measures analysis of variance (RM ANOVA). $P < 0.05$ is significant.

8.2. Objective 2:

Table 3: Mean of RNFL thickness observed in green tea group and control group

RNFL thickness	Group A				Group B				P value
	Baseline	1 month	3 months	6 months	Baseline	1 month	3 months	6 months	
Average									
Superior									
Inferior									
Nasal									
Temporal									

Statistical analyses will be performed with the Paired t-test. $P < 0.05$ is significant.

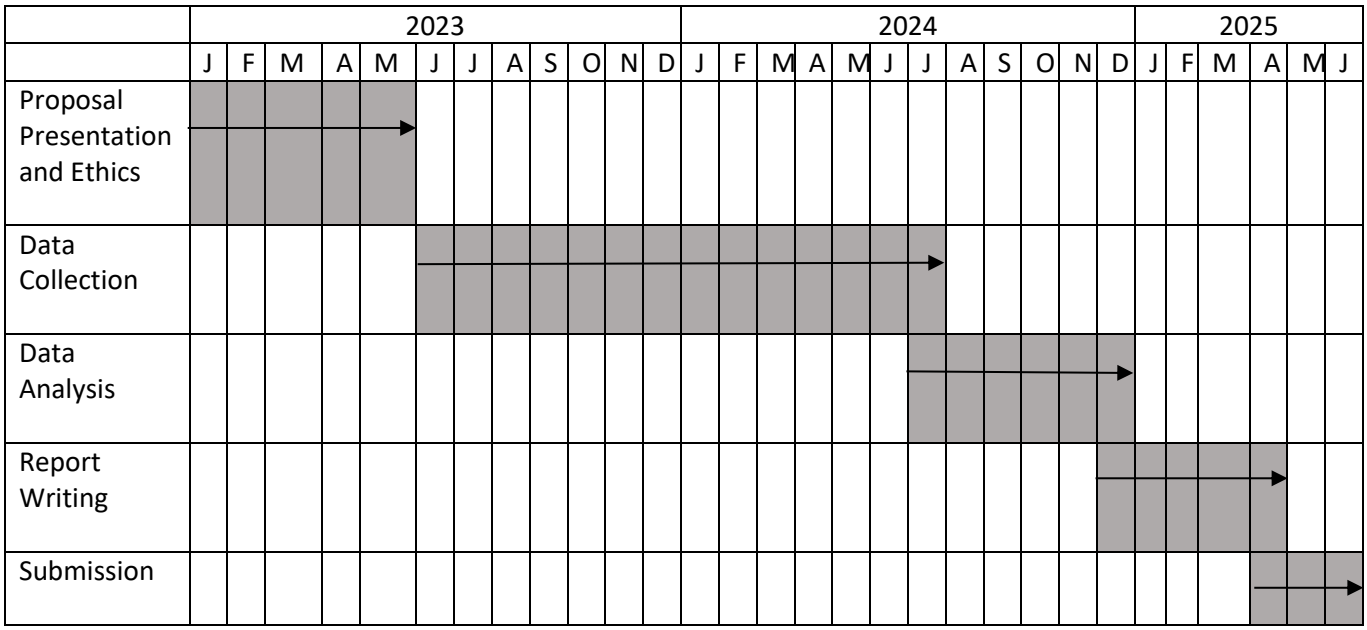
Table 4: Changes of RNFL thickness at every visit from the baseline IOP in green tea group and control group

Duration of study (Month)	Group A				Group B			
	Mean of RNFL differences	95% CI of the difference		P-value	Mean of IOP differences	95% CI of the difference		P-value
		Upper border	Lower border			Upper border	Lower border	
V_0-V_1								
V_0-V_3								
V_0-V_6								

V_0: baseline reading, V_1: visit at 1 month, V_3: visit at 3month, V_6: visit at 6 month

Statistical analyses will be performed with one-way repeated measures analysis of variance (RM ANOVA). $P < 0.05$ is significant.

9. GANNT CHART



10. KEY MILESTONES

Month	Expected Achievement
June 2023	• proposal preparation done • obtained ethical approval
Dec 2023	• 40% data collection
July 2024	• 100% data collection
Dec 2024	• 100% data analysis
May 2025 June 2025	• Complete report writing • submission final report

11. REFERRENCES

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ATTACHMENT A: Dietary Diet

Diari Amalan Minuman

Bil.	Minuman	Jumlah Pengambilan (Nyatakan cawan atau gelas)							Jumlah Pengambilan Seminggu	Kenyataan Lain
		AHAD	ISNIN	SELASA	RABU	KHAMIS	JUMAAT	SABTU		
1.	Teh Hijau									
2.	Kopi									
3.	Nescafe									
4.	Milo									
5.	Vico									
6.	Air Coklat									
7.	Jus Buah									
8.	Lain-lain teh (nyatakan)									

Bulan: _____ Minggu: _____

Sila catatkan jumlah pengambilan minuman harian dalam kuantiti cawan atau gelas.

ATTACHMENT B: Data Collection Form

Data Collection Form

Research Title: **Effects of Green Tea Consumption on Retinal Nerve Fiber Layer Thickness and Intraocular Pressure in Patients with Primary Glaucoma.**

Subject No: _____ Date of examination: _____

A) Demographic Data

Name:_____

Registration No: _____

Age:_____

Gender:_____

Contact No: _____

Smoking/Not smoking

Family History of glaucoma: NO/YES

B) **Comorbid:**

DM	
HPT	
HPL	
IHD	
CKD	

Others :_____

C) Examination

VA	Baseline	1 month	3 month	6 month
RE				
LE				
IOP				
RE				
LE				

D) Investigations

OCT RNFL	Baseline	1 month	3 month	6 month
Average				
Superior				
Inferior				
Nasal				
Temporal				

E) **Treatment**

I. Topical antiglaucoma: Timolol / Xalatan / Travatan / Trusopt / Alphagan/ Lumigan

Other: _____

II. History of glaucoma laser treatment: NO/YES

If YES, type of laser: _____

F) Ocular characteristic

Lens status: _____

Diagnosis: _____

Duration of diagnosis: _____

Stage of glaucoma:_____