ORIGINAL ARTICLE

Effect of Honey Cocktail on Macular Thickness, Retinal Nerve Fiber Layer Thickness and Optic Nerve Head Parameters in Post-Menopausal Women

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ABSTRACT

Introduction: Honey postulated may have an estrogenic effect on the retinal estrogenic receptors. The aim of the study is to compare the mean macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters with and without honey cocktail supplement in post-menopausal women. **Methods:** A randomised interventional study was conducted from March 2014 to July 2015. A total of 60 post-menopausal women were selected and randomised into 2 groups: honey cocktail (20 mg/day) and control. Macular thickness, RNFL thickness and ONH parameters were measured using optical coherence tomography at baseline and at 3 months post honey cocktail supplementation. **Results:** The mean global macular thickness and RNFL thickness were significantly thicker in post-menopausal women with honey cocktail at 3 months post supplement ($p = 0.002$ and 0.033 respectively). There was a significant increase in the mean change of global macular thickness and RNFL thickness in honey cocktail group at 3 months post supplement ($p < 0.001$ and < 0.001 respectively). Although there was no significant difference in the ONH parameters at 3 months post supplement between the two groups but there was significant increase in the mean change of rim area ($p = 0.003$), and significant reduce in the mean change of cup area ($p = 0.001$) and cup-disc-ratio ($p < 0.001$) in honey cocktail group at 3 months post supplement. **Conclusion:** Honey cocktail supplement showed structural changes in the macular thickness, RNFL thickness and OHN parameters of post-menopausal women.

Keywords: Menopause; Honey cocktail; Macular thickness; Retinal nerve fiber layer thickness; Optic nerve head parameters

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INTRODUCTION

Menopause is a natural progression in a women's life and in women's physiology. Menopause typically occurs in a woman's late 40s to early 50s. In Malaysia, the average life expectancy in women ranges from 68 years to 75.2 years. In a study conducted in Singapore found that the mean age of menopause is 49.0 years (1). Post-menopause is a span of lifetime after the last menstrual period (2). Post-menopausal period is 12 consecutive months of amenorrhea after the last menstruation. In post-menopause, a woman's hormone patterns have changed significantly because the ovaries are no longer producing estrogen or progesterone and this result in many of the post-menopausal symptoms experienced by women.

The clinical manifestation of menopause is not only restricted to changes such as vasomotor changes, genitourinary problems, sleep disturbances and mood fluctuations but also include changes in ocular structures such as tear film, lens, intraocular pressure and ocular blood flow (3). There is also evidence to suggest a relationship between estrogen and retinal disorders.

Retina is the inner layer of the eyeball and consist of retinal nerve fiber layer (RNFL). Macula is the central area of the retina and is the most sensitive part of the retina. The normal macular thickness in adult ranges from 203 μm to 335 μm (4). Whereas, the mean RNFL thickness for the normal population is 97.3 ± 9.6 μm (5). The rate of normal age-related retinal thinning is -0.54 ± 0.23 μm/year (6). Disease which affects the macula causes visual disruption and affects the quality of life. The most common form of macular disease is age-related macular degeneration (AMD). AMD is the leading cause of visual loss and blindness among older adults. The prevalence of AMD in the United States is 1.75 million and is expected to increase to 3 million by 2020 (7). Oxidative stress plays an important role in the degeneration of the retina. In women, the antioxidant properties in estrogen may have a protective role against oxidative stress on retina and thus helps promote the survival of the retina. In post-menopausal women, the antioxidant level is low due to decrease in estrogen. The reduction of antioxidant increases the oxidative stress on the retina and therefore makes these post-menopausal women more prone to develop AMD.

The Rotterdam Study showed an increased risk of AMD in those who experienced early menopause following oophorectomy (OR, 3.8) (8). The Blue Mountain Eye Study reported that a long reproductive period was associated with a decreased prevalence of early AMD (9). These findings provide further support for the notion that a shorter duration of estrogen exposure may increase the risk of AMD. Apart from this, researcher has also shown that menopausal females not on hormone replacement therapy (HRT) have a higher risk to develop AMD compared to menopausal females on HRT (10). Hence, female reproductive hormone especially estrogen may have protective effect against AMD.

Optic nerve head (ONH) is a small blind spot on the surface of the retina, located about 3 mm to the nasal side of the macula. It is the point where the retinal nerve fibers leave the eye and become part of the optic nerve. At the center, the porus opticus marks the point of entrance of the central artery and vein of the retina. Evidence supports the hypothesis that estrogen deficiency is involved in the patho-physiology of optic nerve aging and glaucomatous neurodegeneration through several mechanisms (11).

Estradiol regulates smooth muscle tone and vascular resistance and augments the activity of endothelial-based nitric oxide synthase (NOS3) (12). Specifically, estradiol may influence perfusion of the optic nerve, retinal ganglion cells, and their supporting structures. This effect has been demonstrated in animal models and clinical studies (13,14). A 22–45% increase in perfusion to the retina was reported in ovariectomized rats treated with estradiol compared to control ovariectomized rats treated with vehicle (13). In humans, aging and age-related decreases in female sex hormones negatively affect ocular blood flow.

Estrogenic effect on the retina is possible due to the presence of estrogen receptors in the retina. In recent years, studies done in rats, bovine and human have demonstrated the presence of estrogen receptor in the retina (15-18). Based on the study done by Munaut et al. (18), two types of estrogen receptor were identified in the human retina, which were estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). The study demonstrated the presence of ERα and ERβ in the neural retina and RPE-choroid complex. Expression of ERβ was more constant and evenly distributed in the retina, mainly observed in the ganglion cell layer and choroid while expression of ERα was unequally distributed between the retina, especially the ganglion cell layer and the RPE-choroid complex.

With regards to treatment of menopause, HRT has been the mainstay of treatment for decades to aid women in coping with changes in their post-menopausal time frame. This therapy has been found to be beneficial in protecting ocular structural changes related to menopause as well (19). However, in recent years, there's a paradigm shift towards alternative therapy to help women cope with the unpleasant symptoms of menopause and yet lead a normal life. This is due to the concerns that HRT could increase the risk of ischemic stroke, cardiovascular diseases and breast cancer (20). Some of these alternative therapies which are of interest include acupuncture, herbal remedies such as evening prime rose oil, ginseng, black cohosh, red clover and soy supplement and gingko biloba extract (21,22).

Lately, there's an interest in using honey as a modality of treatment for conditions related to menopause and several studies have showed promising results (23-25). Supplementation of honey among postmenopausal breast cancer women significantly increased white blood cell and platelet counts, and creatinine levels (25). Study done by Mohd Effendy et al found that honey reduces osteoclast activity and thus reduce bone loss (26). Outcome of this study demostrated that honey can be used as a treatment for osteoporosis in post-menopausal women (26). Findings on these studies suggest that honey has promising effects on post-menopausal women especially on estrogen sensitive tissues. Therefore, it can be postulated that honey supplement may have an estrogenic effect on the retinal estrogenic receptors and bring about structural changes in retinal parameters in post-menopausal women.

The objective of this study is to compare mean macular thickness, RNFL thickness and ONH parameters between with and without honey cocktail supplement in post-menopausal women.

MATERIALS AND METHODS

A randomised interventional study was carried out on 60 post-menopausal women who attended the Ophthalmology Clinic and Obstetrics & Gynaecology Clinic, Hospital Universiti Sains Malaysia from March 2014 to July 2015. Ethical approval was obtained from Human Research Ethics Committee Universiti Sains Malaysia [USM/ JEPeM/ 277.2.(5): Federal-Wide Assurance (FWA) Reg. No: 00007718; Institutional Review Board (IRB) Reg. No: 00004494]. This study complied with the Declaration of Helsinki.

Post-menopausal women with at least 12 consecutive months of natural amenorrhea, aged between 45 to 65 years old, and with clear media for measurement of macular thickness, RNFL thickness, and ONH parameters were included. Participants that were excluded include those with systemic co-morbidities (diabetes, hypertension, ischemic heart disease), ocular co-morbidities (glaucoma, ocular hypertension, pre-existing macular or retinal disease, optic neuropathy), any past intraocular surgery or ocular trauma, history of any oral natural herbal health products or dietary supplement (regular and constant intake) taken within one month prior to randomisation, patients who are on HRT or was on HRT three months prior to randomisation, any allergies or sensitivity to supplement ingredients, and myopia more than -3.00 dioptre.

Participants who fulfilled the criteria underwent pre-study visit and screening. After the screening procedure was completed and participants who fulfill the selection criteria's were identified, participants were provided with the information sheet regarding the study. Written informed consent was obtained from participants who agreed to be included in the study. The right eye was selected to standardise the examination. If there were ocular pathologies in the right eye, then the left eye was selected.

Baseline parameters of macular thickness, RNFL thickness and ONH parameters were measured using optical coherence tomography (OCT), Cirrus HD-OCT 500 (Carl Zeiss Meditec, Germany) after pupillary dilatation. For macular analysis, a fast 6 mm x 6 mm area macular mapping scanning pattern was used to determine the macular thickness. Macula cube 512 x 218 protocol was used. Macula was divided into nine subfields based on the Early Treatment Diabetic Retinopathy Study (ETDRS) grid (27) which consist of three rings of 1 mm, 3 mm and 6 mm in diameter. Central subfield is the 1 mm ring followed by nasal, inferior, temporal and superior quadrants in the 3 mm inner ring and 6 mm outer ring (Figure 1).

For RNFL analysis, peripapillary ring of a 3.45 mm diameter was measured which consist of nasal, inferior, temporal and superior quadrants (Figure 2A). For ONH analysis, ONH scan was done using the optic disc cube 200 x 200 protocol which defines the disc and cup margins within the 3-dimensional data cube. Parameters that were measured include disc area, rim area, cup-to-disc ratio and cup area (Figure 2B). A signal strength bar shown on the OCT during image acquisition indicates the quality and reliability of the image taken. The signal strength ranges from 0 (poor image quality and reliability) to 10 (best image quality and reliability). Images were included if the signal strength was greater than 6 and there were no saccades in the enface image.

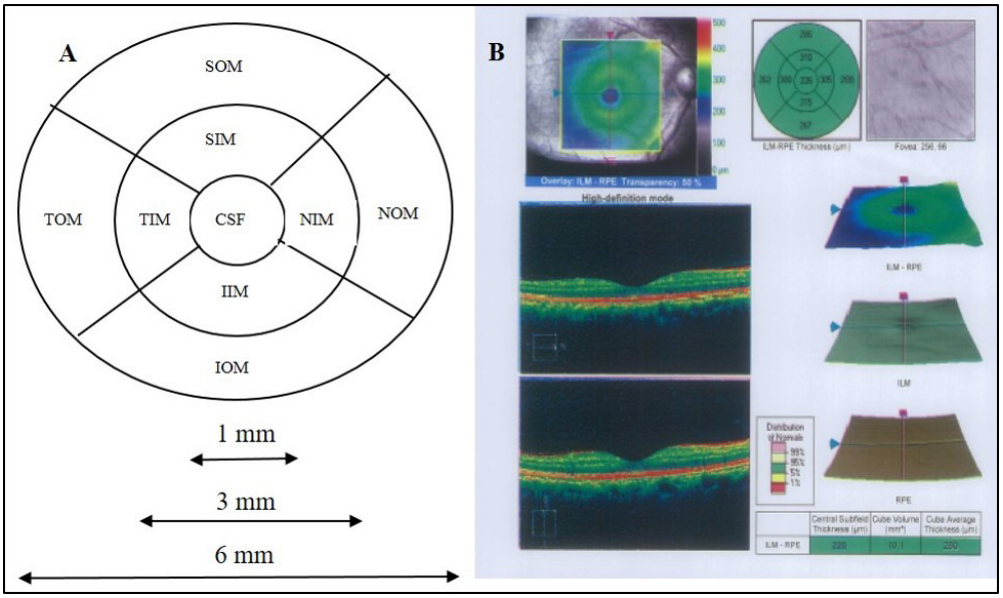

Figure 1: Macular thickness mapping involving nine areas as described by ETDRS (27) (A) and macular thickness mapping taken using Cirrus HD optical coherence tomography (B).
Abbreviation: CSF- central subfield , SIM- superior inner macula, IIM- inferior inner macula, TIM-temporal inner macula, NIM-nasal inner macula, SOM- superior outer macula, IOM-inferior outer macula, NOM- nasal outer macula, TOM- temporal outer macula

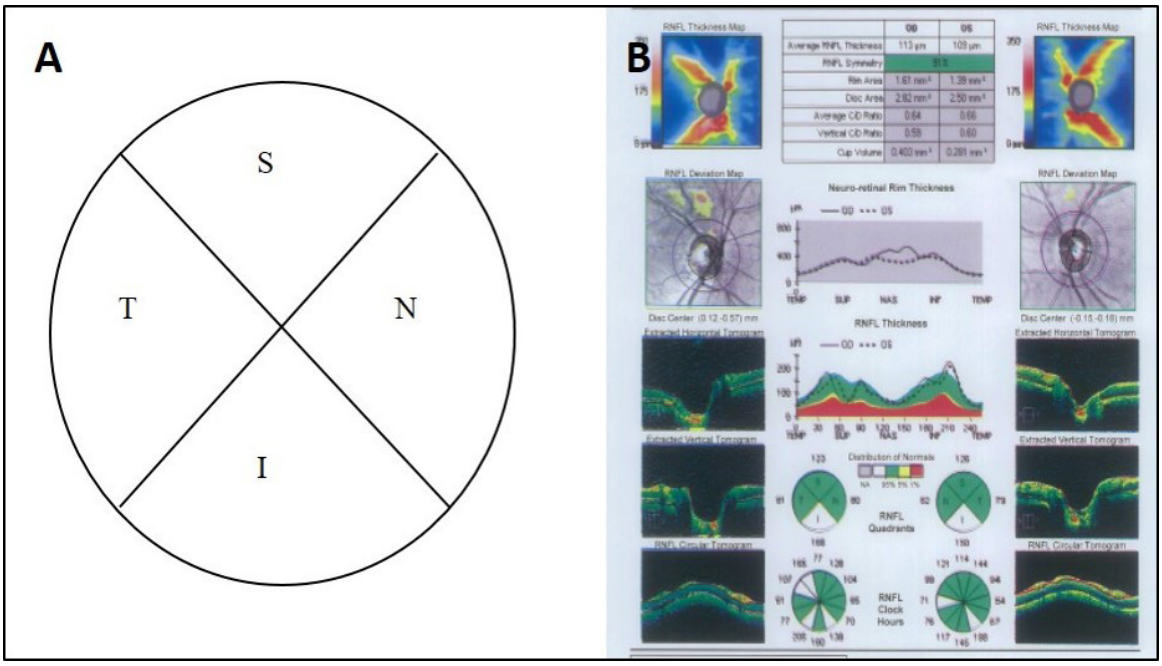

Figure 2: Retinal nerve fiber layer mapping involving 4 quadrants (A) and retinal nerve fiber layer and optic nerve head mapping on Cirrus HD optical coherence tomography (B).
Abbreviation: S-superior, I-inferior, T-temporal, N-nasal

Then, the participants were randomised based on variable randomisation using opaque envelope technique into either 'honey cocktail' or 'no honey' groups. The honey cocktail used in this study is Honey Cocktail 124 (HC124) supplied by Federal Agricultural Marketing Authorities (FAMA), Malaysia. The honey cocktail was initially evaporated to achieve water content of about 20% in FAMA laboratory. Then later, it was sent to Sterile Gamma Company at Shah Alam, Selangor for sterilization via gamma radiation at 25 kGy. After that, it was packed in 20 grams sachet. The project was part of collaboration with School of Pharmaceutical Sciences laboratory, Universiti Sains Malaysia. The dosage used in this study was calculated based on an animal study model (28). The dosage used in that animal study model was 200 mg/kg x 60 kg. In this study, 20 grams of honey cocktail was chosen and is considered as a moderate dose for human being. The content of honey cocktail in a 20 grams sachet are Acasia honey (94.3%), bee pollen (4.7%) and royal jelly (1.0%).

Participants in the 'honey cocktail' group received honey supplement supply for total of three months duration in the form of honey cocktail sachet. Participants were instructed to ingest one sachet of honey cocktail containing 20 grams per day. Participants were informed to take the honey cocktail straight from the sachet without diluting the honey cocktail in water or other liquids. Participants were instructed to take the honey cocktail in the morning before breakfast and after taking the honey cocktail, participants were required to wait at least 30 minutes before taking breakfast or liquids to avoid interaction. The participants in the 'no honey' group did not receive any placebo.

Participants in both groups were reassessed at three months follow-up. For the honey cocktail group, when participants returned at three months, they were required to bring back the consumed honey cocktail sachets and the remaining unused honey cocktail sachets. Compliance was assessed by counting the returned sachets. It was calculated by determining the number of the sachets taken divided by the number of sachets expected to have been taken. They are considered to be compliant if they take the honey cocktail 75% of the time. Participants who were found to be less than 75 % compliant with the study were excluded from the study.

After three months of supplement therapy, the macular thickness, RNFL thickness and the ONH parameters were re-measured. All these parameters were measured using the same machine, with the same techniques and by the same-blinded medical personnel.

Any adverse events associated with honey cocktail used in the study were monitored during follow-up. Participants were also encouraged to self-report any adverse events by telephone. All observed and self-reported adverse events throughout the study were recorded in the case report form.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) Version 22 was used for the analysis of data. Independent t-test was used to compare the parameters (macular thickness, RNFL thickness and ONH) between the two groups. For categorical data, Fisher exact test was used to compare ethnic between the two groups. P value of < 0.05 was taken as significant data.

RESULTS

A total of 60 post-menopausal women were recruited into the study (30 post-menopausal women in honey cocktail group and 30 post-menopausal women in no honey group). Demographic data for both groups is shown in Table I. There were no significant differences in mean age and race between the two groups of post-menopausal women. There were also no significant differences of female reproductive parameters between the two groups.

Table I: Demographic data and female reproductive parameters for both honey cocktail and no honey groups

Characteristic	Overall	Honey Cocktail	No Honey	P value
	n = 60	n = 30	n = 30	
Age (year)				
mean (SD)	60.50 (4.68)	59.83 (4.20)	61.17 (5.09)	*0.273
Race, n (%)				
Malay	59 (98.3)	29 (96.7)	30 (100)	#1.000
Non-Malay	1 (1.7)	1 (3.3)	0 (0)	
Age of menarche (years)				
mean (SD)	13.32 (1.67)	13.43 (1.78)	13.20 (1.58)	*0.593
Age of menopause (years)				
mean (SD)	49.82 (3.76)	49.27 (3.83)	50.37 (4.08)	*0.260
Duration of menopause (years)				
mean (SD)	10.68 (5.28)	10.57 (4.95)	10.80 (5.68)	*0.866
Duration of menstruation (years)				
Mean (SD)	36.50 (3.79)	35.83 (3.50)	37.17 (4.02)	*0.176
Parity (no of children)				
mean (SD	4.53 (2.30)	4.03 (2.34)	5.03 (2.19)	*0.093
Breastfeeding (months)				
Mean (SD)	29.38 (25.52)	24.67 (25.10)	34.10 (43.47)	*0.308
Estrogen Exposure (years)				
Mean (SD)	30.65 (5.49)	30.75 (4.63)	30.55 (6.31)	*0.888

*Independent t test, # Fishers Exact test, p value < 0.05 significant

There was no statistically significant difference in the baseline mean global macular thickness between the two groups (p = 0.347). There was also no statistically significant difference in the baseline mean macular thickness in all quadrants between the two groups except in quadrants of temporal inner macula (TIM) and superior inner macula (SIM) (Table II).

In terms of RNFL, there was also no statistically significant difference in the baseline mean global (p=0.454) and quadrants of RNFL thickness between the two groups. In general, honey cocktail group have thicker macula and RNFL except in nasal nerve fiber layer (NNFL).

The baseline mean disc area and rim area was found to be slightly smaller in honey cocktail group compared to no honey group. The cup area was slightly bigger in the honey cocktail group compared to no honey group.

Table II: Baseline of mean macular thickness, RNFL thickness & ONH parameter for both groups

	Mean (SD)		Mean difference (95 % CI)	t- statistic (df)	P value
	Honey Cocktail n = 30	**No Honey n = 30**			
Macular thickness ((μm)					
Global	282.34 (9.04)	277.31 (12.31)	-5.03 (-10.56, 0.50)	-1.82 (58)	0.347
Quadrant					
CSF	238.87 (17.17)	232.67 (20.08)	-6.20 (-15.86, 3.46)	-1.29 (58)	0.348
TIM	304.17 (14.08)	296.13 (13.23)	-8.03 (-15.10, -0.97)	-2.28 (58)	0.026
NIM	306.37 (14.07)	298.57 (17.30)	-7.80 (-15.99, 0.35)	-1.92 (58)	0.060
SIM	309.87 (11.73)	301.00 (17.79)	-8.87 (-16.65, -1.07)	-2.28 (58)	0.026
IIM	307.37 (12.66)	300.13 (17.00)	-7.23 (-14.97, 0.50)	-1.87 (58)	0.066
TOM	265.93 (20.75)	263.63 (19.94)	-2.30 (-12.81, 8.22)	-0.44 (58)	0.663
NOM	274.10 (22.89)	272.73 (21.44)	-1.37 (-12.83, 10.10)	-0.24 (58)	0.812
SOM	270.90 (11.87)	270.30 (15.90)	-6.00 (-7.85, 6.65)	-0.17 (58)	0.869
IOM	263.50 (15.93)	260.60 (15.72)	-2.90 (-11.08, 5.28)	-0.71 (58)	0.481
RNFL thickness (μm)					
Global	97.14 (7.80)	95.43 (9.64)	-1.71 (-6.24, 2.82)	-0.76 (58)	0.454
Quadrant					
SNFL	120.73 (13.47)	119.67 (11.78)	-1.07 (-7.61, 5.47)	-0.326 (58)	0.745
INFL	127.37 (14.46)	124.90 (15.75)	-2.47 (-10.28, 5.39)	-0.632 (58)	0.530
NNFL	70.63 (9.41)	71.50 (10.67)	0.87 (-4.33, 6.07)	0.334 (58)	0.740
TNFL	69.83 (10.89)	65.67 (10.64)	-4.17 (-9.73, 1.40)	-1.50 (58)	0.139
ONH parameters					
Disc area (mm^2)	2.12 (0.32)	2.15 (0.43)	0.04 (-0.16, 0.23)	0.36 (58)	0.724
Rim area (mm^2)	1.29 (0.23)	1.33 (0.26)	0.05 (-0.08, 0.17)	0.72 (58)	0.473
Cup area (mm^2)	0.83 (0.37)	0.82 (0.39)	-0.11 (-0.21, 0.19)	-0.11 (58)	0.912
Cup-disc-ratio	0.55 (0.12)	0.55 (0.12)	-0.06 (-0.07, 0.05)	-0.19 (58)	0.853

Independent t-test, p value < 0.05 significant
Abbreviation: RNFL-retinal nerve fiber layer, ONH-optic nerve head, CSF-central subfield, TIM-temporal inner macula, NIM-nasal inner macula, SIM-superior inner macula, IIM-inferior inner macula, TOM-temporal outer macula, NOM-nasal outer macula, SOM-superior outer macula, IOM-inferior outer macula, SNFL-superior nerve fiber layer, INFL-inferior nerve fiber layer, NNFL-nasal nerve fiber layer, TNFL-temporal nerve fiber layer.

However, there was no statistically significant difference in all of the ONH parameters between the two groups (Table II) at baseline.

At three months post honey cocktail supplement, there was significantly thicker in the mean global macular thickness in honey cocktail group compared to no honey group (p = 0.002) (Table III). Out of the nine quadrants, three quadrants showed significant thicker in the mean macular thickness; temporal inner macula (TIM), nasal inner macula (NIM), superior inner macula (SIM), and inferior inner macula (IIM).

There was significantly thicker in the mean global RNFL thickness in the honey cocktail group compared to no honey group at three months post honey cocktail supplement (p = 0.033) (Table III). Out of the four quadrants, only temporal nerve fiber layer (TNFL) showed significant thicker in the mean RNFL thickness. Both groups showed no significant difference of ONH parameters at three months post honey cocktail supplement (Table III).

In terms of mean change, there was significant increase in the mean change of global macular thickness in honey cocktail group at three months post honey cocktail supplement (p < 0.001) (Table IV). There was also significant increase in the mean change of macular thickness in all quadrants in honey cocktail group except at inferior inner macula (IIM).

There was significant increase in the mean change of global RNFL thickness in honey cocktail group at three months post honey cocktail supplement (p < 0.001) (Table IV). There was also significant increase in the mean change of RNFL thickness in all quadrants in honey cocktail group except at TNFL.

In terms of ONH, there was significant increase in the mean change of rim area (p = 0.003), and significant reduce in the mean change of cup area (p = 0.001) and cup-disc-ratio (p < 0.001) in honey cocktail group at three months post honey cocktail supplement. There was no significant change in the mean disc area between the two groups.

During the course of the study, there was no detected or reported adverse events such as allergic reaction, diarrhoea or body weakness in participants with honey cocktail supplementation.

DISCUSSION

HRT has been the mainstay of treatment for menopausal related symptoms and ocular structural integrity. The aim of this study was to assess whether alternative therapy such as naturally occurring honey supplement

Table III: Comparison of mean macular thickness, RNFL thickness & ONH parameters at 3 months post honey cocktail supplement between the groups

	Mean (SD)		Mean difference (95% CI)	t-statistic (df)	p-value
	Honey Cocktail n = 30	**No Honey n = 30**			
Macular thickness (μm)					
Global	284.45 (9.16)	275.64 (11.25)	-8.81 (-14.12, -3.51)	-3.33 (58)	0.002
Quadrant					
CSF	239.93 (17.09)	231.23 (19.22)	-8.70 (-18.10, 0.70)	-1.85 (58)	0.069
TIM	306.00 (13.85)	294.73 (13.07)	-11.23 (-18.19, -4.27)	-3.23 (58)	0.002
NIM	308.37 (14.10)	296.57 (17.80)	-11.80 (-20.09, -3.50)	-2.85 (58)	0.006
SIM	312.23 (12.47)	299.10 (17.25)	-13.13 (-20.91, -5.36)	-3.38 (58)	0.001
IIM	308.03 (13.18)	296.97 (14.38)	-11.07 (-18.19, -3.94)	-3.11 (58)	0.003
TOM	268.53 (20.25)	263.10 (20.23)	-5.43 (-15.89, 5.02)	-1.04 (58)	0.303
NOM	276.37 (22.92)	271.33 (21.40)	-5.03 (-16.49, 6.43)	-0.88 (58)	0.383
SOM	274.97 (11.72)	269.03 (16.76)	-5.93 (-13.41, 1.54)	-1.59 (58)	0.117
IOM	265.67 (15.05)	258.67 (15.37)	-7.00 (-14.86, 0.86)	-1.78 (58)	0.080
RNFL thickness (μm)					
Global	98.54 (8.60)	93.53 (9.19)	-5.01 (-9.61, -0.41)	-2.18 (58)	0.033
Quadrant					
SNFL	122.17 (12.26)	117.47 (11.20)	-4.70 (-10.77, 1.37)	-1.55 (58)	0.126
INFL	129.37 (15.56)	122.93 (15.66)	-6.43 (-14.50, 1.63)	-1.60 (58)	0.116
NNFL	72.60 (11.60)	69.03 (10.44)	-3.57 (-9.27, 2.14)	-1.25 (58)	0.216
TNFL	70.03 (10.57)	64.70 (9.32)	-5.33 (-10.49, -0.18)	-2.07 (58)	0.043
ONH parameters					
Disc area (mm^2)	2.11 (0.32)	2.16 (0.44)	0.04 (-0.16, 0.24)	0.42 (58)	0.677
Rim area (mm^2)	1.32 (0.23)	1.32 (0.25)	-0.00 (-0.13, 0.12)	-0.03 (58)	0.979
Cup area (mm^2)	0.80 (0.36)	0.84 (0.39)	0.04 (-0.15, 0.24)	0.44 (58)	0.659
Cup-disc-ratio	0.54 (0.12)	0.56 (0.12)	0.02 (-0.05, 0.08)	0.50 (58)	0.618

Independent t-test, p value < 0.05 significant
Abbreviation: RNFL-retinal nerve fiber layer, ONH-optic nerve head, CSF-central subfield, TIM-temporal inner macula, NIM-nasal inner macula, SIM-superior inner macula, IIM-inferior inner macula, TOM-temporal outer macula, NOM-nasal outer macula, SOM-superior outer macula, IOM-inferior outer macula, SNFL-superior nerve fiber layer, INFL-inferior nerve fiber layer, NNFL-nasal nerve fiber layer, TNFL-temporal nerve fiber layer.

Table IV: Comparison of mean change in macular thickness, RNFL thickness & ONH parameters between the groups

	Mean change (SD)		Mean difference (95% CI)	t-statistic (df)	P value
	Honey Cocktail n = 30	No Honey n = 30			
Macular thickness (µm)					
Global	2.11 (2.35)	-1.67 (3.10)	-3.78 (-5.20, -2.36)	-5.33 (58)	< 0.001
Quadrant					
CSF	1.07 (5.00)	-1.43 (2.86)	-2.50 (-4.60, -0.41)	-2.39 (58)	0.020
TIM	1.80 (3.60)	-1.40 (2.70)	-3.20 (-4.84, -1.56)	-3.90 (58)	< 0.001
NIM	2.00 (6.70)	2.00 (3.81)	-4.00 (-6.82, -1.18)	-2.84 (58)	0.006
SIM	2.37 (2.88)	-1.90 (3.96)	-4.27 (-6.05, -2.48)	-4.77 (58)	< 0.001
IIM	0.67 (3.03)	-3.17 (15.28)	-3.83 (-9.63, 1.96)	-1.35 (31)	0.187
TOM	2.60 (5.57)	-0.53 (2.13)	-3.13 (-5.31, -0.96)	-2.88 (58)	0.006
NOM	2.27 (7.09)	-1.40 (2.09)	-3.67 (-6.37, -0.96)	-2.72 (58)	0.009
SOM	4.07 (4.68)	-1.27 (7.25)	-5.33 (-8.49, -2.18)	-3.38 (58)	0.001
IOM	2.17 (5.34)	-1.93 (4.88)	-4.10 (-6.74, -1.46)	-3.10 (58)	0.003
RNFL thickness (µm)					
Global	1.40 (2.82)	-1.90 (2.60)	-3.30 (-4.70, -1.90)	-4.71 (58)	< 0.001
Quadrant					
SNFL	1.43 (4.34)	-2.20 (4.00)	-3.63 (-5.79, -1.47)	-3.37 (58)	0.001
INFL	2.00 (6.42)	-1.97 (4.32)	-3.97 (-6.78, -1.14)	-2.81 (58)	0.007
NNFL	1.97 (7.50)	-2.47 (4.20)	-4.43 (-7.54, -1.32)	-2.85 (58)	0.006
TNFL	0.20 (5.30)	-0.97 (2.39)	-1.17 (-3.29, 0.96)	-1.10 (58)	0.276
ONH parameters					
Disc area (mm^2)	0.00 (0.02)	0.00 (0.02)	0.01 (-0.00, 0.02)	1.43 (58)	0.159
Rim area (mm^2)	0.03 (0.08)	-0.01 (0.02)	-0.05 (-0.08, -0.02)	-3.05 (58)	0.003
Cup area (mm^2)	-0.03 (0.08)	0.02 (0.02)	0.05 (0.02, 0.09)	3.52 (58)	0.001
Cup-disc-ratio	-0.01 (0.02)	0.01 (0.02)	0.02 (0.01, 0.03)	3.90 (58)	< 0.001

Independent t-test, p value < 0.05 significant

Abbreviation: RNFL-retinal nerve fiber layer, ONH-optic nerve head, CSF-central subfield, TIM-temporal inner macula, NIM-nasal inner macula, SIM-superior inner macula, IIM-inferior inner macula, TOM-temporal outer macula, NOM-nasal outer macula, SOM-superior outer macula, IOM-inferior outer macula, SNFL-superior nerve fiber layer, INFL-inferior nerve fiber layer, NNFL-nasal nerve fiber layer, TNFL-temporal nerve fiber layer.

is beneficial to the ocular structures especially the retina of post-menopausal women. Based on literature search, till date, there are no studies done assessing the effect of honey or honey cocktail on macular thickness, RNFL and ONH parameters in post-menopausal women.

At baseline, although we observed that the mean macular thickness was thicker in the honey cocktail group in global but it was not significant. Comparison between the two groups at three months review showed that the mean global and several quadrant macular thickness was significantly thicker in the honey cocktail group compared to group without honey cocktail supplementation. The mean change in the macular thickness between the two groups at three months review was also significant except for IIM.

There are several reasons for this positive observation. Studies have demonstrated the presence of estrogen receptor in the retina (15-18), which are ERα and ERβ. These receptors were found to be unevenly distributed in the ganglion cell layer and the RPE-choroid complex (18). Administration of HRT, which contains estrogen to post-menopausal women showed improvement in ocular blood flow due to vasodilatory effect and improved blood viscosity (13). Researchers have also demonstrated that honey has biologically active estrogen-like molecules or phytoestrogens and thus they are able to exhibit estrogenic activities (29,30). Therefore, it is postulated that the increase in mean macular thickness seen in the honey cocktail group is due to direct effect of estrogenic like action of honey on estrogen receptor. Apart from this, it is also due to the effect of estrogenic like action of honey on vascular endothelial cell causing vasodilatation and increased retinal vascular blood flow. However, not all quadrants of macular showed increase in thickness. This variation is probably due to uneven distribution of estrogen receptors in the different areas of the retina and therefore the effect of honey on these receptors is also unequal.

In comparison, the mean macular thickness was significantly thinner in the no honey group in global and in several other macular quadrants. It is postulated that this thinning is due to possible degeneration of the macula with time compounded by the lack of estrogenic like activity in the retina. Besides this, the lack of estrogen activity also reduces blood flow in the retina and relatively causes macular thinning. The reduction in the macular thickness could also be due to age related atrophy.

The other reason for the positive effect of honey on mean macular thickness observed in honey cocktail post-menopausal women is the effect of honey as antioxidant. Oxidative stress occurs in the eye of post-menopausal women due to aging compounded by lack of estrogen (which is also an antioxidant) and other

antioxidants (31). Honey contains potent antioxidants such as vitamin C, monophenolics, flavonoids, and polyphenolics (32). The similarities between estrogen and flavonoids in honey compounds are shown in Figure 3. Among the flavinoids, quercetin is a more powerful antioxidant and free radical scavenging substance (31). It has also been proven to have anti apoptotic activity on retinal ischemic-reperfusion injury and prevent retinal damage (31). Therefore, the increase in macular thickness in post-menopausal women with honey cocktail supplement is possible due to the antioxidant effect of honey in preventing macular ischemia and degeneration. However, not all quadrants of macula showed increase in thickness. This finding is probably due to variable antioxidant effect of honey in different quadrants of the macula.

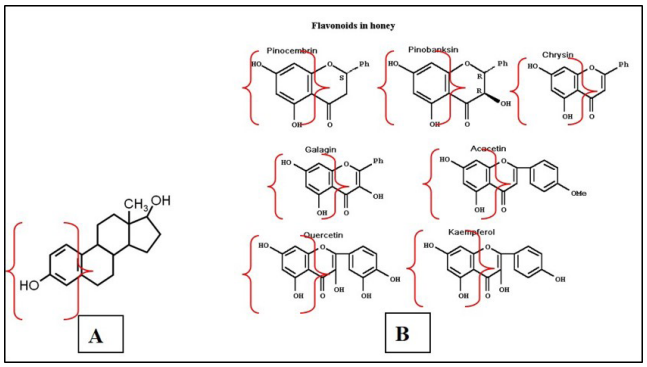

Figure 3: Chemical structure of estrogen (A) compared to flavonoids chemical structures in honey (B). Red brackets show similarities of phenol found in estrogen and flavonoids in honey [Adapted from Ogueta et al (15)].

Other causes of increased macular thickness include presence of macular oedema, epiretinal membrane or presence of vitreo-macular traction. In this study, the increase in mean macular thickness in honey treated post-menopausal women is not due to these causes. In macular odema, there is abnormal fluid accumulation within the retina. This is demonstrated as presence of intra-retinal cystic appearance and the normal contour of the macular will appear distorted on the OCT images. Epiretinal membrane is a thin sheet of tissue which develops on the surface of the retina. It is associated with retinal thickening. OCT shows retinal thickening but there is a membrane overlying the macula with distorted macular contour and sometimes with loss of foveal depression and presence of posterior vitreous detachment (33). Vitreo-macular traction is characterized by presence of incomplete posterior vitreous detachment, strong adherence of the posterior hyaloid face to the macula and antero-posterior traction. It is usually present in aging patients with liquefaction of the vitreous and with patients with other ocular disease such as diabetic retinopathy, and other macular diseases. OCT will show presence of vitreo-macular traction at the vitreo macular interface and incomplete posterior vitreous detachment leading to a pseudo macular thickening. In our study, OCT showed normal contour in all participants and none of these features were demonstrable on the OCT images.

Other than the effect of honey alone, this study used honey cocktail, which comprises of bee pollen and royal jelly. Both of these substances have similar efficacy and function as honey, which include estrogenic effect on estrogen receptors, vasodilatory and increase retinal perfusion, antioxidant effect and anti-inflammatory effect (34).

Based on the analysis of the RNFL thickness of this current study, not only the mean global RNFL thickness but also quadrant RNFL thickness was thicker from baseline to at three months review in the honey cocktail group. In comparison, the mean RNFL thickness was thinner in global and all RNFL quadrants from baseline to at three months review in the no honey group.

Estrogen has been proven to increase retinal blood flow by its vasodilator properties. Estrogen affects vascular reactivity through direct effects on endothelial cells and increases estrogen receptor mediated nitrous oxide (NO) production (35). Since NO is also a potent vasodilator, this combination cause marked vasodilatation. A study done to analyse the effect of estrogen therapy in ovariectomised rats showed significant increased retinal perfusion in the range of 22% to 45% (36). In the retina, the arteries and veins are found to be situated within the nerve fiber layer whereas capillaries are found within the ganglion cell layer and the inner nuclear layer. Since there's increased blood flow through the arteries and veins in the RNFL, this may explains the increase in RNFL thickness in the honey cocktail group.

Based on the analysis of ONH parameters of this current study, there was increase in the rim area and reduction of the cup area, cup-disc-ratio and disc area from baseline to at three months post honey cocktail supplement group. In comparison, all four parameters were also affected in the no honey group whereby there was increase in the disc area, cup area and cup to disc ratio and reduction in the rim area from baseline to at three months review. These findings that we observe from the ONH parameters were correlated with other studies (13, 37).

According to a study done by Akar et al (37) to assess the effect of menstrual cycle on ONH parameters, they found that there's significant difference in the ONH parameters between follicular, ovulatory and luteal phase. In that study, the neuro-retinal rim area decreased significantly in the luteal phase whereas cup area and cup-to-disc ratio were found to be increased in the same phase. There was no significant difference in the ONH parameters in the follicular and ovulatory phase. The luteal phase is a phase in the menstrual cycle where the estrogen level is low. It was postulated that estrogen a potent vasodilator was responsible for the blood flow of the ONH during

the different stages of menstrual cycle thus causing the changes at the ONH in relation to estrogen levels. Animal study done by Vajaranant and Pasquale (11), showed estrogen therapy in ovariectomised rats increased retinal blood flow to ONH and protects the ONH topography. Therefore, honey cocktail through its estrogenic effect probably works in a similar manner to cause changes in ONH parameters. Besides this, ONH is also very much affected by oxidative stress and recent study has demonstrated that ONH damage is also associated with immune system activation. Oxidative stress stimulates a cascade of events leading to T-cell activation and tumor necrosis factor alpha (TNFα) activation. Honey and bee products such as bee pollen and royal jelly, which that have been used in this study, have good, protective immune-modulator response (38). Therefore, they may protect the ONH from these immune related damages.

This study has several limitations. The effect of honey cocktail on the retina in post-menopausal women was not compared with HRT, which is the current gold standard treatment for menopause. A comparison between HRT and honey on posterior segment structures in post-menopausal women would provide a better overview weighing the pros and cons between these two therapies. Besides that, this study used honey cocktail. A comparison between honey and honey cocktail on the evaluation of macular thickness, RNFL thickness and ONH parameters would have provided a better comparison of the effects honey and other bee products. The effect of honey cocktail in this study was only assessed at three months. Therefore the short terms effect and the long terms effect of honey cocktail on the macular thickness, RNFL thickness and ONH parameters are not known. A longer study duration assessing the effect of honey cocktail on a monthly basis would provide a more detailed measure on the effect of honey cocktail on posterior segment structures.

Other limitation in this study is the physiological data such as body weight and body height were not assessed among the participants. We recommended that in future study, systemic physiological data should be included beside other parameters for ocular changes.

CONCLUSION

Honey cocktail supplementation demonstrated structural changes in the macula, RNFL, and on the ONH parameters of post-menopausal women. However, this is a preliminary study and therefore future larger studies are needed to fully understand the effect of honey cocktail on retinal parameters.

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