Research Article

Protective effect of aqueous extracts from *Canarium odontophyllum* Miq. leaf on liver in streptozotocin-induced diabetic ratsSiti Balkis Budin¹, Shashi Kumar¹, Nor Malia Abd Warif¹, Shafikha Mohd Saari¹, Dayang Fredalina Basri^{1*}¹ School of Diagnostic & Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia<https://doi.org/10.28916/lsmb.2.1.2018.5>

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Abstract

The fruit of *Canarium odontophyllum* Miq. is a traditional delicacy in Borneo for its anti-aging benefit. This study evaluated the protective effect of *C. odontophyllum* leaf aqueous extract on damaged liver in streptozotocin-induced diabetic rats. A total of 30 male Sprague-Dawley rats (150-250g) were randomly divided into three groups: control group, diabetic without treatment and diabetic treated with 300 mg/kg aqueous extract of *C. odontophyllum* for 28 consecutive days. The diabetic condition was induced by intraperitoneal injection of streptozotocin at 65 mg/kg body weight. At the end of study period, blood was collected to assess the biochemical changes and the oxidative stress markers whereas the liver section was examined for morphological changes. Result showed that the level of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) in diabetic rats treated with *C. odontophyllum* were significantly reduced ($p < 0.05$) compared to untreated diabetic group. Results showed an improvement in oxidative stress markers as presented by lower level of malondialdehyde (MDA) and protein carbonyl as well as higher level of reduced glutathione (GSH) and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in *C. odontophyllum* treated group compared to untreated diabetic group. Histologically, there were no obvious morphological changes in both diabetic groups. These findings demonstrated that the aqueous extract of *C. odontophyllum* has the potential to reduce oxidative stress in liver of streptozotocin-induced diabetic rat.

Keywords: *Canarium odontophyllum* Miq., antidiabetic, medicinal plant, liver damage, protective effect

1.0 Introduction

According to World Health Organization report, diabetes mellitus is a chronic disease caused by failure of pancreas to produce sufficient insulin, or when the body is unable to use the produced insulin effectively (WHO 1999). Under normal circumstances, blood glucose levels are regulated by insulin. Diabetes is widespread throughout the world and the prevalence of diabetes worldwide is expected to be 2.8% in 2000 and expected to increase to 4.4 % in 2030 (Wild et al. (2004).

In diabetic condition, either the increase of prooxidant or the decrease of antioxidant status may occur, thus leading to oxidative stress (Forbes et al., 2003). The increased of oxidative stress in diabetes is multifactorial, whereby the autooxidation of glucose plays an important role in the formation of reactive oxygen species (ROS). ROS may attack the tissues and organs in the body that will give rise to various clinical abnormalities (Bagri et al. 2009). Antioxidant promotes the elimination of free radicals and disturbances in its activity may further cause oxidative stress (Rahimi et al., 2005). Oxidative stress condition in diabetic patients play a role in pathogenesis of long-term complications, such as neuropathy, nephropathy and microangiopathy which lead to higher risk of morbidity and mortality (Kuyvenhoven & Meinders 1999; Lipinski 2001; Rains and Jain 2011).

Canarium odontophyllum Miq. is classified under the Burseraceae family and can be found in the tropical rain forest of Sarawak, Malaysia often being consumed as nutritious ingredient by the local community (Chew et al., 2012). Previous study done on other *Canarium* species has revealed that the leaves of *C. schweinfurthii* Engl. has high antioxidant activity (Ngbede et al., 2008). According to Chew et al. (2012), phytochemical analysis of the leaves of *C. patentinervium* Miq. showed the presence of tannin and flavonoid which act as antimicrobial and free radical scavenger. Tannin extracted from the leaves, twigs and stem bark of *C. album* showed 1,1-depheny-2-picrylhydrazyl (DPPH) activity (Zhang & Lin, 2008). *C. subulatum*, *C. zeylanicum*, *C. boivinii* and *C. manii* contained the chemical components such as terpenoids, tannins and bioflavonoids (Mogana et al., 2011). Biological studies shown that certain *Canarium* species have hepatoprotective, antidiabetic (Mokiran et al. 2014) and antioxidant properties (Anand et al., 1992). Azlan et al. (2010) reported that the fruit of *C. odontophyllum* especially its outer skin contains high level of antioxidant compounds such as phenolic, flavonoid and anthocyanin.

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To date, no studies have been conducted on the *C. odontophyllum* leaves as an alternative treatment to reduce the complications associated with diabetes mellitus. The objective of this study was to identify the potential of *C. odontophyllum* to be used in the prevention against liver damage in streptozotocin-induced diabetic rat.

2.0 Material and methods

2.1 Plant materials

Fresh *C. odontophyllum* leaves were collected from Kuching, Sarawak, Malaysia. The leaves were deposited in the Herbarium University Kebangsaan Malaysia in Bangi, Malaysia with voucher specimen number UKMB40052.

2.2 Preparation of aqueous extract from *C. odontophyllum* leaves

The *C. odontophyllum* leaves were washed and dried in an oven at 50°C until constant weight was obtained. The dried leaves were grounded into fine powder using an electric grinder. About 100 g of powdered leaves were soaked in 500 ml sterile distilled water at a ratio of 1:5 (w/v) at room temperature and shaken on an orbital shaker at 100 rpm overnight. The mixture was centrifuged at 3000 rpm for 5 min and the supernatant was collected. The supernatant was then filtered through Whatman No. 1 filter paper and freeze-dried under vacuum at -50°C to produce a fine crystal-like crude aqueous extract. The powdered extract was kept in an air-tight container and stored at 4°C until further use.

2.3 Experimental animals

Thirty male Sprague-Dawley rats weighing between 200 to 250 g obtained from the Animal Unit, Universiti Kebangsaan Malaysia were used for this study. They were kept in polycarbonate cages and allowed to acclimatize for 7 days before experiment started and maintained under laboratory conditions of temperature, humidity and light. Standard rat pellet and tap water were provided *ad libitum* throughout the study period. The rats were randomly divided into three groups and each group comprised 10 rats: normal (N), diabetic control (STZ group) and *C. odontophyllum* -treated diabetic group (STZ+CO group).

The rats were fasted overnight prior to diabetes induction. Diabetes was induced by injection of a single intraperitoneal at the dose of 65 mg/kg body weight (Saari et al. 2017). Three days after STZ injection, fasting blood glucose were determined using a glucometer and rats with fasting blood glucose level above 15 mmol/L were considered for this study (Budin et al., 2013). (At the end of the study period, the diabetic was confirmed again by analyzing blood glucose level using hexokinase assay).

The extract was administered orally by force feeding daily at the dose of 300 mg/kg for 28 days. The choice of 300mg/kg as the dose of the extract was based on Kamtchouing et al. (2006) which reported that the dose (from the stem bark extract of *Canarium schweinfurthii* Engl) showed anti-diabetic activity. At the end of the experimental period, the rats were fasted overnight, anesthetized using chloroform and blood was drawn by cardiac puncture. The liver was immediately excised and washed with 0.9% cold normal saline before kept frozen at -80°C until used.

All experiments were performed in accordance with the procedures approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC), (Approval No. FSK/BIOMED/2013/MALIA/13-NOV/554-NOV-2013-AUG-2015).

2.4 Preparation of liver homogenate

Liver sample (1g) was homogenized using ultra-Turrax homogenizer in 10 ml of 1.15% KCl and centrifuged at 13 000 rpm for 15 min at 4°C and the same process was repeated twice. The supernatant was then removed and stored in an eppendorf tube and kept at -40 °C. The supernatant was used for oxidative stress marker evaluation and the whole process was conducted in cold condition.

2.5 Biochemical assays

The blood was centrifuged at 4000 rpm for 5 min at 4°C to obtained the serum. Serum liver biomarkers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and gamma glutamyltransferase (GGT)) were measured using automated chemical analyzer (Beckam Coulter AU400).

2.6 Measurement of antioxidant activity

Malondialdehyde (MDA) level was measured with Ledwozyw et al. (1986) method as modified by Gwarzo et al. (2014). Protein carbonyl was measured based on its reaction with 2,4-dinitrophenylhydrazin (DNPH) as demonstrated by Levine et al. (1990). Superoxide dismutase (SOD) activity was determined in liver homogenate according to the method of Beyer and Fridovich (1987). Reduced glutathione (GSH) was measured with the method according to Ellman (1959) as described by Giustarini et al. (2014). Glutathione peroxidase (GPx) activity in liver homogenate was determined according to Flohe & Gunzler (1984).

2.7 Histological examination

The liver tissues were immediately washed in normal saline and fixed in 10% formalin solution. The sections were analyzed via haematoxylin and eosin staining (H&E staining) and examined under a light microscope. Histopathological changes were studied by evaluating the tissue slides.

2.8 Statistical analysis

The Statistical Packager for the Social Science 21 (SPSS 21), was used for statistical analysis. All variables were checked for normality and homogeneity of variance. The data obtained were tested using one-way ANOVA followed by Tukey's post hoc multiple comparison test. The value of $p < 0.05$ was considered statistically significant. All data were expressed as mean $\pm$ SEM.

3.0 Results

3.1 Effect of aqueous extract from *C. odontophyllum* on body weight

As shown in Table 1, the body weight of both STZ group and STZ+CO group showed a significant drop compared to the normal (N) group. The *C. odontophyllum* extract at 300mg/kg did not improve body weight loss in diabetic rat.

3.2 Effect of *C. odontophyllum* extract on the liver injury biomarkers

The effect of aqueous extract of *C. odontophyllum* on the liver injury biomarkers in diabetic rats are shown in Table 2. The level of ALT, AST, ALP, total bilirubin and GGT in serum of STZ group were found to be significantly increased compared to N group ($p < 0.05$). It was observed that the *C. odontophyllum* extract was capable to reduce all liver injury biomarkers in STZ + CO group.

Table 1 The body weight of all experimental group.

Group	Body weight (g)
N	341.08 $\pm$ 25.4
STZ	232.65 $\pm$ 10.51 ^a
STZ + <i>C. odontophyllum</i> 300mg/kg	214.17 $\pm$ 16.7 ^a

Value expressed as mean $\pm$ SEM.

^a significant difference compared with N group

Table 2 The effect *Canarium odontophyllum* leaves at 300mg/kg on liver injury biomarkers

Parameters	Groups		
	N	STZ	STZ + <i>C. odontophyllum</i> 300mg/kg
ALT (U/L)	61 $\pm$ 1.8	380 $\pm$ 258 ^a	319 $\pm$ 81
AST (U/L)	108 $\pm$ 21	397 $\pm$ 268 ^a	325 $\pm$ 81
ALP (U/L)	204 $\pm$ 47	1559 $\pm$ 502 ^a	1287 $\pm$ 452
TBIL (mmol/L)	2.35 $\pm$ 0.25	4.11 $\pm$ 1.24 ^a	3.6 $\pm$ 1.03
GGT (U/L)	1.18 $\pm$ 0.15	4.05 $\pm$ 0.65 ^a	3.15 $\pm$ 1.33

Value expressed as mean $\pm$ SEM.

^a significant difference compared with N group

3.3 Effect of *C. odontophyllum* extract on oxidative stress markers

The level of MDA, protein carbonyl and GSH as well as GPx and SOD activity in the liver homogenate are shown in Table 3. In STZ group, the SOD, GPx and GSH status were significantly reduced, while the protein carbonyl and MDA level were significantly higher when compared with N group ($p < 0.05$). The aqueous extract of *C. odontophyllum* significantly decreased the protein carbonyl level of the diabetic rat. On the other hand, the extract improved the antioxidant status in STZ+CO group compared to untreated diabetic group. However, the improvement in oxidative stress biomarkers were not significant.

Table 3 The effect of *Canarium odontophyllum* leaves at 300mg/kg on oxidative stress markers of the liver

Parameters	Groups		
	N	STZ	STZ + <i>C. odontophyllum</i> 300mg/kg
MDA (nmol/mg protein)	3.17 $\pm$ 0.571	4.68 $\pm$ 0.90 ^a	3.96 $\pm$ 0.485
Protein carbonyl (mg/ml)	0.066 $\pm$ 0.012	0.173 $\pm$ 0.029 ^a	0.112 $\pm$ 0.025 ^b
GSH (mM/mg protein)	0.011 $\pm$ 0.011	0.008 $\pm$ 0.002 ^a	0.009 $\pm$ 0.001
SOD (U/min/mg protein)	0.69 $\pm$ 0.05	0.345 $\pm$ 0.18 ^a	0.415 $\pm$ 0.116
GPx (nmol/min/mg protein)	0.025 $\pm$ 0.005	0.019 $\pm$ 0.005 ^a	0.021 $\pm$ 0.003

Value expressed as mean $\pm$ SEM.

^a significant difference compared with N group and ^b significant difference compared with STZ group

3.4 Effect of *C. odontophyllum* extract on morphological changes of liver

Histopathological photographs from Figure 1 showed that there were no obvious morphological changes observed under microscopic observation in both the STZ and STZ + CO group. The hepatocytes were well organized in plates and no necrosis and inflammatory cells were noted.

Figure 1 Microscopic photographs of the liver tissue in (A) N group (B) STZ group and (C) STZ + CO. Note that there were no obvious morphological changes in both diabetic groups (Magnification X40)

4.0 Discussion

There was a significant decreased in the body weight of diabetic rats. In insulin deficiency, peripheral cells are unable to use glucose as energy source. Body weight progressively reduced due to energy source being rerouted from glucose metabolism to fat and muscle from the process of lipolysis and proteolysis respectively. The diabetic rats treated with *C. odontophyllum* showed no signs of weight elevation after 28 days of treatment which was in accordance with Mokiran et al., (2014).

This study found that there was an increased in the level of all liver injury biomarkers in STZ group indicate the occurrence of liver injury in diabetic rats. These findings are consistent with He et al. (2009) which showed an increased in liver enzyme level in diabetic rats. Diabetes also caused the fat accumulation in the liver and acts as substrates for lipid peroxidation (Gutteridge, 1995) which causes damage to the membrane and initiates liver injury. There was an improvement in liver biomarkers following treatment with *C. odontophyllum* indicating the potential of extract in minimizing the liver damage in diabetic condition. Previous study showed that the usage of aqueous extract from the leaves of *Moringa oleifera* which has high antioxidant properties was found to offer protection for liver in diabetic condition (Jaiswal et al. 2013).

Diabetes cause excess in generation and decrease in destruction of ROS which subsequently increase MDA and protein carbonyl level (Yoshikawa and Naito, 2002) and altered the antioxidant status (Aragno et al., 1999). According to Cai et al., (2017) the antioxidant activity will be decreased after the exposure of cell to high glucose level. *C. The reduction in antioxidant status diabetic rats is in line with Abolfathi et al., (2012) hence, suggesting that the induction of STZ can cause oxidative stress in the liver.*

C. odontophyllum treatment was able to reduce MDA and protein carbonyl besides improving antioxidant defense system in diabetic rat. It shows that aqueous *C. odontophyllum* leaf extract has the capacity to reduce oxidative stress in diabetic condition and demonstrate its potential as a protective agent from free radical damage. The increase in antioxidant enzymes in the group treated with *C. odontophyllum* may be due to rich phenolic content in the leaves of *C. odontophyllum* (Chew et al., 2012). Furthermore, Mokiran et al. (2014) also supported that *Canarium* species has the hepatoprotective and antidiabetic effects. *Canarium* species has high antioxidant properties and rich with antioxidant compound such as phenolic, flavonoid and anthocyanin (Anand et al., 1992; Azlan et al., 2010). The result of this study corresponded with Prabakaran & Ashokkumar (2013) which observed an increased antioxidant activity in diabetic rats treated with esculetin which is a coumarin derivative with high antioxidant activity (Witaicenis et al. 2013).

Based on the histological observation of liver tissues in this study, there were no obvious morphological alteration in both diabetic groups which indicated that the increase in liver injury oxidative stress biomarkers did not correlate with the histopathological findings. The liver injury that occurs in the present study was not severe enough to cause significant morphological changes from the histopathological observation.

Histological observation was performed on the liver sections to examine any abnormality in the tissue. In this study, no abnormal cells are seen in all experimental groups. This result was not consistent with Abolfathi et al., (2012) where the group which was exposed to STZ for 32 days can lead to the formation of hepatocytes with various sizes and with the presence of inflammatory cells and fat globule.

5.0 Conclusion

The findings from the present study provide evidence that the leaves from *C. odontophyllum* leaves can be utilized as an alternative treatment to reduce the complications associated with diabetes mellitus specifically in the prevention of liver damage on streptozotocin-induced diabetic rat by providing protection against oxidative stress with significant antioxidant effect.

6.0 Acknowledgements

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7.0 References

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