

Original Article

Hepatoprotective effect of tualang honey supplementation in streptozotocin-induced diabetic rats

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Summary: Hepatic dysfunction such as elevations of transaminases and alkaline phosphatase is associated with diabetes. We investigated the hepatoprotective effect of Malaysian tualang honey in streptozotocin (STZ; 60 mg/kg; ip)-induced diabetic rats by measuring serum AST, ALT and ALP activities. Non-diabetic and diabetic rats were administered distilled water or tualang honey orally for four weeks. Serum AST, ALP and ALT activities were significantly ($p < 0.01$) elevated in diabetic control rats. Tualang honey significantly ($p < 0.01$ or $p < 0.05$) reduced AST, ALT and ALP activities in diabetic rats. These results suggest that tualang honey may produce hepatoprotective effect in diabetic rats.

Industrial relevance: Natural products are generally recognized for their enriched chemical and bioactive constituents which are the main contributors of their biological activities or therapeutic effects. This study provides evidence for hepatoprotective effect of tualang honey in diabetic rats. Considering the increasing prevalence of liver related disorders, most of which are secondary effects of exogenously ingested/administered substances, these results suggest that tualang honey may be effective in the treatment of hepatic damage or perhaps co-administered with other therapeutic agents to minimize their side effects.

Keywords: Streptozotocin; aspartate aminotransferase; alanine transaminase; alkaline phosphatase; tualang honey

INTRODUCTION

Hepatic abnormalities such as elevations of transaminases and alkaline phosphatase (ALP) are common in diabetes mellitus (Erbey *et al.*, 2000, Vozarova *et al.*, 2002, Meybodi *et al.*, 2008, Leeds *et al.*, 2009). The measurement of these enzymes and other biochemical markers such as albumin, total bilirubin in serum constitutes the liver function tests (Coates, 2011). These liver function tests are frequently utilized to diagnose or screen for hepatobiliary disease, examine the progression of a disease as well as to monitor or detect the hepatotoxicity that may arise from the use of drugs or substances (Elizabeth and Harris, 2005, Senior, 2009, Coates, 2011). Measurement of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reflects the concentrations of intracellular AST and ALT that have leaked into the general circulation and thus, serves as an indicator of hepatotoxicity (Elizabeth and Harris, 2005).

In our previous study, we have reported that Malaysian tualang honey exerts hypoglycemic and antioxidant effects in STZ-induced diabetic rats (Erejuwa *et al.*, 2010a, 2010c). We have also showed that oral hypoglycemic agents (metformin and/or glibenclamide) combined with tualang honey improves glycemic control (Erejuwa *et al.*, 2011) and are more effective in ameliorating oxidative stress in pancreas and kidney of diabetic rats than either drug alone (Erejuwa *et al.*, 2010b, 2011a, 2011b). The main aim of this study was to

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investigate the potential hepatoprotective effect of tualang honey in STZ-induced diabetic rats by measuring the activities of serum AST, ALT and ALP.

MATERIALS AND METHODS

Animals: Adult male Sprague-Dawley rats weighing 250 – 300g were obtained from the Laboratory Animal Research Unit of Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia. Prior to the experiment, the rats were acclimatized in the animal room at $25\pm 2^{\circ}\text{C}$ with 12 hour light/dark cycle for at least a week. All animals had free access to commercial pellet, except otherwise stated, and water *ad libitum*. Approval for the use of rats was obtained from the Animal Ethics Committee of Universiti Sains Malaysia [USM / Animal Ethics Approval / 2007 / (28) (095)]. All the experimental procedures were performed in accordance with the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes and in accordance with the Recommendations from Helsinki Declaration.

Induction of diabetes: Prior to induction of diabetes, the rats were fasted for at least 16 hours. Diabetes was induced in rats by intraperitoneal administration of STZ (60 mg/kg body weight) dissolved in 0.1M citrate buffer, pH 4.5. Another group of rats which served as control was injected with citrate buffer alone without STZ. 48 hours after STZ injection, diabetes was confirmed by measuring blood glucose concentrations (using an Accu-Chek Glucometer, Roche, Germany) in blood samples taken from tail vein. Rats with blood glucose levels of 12 mmol/L or more were considered to be diabetic and included in the study.

Preparation of tualang honey: Tualang honey (AgroMas®, Malaysia) was provided by Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia. The tualang honey was previously evaporated at 40°C to achieve 20% water content. Tualang honey has the following composition: total reducing sugar (67.5%) [fructose (29.6 %), glucose (30.0 %), maltose (7.9 %); fructose/glucose ratio (0.99)], sucrose (0.6 %) and water (20.0 %). It was freshly prepared by diluting in distilled water prior to administration to the rats.

Treatment: The animals were randomly divided into four groups. Each group comprised five to six rats. Distilled water or tualang honey was administered once daily by oral gavage for 4 weeks as follows:

- Group 1: Normal + Distilled water (0.5 ml)
- Group 2: Normal + Tualang honey (1.0 g/kg body weight)
- Group 3: Diabetic + Distilled water (0.5 ml)
- Group 4: Diabetic + Tualang honey (1.0 g/kg body weight)

After treatment for four weeks, overnight fasted rats were sacrificed by decapitation and trunk blood samples were collected in centrifuge tubes without anticoagulants and allowed to clot. The blood samples were centrifuged at $3,000 \times g$ for 20 minutes, serum was prepared and stored at -80°C until use.

Assay of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP): The activities of serum AST, ALT and ALP were assayed using kits (Randox Laboratories Antrim, UK). The assays were carried out in an autoanalyser.

Statistical analysis: Statistical analysis was carried out using SPSS 18.0.1. Data are expressed as median (range). Each group consisted of 5–6 rats. Groups were compared by Kruskal-Wallis test. Differences between two groups were identified by Mann-Whitney test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of tualang honey on blood glucose in normal and STZ-induced diabetic rats: Figure 1 shows the effect of tualang honey on blood glucose in normal and STZ-induced diabetic rats. The figure indicated that blood glucose concentrations in the diabetic control rats were significantly ($p < 0.01$) higher than those in the non-diabetic control rats. The results of the average weekly measurements of blood glucose revealed that administration of tualang honey to diabetic rats significantly ($p < 0.05$) reduced blood glucose levels by the end of first week. Compared with the glucose levels before treatment (week 0), this hypoglycemic effect of tualang honey was sustained till the end of the study (week 4). However, the data showed that the honey-treated diabetic rats compared with the diabetic control rats exhibited significantly reduced blood glucose at 4th week only (week 1, $p = 0.262$; week 2, $p = 0.229$; week 3, $p = 0.077$; week 4, $p = 0.011$). The blood glucose concentrations in normal rats treated with tualang honey were not significantly different from those in normal control rats.

Effect of tualang honey on the activities of serum AST, ALT and ALP in normal and STZ-induced diabetic rats: Table 1 shows the effect of tualang honey on the activities of AST, ALT and ALP in serum of normal and STZ-induced diabetic rats. The Kruskal-Wallis test showed that there was a significant ($p < 0.0001$ or $p < 0.001$) difference in the activities of AST, ALT and ALP among all the four groups. The activities of AST, ALT and ALP were significantly ($p < 0.01$) elevated in the diabetic control rats compared with normal control rats. Administration of tualang honey significantly ($p < 0.01$ or $p < 0.05$) reduced the elevated activities of AST, ALT and ALP in STZ-induced diabetic rats. The activities of AST, ALT and ALP in normal rats treated with tualang honey were not significantly different from those in normal control group.

Table 1. Effect of tualang honey on activities of AST, ALP and ALT in serum of STZ-induced diabetic rats

Group	AST (IU/L) Median (range)	ALT (IU/L) Median (range)	ALP (IU/L) Median (range)
Non-diabetic control	121.5 (106.0–137.0)	47.0 (42.0–57.0)	83.0 (47.0–130.0)
Non-diabetic + Tualang honey	131.0 (92.0–166.0)	41.0 (39.0–55.0)	78.0 (66.0–95.0)
Diabetic control	302.0 (221.0–616.0) *	153.0 (123.0–199.0) **	730.0 (563.0–1159.0)**
Diabetic + Tualang honey	170.0 (87.0–192.0) ††	100.0 (50.0–142.0) *,†	470.0 (224.0–598.0)**,†
	Kruskal-Wallis, $p < 0.001$	Kruskal-Wallis, $p < 0.0001$	Kruskal-Wallis, $p < 0.0001$

* $p < 0.05$, ** $p < 0.01$ versus non-diabetic control rats; † $p < 0.05$, †† $p < 0.01$ versus diabetic control rats

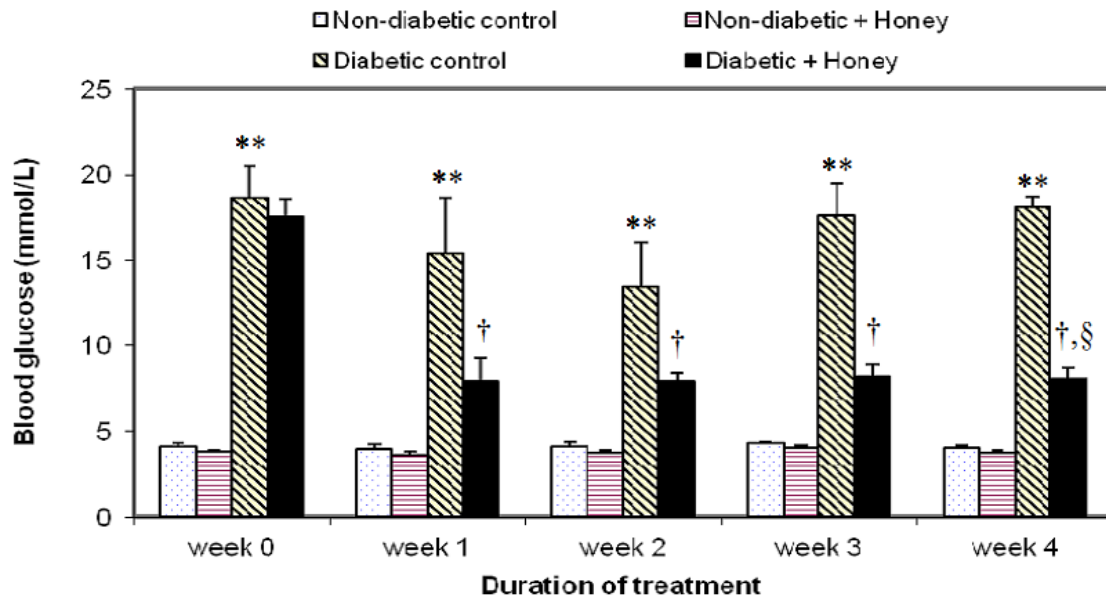


Figure 1. Effect of tualang honey on fasting blood glucose of STZ-induced diabetic rats

** $p < 0.01$ versus non-diabetic control at the same week; †† $p < 0.01$ versus the same group at week 0; § $p < 0.05$ versus diabetic control at week 4

DISCUSSION

The data indicated that tualang honey supplementation in diabetic rats resulted in reduced blood glucose concentrations. This hypoglycemic effect was statistically significant (compared with the diabetic control rats) at the end of the study. In contrast, no such hypoglycemic effect was observed in non-diabetic rats. These results are similar to what we found in our previous studies (Erejuwa et al., 2009, 2010a). The results showed that the activities of AST and ALT in serum of STZ-induced diabetic rats were markedly elevated. These enzymes are usually found in large quantities in the liver where they play an important role in the metabolism of amino acid (Whitehead et al., 1999). However, as a result of damage or toxicity to the liver, these enzymes may leak from the hepatocytes into the circulation where their levels become elevated (Whitehead et al., 1999, Elizabeth and Harris, 2005). Therefore, the elevated levels of AST and ALT in serum of STZ-induced diabetic rats suggest hepatocellular damage. Besides, the data showed an increased activity of ALP in serum of STZ-induced diabetic rats. ALP, predominantly found in the bile duct of the liver, is considered an indicator of biliary function, cholestasis and hepatic function (Whitehead et al., 1999, Elizabeth and Harris, 2005). The reduced levels of insulin in STZ-induced diabetic rats (Erejuwa et al., 2011c) might contribute to the elevated levels of these enzymes (O'Brien and Granner, 1991). These results corroborate those of other authors who also reported

increased AST, ALT and ALP in STZ-induced diabetic rats (Saeed et al., 2008, Zheng et al., 2010). Treatment with tualang honey considerably reduced the elevated levels of AST, ALT and ALP in serum of STZ-induced diabetic rats. This indicates that tualang honey exerts hepatoprotective effect in STZ-induced diabetic rats.

The actual mechanisms by which tualang honey reduced elevated serum levels of AST, ALT and ALP in STZ-induced diabetic rats remain unclear. Considering the evidence that implicates hyperglycemia or diabetes mellitus in hepatic damage or dysfunction (O'Brien and Granner, 1991, Vozarova et al., 2002), this hepatoprotective effect may be due to effect of tualang honey on hyperglycemia as reported in this study. In our previous study, we have shown that tualang honey reduces hyperglycemia in a dose-dependent response (Figure 1. Effect of tualang honey on fasting blood glucose of STZ-induced diabetic rats (Erejuwa et al., 2010a). Recently, we have reported that besides improving glycemic control, tualang honey also improves lipid abnormalities (Erejuwa *et al.*, 2011c). In view of the role of liver in glucose and lipid homeostasis, the hepatoprotective effect of tualang honey may contribute to amelioration of glucose and lipid disturbances reported in STZ-induced diabetic rats (Erejuwa *et al.*, 2011c). This view is further corroborated by findings which showed that an elevated activity of ALT correlated with poor glycemic control and elevated triglycerides in type 1 diabetes, an equivalent of STZ-induced diabetes in rodents (Leeds et al., 2009).

In our previous studies, we have shown that tualang honey ameliorates oxidative stress in pancreas (Erejuwa et al., 2010b, 2010c, 2011b) and kidney (Erejuwa et al., 2009, 2010a, 2011a). In view of recent evidence which showed that pine honey ameliorated hepatic oxidative stress and improved hepatic function (AST, ALT and ALP) in trichlorfon-induced biochemical alterations in mice (Eraslan et al., 2010), tualang honey may elicit a hepatoprotective effect via amelioration of hepatic oxidative stress. In addition, a study by Al-Waili showed that intravenous administration of honey produced a hepatoprotective effect against carbon tetrachloride-induced liver damage in sheep (Al-Waili, 2003). Similarly, amelioration of hepatic function (AST, ALT and ALP) was also reported after honey feeding in rats that underwent total food restriction (Al-Waili et al., 2006a, 2006b).

CONCLUSIONS

The findings in this study indicate that tualang honey supplementation in STZ-induced diabetic rats reduces elevated levels of AST, ALT and ALP. These data, thus, suggest that besides its antidiabetic and antioxidant effects, tualang honey also produces hepatoprotective effect in STZ-induced diabetic rats. In addition, it may be suggested that co-administration of tualang honey with other therapeutic agents may be effective in minimizing the side effects of synthetic drugs.

ACKNOWLEDGEMENTS

This work was funded by a grant (1001/PPSP/81202020) from Universiti Sains Malaysia (USM). The first author acknowledges USM Fellowship. We also express our appreciation to Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia for supplying tualang honey.

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