

Renoprotective Role of Tualang Honey against High Cholesterol Diet Induced Acute Kidney Diseases in an Animal Model

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ABSTRACT

The aim of this study is to determine the renoprotective effects of tualang honey against high cholesterol diet (HCD) induced acute and subacute kidney injuries in an animal model. **Methodology:** Ten female Sprague-Dawley rats were divided into two groups: the HCD group, fed with 12% cholesterol diet, and the HCD with tualang honey (HCD+TH) group, fed with HCD and daily dose of 1.4g/kg/day of tualang honey. Biochemical analysis of lipid and renal profiles were performed at 48 hours, 7 days, and 6 weeks. The animals were sacrificed after 6 weeks and the kidneys were harvested for histological examination. **Results:** The serum cholesterol and triglyceride levels were significantly lower in the HCD+TH group as compared to the HCD group at 7 days with p values of 0.025 and 0.031 respectively. The HCD+TH group showed significantly lower serum creatinine level than the HCD group at 48 hours (p=0.018). The histological sections of the renal tissue of the HCD and HCD+TH groups both revealed segmental mesangial hypercellularity and mesangial matrix expansion of the glomeruli. **Conclusion:** Our study revealed that tualang honey exhibited some degree of renoprotective effect biochemically but not histologically in the rat models fed with high cholesterol diet.

INTRODUCTION

The incidence of acute kidney disease and disorders (AKD) is on the rise in both developed and developing countries (Lameire *et al.*, 2013). It is associated with severe complications where it increases the risk of short-term and long-term mortality, development of chronic kidney disease and rapid progression to end-stage renal disease (Rewa and Bagshaw, 2014). Also although there is a growing insight into the causes and mechanisms of kidney diseases, preventive and therapeutic measures are still few (Lameire *et al.*, 2013). Natural products have been used for medicinal purposes long before recorded history and presently their use coupled with other alternative

treatment options have accelerated due to the rise in prevalence of diseases, increasing costs of medical care and public concerns on the rising adverse effects of conventional medicine (Mohd Effendy *et al.*, 2012). Honey which is one of these natural products, has been deemed to be of medicinal importance and used since ancient times to treat several diseases (Gan *et al.*, 2009). Amongst the various types of Malaysian honey, tualang honey is a type of polyfloral wild honey produced by the rock bee (*Apis dorsata*) (Othman *et al.*, 2015). It has been shown to be rich in phenolic acids and flavonoid compounds (Khalil *et al.*, 2011). In addition a number of compounds that have been determined in tualang honey have not been previously reported in other types of honey. They include stearic acids, 2-cyclopentene-1, 4,-dione, 2[3H]-furanone or dihydro-butyrolactone, gamma-crotonolactone or 2[5H]-furanone, 2-hydroxy-2-cyclopenten-1-one, hyacinthin, 2, 4-dihydroxy-2, 5-dimethyl-3[2H]-furan-3-one, and phenylethanol (Ahmed and Othman, 2013).

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Tualang honey has been documented to have a good anti-bacterial (Shehu *et al.*, 2015) and anti-inflammatory activities (Al-Waili and Boni, 2003). It also has a considerable effect on the healing process of different types of wounds (Khoo *et al.*, 2010) and has been used in the treatment of diabetic foot (Imran *et al.*, 2011). It demonstrates a significant antioxidant activity due to its high constituents of phenolics and flavonoids (Shehu *et al.*, 2015). Additionally it has been shown to have anti-neoplastic activity (Yaacob *et al.*, 2013) and a potential role in the improvement of learning and memory (Othman *et al.*, 2015). In an experimental study, tualang honey produced a hepatoprotective effect (Erejuwa *et al.*, 2011). We have previously demonstrated the ability of HCD to induce acute and subacute kidney injuries in animal model (Zenab *et al.*, 2017) and as a continuation we aimed to determine the lipid lowering and renoprotective effects of tualang honey in this animal model.

MATERIALS AND METHODS

Animals

Ten female Sprague-Dawley rats (age 6-8 weeks) weighing 140-170 grams were used in this study. The rats were purchased from A-Sapphire Enterprise, Seri Kembangan, Selangor. Each cage housed two rats under standard experimental conditions of 20-26°C at 50-70% humidity with 12 hours light/dark cycles. They had free access of water and food throughout the experiment. The animal handling procedures, treatment and experimental protocols were approved by the Institutional Animal Care and Use Committee, International Islamic University Malaysia IACUC-IIUM) No. of IACUC Approval : IIUM / IACUC Approval / 2016/ (12) (83).

High cholesterol diet

Twelve percent cholesterol diet with 3% cholic acid was prepared by mixing of 1kg of commercial rat pellet in powder form with 120 grams of analytical pure cholesterol powder (Nacalai-Tesque, KYOTO, JAPAN. Lot No. M4T5494. Code 08721-75) and 3 grams of cholic acid (Nacalai-Tesque, KYOTO, JAPAN. Lot No. M6H9123. Code 08805-56) in order to produce stable hypercholesterolemia in rats that are resistant to increase in plasma cholesterol level (Monte and Jimenez, 1993).

The preparation was carried out weekly to avoid oxidative modification of the cholesterol.

Tualang honey

Tualang honey (AgroMas, Malaysia) was supplied by Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia. The nutritional composition and specifications of tualang honey are as shown in table 1. The honey dose was calculated by conversion of human equivalent dose to rat dose using Km factor according to Reagan-Shaw *et al.* (2008) (Reagan-Shaw *et al.* 2008) as the following:

$$\text{Human equivalent dose (HED)} = \text{Animal dose} \times \frac{\text{Animal Km factor}}{\text{Human Km factor}}$$

Experimental design

After 10 days of acclimatization, the rats were randomly divided into two groups. Group I served as the HCD group (n=5) and was fed with the 12% cholesterol diet. Group II served as HCD with tualang honey (HCD+TH) group (n=5) and was fed with 12% cholesterol diet along with oral daily dose of 1.4 g/kg/day of tualang honey by gavage. The experimental diets were administered for 6 consecutive weeks.

Biochemical study

Blood specimens collected at completed 48 hours, 7 days and 6 weeks were analysed for lipid profile and renal function test (Siemen Xpand Plus, USA).

Histological study

At the completed 6 weeks, the rats were euthanized and both kidneys were harvested and fixed in 10% neutral buffered formalin for histological examination. The kidneys were processed using automated tissue processor (Leica TP 1020). The tissues were embedded into paraffin blocks (Leica EG1160).

The tissue blocks were subsequently sectioned at 3 µm thickness and stained with hematoxylin and eosin (HandE) and Masson trichrome.

Statistical analysis

Statistical analysis was performed using Student's t-test available in the statistical programme SPSS version 20.0 to compare lipid profile and renal function test parameters of the study groups. A p value of <0.05 was considered to be significant. The histological sections were analysed by two pathologists.

Table 1: Nutritional composition and specifications of tualang honey.

Parameter, Unit	Result	Standard (Food Reg 1985, Reg. 130)
Reducing Sugar (g/100g):		>60.0
Fructose	38.0	
Glucose	36.9	
Sucrose (g/100g)	Not detected (<0.01)	<10.0
Ash (g/100g)	0.02	<1.0
Moisture (g/100g)	23.1	<20.0

Table 2: Lipid profile in the HCD and HCD+TH groups of rats.

Lipid Profile parameter (mmol/L)	48-hours		7 Days		6 week	
	HCD	HCD+TH	HCD	HCD+TH	HCD	HCD+TH
Cholesterol	2.30±0.40	2.40±0.69	2.84±0.68	1.83±0.71*	7.38±2.93	8.76±4.07
HDL-c	1.88±0.21	2.03±0.32	2.45±0.65	2.11±0.95	0.23±0.10	0.18±0.08
Triglyceride	0.50±0.20	0.41±0.17	0.44±0.10	0.28±0.11*	0.53±0.15	0.53±0.11
vLDL-c	0.10±0.04	0.08±0.03	0.08±0.02	0.05±0.02	0.11±0.03	0.11±0.02

Values are given as means ± sd. Significant differences were analysed using Student's t-test, and indicated as * $p < 0.05$ when comparing HCD with HCD+TH rats.

Table 3: Renal profile in the HCD and HCD+TH groups of rats.

Renal Profile parameter	48-hours		7 Days		6 week	
	HCD	HCD+TH	HCD	HCD+TH	HCD	HCD+TH
Creatinine ($\mu\text{mol/L}$)	33.40±9.15	15.60±9.9*	25.20±12.80	25.40±10.03	40.60±4.28	34.80±3.83
Urea (mmol/L)	4.56±1.05	4.46±0.87	9.24±4.07	6.26±1.15	9.76±4.20	7.98±0.68
Sodium (mmol/L)	137.40±0.55	137.60±1.67	136.80±2.17	138.20±1.30	139.20±2.05	138.80±0.84
Potassium (mmol/L)	5.38±0.29	5.54±0.88	7.18±1.86	5.96±0.78	4.40±0.48	5.10±0.38
Chloride (mmol/L)	100.40±0.55	101.00±1.22	100.20±0.84	100.00±1.22	99.80±1.48	99.40±0.55
Uric acid (mmol/L)	0.11±0.05	0.26±0.26	0.16±0.04	0.19±0.07	0.13±0.03	0.13±0.02

Values are given as means ± sd. Significant differences were analysed using student t-test, and indicated as * $p < 0.05$ when comparing HCD with HCD+TH groups of rats.

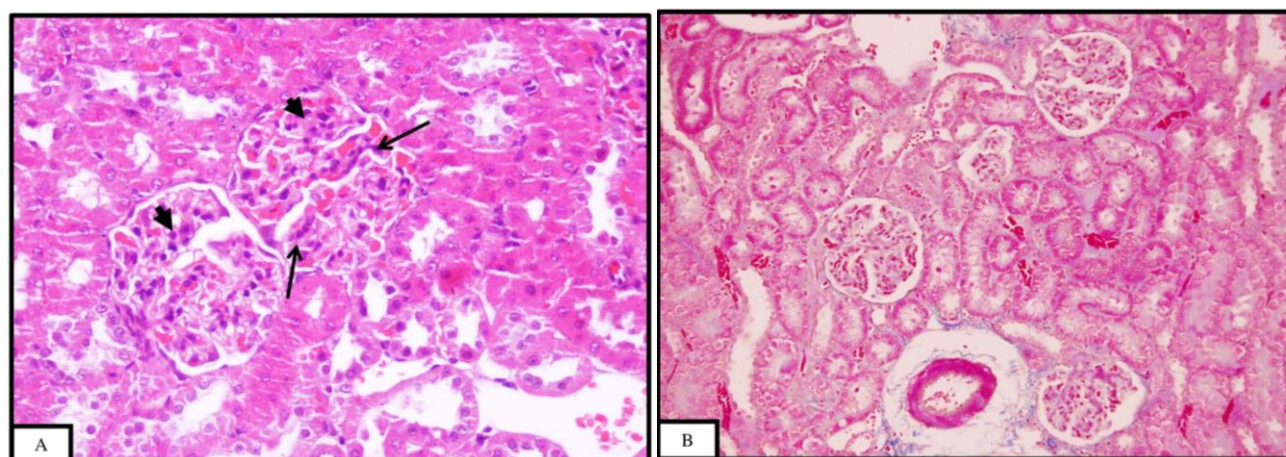


Fig. 1: Pictomicrograph of a kidney section of the high cholesterol diet (HCD) group. Representative (A) HandE – stained section (x40 objective) showing segmental mesangial hypercellularity (arrow) with mesangial matrix expansion of the glomeruli (arrow head) and (B) Masson Trichrome-stained section (x40 objective) exhibiting of no area of increased amount of periglomerular peritubular fibrous tissue formation.

RESULTS

The lipid profile

The lipid profile results for the HCD group and HCD+TH group are as shown in table 2. There was a significant difference in the level of total cholesterol (TC) between the HCD and the HCD+TH groups at 7 days ($p = 0.025$). The mean serum triglyceride (TG) at the completed 7 days for the HCD+TH group were also significantly lower as compared to the HCD group ($p=0.031$). There was no significant difference observed in the HDL-c level between the HCD group and the HCD+TH group at all intervals of measurements.

The renal function test

Table 3 shows the renal profile results of the two experimental groups. The only significant difference in renal function test observed was in the serum creatinine level between HCD and HCD+TH groups at completed 48 hours where p value

was 0.018 with a mean difference of 17.80. There was no significant difference in the serum creatinine between the groups at 6 weeks ($p=0.054$). None of the other renal profile parameters (urea, sodium, potassium, chloride and uric acid) showed significant difference at 48 hours, 7 days and 6 weeks.

The renal histology

The sections of the kidneys from the HCD group showed segmental mesangial hypercellularity with segmental mesangial matrix expansion of most glomeruli in both kidneys. No other renal histopathological changes were seen in this group (Figure 1A). As for the Masson trichrome stained sections, the kidney showed no evidence of increased amount of periglomerular or peritubular fibrous tissue (Figure 1B). The sections of the kidneys from the HCD + TH group showed similar features to that of HCD group both in sections stained with haematoxylin and eosin (Figure 2A) and in the sections stained with Masson trichrome (Figure 2B).

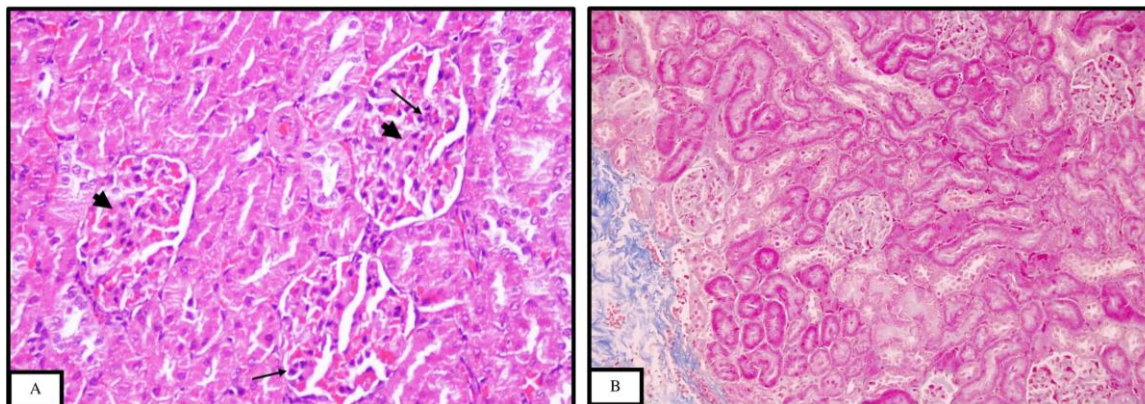


Fig. 2: Pictomicrograph of a kidney section of the high cholesterol diet with tualang honey (HCD+TH) group. Representative (A) HandE-stained section (x40 objective) showing segmental mesangial hypercellularity (arrow) with mesangial matrix expansion of the glomeruli (arrow head) and (B) masson trichrome-stained section (x20 objective) exhibiting of no area of increased amount of periglomerular of peritubular fibrous tissue formation.

DISCUSSION

In our earlier study, we demonstrated the ability of 12% cholesterol diet to induce dyslipidaemia (Zenab *et al.*, 2017; Azril Shahreez *et al.*, 2016) and acute and subacute kidney injuries in female Sprague Dawley rats (Zenab *et al.*, 2017). In this study, we investigated the lipid lowering and renoprotective activities of tualang honey. We have chosen the measurements intervals at 48 hours, 7 days and 6 weeks as they meet the defined periods for acute and subacute kidney injuries as stipulated by KDIGO guidelines (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, 2012). KDIGO guidelines proposed the term AKI to include both acute and subacute pathologies, in which changes that occur within 48 hours are termed as acute while those that evolve over more than 48 hours but generally under than three months are referred to as subacute kidney injury. However since it has been documented in the literature that a period of 6 weeks is sufficient to induce sub-acute kidney injury by using HCD, we thus have confined the study to a period of 6 weeks.

The concurrent supplementation of tualang honey with 12% cholesterol diet to female Sprague Dawley rats for six consecutive weeks in this study has revealed that honey supplementation significantly reduced both the mean serum the TC and TG levels at the completed 7 days in comparison to HCD group. There was however no significant reduction at 48 hours which maybe too early for honey to take effect while the absence of lipid lowering effects at 6 weeks may be related to the inadequate dose of honey utilized in the study. At 6 weeks Erejuwa *et al.* (2011) however reported a reduction in the level of TG (Erejuwa *et al.*, 2011). Dyslipidaemia is a recognized cause of renal damage and it is also known to worsen the already compromised renal function in patients with pre-existing nephropathies (Balarini *et al.*, 2011). We did not report the LDL-c level in this study as the Friedewald formula used for calculation of LDL-c is not advisable to be utilised in hypercholesterolemic

rats since it will overestimate the LDL-c (Sanchez-Muniz and Bastida, 2008).

The honey supplementation in the animal model also has demonstrated some renoprotective effects as evident by the significant reduction of the mean creatinine level at 48 hours and at 6 weeks although not significant. Additionally although not attaining significant levels the mean blood urea levels were generally lower in the tualang honey supplemented group. Also the mean serum uric acid levels in the group showed a decreasing trend throughout the experiment. Erejuwa *et al.*, (2011) reported a reduction in the level of serum creatinine when tualang honey was used in combination with oral hypoglycaemic agents (metformin) in diabetic rats. He suggested that, combination of tualang honey with oral hypoglycaemic drugs may have beneficial effect in preventing renal damage (Erejuwa *et al.*, 2011).

The present study also revealed that the 12% cholesterol diet induced histologically segmental mesangial hypercellularity with mesangial matrix expansion of the renal glomeruli indicating its ability to induce acute and subacute kidney injuries which conformed to that reported by Ghada *et al.* (2014) in addition to other histopathological changes not observed in ours (Ghada *et al.*, 2014). Burneval *et al.*, (2002) also noted that severe hyperlipidaemia in Apo-E null mice caused injury to the renal glomeruli, characterized by mesangial matrix expansion while hypercellular glomeruli with mesangial proliferation, was reported by Al-Rejaie *et al.* (2012) in his experiment on gender difference in renal injury induced by high cholesterol diet (Bruneval *et al.*, 2002); Al-Rejaie *et al.* 2012). Mesangial cells are target cells of hyperlipidaemia and they have a role in inducing glomerular injury due to their major roles in the production of extracellular matrix. Mesangial cells also have the ability to bind lipoproteins through receptors expressed and this can in turn lead to lipid accumulation and subsequently dysfunctional glomeruli (Bruneval *et al.*, 2002). The honey supplemented groups also exhibited similar histological findings to that of the non-supplemented group suggesting therefore that tualang honey in this study did not prevent the renal

histopathological changes caused by HCD when administered concurrently.

Overall the non-sustainable renoprotective effects of tualang honey on the kidney against the HCD in term of the renal function and the absence of its protective effects against the renal histological changes is most probably attributed to the dose of tualang honey used in this study. Different doses of tualang honey have been used in different studies as in a study by Khalil *et al.* (2015) a dose of 3g/kg/day for 45 days was used to examine its cardioprotective effects (Khalil *et al.*, 2015). Erejuwa *et al.*, (2011) however used a lower dose compared to ours, where the dose used is 1g/kg body weight, once daily for 4 weeks to study its hepatoprotective effects (Erejuwa *et al.*, 2011).

CONCLUSION

Our study revealed that tualang honey exhibited some degree of renoprotective effect biochemically but not histologically in the rat models fed with high cholesterol diet. These findings warrant further investigations to look at the optimum dose of tualang honey that may exert maximum protective effects against dyslipidaemia and hence increasing the probability of conferring protective effects against high cholesterol diet acute and subacute kidney injuries.

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Conflict of Interests: There are no conflicts of interest.

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