**DISSERTATION PROPOSAL**

# SUPPLEMENTARY KELULUT HONEY THERAPY IN JUVENILE OPEN-ANGLE GLAUCOMA: EFFECTS ON IL-6, RNFL AND DRY EYE

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# CHAPTER 1: INTRODUCTION

## 1.1 STUDY INTRODUCTION

Glaucomatous optic neuropathy (GON) is a group of eye disease, characterized by progressive by loss of retinal ganglion cells (RGC) and axonal degeneration with concomitant optic disc cupping, thinning of the retinal nerve fiber layer (RNFL) and clinically detectable early visual field defects.

It represents a leading cause of irreversible visual impairment and blindness worldwide, estimated to affect 111.8 million people worldwide in 2040 (Tham YC et al, 2014). According to National Eye Survey II, glaucoma is the third leading causes of blindness and low vision in Malaysia (Chew et al, 2018).

Glaucoma can be further divided into two main categories, which are open or closed angle glaucoma. It can be subdivided into primary or secondary. Primary open angle glaucoma (POAG) is the most common type of glaucoma and occurs in elderly individuals with an open angle without gonioscopic abnormalities. Juvenile open angle glaucoma (JOAG) affects the younger population age ranging from 3 to 40 years old.

Pro-inflammatory cytokines such as Interleukin-6 have been linked to play an active role in the immunomodulation of the nerve layer, leading to glaucomatous optic neuropathy. Stingless bee honey (Kelulut) was found to have antioxidant, anti-inflammatory and free radical scavenging properties. The beneficiary effects of honey confer protective effects on neurons against oxidative damage as well as reducing the pro-inflammatory cytokine such as Interleukin-6. We believe that this non-invasive study which involves evaluation of honey Kelulut on the mean

serum level of Interleukin-6 and retinal nerve fiber layer thickness will provide a potential supplementary therapy for JOAG.

## 1.2 STUDY RATIONALE

Juvenile open-angle glaucoma (JOAG) is a rare form of primary open-angle glaucoma that manifests between the ages of 3 and 40. It is often associated with mutations in the myocilin (MYOC) gene, leading to improper drainage of aqueous humor and resulting in elevated intraocular pressure (IOP). This increased IOP can cause optic nerve damage, leading to irreversible vision loss. JOAG typically follows an autosomal dominant inheritance pattern and often requires surgical intervention due to its resistance to medical therapies.

Kelulut honey, produced by stingless bees, has demonstrated significant anti-inflammatory and antioxidant properties, attributed to its high polyphenolic content. These properties suggest potential neuroprotective effects, which could be beneficial in managing neurodegenerative conditions. In view of oxidative stress and inflammation are key factors in the pathogenesis of glaucoma, Kelulut honey may serve as a valuable adjunct to conventional antiglaucoma therapies.

Monitoring serum levels of interleukin-6 (IL-6), peripapillary retinal nerve fiber layer (RNFL) thickness and its effect on dry eye parameters are established methods for evaluating inflammatory status and glaucomatous damage, respectively. The immunomodulatory effects of Kelulut honey could potentially modulate these biomarkers, leading to improved clinical outcomes for JOAG patients. Findings from research into Kelulut honey's therapeutic potential may lead to the development of more effective treatment strategies for JOAG, ultimately enhancing patient quality of life.

JOAG is a progressive condition that can lead to irreversible damage to the optic nerve if not treated promptly. Given its early onset (often in individuals under 40), the risk of significant vision loss over a long period is high if IOP is not controlled. Since JOAG typically affects younger individuals, if left untreated, the cumulative loss of vision can be substantial, potentially affecting the patient's ability to work, drive, and perform daily activities. The psychological burden of losing vision at a young age can be immense, potentially leading to depression and anxiety, especially for those who are still in school or early adulthood. The role of immunomodulation via the usage of Kelulut honey can prevent the disease from progressing to advanced stages where treatment becomes more complex and reduces the need for invasive interventions.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 JUVENILE OPEN ANGLE GLAUCOMA

Juvenile open angle glaucoma (JOAG) is a type of primary open angle glaucoma, where the onset of disease ranges from 3-40 years of age (Elgin U et al, 2014). Genetic factors have been implicated in the pathophysiology of POAG. JOAG is most commonly inherited in an autosomal dominant fashion with incomplete penetrance. This inheritance pattern has been documented in various studies of familial glaucoma cases (Wiggs & Pasquale, 2017).

Among the major genes associated with JOAG includes Myocillin (MYOC), Cytochrome P450 1B1 (CYP1B1), Forkhead Box C1 (FOXC1), Optineurin (OPTN). Myocilin, *MYOC* gene mutations are associated with 2% to 4% of POAG, and this mutation are reported to be higher in JOAG (Fingert JH et al, 2002). The MYOC gene is located on chromosome 1q24.3 (Stone et al., 1997). It encodes myocilin, a protein thought to be involved in the regulation of intraocular

pressure (IOP) by modulating aqueous humor outflow through the trabecular meshwork. MYOC mutations account for 10–30% of JOAG cases (Sarfarazi & Stoilov, 2000). Mutation of myocilin results inprotein misfolding and accumulation in the trabecular meshwork cells, causing impaired aqueous outflow and increased IOP. MYOC mutations often result in early-onset, aggressive disease and genetic screening for MYOC mutations is recommended in families with a history of JOAG (Wiggs & Pasquale, 2017).

CYP1B1 is located on chromosome 2p22.2 (Kaur, Mandal, & Chakrabarti, 2008). It plays a role in ocular development and metabolism of endogenous compounds. Homozygous or compound heterozygous mutations in CYP1B1 can lead to structural abnormalities in the trabecular meshwork. Higher prevalence are seen in Middle Eastern, Indian, and other consanguineous populations.

Forkhead Box C1 is located on chromosome 6p25 (Wiggs & Pasquale, 2017). It is involved in the regulation of ocular embryogenesis. Mutations are associated with anterior segment dysgenesis and increased risk of JOAG.

Optineurin (OPTN) is located on chromosome 10p13 (Fingert, 2011), a gene responsible in neuroprotection and the regulation of apoptosis in retinal ganglion cells. Some mutations have been linked to both normal-tension glaucoma and JOAG.

While specific genes like MYOC and CYP1B1 have been implicated, JOAG is likely influenced by multiple genetic loci, each contributing a small effect. Genome-Wide Association Studies (GWAS) have identified additional loci associated with glaucoma risk, including regions near TMCO1, CDKN2B-AS1, and SIX6 (Tham et al., 2014). These loci highlight pathways related to trabecular meshwork function, retinal ganglion cell survival, and anterior chamber development.

While comprehensive epidemiological data specific to Malaysia is limited, studies from neighboring countries and regional research provide valuable insights into its prevalence and characteristics. A recent population-based study in South Korea reported that the incidence of JOAG was 5.02 per 100,000 residents and average prevalence of JOAG was 14.17 per 100,000 residents (Baek Su et al, 2024). In a study conducted in Singapore, JOAG accounted for 3.3% of all newly diagnosed glaucomas, with a mean age at diagnosis of 26 ± 9.8 year (Selvan H et al, 2022).

JOAG is distinct from congenital glaucoma which presents with buphthalmos, megalocornea, Haab's striae, and ocular or other systemic developmental anomalies. JOAG is distinguished by its elevation in intraocular pressure with large fluctuations, faster progression and severity of visual field loss compared to adult POAG. Managing JOAG patients is challenging as medical treatment is often inadequate where most patient eventually requires surgical intervention for effective management (Ciociola EC et al 2022). The resulting visual impairment and blindness can significantly impair the patient's quality of life and limit daily living activities.

## 2.2 IMMUNOMODULATION

Recent studies suggest that immune system and neuroinflammation plays a major contributing factor in the pathogenesis of optic neuropathy in glaucoma (Adornetto A et al, 2019). Adaptive and protective responses of resident or systemic immune cells can support neurons and promote tissue repair mechanisms after injurious insults, however prolonged inflammatory processes can also produce neurotoxic mediators (Tezel G, 2013). In glaucoma, chronic glial activation along with a failure in the regulation of immune response pathways may compromise the immune homeostasis resulting in neurodegeneration and promote secondary injury to neurons. Both

astroglia and microglia resident in the retina and optic nerve (head) profoundly respond in glaucomatous human donor eyes and animal models with glaucoma.

Astroglia (retina and optic nerve astrocytes, and retinal Müller cells) normally preserve tissue homeostasis, maintain synapses, recycle neurotransmitters, deliver neurotrophic factors and metabolites, control blood flow and vascular barriers, and participate neurogenesis. These neurosupportive cells quickly recognize and respond to stress and injury signals as a neuronal defense and recovery mechanism. In glaucomatous optic nerve head, astroglia responses leads to tissue remodeling and glial scarring, creating biomechanical stress on optic nerve axons. Microglia cells normally provide neurotrophic support, and their scavenger and phagocytosing functions promote tissue cleaning and healing. Similar to astroglia cells, its chronic activation leads to injury to optic nerve axons (Barış M, 2019)

Other cells such as myelin-producing oligodendrocytes and blood-borne monocytes are also linked to inflammatory component in glaucoma. Thus, chronic tissue stress, neuron injury, glial and systemic immune responses, and sustained release of neurotoxic mediators may create a vicious cycle that may promote progressing neuron injury. Treatments targeting these neurodestructive outcomes is critical for the survival of neuronal tissue while avoiding neurodegenerative inflammation.

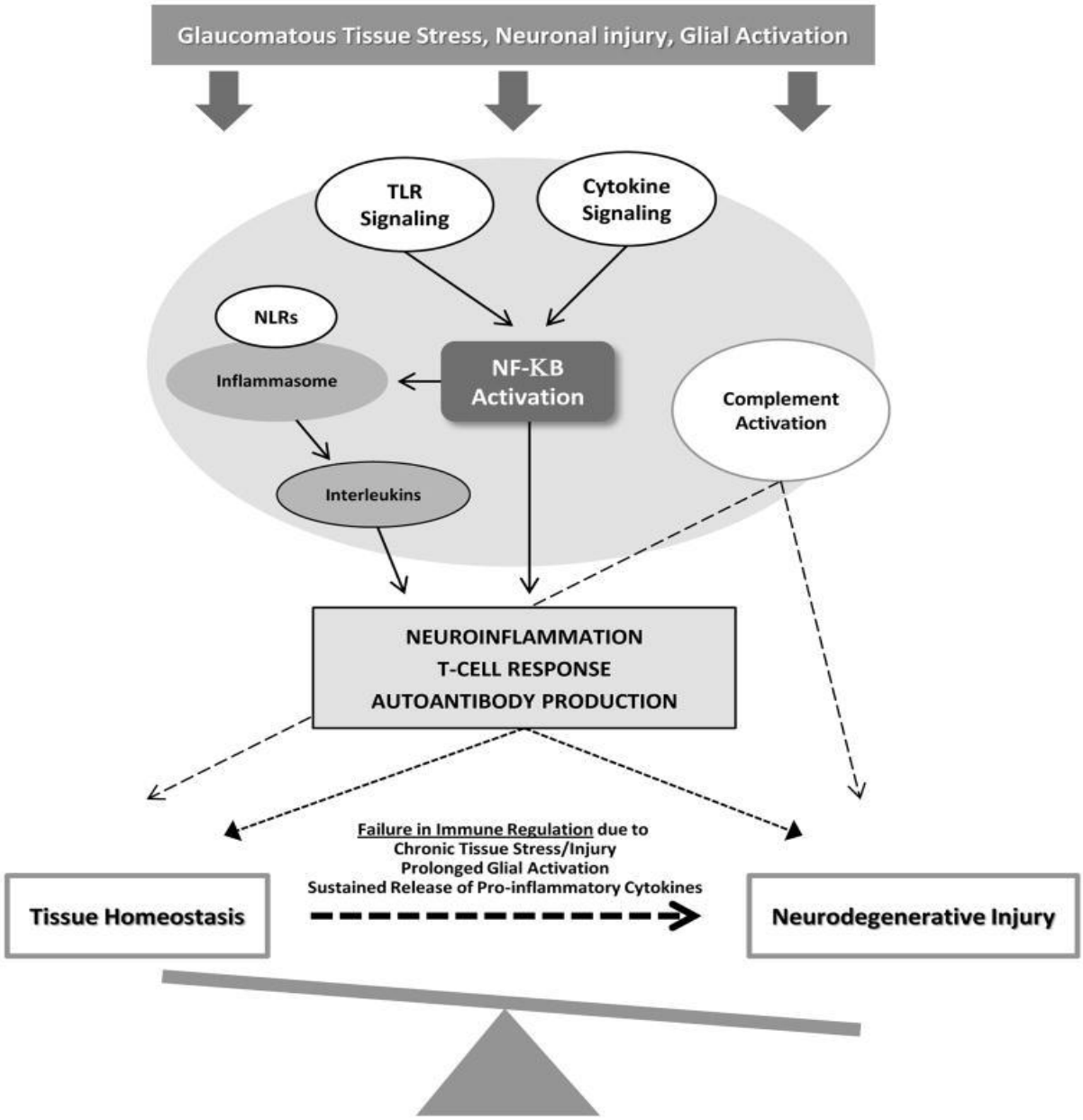


Figure 1: Immune regulation in glaucoma. Various pathways, such as pro-inflammatory cytokine signaling (including TNF-α/TNFR signaling), TLR signaling, NF-κB activation, NLRs and inflammasome assembly, and complement activation are co-players of inflammatory responses in glaucomatous tissues. A failure in the regulation of immune response pathways may shift the physiological equilibrium toward an inflammatory neurodegenerative process.

## 2.3 INTERLEUKIN-6

IL-6 is a proinflammatory cytokines that plays an important role in mediating inflammation in glaucomatous eye. Retinal ganglion cells (RGC) do not normally regenerate axons after injury, instead undergo apoptotic cell death (Leibinger M et al, 2016). IL-6 is synthesized by injured retinal ganglion cells following elevation of intraocular pressure and transported in an orthograde fashion along the nerve, accumulating at axonal disruption in the optic nerve head (Chidlow G et al, 2012). This results in inflammation and apoptosis of RGC.

While direct studies on serum IL-6 levels in JOAG are lacking, existing research on POAG and NTG suggests a potential association between IL-6 and glaucoma. Studies also suggests that higher serum levels of IL-6 are associated with primary open angle glaucoma and its severity (Huang P et al, 2010, Ulhaq ZS et al, 2021). Another study found that serum IL-6 levels were borderline higher in NTG patients compared to controls, suggesting a potential association between serum IL-6 alterations and glaucoma. (Lin KH et al, 2014).

## 2.4 RETINAL NERVE FIBER LAYER

The retinal nerve fiber layer (RNFL) contains the non-myelinated axons of the RGCs that form the optic nerve. RNFL thinning reflects the axonal loss within the optic nerve. In normal discs, the RNFL is thicker in the inferior and superior quadrants of the disc, and thinner in the nasal and temporal quadrants of the disc. The mean RNFL thickness for the normal population is 97.3 ± 9.6 µm (Alasil T et al, 2013) whereas the rate of normal age-related retinal thinning is -0.54 ± 0.23 µm/year (Wu Z et al, 2017).

Structural damage almost always precedes functional damage. Damage to the RNFL precedes observable changes in the optic nerve head and significant RNFL loss can occur before reproducible functional loss can be demonstrated (Choplin NT et al, 2015). Using light from a low coherence source, optical coherence tomography (OCT) precisely measures the RNFL surrounding the optic nerve head (peripapillary RNFL). A normative database of the RNFL thickness has been obtained to identify and monitor the changes during follow up.

RNFL thinning often precedes visual field loss, which is why monitoring RNFL changes is so important. In the early stages, patients with JOAG may not notice significant changes in their vision, but OCT can detect the thinning of the RNFL long before visual field defects appear. The rate at which the RNFL thins over time is a critical factor in determining the progression of JOAG. A rapid rate of RNFL loss may indicate that the glaucoma is progressing more quickly, and treatment may need to be adjusted to prevent further damage.

## 2.5 STINGLESS BEE HONEY (KELULUT)

With the advent of recent scientific approaches, honey is being widely recognized as treatment adjuvant for many diseases owing to its anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer and wound healing properties (Al-Hatamleh MA et al, 2020). Honey possesses a wide variety of bioactive components, approximately 200 different compounds. It beneficiary effects is attributed particularly by flavonoids and polyphenols, which protects neurons against oxidative damage and neurotoxicity, improve neuronal function and enhance regeneration (Iftikhar A et al, 2022).

Stingless bee honey (Kelulut) is a tropical rainforest honey produced by tropical Meliponini bee species. It is postulated that Kelulut has better antioxidant, anti-inflammatory and free radical

scavenging properties than other local honey, owing to the higher contents of phenolic acid and flavonoid substance (Kek SP et al, 2017).

Recent evidence indicates stingless bee honey decreased the IL-6 secretion by macrophages after lipopolysaccharide (LPS) stimulation, reaching a reduction of 43.9% (Biluca FC et al, 2020). In support of these findings, stingless bee honey decreased the circulating levels IL-6 in different tissues of lipopolysaccharide-induced rats (Ranneh Y et al, 2021). Trigona honey (Madu Kelulut) significantly reduced the mean IL-6 level among ventilated pneumonia patient in intensive care unit compared to control group (p value < 0.001). The reduction was observed between Day 0 and Day 6 of treatment (Omar SC et al, 2020). In Malaysia, the domestic cultivation of Kelulut honey on a large scale is made possible in a controlled farm environment, hence adding to the cost effectiveness (Mustafa MZ et al, 2018). The majority of honey's health-promoting qualities can only be obtained by applying large dosages of the substance, between 50 and 80 g per intake (Bogdanov S et al, 2018).

Another study evaluated the effect of honey on macular thickness, retinal nerve fiber layer thickness and optic nerve head parameters in post-menopausal women with honey cocktail of 20g/day for 3 months showed significant increase in the mean change of global RNFL thickness in honey cocktail group at three months post honey cocktail supplement, with p value of < 0.001 (Sivagurunathan et al, 2019).

The article titled "Therapeutic Potential of Honey and Propolis on Ocular Disease" by Norhashima et al published in *Pharmaceuticals* in November 2022, provides a comprehensive review of the pharmacological properties of honey and propolis in the context of ocular health. A clinical study Jung Won Park et al. highlights the neuroprotective effects of Brazilian Green Propolis (BGP) on retinal ganglion cells (RGCs) in an ischemic mouse retina model. Results suggest that BGP has a neuroprotective effect on RGCs through the upregulation of histone acetylation, downregulation of apoptotic stimuli, and suppression of NF-κB mediated

inflammatory pathway in ischemic retina. Kelulut honey which also known for its potent anti-inflammatory and antioxidant properties, might have similar effects on the retina. This study is proposed to evaluate whether *Kelulut* honey reduces IL-6 levels and protects against neurodegeneration in JOAG patients, paralleling the effects seen with propolis in the mouse model.

# CHAPTER 3: STUDY OBJECTIVES, RESEARCH QUESTIONS, RESEARCH HYPOTHESIS

## 3.1. STUDY OBJECTIVES

### 3.1.1. GENERAL OBJECTIVES

**To evaluate the effects of supplementary therapy of stingless bee honey (Kelulut) on the mean serum level of Interleukin-6, retinal nerve fiber layer thickness and dry eye disease parameters in patients with JOAG.**

### 3.1.2. SPECIFIC OBJECTIVES

1. To compare the mean serum level of Interleukin-6 in patients with JOAG with and without honey Kelulut consumption for 3 months.
2. To compare the mean retinal nerve fiber layer thickness in patients with JOAG with and without honey Kelulut consumption for 3 months.

**3. To compare the tear break-up time (TBUT) and Schirmer test in patients with JOAG with and without honey Kelulut consumption for 3 months.**

**4. To compare the level of glycated hemoglobin (HbA1c) level in patients with JOAG with and without honey Kelulut consumption for 3 months.**

## 3.2. RESEARCH QUESTIONS

3.2.1. Does the mean serum level of interleukin-6 differs with and without

consumption of honey Kelulut for 3 months among JOAG patients?

3.2.2. Does the mean thickness of retinal nerve fiber layer increases with consumption of honey Kelulut for 3 months among patients with JOAG?

**3.2.3. Does the tear break-up time (TBUT) and Schirmer test increases with consumption of honey Kelulut for 3 months among patients with JOAG?**

**3.2.4. Does the level of glycated hemoglobin (HbA1c) increases with consumption of honey Kelulut for 3 months among patients with JOAG?**

## 3.2. RESEARCH HYPOTHESIS

3.2.1. The mean serum level of Interleukin-6 reduces after consumption of honey Kelulut for 3 months among patients with JOAG.

3.2.2. The mean thickness of retinal nerve fiber layer increases after consumption of Honey Kelulut for 3 months among patients with JOAG.

**3.2.3 The tear break-up time (TBUT) and Schirmer test increases after consumption of honey Kelulut for 3 months among patients with JOAG.**

**3.2.4 The level of glycated hemoglobin (HbA1c) does not increase with the consumption of honey Kelulut for 3 months among patients with JOAG.**

## 3.3. NULL HYPOTHESIS

3.3.1. For Interleukin-6 (IL-6) levels:
Null Hypothesis:
There is no significant difference in the mean serum level of Interleukin-6 (IL-6) before and after 3 months of *Kelulut* honey consumption among patients with juvenile-onset open-angle glaucoma (JOAG).

3.3.2. For retinal nerve fiber layer (RNFL) thickness:
Null Hypothesis:
There is no significant difference in the mean retinal nerve fiber layer (RNFL)

thickness before and after 3 months of *Kelulut* honey consumption among patients with juvenile-onset open-angle glaucoma (JOAG).

**3.3.3 For dry eye disease parameters**
**Null Hypothesis:**
**There is no siginificance difference in tear break-up time (TBUT) and Schirmer test increases after consumption of honey Kelulut for 3 months among patients with juvenile-onset open-angle glaucoma (JOAG).**

**3.3.4 For level of glycated hemoglobin**
**Null Hypothesis:**
**There is no siginificance difference in the level of glycated hemoglobin level after consumption of honey Kelulut for 3 months among patients with juvenile-onset open-angle glaucoma (JOAG).**

## CHAPTER 4: METHODOLOGY

### 4.1. STUDY DESIGN

Randomised controlled trial

### 4.2. STUDY POPULATION, LOCATIONS AND DURATION

Study population:

Patients diagnosed with Juvenile Open Angle Glaucoma (JOAG)

Study site:

Department of Ophthalmology Hospital Universiti Sains Malaysia (HUSM)

Department of Ophthalmology Hospital Raja Perempuan Zainab II (HRPZ II)

Department of Ophthalmology Hospital Sultanah Nur Zahirah (HSNZ)

Department of Ophthalmology Hospital Sultan Ismail Petra (HSIP)

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Study site involves patients from the Department of Ophthalmology attending Glaucoma follow up clinic, under the supervision of Glaucoma Specialist. These centers have the appropriate expertise, facilities and patient populations.

All recruited patients are managed by trained Glaucoma specialists following a standardized protocol for the administration of Kelulut honey, clinical assessments, and data collection to minimize inter-site variability. Further measures include standardize procedures for IL-6 sample collection, RNFL imaging, and standardized clinical evaluations across all sites.

Study duration:

Feburary 2026– June 2026

End point: Completion of honey supplement for 3 months, followed by comprehensive ophthalmic examination, blood sampling collection and OCT RNFL measurement.

## 4.3. SAMPLING METHOD

Sampling method

1. All JOAG patients that fulfilled selection criteria

2. Patients who are treated at Ophthalmologyy Clinic HUSM, HPRZ II, HSNZ and HSIP from February 2026 – November 2026.

## 4.4. SAMPLE SIZE

Sample size calculation

GPower 3.1.9.7 software was used to calculate the sample size.

The power of the study will be set at 80% with a significance level of 5%.

For the sample size calculation was statistical data used was repeated measures ANOVA within and within between.

The largest sample size was 24.

To account for the attrition rate, 20% was added, which gives a total estimated minimum sample size of 30 per group.

Total sample size: 60 (30 per group)

Objective 1:

To compare the mean serum level of Interleukin-6 before and after consumption of honey Kelulut for 3 months among patients with JOAG.

Calculated sample size: 24

By the addition of 20% drop outs, the sample size that needs to be collected for each group is 30 subjects

Total sample size: 60

Objective 2:

To compare the mean RNFL thickness before and after consumption of honey Kelulut for 3 months among patients with JOAG**.**

Calculated sample size: 24

By the addition of 20% drop outs, the sample size that needs to be collected for each group is 27 subjects.

Total sample size: 60

## 4.5. SELECTION CRITERIA

Inclusion criteria

All JOAG patients age 15 years old and above – 40 years old at the time of diagnosis

Compliant to conventional treatment and achieved target intraocular pressure

Exclusion criteria

Patients with allergy to honey or honey-based products

OCT signal less than 6/10

Concurrent retinal diseases and other optic neuropathies

Systemic neurodegenerative disease

Systemic comorbidities such as diabetes mellitus and autoimmune diseases

Patients on other honey-based products for the last 3 months

Patient who consumes honey-based products during the study duration of 3 months (monitoring via honey-diary, return of the used sachet during the follow up visits)

Patients on anti-oxidants, traditional medications or herbal products for the last 3 months

Topical usage of steroids, systemic steroids and non-steroidal anti-inflammatory drugs.

Sepsis

Compliance less than 75%.

The duration of treatment with honey will be 3 months (90 days), patient who missed more than 22 sachets of honey will be excluded.

Compliancy will be assessed through regular monitoring via monitoring of honey-diary and patients are required to return the used honey sachets during clinic visits.

Withdrawal criteria

All patients who do not receive standard treatment for JOAG

All candidates who develops sudden unexpected severe allergic reaction (SUSAR) to the investigated product.

Participants who withdraw or drop out of the study will not be replaced**.** Follow-up assessments will continue for all participants, including those who withdraw from the honey supplementation, to allow for comprehensive data collection and minimize loss to follow-up. Throughout the study period, participants will continue their conventional glaucoma medications without interruption to ensure standard clinical care is maintained.

## 4.6. RANDOMISATION

The design for this study includes four centers, with two centers randomly assigned to the treatment "honey" group and the remaining two to the control "no-honey" group.

Individual randomization poses practical limitations due to the rarity of juvenile-onset open-angle glaucoma (JOAG), In this context, such randomization is more feasible and logistically advantageous, particularly in terms of coordinating the supply *Kelulut* honey to patients and simplifies distribution of *Kelulut* honey. The *Kelulut* honey is supplied to participants on a monthly basis. During each monthly visit, compliance with the honey

consumption regimen is reviewed, and any potential side effects are systematically monitored and documented. This regular follow-up ensures adherence to the intervention and allows for timely identification of adverse events, thereby enhancing the safety and reliability of the study.

Blood samples for the measurement of serum Interleukin-6 (IL-6) levels will be collected and these samples will then be transported under controlled conditions to the HUSM (Hospital Universiti Sains Malaysia) laboratory for analysis. This ensures a coordinated transportation of blood samples to HUSM laboratory.

## 4.7. HONEY SUPPLEMENT

The honey used in this study is Brainey Madu Kelulut, harvested from Heterotrigona Itama. It will be supplied for a total of three months after the recruitment and completion of initial examination and blood sampling. The participants in the ‘no honey’ group did not receive any placebo. Patient in the ‘no-honey’ group is prohibited from consuming products that is based on honey Kelulut or other honey-based products throughout the study period. The control group will consist of matched JOAG patients receiving standard treatment without honey.

Both the patients and the Principal Investigator will not be blinded by the modality of treatment.

Participants were instructed regarding the consumption of the honey by the Principal Investigator. The honey will be supplied in the form of sachet, dosage of 30g per sachet per day for 3 months. The participants are advised to consume the honey at least 30 minutes prior to meals or other medications to avoid any interactions. Participants are also advised not to mix or dilute the honey using other solutions or food products. Participants are also prohibited from consuming other honey-based products throughout

the duration of the study. Those who develop any allergic reaction such as rashes, shortness of breath, wheezing, dysphagia or anaphylactic reactions are advised to immediately discontinue the product and seek immediate treatment from the nearest Emergency Department and contact the Principal Investigator regarding the side effects.

The principal investigator will also advise the patients regarding the compliance to treatment. The compliance will be monitored via a printed diary that will be given upon enrolment. Patients are required to tick on the diary upon completion of the honey sachets daily.

## 4.8. DEFINITION OF TERMS

Juvenile open angle glaucoma

Juvenile-onset open-angle glaucoma (JOAG) is a subset of primary open-angle glaucoma that is diagnosed before 40 years of age. *Myocilin* gene mutations are the most commonly associated. JOAG is characterized by high intraocular pressures (IOP elevation in intraocular pressure with large fluctuations, faster progression and severity of visual field loss compared to adult POAG.

Interleukin-6

IL-6 belongs to the class of four-helical cytokines. The cytokine can be synthesized and secreted by many cells. It acts via a cell surface-expressed interleukin-6 receptor, which is not signaling competent. This receptor, when complexed with interleukin-6, associates with the signaling receptor glycoprotein 130 kDa (gp130), which becomes dimerized and initiates intracellular signaling via the Janus kinase/signal transducer and activator

of transcription and rat sarcoma proto oncogene/mitogen-activated protein kinase/phosphoinositide-3 kinase pathways.

Retinal nerve fiber layer

The retinal nerve fiber layer (RNFL) or nerve fiber layer, stratum opticum, is formed by the expansion of the fibers of the optic nerve; it is thickest near the optic disc, gradually diminishing toward the ora serrata. As the nerve fibers pass through the lamina cribrosa sclerae they lose their medullary sheaths and are continued onward through the choroid and retina as simple axis-cylinders. Upon reaching the internal surface of the retina they radiate from their point of entrance over this surface grouped in bundles, and in many places arranged in plexuses.

Honey Kelulut

Stingless bee honey (Kelulut) is a tropical rainforest honey produced by tropical Meliponini bee species, bees are from genus Trigona. stingless bees (*Meliponini* sp.) or *lebah Kelulut* which do not have stingers, build nests in already existing cavities or hollowed out areas of trees, buildings and hives. The major composition of stingless bee honey includes sugars (fructose and glucose) with nearly zero hydroxymethylfurfural (HMF). It also contains small amounts of other compounds, such as organic acids, phenolic compounds (eg., phenolic acids and flavonoids), proteins, amino acids (eg., phenylalanine, alanine, tyrosine, valine, acetate and trigonelline), enzymes, vitamins and minerals.

## 4.9. STUDY INSTRUMENTS

#### 4.9.1. SLITLAMP BIOMICROSCOPY

Slit lamp biomicroscope (Topcon Corp, Japan) was used for ocular examination. It is used for assessment of the patients' eye through binocular, stereoscopic magnified views of the anterior segment and posterior segment. Posterior segment examination was conducted with the aid of + 78 or +90 Diopter lens.

#### 4.9.2. GOLDMAN APPLANATION TONOMETER (HAAG STREIT, GERMANY)

Goldman applanation tonometer (Haag Streit, Germany) has been regard as a gold standard to measure intraocular pressure (IOP), based on Imbert-Fick principle.

#### 4.9.3. GOLDMAN GONIOSCOPE

The status of angle structure in the anterior segment of the eye was determined by using single mirror Goldman gonioscopy lens.

#### 4.9.4. HUMPHREY VISUAL FIELD ANALYSER II MODEL 750

It is an automated, static, computerized perimetry machine used as the gold standard perimetry to assess patients' visual field. Swedish Interactive Threshold Algorithm Standard (SITA-STANDARD) with test pattern 10-2 or 24-2 full threshold was used as strategy in assessment of visual field.

#### 4.9.5. ELISA KIT

The BioLegend LEGEND MAX™ Human IL-6 ELISA Kit is a Sandwich Enzyme Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is precoated with a rat monoclonal anti-human IL-6 antibody. The detection antibody is a biotinylated rat monoclonal anti-human IL-6 antibody. This kit is specifically designed for the accurate

quantitation of human IL-6 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

4.9.6. OPTICAL COHERENCE TOMOGRAPHY Cirrus HD-OCT 500

(Carl Zeiss Meditec, Germany)

OCT is a noninvasive in vivo cross-sectional imaging technology using low-coherence interferometry. OCT is the optical analogue of ultrasound imaging; it generates high-resolution cross-sectional 2D images of internal tissue microstructures. Specifically pertaining to glaucoma, OCT enables the objective quantitative evaluation of critical neural structures, including the RNFL, the optic nerve head (ONH), and the macula. By comparing these measurements with established normative databases, clinicians can determine whether structures are borderline or outside normal limits, improving detection of disease and its progression.

The eyes are dilated with Gutt Tropicamide 1% and Gutt Phenylephrine 5% before recording the images. Internal fixation was chosen (subject fixation with the eye being studied because of better reproducibility). The average thickness for each composite scan was calculated by taking the arithmetic mean of the 100 data points. In addition, the peripapillary RNFL thickness was divided into four quadrants defined as superior (46 to 135 degrees), nasal (136 to 225 degrees), inferior (226 to 315 degrees), and temporal (316 to 45 degrees).

## 4.10. DATA COLLECTION AND ANALYSIS

### 4.10.1. PATIENT RECRUITMENT

The study will be conducted after obtaining approval from the Universiti Sains Malaysia Ethical Committee. Patient from the Department of Ophthalmology Hospital Universiti Sains Malaysia (HUSM), Department of Ophthalmology Hospital Raja Perempuan Zainab II (HRPZ II), Department of Ophthalmology Hospital Sultanah Nur Zahirah (HSNZ) and Department of Ophthalmology Hospital Sultan Ismail Petra (HSIP) will be selected according to our inclusion and exclusion criteria. Participants who fulfilled the selection criteria are identified and provided with the information sheet regarding the study. Written informed consent is obtained from all participants or legal guardian.

The eye with the better visual acuity will be selected to standardize the examination. A comprehensive ophthalmic assessment is done which includes visual acuity, Schirmer test, Tear break-up time (TBUT), intraocular pressure measurement by Goldmann applanation tonometry, slitlamp and fundus examination and gonioscopy using Goldmann three mirror contact lens. OCT macula and RNFL thickness measurement using optical coherence tomography is done after pupillary dilatation by some identified single-blinded trained personnel.

Blood sampling will be taken from all participants, serum separated, stored and transported to HUSM Laboratory and assayed. Quantification of cytokine IL-6 performed using IL-6 enzyme-linked immunosorbent assay (ELISA) kit. The participants were randomized into either ‘honey’ or ‘non-honey’ groups. The honey used in this study is Brainey Madu Kelulut, harvested from *Heterotrigona Itama* supplied for a total of three months after the recruitment and completion of initial examination and

blood sampling. The participants in the 'no honey' group did not receive any placebo. After 3 months of honey supplement therapy, participants in both groups were reassessed during follow-up. The mean serum IL-6 and RNFL thickness were re-measured, using the same OCT machine and IL-6 ELISA test kit. Participants who were not compliant to the honey supplement were excluded from the study.

### 4.10.2. COLLECTION OF BLOOD SAMPLE

Approximately 3-4 milliliters of venous blood will be taken from all participants and collected by identified trained blinded medical personnel into a plain tube, sample will be labelled. Similar amount is collected into EDTA tube for HbA1c analysis. The sample is allowed to be at room temperature about 20-30 minutes to allow for clot formation. Centrifugation is then done for 10 minutes; the resultant supernatant is the serum. The serum formed will be transferred into a clean polypropylene tube using Pasteur pipette, the serum is later stored and transported at temperature -70°C or lower.

The sample will be transported to the Immunology Laboratory at Hospital Universiti Sains Malaysia for quantification and analysis of IL-6, processed by a identified trained blinded laboratory personnel.

### 4.10.3. QUANTIFICATION OF IL-6

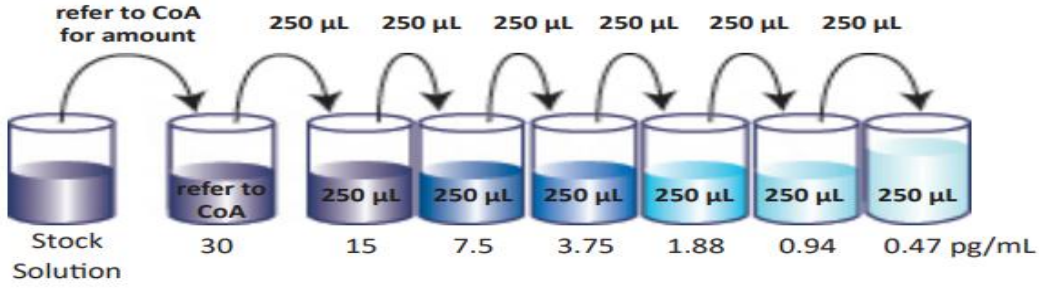
The serum level of Interleukin-6 is measured using the Human IL-6 ELISA kit with pre-coated serum (LEGEND MAXTM, BioLegend, Inc). The sampling, storage, assay procedures were done according to the kit protocol. The analysis will be conducted in the Biomedical Laboratory and Central Research Laboratory, USM.

4.10.4. REAGENT AND SAMPLE PREPARATION

The 20X Wash Buffer was diluted to with 1X deionized water. Any crystals formed within the 20X Wash buffer will be dissolved by bringing to room temperature and vortex until dissolved. Reconstitute the lyophilized Human IL-6 Standard by adding the volume of Assay Buffer A to make the standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely. Initial testing of biological samples should be performed to determine an optimal concentration that is within the linear range of the standard curve. Dilute samples using Assay Buffer A.

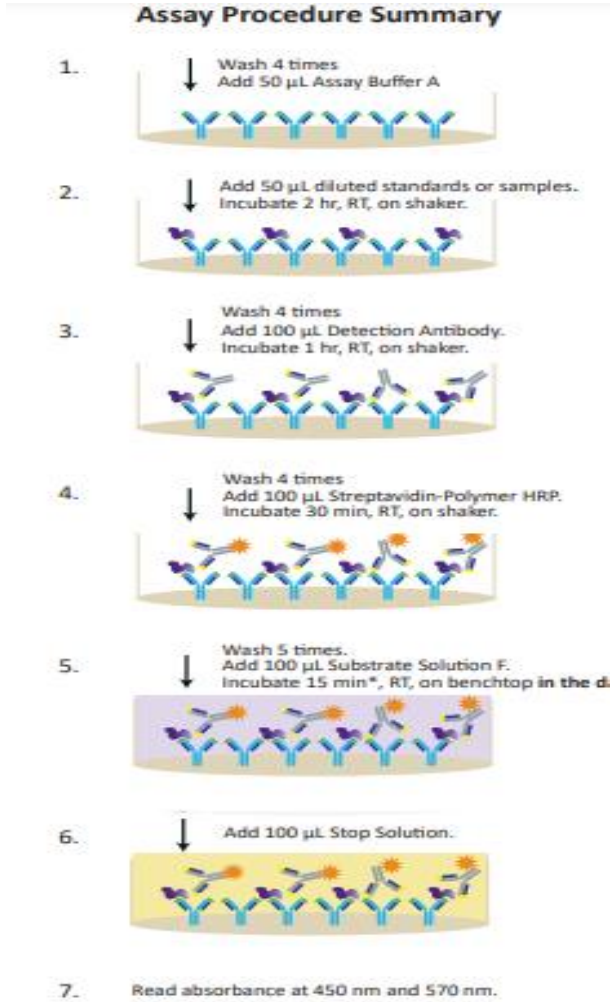
4.10.5. ASSAY PROCEDURES

Dilute 12.5 µL of the standard stock solution in 487.5 µL of Assay Buffer to create 500 µL of the 500 pg/mL top standard. Next, Assay Buffer A is used as the diluent and six two-fold serial dilutions are made using the 500 pg/mL top standard in different tubes. Assay Buffer A acts as the zero standard (0 pg/mL), and the human IL-6 standard concentrations in the tubes are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. Next, at least 300 µL of 1X Wash Buffer is used to wash the microplates four times.




50 µL of Assay Buffer A and 50 µL of standard dilutions or samples are added to the corresponding wells in each well. After that, the plates are sealed with a plate sealer and

incubated for two hours at room temperature with 200 rpm shaking. After discarding the contents, the plate is cleaned four times using 1X Wash Buffer. After adding 100 µL of Human IL-6 Detection Antibody solution to each well, the plate is sealed and left to incubate for an hour at room temperature while being shaken. After discarding the contents, the plate is cleaned four times using 1X Wash Buffer. Following that, 100 µL of Avidin-HRP After adding a solution, the plate is sealed and allowed to sit at room temperature for half an hour while being shaken. After discarding the contents, the plate is cleaned five times using 1X Wash Buffer. After adding 100 µL of Substrate Solution F to each well, each one is left in the dark for 15 minutes. Human IL-6-containing wells ought to become blue in a way that is proportionate to the amount of the substance present. Each well should contain 100 µL of Stop Solution to stop the reaction, and the resultant solution should turn yellow instead of blue. After 30 minutes, the absorbance at 450 nm wavelength is measured, and the findings are recorded. Sample will be discarded after the completion of data collection and analysis.




4.10.6. DATA COLLECTION AND ANALYSIS

Data recorded in data collection sheet transferred into electronic data and analyzed using Statistical Package for Social Sciences (SPSS), the latest version. The mean serum IL-6 and mean RNFL thickness of both groups will be analyzed using ANOVA repeated measures; within factors and within-between factors. All the p-value of less than 0.05 was considered statistically significant.

4.10.7. FOLLOW UP

Patients are assessed after a period of 3 months. RNFL thickness and serum IL-6 level will be measured. All these parameters will be measured using the same machine using same techniques. Compliance to the honey supplement will also be tested, by evaluating the diary filled by the patients and the consumed honey packages. Participants who were found to be less than 75 % compliant with the study were excluded from the study.

# CHAPTER 5: RESULTS

## 5.1 DEMOGRAPHIC DATA

| Variables | Honey group (n = 43) | Control group (n = 43) | *p* value |
|---|---|---|---|
| Age (years) | | | |
| Sex<br>Male, n (%)<br>Female, n (%) | | | |
| Race<br>Malay, n (%)<br>Chinese, n (%)<br>Indian, n (%)<br>Others, n (%) | | | |
| BCVA (Log MAR) | | | |
| Duration of JOAG (years) | | | |
| Number of topical antiglaucoma medications | | | |
| Received surgical intervention for IOP control | | | |

## 5.2 COMPARISON OF MEAN SERUM LEVEL OF IL-6 PRE AND POST 3 MONTHS OF HONEY (KELULUT) TREATMENT AMONG PATIENTS WITH JOAG

Comparison of mean serum level of IL-6 pre and post 3 months of honey (Kelulut) treatment among patients with JOAG

| **Mean serum IL-6 level** | Group | Mean diff (95% CI) | p value |
|---|---|---|---|
| Time | | | |
| Baseline | Honey | | |
| | No honey | | |
| 3 months' post treatment | Honey | | |
| | No honey | | |

## 5.3 COMPARISON OF MEAN RETINAL NERVE FIBER LAYER THICKNESS PRE AND POST 3 MONTHS OF HONEY (KELULUT) TREATMENT AMONG PATIENTS WITH JOAG

| **RNFL thickness** | Group | Mean diff (95% CI) | p value |
|---|---|---|---|
| Time | | | |
| Baseline | Honey | | |
| | No honey | | |
| 3 months' post treatment | Honey | | |
| | No honey | | |

## 5.4 COMPARISON OF DRY EYE DISEASE PARAMETERS PRE AND POST 3 MONTHS OF HONEY (KELULUT) TREATMENT AMONG PATIENTS WITH JOAG

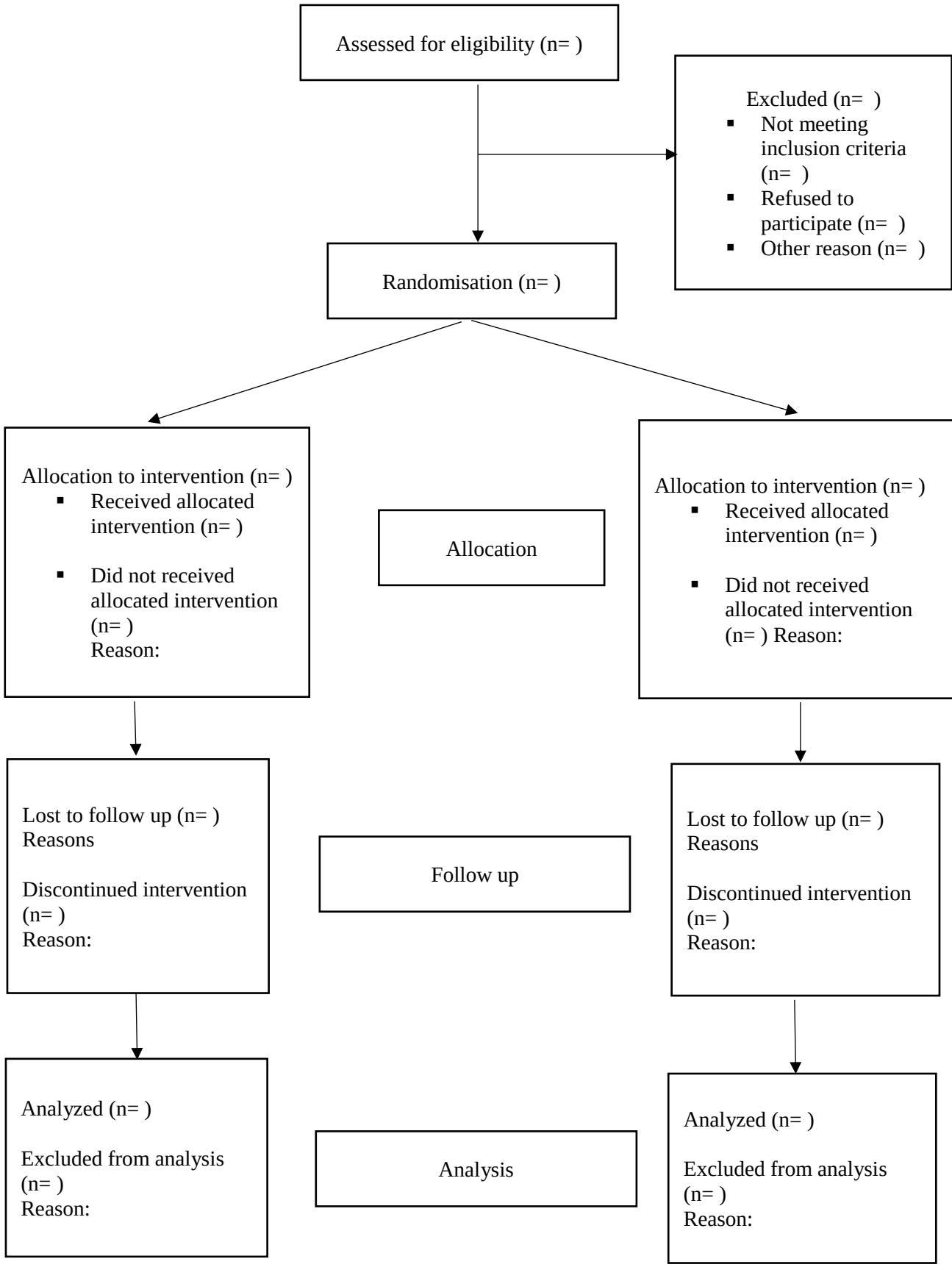
| Comparison of **TBUT** | Group | Mean diff (95% CI) | p value |
|---|---|---|---|
| Time | | | |
| Baseline | Honey | | |
| | No honey | | |
| 3 months' post treatment | Honey | | |
| | No honey | | |

| Comparison of **Schirmer test** | Group | Mean diff (95% CI) | p value |
|---|---|---|---|
| Time | | | |
| Baseline | Honey | | |
| | No honey | | |
| 3 months' post treatment | Honey | | |
| | No honey | | |

## 5.5 COMPARISON OF LEVEL OF GLYCATED HEMOGLOBIN PRE AND POST 3 MONTHS OF HONEY (KELULUT) TREATMENT AMONG PATIENTS WITH JOAG

| **HbA1c `** | Group | Mean diff (95% CI) | p value |
|---|---|---|---|
| Time | | | |
| Baseline | Honey | | |
| | No honey | | |
| 3 months' post treatment | Honey | | |
| | No honey | | |

**CHAPTER 6: FLOW CHART**



## CHAPTER 7: GANTT CHART

| Year | 2024 | 2024-2025 | | 2025-2026 | | 2026 |
|---|---|---|---|---|---|---|
| Months | Jan-May | Jun-Nov | Dec-May | Jun-Nov | Dec-May | Jun |
| Proposal preparation and ethical approval | → | → | → | | | |
| Data collection | | | | | → | → |
| Data analysis/ interpretation | | | | | → | → |
| Report writing | | | | | → | → |
| Submission of research book | | | | | | → |

## CHAPTER 8: MILESTONE

| Data | Expected achievement |
|---|---|
| Jan 2024 – November 2024 | Proposal presentation (Department of Ophthalmology USM)<br>Jawatankuasa Etika Penyelidikan Manusia (JEPeM)<br>USM presentation<br>NMRR application |
| December 2024-February 2025 | JEPeM and NMRR approval |
| March 2025 – May 2025 | 30% of data collection |
| June 2025 – November 2025 | 80% of data collection<br>30% of report writing |
| December 2025 – May 2026 | 100% data collection<br>100% of data analysis<br>100% of report writing |
| June 2026 | Submission of research book |

# CHAPTER 9: ETHICAL CONSIDERATION

## 9.1. ETHICS

This study will be conducted after the approval from the Ethical Committee USM, in accordance to the Declaration of Helsinki and International Conference of Harmonisation (ICH). All researchers of this study were GCP-certified.

## 9.2. VULNERABILITY

The subjects for this study involve subjects with JOAG attending the Ophthalmology Clinic HUSM, HRPZ II, HSNZ and HSIP that fulfills the study requirement and capable of giving their own consent. Legal age in Malaysia is 18 years old.

For participant aged 15 to-under 18 years old: Co-sign informed consent with parents are obtained.

They will be provided full information about the study's purpose, duration, procedure, benefit, risk and institutional approval. They will be given full authority to decide and full freedom to participate in the study without affecting his/her condition management and care. The investigator could withdraw the subject, or the subject could withdraw from participation in the study any time without penalty or any loss of benefits. The reason for withdrawal will be recorded. In cases of developing allergic reaction or severe adverse effects, appropriate treatment will be provided accordingly and patients will be followed up in ophthalmology clinic for further management and treatment.

## 9.3. CONFLICT OF INTEREST

There is no conflict of interest in any parts of this study, including towards the medical devices and pharmaceutical companies.

## 9.4. INFORMED CONSENT

Patients will be briefed regarding the study design, any adverse reactions related to the procedures and the anticipated benefits of the study. A summary of this information will be presented in written consent form. Consent is obtained from patients or guardians who agrees to participate in the study. A copy of this will be given to the subjects. A list of contact number of the investigators will also be included in the patient information leaflet. Any further information regarding the study will be informed to patient throughout the study period and patients will be informed regarding the result at the end of the study.

All the subjects will be informed regarding the study findings. This information will be shared as part of the informed consent process, ensuring participants are well-informed about the scientific basis for the study and any relevant outcomes from similar studies, including those that suggest potential benefits for retinal health. This ensures transparency and gives participants a comprehensive understanding of the context of the study.

## 9.5. PATIENT WITHDRAWAL

The investigators could withdraw the subject, or the subject could withdraw their participation from the study without any penalty or loss of benefits. The reason for withdrawal would be recorded in the Case Report form. Reasons for cessation of study subjects by investigators includes non-compliance with protocol and study requirement and adverse effect towards the drug.

## 9.6. RISK AND BENEFITS TO STUDY PARTICIPANTS

There are no serious adverse effects by the study procedures such as slit lamp examination/ RNFL measurement/venipuncture for serum IL 6 measurement. Dosage of the honey provided to the patient is within the therapeutic dosage. Patient will be periodically for adverse effect during monthly follow up. In case of developing adverse effects to the honey, patients are advised to immediately stop the honey, contact the study doctor and seek immediate treatment from the emergency service at the nearest hospital.

## 9.7. DATA HANDLING AND RECORD KEEPING

Participants' medical records will be kept in the study site (Ophthalmology Clinic and Visual Sciences, School of Medical Hospital Universiti Sains Malaysia. Data obtained from the study will be kept by the principal investigator in a password locked computer for up to 3 years after the study has ended after which the study data will be subsequently destroyed. No future research will be done using the similar data or biospecimens.

## 9.8. PRIVATE AND CONFIDENTIALITY

All participants will be given a subject identification number to maintain privacy and confidentiality. Participants will be informed of their clinical findings and will be managed accordingly during the study. Medical information will be kept confidential unless disclosed by law, the researcher, the ethical board for this study and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. Data collection forms are anonymous and the data will be entered into SPSS software. Only the research team members have access to the data. In the event that this study is published, study result will be published in a collective data format with no identification

of any individual or disclosure of the participants' personal information. If the study being published, relevant permissions will be obtained prior to publication.

## 9.9. COMMUNITY SENSITIVITIES AND BENEFITS

There is no element of sensitivity to the community in this research study. The findings from this study will greatly improve future management of JOAG both from the local and global community.

## 9.10. INCENTIVES AND HONORARIUM

All participants will be given a leaflet/ brochure that contains information regarding the juvenile open angle glaucoma and beneficial effects of honey on the neuromodulation involved in the progression of glaucoma disease. This study is fully funded, therefore there is no cost for the participant. Additionally, participants will continue to receive their standard conventional medications for glaucoma or other underlying conditions as prescribed by their treating ophthalmologist or healthcare provider, and these will be provided at their usual cost or as per their existing healthcare arrangements. An honorarium will be provided to participants as a token of appreciation for their involvement in the study. The honorarium will be issued once the MSO (Medical and Scientific Office) grant is approved**.**

## **9.11. PARENTAL ROLE**

Parents play a pivotal role in monitoring their child's compliance with both the conventional treatment and the investigational product; they also must ensure the child

follows the instructions that must be followed throughout the study period. The consumption of the product should be monitored directly by parents and be recorded in a honey diary provided to the participants, which will be handed to the investigator at the end of the study period.

### 9.12. ROLE OF PRINCIPAL INVESTIGATOR

Principal investigator will not be the direct service provider of the recruited patients. The principal investigator will be in charge if the subjects may have any enquiries or develop health issues throughout the study.

## CHAPTER 10: RESEARCH REGISTRATION

10.1 Registration

Registration number: RSCH ID-24-04301-DXV

Name of trial registry: National Medical Research Register (NMRR)

10.2 Protocol

Research Protocol is available for review at NMRR website

10.3 Funding

This research is funded by a research grant

Grant funding: Fundamental Research Grant Scheme, FRGS

Grant number: 203.PPSP.6171354

Ref Number KPT: FRGS/1/2022/STG02/USM/02/7

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# CHAPTER 12: APPENDICES

12.1 PATIENT INFORMATION AND CONSENT FORM

12.2 DATA COLLECTION FORM

12.3 HONEY TREATMENT DIARY

# RESEARCH INFORMATION

**Research Title:**
Supplementary Kelulut Honey Therapy In Juvenile Open-Angle Glaucoma: Effects On Il-6, RNFL And Dry Eye

**Name of main and co-Researcher:**
Dr Thamarai A/P Munirathinam MPM 67381 (HUSM)
Dr Azhany Binti Yaakub MPM 34859 (HUSM)
Dr Munira Binti Yusoff MPM 39594 (HRPZ II)
Dr Nor Anita binti Che Omar MPM 33552 (HSNZ)
Dr Norihan Binti Ibrahim MPM 49484 (HSIP)

## INTRODUCTION

You or the person under your care are invited to take part voluntarily in a interventional research. This research is about the evaluation of supplementary therapy of stingless bee honey (Kelulut) on patients with juvenile open angle glaucoma.

JOAG is a subset of primary glaucoma that affects the younger patients and the resulting disability from this disease is higher. It has an important impact on the quality of life of young adults whom represent the socioeconomically productive subgroup of population. It has a faster progression, higher magnitude of IOP and it is often refractory to medical treatment. Therefore, Kelulut may represent a possible adjuvant treatment to antiglaucoma medications owing to its anti-inflammatory and anti-oxidant properties. Serum level of IL-6 and peripapillary measurement of RNFL may possibly be used to monitor the changes. The immunomodulatory effects of Kelulut may possibly give beneficial effect to JOAG patients and results from this research can be used as a reference for development of better treatment outcomes.

It is important that you read and understand this research information before agreeing to participate in this study. You will receive a copy of this form to keep for your records if you agree to participate.

Your participation in this study is expected to be 3 months from the recruitment of participants until completion of the honey therapy and ophthalmic examinationsThis study is estimated to include up to **60** participants.

## PURPOSE OF THE STUDY

The purpose of this study are to determine the effects of supplementary therapy of stingless bee honey (Kelulut) on the serum level of Interleukin-6, retinal nerve fiber layer thickness and dry eye paarmeters in patients with juvenile open angle glaucoma.

## PARTICIPANTS CRITERIA

The research team members will discussed your eligibility to participate in this study. It is important that you are completely truthful with the staff including your health history.

This study will include individual who are:

1. All JOAG patients above 15 years – 40 years old
2. All Compliant to treatment and achieved target intraocular pressure

This study will not incude individual who are:

1. Patients with allergy to honey or honey-based products
2. OCT signal less than 6/10
3. Concurrent retinal diseases and other optic neuropathies

4. Systemic neurodegenerative disease
5. Systemic comorbidities such as diabetes mellitus and autoimmune diseases
6. Patients on anti-oxidants, traditional medications or herbal products for the last 3 months
7. Topical usage of steroids, systemic steroids and non-steroidal anti-inflammatory drugs.
8. Sepsis

**STUDY PROCEDURES**

Participants who fulfilled the selection criteria and agreed to participate are identified and provided with the information sheet regarding the study. Written informed consent is obtained from all participants at the beginning of the study. A comprehensive ophthalmic assessment is done which includes optical coherence tomography of RNFL thickness followed by blood sampling for the quantification of IL-6 using ELISA kit and HbA1c. The participants were randomized based on simple randomization technique into either 'honey' or 'non-honey' groups. The honey used in this study is Brainey Madu Kelulut, supplied for a total of three months after the recruitment and completion of initial examination and blood sampling. The participants in the 'no honey' group did not receive any placebo. After 3 months of honey supplement therapy, participants in both groups were reassessed during follow-up. The mean serum IL-6 and RNFL thickness were re-measured, using the same OCT machine and IL-6 ELISA test kit. Participants who were not compliant to the honey supplement were excluded from the study. Data entry and statistical analysis is done using Statistical Package for Social Sciences (SPSS) Version 26.0. Comparison of the total mean RNFL thickness and serum IL-6 level before and after consumption of honey will be analyzed.

**RISKS**

Honey Kelulut has been identified as a safe supplement, 30g per day for 90 days. The dosage of the honey given is within the therapeutic level. Honey kelulut is also given as a supplementary therapy in conjunction with the conventional anti- glaucoma medications and treatment.

**REPORTING HEALTH EXPERIENCES.**

Please contact, at any time, the following researcher if you experience any health problem either directly or indirectly related to this study.

Dr. Thamarai A/P Munirathinam ,MMC Registration No.67381 at H/P No +60177712935.

**PARTICIPATION IN THE STUDY**

Your taking part in this study is entirely voluntary. You may refuse to take part in the study or you may stop your participation in the study at anytime, without any penalty or loss of benefits to which you are otherwise entitled. Your participation also may be stopped by the research team without your consent if in any form you have violated the study eligibility criteria. The research team member will discussed with you if the matter arises. **This study is** fully funded, therefore there is no cost for the participant.

Parents play a pivotal role in monitoring their child's compliance with both the conventional treatment and the investigational product; they also must ensure the child follows the instructions that must be followed throughout the study period. The consumption of the product should be monitored directly by parents and be recorded in a honey diary provided to the participants, which will be handed to the investigator at the end of the study period.

**POSSIBLE BENEFITS [Benefit to Individual, Community, University]**

You may obtain information regarding your eye's health condition and laboratory test that may be provided in the study. Information obtained from this study will benefit patients and the community in the future. The immunomodulatory effects of Kelulut ay possibly give beneficial effect to JOAG patients and the result from this research can be used as reference for developing better treatment outcomes.

**QUESTIONS**

If you have any question about this study or your rights, please contact;

Dr Thamarai A/P Munirathinam MMC 67381
Department of Ophthalmology and Visual Sciences
Health Campus, Universiti Sains Malaysia
Kubang Kerian, Kelantan
Tel: +60177712935

Assoc. Prof. Dr Azhany Yaakub MMC 34859
Department of Ophthalmology and Visual Sciences
Health Campus, Universiti Sains Malaysia
Kubang Kerian, Kelantan
Tel: +60199101993

If you have any questions regarding the Ethical Approval or any issue / problem related to this study, please contact;

Mr. Mohd Bazlan Hafidz Mukrim
Secretary of Human Research Ethics Committee USM
Division of Research & Innovation (R&I)
USM Health Campus
Tel. No. : 09-767 2354 / 09-767 2362
Email : bazlan@usm.my

OR

Miss Nor Amira Khurshid Ahmed
Secretariat of Human Research Ethics Committee USM
Research Creativity & Management Office (RCMO)
USM Main Campus, Penang
Tel. No. : 04-6536537
Email : noramira@usm.my

**CONFIDENTIALITY**

Your information will be kept confidential by the researchers and will not be made publicly available unless disclosure is required by law.

Data obtained from this study that does not identify you individually will be published for knowledge purposes.

Your original records may be reviewed by the researcher, the Ethical Review Board for this study, and regulatory authorities for the purpose of verifying the study procedures and/or data. Your information may be held and processed on a computer. Only research team members are authorized to access your information.

By signing this consent form, you authorize the record review, information storage and data process described above.

**SIGNATURES**

To be entered into the study, you or a legal representative must sign and data the signature page [ATTACHMENT S or ATTACHMENT G (for genetic sample only) or ATTACHMENT P]

ATTACHMENT S

**Subject Information and Consent Form**
**(Signature Page)**

***Research Title:***
Supplementary Kelulut Honey Therapy In Juvenile Open-Angle Glaucoma: Effects On Il-6, RNFL And Dry Eye

***Name of main and co-Researcher:***
Dr Thamarai A/P Munirathinam MPM 67381 (HUSM)
Dr Azhany Binti Yaakub MPM 34859 (HUSM)
Dr Munira Binti Yusoff MPM 39594 (HRPZ II)
Dr Nor Anita binti Che Omar MPM 33552 (HSNZ)
Dr Norihan Binti Ibrahim MPM 49484 (HSIP)

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Participant Information and Consent Form to keep for myself.
- This study is fully funded, therefore there is no cost for the participants

**Participant Name**

**Participant I.C No**

**Signature of Participant** or Legal Representative

**Date** (dd/MM/yy)

**Name of Individual**
Conducting Consent Discussion

**Signature of Individual**
Conducting Consent Discussion

**Date** (dd/MM/yy)

**Name & Signature of Witness**

**Date** (dd/MM/yy)

Note: i) All participants who are involved in this study will not be covered by insurance.

ATTACHMENT G

**Subject Information and Consent Form**
**(Signature Page – Genetic Sample)**

***Research Title:***
Supplementary Kelulut Honey Therapy In Juvenile Open-Angle Glaucoma: Effects On Il-6, RNFL And Dry Eye

***Name of main and co-Researcher:***
Dr Thamarai A/P Munirathinam MPM 67381 (HUSM)
Dr Azhany Binti Yaakub MPM 34859 (HUSM)
Dr Munira Binti Yusoff MPM 39594 (HRPZ II)
Dr Nor Anita binti Che Omar MPM 33552 (HSNZ)
Dr Norihan Binti Ibrahim MPM 49484 (HSIP)

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Participant Information and Consent Form to keep for myself.

_____
Participant Name

_____
Participant I.C No.

_____ _____
Signature of Participant or Legal Representative Date (dd/MM/yy)

_____
Name of Individual
conducting Consent Discussion

_____ _____
Signature of Individual Date (dd/MM/yy)

Conducting Consent Discussion

| | |
|---|---|
| Name & Signature of Witness | Date (dd/MM/yy) |

Note: i) All participants who are involved in this study will not be covered by insurance.
ii) Excess samples from this research will not be used for other reasons and will be destroyed with the consent from the Human Research Ethics Committee, USM.

*ATTACHMENT P*

**Participant's Material Publication Consent Form**
**Signature Page**

***Research Title:***
Supplementary Kelulut Honey Therapy In Juvenile Open-Angle Glaucoma: Effects On Il-6, RNFL And Dry Eye

***Name of main and co-Researcher:***
Dr Thamarai A/P Munirathinam MPM 67381 (HUSM)
Dr Azhany Binti Yaakub MPM 34859 (HUSM)
Dr Munira Binti Yusoff MPM 39594 (HRPZ II)
Dr Nor Anita binti Che Omar MPM 33552 (HSNZ)
Dr Norihan Binti Ibrahim MPM 49484 (HSIP)

To become a part this study, you or your legal representative must sign this page.

By signing this page, I am confirming the following:

- I understood that my name will not appear on the materials published and there have been efforts to make sure that the privacy of my name is kept confidential although the confidentiality is not completely guaranteed due to unexpected circumstances.
- I have read the materials or general description of what the material contains and reviewed all photographs and figures in which I am included that could be published.
- I have been offered the opportunity to read the manuscript and to see all materials in which I am included but have waived my right to do so.
- All the published materials will be shared among the medical practitioners, scientists and journalist worldwide.
- The materials will also be used in local publications, book publications and accessed by many local and international doctor's worldwide.
- I hereby agree and allow the materials to be used in other publications required by other publishers with these conditions:
- The materials will not be used as advertisement purposes nor as packaging materials.
- The materials will not be used out of context – i.e.: Sample pictures will not be used in an article which is unrelated subject to the picture.

**Participant Name**

**Participant I.C No**. **Participant's Signature** **Date** (dd/MM/yy)

**Name and Signature of Individual** **Date** (dd/MM/yy)
Conducting Consent Discussion

Note: i) All participants who are involved **in this study will not be covered by insurance.**

| | |
|---|---|
| Serial number | |
| MRN | |
| Group | |

UNIVERSITI SAINS MALAYSIA

**DATA COLLECTION FORM**

**Supplementary Kelulut Honey Therapy In Juvenile Open-Angle Glaucoma: Effects On Il-6, RNFL And Dry Eye**

## A. BIODATA OF PATIENT:

| | |
|---|---|
| Date of birth | |
| MRN | |
| Age | |
| Gender | |
| Race | |

## B. SYSTEMIC DISEASES:

| | |
|---|---|
| Diabetes mellitus Type I | |
| Diabetes mellitus Type II | |
| Hypertension | |
| Hypercholesterolemia | |
| Autoimmune disease | |
| Malignancy | |
| Tuberculosis | |
| Systemic lupus erythematosis | |
| Allergy | |
| Others | |

## C. DIAGNOSIS:

| | RIGHT EYE | | LEFT EYE | |
|---|---|---|---|---|
| Type of glaucoma | JOAG | POAG | JOAG | POAG |
| Date of diagnosis | | | | |
| Onset of disease | | | | |
| Duration of disease | | | | |
| Severity of glaucoma | Mild | | Mild | |
| | Moderate | | Moderate | |
| | Severe | | Severe | |

## D. OCULAR EXAMINATION

**PRE-HONEY** Date of examination:

| | | RIGHT EYE | | LEFT EYE | |
|---|---|---|---|---|---|
| Visual acuity | | unaided | | unaided | |
| | | pinhole | | pinhole | |
| Intraocular pressure | | RE | | | |
| | | LE | | | |
| Goniscopy (Shaffer Grading) | | | | | |
| Fundus | | | | | |
| Humprey visual field test | Pattern | | | | |
| | MD | | | | |
| | VFI | | | | |
| Antiglaucoma eyedrops | | | | | |
| Types of ocular surgery | | | | | |

**POST HONEY** Date of examination:

| | | RIGHT EYE | | LEFT EYE | |
|---|---|---|---|---|---|
| Visual acuity | | unaided | | unaided | |
| | | pinhole | | pinhole | |
| Intraocular pressure | | RE | | | |
| | | LE | | | |
| Goniscopy (Shaffer Grading) | | | | | |
| Fundus | | | | | |
| Humprey visual field test | Pattern | | | | |
| | MD | | | | |
| | VFI | | | | |
| Antiglaucoma eyedrops | | | | | |
| Types of ocular surgery | | | | | |

## E. Investigated parameters

| | | PRE-HONEY | POST HONEY |
|---|---|---|---|
| Serum IL-6 level (pg/mL) | | | |
| Retinal nerve fiber layer thickness (μm) | Mean | | |
| | Superior | | |
| | Inferior | | |
| | Nasal | | |
| | Temporal | | |
| Tear break up time (seconds) | | | |
| Schirmer test (mm) | | | |
| Glycated haemoglobin HbA1c(%) | | | |

**TREATMENT DIARY**

| | |
|---|---|
| **Name** | |
| **IC** | |
| **RN** | |

**HONEY TREATMENT DIARY (to be completed by patient)**

| DAY | DATE | HONEY USAGE (√ FOR YES, X FOR NO) | NOTES (SIDE EFFECTS) |
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**MONTHLY SUMMARY TABLE** (to be completed by principal investigator)

| MONTH | NUMBER OF SACHET CONSUMED | SIDE EFFECTS | COMPLIANCE (%) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |