

## Comparative effects of lime juice with brown sugar versus lime juice with honey supplementation on liver function tests and digestive health of healthy adults

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### Abstract

Honey and lime (*Citrus microcarpa*) are known to have antioxidant properties and have been used for traditional medical purposes. Honey and lime juice have shown positive hepatoprotective effects in animals and human studies, respectively. No studies have documented the effects of a combination of honey and lime juice on liver function tests. The objective of the present study was to compare the effect of lime juice mixed with brown sugar vs lime juice mixed with honey on liver function tests and digestive health among healthy adults. A randomized study was carried out involving 34 healthy adults (14 males and 20 females) aged between 20 and 50 years. Face-to-face interviews for health screening were conducted and data were collected using a questionnaire. The supplementation was taken daily for 30 days early in the morning at the restaurant of the School of Food Science and Technology, Universiti Malaysia Terengganu. Venous blood was collected before and after supplementation, to determine any changes of the liver function test enzymes. Most serum enzymes in the lime juice mixed with honey group did not change significantly. However, there were significant decreases in lactate dehydrogenase (LDH) in both groups in both males (-46.1%) and females (-35.6%). In terms of digestive health that was measured using questionnaires, lime juice is more beneficial than honey mixed with lime juice. Glutamic oxalate transaminase (GOT) was decreased with lime juice mixed with honey (-5.1%) but increased with lime juice mixed with brown sugar (13.5%). Based on the present study, it may be suggested that the beneficial effects observed might be influenced by the baseline levels of the parameters. This study provides support for the use of lime juice for improving digestive health among healthy adults.

**Keywords:** Glutamic oxalate transaminase, Honey, Lactate dehydrogenase, Lime juice

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## Introduction

The liver is one of the most important organs for metabolism, and liver dysfunction is intimately linked to adverse metabolic consequences as seen in type 2 diabetes and non-alcoholic fatty liver disease. Liver function can be tested by measuring the concentration of several enzymes in the bloodstream, termed a liver function test (LFT) (Park et al., 2013; Hall and Cash, 2012). These enzymes are normally found within the hepatocytes but with liver dysfunction and damage they appear in the circulation. Oxidative stress can cause liver damage (Li et al., 2015). Lime is an important nutrient source in China (Zou et al., 2015) and been used as a cough relieving medicine by mixing lime juice with sugar or honey (Aibinu et al., 2007). Lime and lime juice contain a high amount of antioxidants (Quaiquil et al., 2001). Therefore, liver function might be protected or improved by consuming lime juice. Honey has been used as a traditional medicine for centuries around the world due to its various beneficial functions such as antioxidant, anti-inflammatory, and anti-ulcer activity as well as promotion of wound healing (Erguder et al., 2008). Honey has been reported to improve liver function (Chandane et al., 2013) but an overdose of honey might cause adverse effects (Wilson et al., 2011; Avwioro et al., 2012) due to excess fructose converting into fat and being stored in the liver, causing non-alcoholic fatty liver disease (NAFLD). The purpose of this study is to investigate the effect of consuming the combination of lime juice and honey, in comparison with lime juice and brown sugar, in the daily diet on LFT in healthy humans. This study has been done due to a lack of human studies on the effect of honey and lime on LFT

## Material and Methods

### Material

Honey was purchased from a local supplier and lime (*Citrus microcarpa*) was purchased from a local market.

### Mixture preparation

Control group: 5 g fresh lime juice was mixed with 10 g of brown sugar with warm water at 40 to 50 degrees Celsius to a final volume of 150 ml.

Supplementation group: 5 g fresh lime juice was mixed with 10 g of honey with warm water at 40 to 50 degrees Celsius to a final volume of 150ml.

## Subject selection and study design

40 adult subjects were screened for eligibility criteria. Inclusion criteria were as follows: 20 to 50 years old; male or female; not suffering from cancer or other chronic disease or mental disorder; had not participated in any clinical trial three months prior to the intervention; and body mass index (BMI) range from 18.5 – 30.0 kg/m<sup>2</sup>. This study was approved by the Universiti Sultan Zainal Abidin (UNiSZA) Human Research Ethics Committee (UHREC-628-1 jld.2 (16)) following the ethical consideration code for human.

Eligible subjects were screened with a Health and Lifestyle Questionnaire. 34 subjects were divided into two groups as shown in Fig. 1: (i) Control group to receive 10 gram of brown sugar with 5 gram of lime juice daily, (ii) Supplementation group to receive 10 gram of honey with 5 gram of lime juice daily; both groups to be treated for 30 days. About 3-4 ml blood sample was drawn twice (baseline and endline) after an overnight fasting (12 hours) by nurses at University Health Centre. Measurements included LFT using Spotchem EZ SP-4430 from Japan for total protein, albumin, total bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase. Digestive health was measured using questionnaire with five Likert scale; body weight, body fat percentage were obtained using Tanita Body Fat Monitor. Baseline data was taken a day before the supplementation started and endline data was taken after completion of 30 days' supplementation.

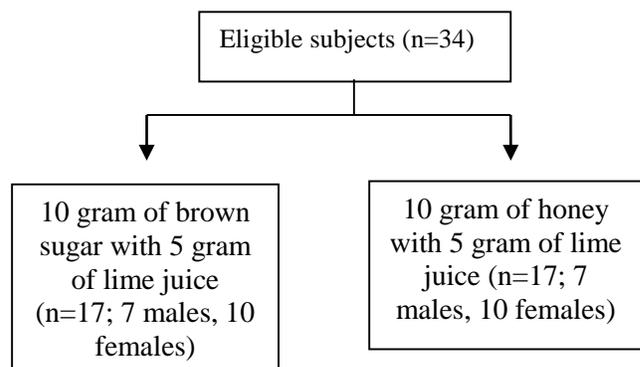


Figure-1: Experimental design of this study

### Statistical analysis

The data collected were analyzed with the Statistical Package for Social Science (SPSS) version 20.0. Normality test was carried out using Shapiro-Wilk test. The significant difference ( $p < 0.05$ ) was tested



within (paired t-test or Wilcoxon Signed Rank test) and between (independent t-test or Mann-Whitney U test) groups pre- and post-intervention.

## Results and Discussion

Table 1 shows the subject characteristics for both groups. At baseline, it was found that all parameters were within normal range, apart from LDH which shown significant different between groups. Overall, compliance among all participants was good (more than 85%) since they need to come to the Restaurant in the school to receive the supplementation. The control group showed a significant decrease in

LDH by a mean of 19.3%. This is supported by Solomon et al. (2015), who found that a high concentration of lemon juice significantly decreases GOT and GPT as compared to control and did not impair biochemical activities of liver or have a deleterious effect on the liver function of 24 growing rabbits. In the present study, a significant change in control group in LDH may be due to the baseline LDH enzyme of control group being much higher as compared to supplemental group, even still in the normal range (105- 333 IU/L). At similar baseline value of GOT for both groups, resulted indicated an increase following lime juice + brown sugar, however no effect in another group.

**Table-1: Subject characteristics for both groups**

	<b>Lime juice + sugar (control, n=17)</b>	<b>Lime juice + honey (n=17)</b>	<b>p value</b>
Age (years)	26.00 (7.50)	25.00 (8.00)	0.496
Weight (kg)	63.52 ± 12.62	61.40 (14.00)	0.882
Height (cm)	159.84 ± 8.14	159.99 ± 9.10	0.961
Body Mass Index, (BMI) (kg/m <sup>2</sup> )	24.81 ± 4.15	24.55 ± 3.46	0.845
Body Fat Percentage (%)	25.21 ± 14.26	24.45 ± 8.74	0.854
Total Protein (g/dL)	7.20 (0.75)	7.04 ± 0.72	0.270
Albumin (g/dL)	4.06 ± 0.41	4.06 ± 0.36	0.965
Total Bilirubin (mg/dL)	0.41 ± 0.16	0.42 ± 0.27	0.816
GOT (IU/L)	17.00 (7.00)	17.00 (15.00)	0.238
GPT (IU/L)	13.00 (8.00)	11.00 (5.50)	0.333
LDH (IU/L)	271.35 ± 55.98	194.94 ± 65.93	0.001

Data are presented as mean ± SD or median (IQR). P\* value < 0.05 indicates significant difference by independent t-test.

Abbreviation: GOT= glutamic oxaloacetic transaminase; GPT= glutamic pyruvic transaminase; LDH= lactate dehydrogenase

**Table-2: Effect of supplementations on liver function test**

	<b>Lime juice + brown sugar</b>			<b>Lime juice + honey</b>		
	<b>Baseline</b>	<b>After</b>	<b>p value</b>	<b>Baseline</b>	<b>After</b>	<b>p value</b>
T-Protein (g/dL)	7.20 (0.75)	7.18 ± 0.48	0.571	7.04 ± 0.72	7.00 (0.60)	0.812
Albumin (g/dL)	4.06 ± 0.41	4.16 ± 0.31	0.190	4.06 ± 0.36	4.12 ± 0.41	0.498
T-Bilirubin (mg/dL)	0.41 ± 0.16	0.40 (0.15)	0.749	0.42 ± 0.27	0.40 (0.20)	0.628
GOT (IU/L)	17.00(7.00)	21.12 ± 5.24	0.021 <sup>*b</sup>	17.00 (15.00)	16.00 (17.00)	0.261
GPT (IU/L)	13.00 (8.00)	14.71 ± 3.33	0.154	11.00 (5.50)	9.00 (11.50)	0.553
LDH (IU/L)	271.35 ± 55.98	229.06 ± 64.58	0.002 <sup>*a</sup>	194.94 ± 65.93	162.12 ± 50.35	0.079

Data are presented as mean± SD or median (IQR). P\* value < 0.05 indicates significant difference by <sup>a</sup>paired t-test or <sup>b</sup>Wilcoxon-Signed Rank test.

Abbreviation: GOT= glutamic oxaloacetic transaminase; GPT= glutamic pyruvic transaminase; LDH= lactate dehydrogenase



**Table-3: Influence of sex differences on the effect of supplementation on liver function test**

		Male (n=7)			Female (n=10)		
		Before	After	P value	Before	After	P value
Effect of brown sugar and honey	Total Protein (g/dL)	7.37 ± 0.42	7.24 ± 0.51	0.507	7.20 (0.58)	7.15 ± 0.48	0.889
	Albumin (g/dL)	4.43 ± 0.14	4.40 ± 0.16	0.604	3.80 ± 0.32	4.00 ± 0.27	0.130
	Total Bilirubin (mg/dL)	0.53 ± 0.14	0.50 (0.70)	0.500	0.32 ± 0.10	0.30 (0.13)	1.000
	GOT (IU/L)	19.71 ± 3.64	21.00 ± 3.70	0.368	16.00 (8.50)	21.20 ± 6.30	0.032 <sup>ab</sup>
	GPT (IU/L)	14.71 ± 4.42	14.86 ± 3.76	0.934	11.00 (4.25)	16.00 (4.25)	0.067
	LDH (IU/L)	294.14 ± 57.43	248.00 ± 78.59	0.010 <sup>ab</sup>	255.40 ± 51.82	215.80 ± 53.12	0.048 <sup>ab</sup>
Effect of honey and lime juice	Total Protein (g/dL)	6.77 ± 0.43	6.97 ± 0.18	0.172	7.23 ± 0.84	7.14 ± 0.95	0.583
	Albumin (g/dL)	4.00 (0.70)	4.19 ± 0.36	0.865	4.00 ± 0.39	4.08 ± 0.45	0.505
	Total Bilirubin (mg/dL)	0.50 (0.30)	0.50 (0.10)	0.335	0.30 ± 0.22	0.31 ± 0.10	0.811
	GOT (IU/L)	21.00 (19.00)	26.00 ± 13.58	0.233	14.00 (5.75)	15.00 (5.50)	0.607
	GPT (IU/L)	14.00 (23.00)	19.00 ± 10.91	0.463	9.00 (3.00)	9.00 (3.25)	0.914
	LDH (IU/L)	198.71 ± 78.47	162.43 ± 62.46	0.219	192.30 ± 60.03	161.90 ± 43.67	0.245

Data are presented as mean ± SD or median (IQR). P value < 0.05 indicates significant difference by p<sup>\*a</sup> < 0.05 using paired t-test and p<sup>\*b</sup> < 0.05 using Wilcoxon Sign rank test.

For the lime juice + honey group, the result shows no significant effect in all parameters. However, according to Erguder et al. (2008), 10 mg/kg of honey supplementation to jaundiced male rats for 7 days lead to a significant decrease in alanine transaminase (ALT) and adenosine deaminase (ADA) activities compared to other control groups.

GOT, GPT and LDH in the lime juice + honey group showed a tendency of slight decrease as compared to the control group. A low dosage of honey might cause no significant difference, and the subjects were not jaundiced as previously reported. A previous study reported that daily consumption of lemon juice from 5 g to 15 g in human can improve liver function (Pole, 2006).

For the control group, the results show a significant decrease for LDH in both males and females. Jaiswal et al. (2015) reported that pre-treatment of rats with 0.5 ml of lime juice extract before exposure to carbofuran

lead to a significant decrease in serum LDH, which is in agreement with these results. LDH showed significant decrease in the control male (by 17.1%) and control female (by 12.6%) groups, while total protein, total bilirubin, albumin and GPT did not show any significant changes. A previous study by Kim et al. (2015) showed that lime consumption decreased serum total protein and albumin level significantly in overweight Korean women over 11 days. However, the subjects selected in present study were all healthy, and there may be different effects between normal weight and obese subjects. Only the female control group showed an increase in GOT (17.3%). This may be related to the increased in body fat percentage, as shown in Table 6. It was explained by Stranges et al. (2004) that increased body fat distribution increased level of hepatic enzyme, likely as a result of unrecognized fatty liver.

**Table-4: Absolute change and percent change of the effect of honey with lime juice and brown sugar with honey on liver function test**

	Lime juice + brown sugar (control, n=17)		Lime juice + honey (n=17)		p value	
	Absolute change	Percentage change (%)	Absolute change	Percentage change (%)	Absolute change	Percentage change (%)
Total Protein (g/dL)	-0.09 ± 0.50	-1.02 ± 6.90	0.03 ± 0.45	0.61 ± 6.72	0.458	0.488
Albumin (g/dL)	0.11 ± 0.32	0.00 (8.97)	0.06 ± 0.35	1.71 ± 9.07	0.685	0.876
Total Bilirubin (mg/dL)	0.04 ± 0.23	14.29 ± 50.45	0.00 (0.25)	-2.01 ± 24.11	0.712	0.263
GOT (IU/L)	1.94 ± 3.19	13.54 ± 20.29	-1.00 (6.00)	-5.41 ± 22.69	0.025 <sup>ab</sup>	0.015 <sup>ab</sup>
GPT (IU/L)	1.53 ± 4.09	18.21 ± 34.86	0.00 (4.50)	0.00 (38.84)	0.128	0.105
LDH (IU/L)	-42.29 ± 45.88	-15.28 ± 17.62	-32.82 ± 72.19	-21.76 (37.03)	0.651	0.850

Data are presented as mean± SD or median (IQR). P\* value < 0.05 indicates significant difference by <sup>\*a</sup>Wilcoxon Sign rank test or <sup>\*b</sup>< Mann-Whitney U test.



**Table-5: Absolute change and percentage change of the effect of supplementation on liver function test among male and female group**

		Lime juice + brown sugar			Lime juice + honey male (n=7)		
		male (n=7)	female (n=10)	p value	male (n=7)	female (n=10)	p value
Absolute change	T-Protein (g/dL)	-0.13 ± 0.48	-0.07 ± 0.54	0.822	0.20 ± 0.34	-0.09 ± 0.50	0.204
	Albumin (g/dL)	-0.03 ± 0.14	0.20 ± 0.38	0.152	0.029 ± 0.35	0.08 ± 0.36	0.776
	T-Bilirubin (mg/dL)	0.10 ± 0.34	0.00 ± 0.12	0.399	0.00 (0.30)	0.01 ± 0.13	0.601
	GOT (IU/L)	1.29 ± 3.50	2.40 ± 3.06	0.496	-4.29 ± 9.16	-1.00 (6.50)	0.669
	GPT (IU/L)	0.14 ± 4.38	2.50 ± 3.81	0.255	-2.14 ± 10.14	-0.10 ± 2.92	0.551
	LDH (IU/L)	-46.14 ± 32.86	-39.6 ± 54.81	0.783	-36.29 ± 69.96	-30.4 ± 77.37	0.875
Percentage change (%)	T-Protein (%)	-1.63 ± 6.37	-0.59 ± 7.55	0.770	3.22 ± 5.27	-1.21 ± 7.26	0.190
	Albumin (%)	-0.62 ± 3.09	5.88 ± 10.77	0.145	-0.95 ± 8.63	2.24 ± 9.78	0.783
	T-Bilirubin (%)	19.69 ± 58.78	10.50 ± 46.72	0.724	-1.44 ± 25.47	-2.50 ± 24.61	0.936
	GOT (%)	8.16 ± 18.42	17.30 ± 21.62	0.377	-10.85 ± 25.30	-1.60 ± 21.19	0.426
	GPT (%)	6.93 ± 35.74	26.10 ± 33.78	0.278	-2.67 ± 50.71	2.67 ± 23.42	1.000
	LDH (%)	-17.08 ± 12.76	-14.01 ± 20.95	0.736	-9.80 ± 44.41	-6.82 ± 48.55	0.899

Data are presented as mean± SD or median (IQR). P value > 0.05 indicates no significant difference.

Abbreviation: GOT= glutamic oxaloacetic transaminase; GPT= glutamic pyruvic transaminase; LDH= lactate dehydrogenase

**Table-6: Effect of supplementations on total score of digestive health in total, male and female**

		Lime juice + brown sugar	Lime juice + honey
Total (n=17)	Before	71.88 ± 13.33	73.06 ± 13.27
	After	77.88 ± 12.44	73.29 ± 11.53
	P value	0.025*	0.941
Male (n=7)	Before	79.71 ± 13.54	81.71 ± 11.51
	After	79.71 ± 13.87	76.86 ± 9.01
	P value	1.000	0.347
Female (n=10)	Before	66.40 ± 10.62	67.00 ± 11.21
	After	76.60 ± 11.93	70.80 ± 12.87
	P value	0.008*	0.365

Data are presented as mean± SD. P\* value < 0.05 indicates significant difference by paired t test.

No favourable effect was shown during and after intervention in the control group, perhaps due to the short duration and the subjects being healthy. Hence, the effects in improving liver function may not be significant.

For the lime juice + honey group, the results did not indicate significant effects on liver enzymes. However, Chandane et al. (2013) reported that a high dose of 5 g/kg/day of honey in rats for 15 and 30 days significantly prevented, and indeed reversed levels of AST and ALT. Furthermore, 30 days of honey

treatment lead to reduced degeneration and increased regeneration of the liver. Therefore, the non-significant result may be due to low dose of honey (0.16 g/kg/day based on mean body weight 61.4 kg) and low dose of lime juice (0.08 g/kg/day), which is supported by Al-Waili and Noori (2003) that the effect was greater with a higher concentration of inhaled honey in sheep with carbon tetrachloride-induced liver injury affecting blood sugar, renal and liver function. From Table 4, it can be seen that the control group showed a decrease in values for total protein and LDH, while the supplemental group showed decreased total bilirubin, GOT, and LDH. Abdul-Ghani *et al.* (2008) reported ingestion of honey induces spermatogenesis in rats and reduced LDH activity but since both groups showed decrease in LDH, hence the effect might not due to honey but lime juice. Among the parameters shown in Table 4, only GOT showed significant difference in terms of both absolute change and percent change between control and supplemental group, demonstrating the positive effect of honey with lime juice.

In short, the present study demonstrates that honey with lime juice decreased serum enzymes more than brown sugar with lime juice.

Table 5 shows no significant difference in absolute change and percent change between male and female in both control and supplemental group. Although



insignificant, the result did show that honey with lime juice improved or decreased serum enzyme levels compared to brown sugar with lime juice in the study. Hence, honey with lime juice show better effects in terms of liver function. Since there is no significant difference in absolute change and percentage change between sexes, it can be concluded that there is no effect of sex difference on supplementation.

There was a significant improvement in the control group. According to Mohanapriya et al. (2013), lime scent induces saliva secretion, which aids in primary digestion. Additionally, its acid helps in the breakdown of macromolecules in food. Its flavonoids stimulate the digestive system and increase the secretion of bile and gastric juice as well as peristaltic motion. Lime’s flavonoids also function as direct antioxidants and free radical scavengers and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie and Crozier, 2000). In the lime + honey group, there was no significant improvement. Based on this study, combination of lime juice with honey does not have positive effect on digestive health, while lime juice without honey plays a major role in improving digestive health. Between gender, only control group female shows significant improvement in digestive score to 76.6%. A study by Ganguly and Roy (2015) stated that lime is vital in treatment for gastric disorders like indigestion, constipation and peptic ulcer, burning in the chest, abrupt bilious vomiting and excessive accumulation of saliva in mouth. This may explain the improvement in the female control group.

The results showed significant decreases in body fat in control group and no any significant changes in all the

parameters in lime juice + honey group. The change in body fat percentage, particularly in female (but not in male) in control group was explained by Nakajima et al. (2014) as the consumption of lime with phenolic compound being linked to adipocytes apoptosis. This reduces the number of adipose cell and helps in weight loss and the prevention of weight cycling. However, according to Lin et al. (2012), daily exercise with combine or separate calorie control will result in significant reduction in body weight and body fat percentages, as well as improved body composition. This means that exercise should be done regularly along with the consumption of lime juice and honey mixture to provide better results in weight loss.

Male control group showed no significant changes while female control group show only significant increase in body fat percentage. This may be due to the limitations of bioelectrical impedance analyser (BIA), as it uses electric current flows at different rates through the body fluid and is influenced by the factors such as environment, ethnicity, phase of menstrual cycle, and underlying medical conditions (Dehghan and Merchant, 2008). Another study showed that body fat percentage varied in BIA by 8.8% and 9.9% from the highest to the lowest measurement in women and men, respectively (Slinde and Rossander, 2001).

For the lime juice + honey group, there were no significant changes in both male and female groups, although there were slight but insignificant increases in BMI, body weight and body fat percentage. This may be due to the subjects were in healthy state in baseline. Hence, no reduction in those parameters is required for the body to be healthy.

**Table-7: Effect of supplementations on body weight, body fat percentage and body mass index (BMI)**

		Lime juice + brown sugar			Lime juice + honey		
		Weight (kg)	Body Fat (%)	BMI (kg/m <sup>2</sup> )	Weight (kg)	Body Fat (%)	BMI (kg/m <sup>2</sup> )
Total (n=17)	Before	63.52 ± 12.62	25.21 ± 14.26	24.81 ± 4.25	66.00 (20.15)	24.45 ± 8.75	24.55 ± 3.46
	After	63.23 ± 12.39	22.50 (27.30)	24.73 ± 4.12	64.60 (19.60)	25.82 ± 10.03	24.65 ± 3.53
	P value	0.165	0.006*	0.251	0.641	0.073	0.379
Male (n=7)	Before	66.09 ± 10.28	18.10 ± 9.37	23.94 ± 3.30	67.31 ± 15.00	17.83 ± 9.50	23.77 ± 4.49
	After	65.70 ± 9.95	19.27 ± 8.05	23.81 ± 3.27	67.11 ± 14.89	19.09 ± 12.12	23.70 ± 4.45
	P value	0.327	0.226	0.381	0.403	0.426	0.413
Female (n=10)	Before	61.72 ± 14.29	33.60 (32.30)	26.33 (9.61)	59.81 ± 7.68	29.10 ± 4.21	25.11 ± 2.65
	After	61.50 ± 14.10	31.53 ± 14.28	26.50 (9.25)	60.32 ± 7.90	30.53 ± 4.60	25.32 ± 2.79
	P value	0.379	0.017*	0.646	0.223	0.088	0.238

Data are presented as mean ± SD or median (IQR). P\* value < 0.05 indicates significant difference by Wilcoxon-signed ranked test.



## Conclusion

The lime juice + honey group did not change significantly in most of the serum enzyme but showed better improvement in GOT compared to control group. This indicates honey demonstrated a better positive effect than brown sugar in terms of GOT improvement. In the control group, there was a significant reduction of LDH enzyme for both males and females. A significant improvement in digestive health was seen in female control group, and lime juice itself is beneficial in managing digestive health with or without honey or brown sugar added. Consumption of 10 g of honey with 5 g of lime juice is insufficient to provide an adequate protective effect on liver.

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## Contribution of Authors

Edmund CCK: Data collection and manuscript writing  
Ainie R: Data collection  
Chong KH: Statistical analysis  
Norhayati H: Manuscript writing  
Anam A: Literature search  
Asma A: Manuscript final reading  
Philip CC: Manuscript checking and final approval  
Yusof HM: Conceived idea, data interpretation and manuscript final approval

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