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Research Article

HYPOGLYCEMIC EFFECT AND IN VITRO ANTIOXIDANT ACTIVITY OF THE DICHLOROMETHANE FRACTION FROM THE LEAVES OF FICUS ODORATA (BLANCO) MERR. (MORACEAE)

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ABSTRACT

This study aimed to evaluate the hypoglycemic and antioxidant activity of the leaves of Ficus odorata. The methanolic crude extract was fractionated by liquid-liquid partitioning using organic solvents with increasing polarity. Four fractions were obtained such as, hexane, dichloromethane (DCM), ethyl acetate and water. Of the four fractions tested, DCM fraction yielded the highest amount of flavonoid content at 335.63 mg QE/g sample. TLC and HPLC analyses of DCM fraction revealed that quercetin may be present in the fraction. In acute oral toxicity test, zero mortality rate was observed after 14 days of treatment. Gross necropsies of selected rat organs were normal. Histopathological analyses of liver and kidneys were unremarkable. In hypoglycemic study, induction of diabetes was successfully completed in 28 days using single dose of streptozotocin (30 mg/kg body weight) and high fat diet (Srinivasan et al., 2005). Daily oral administration of DCM fraction showed that at a dose of 50 mg/kg ($p<0.001$) and 200 mg/Kg ($p=0.002$) produced significant decrease in their mean blood sugar after the 14-day treatment. The same effect was observed with the positive control at 10 mg/kg of glyburide ($p<0.001$). On the other hand, the results in the in vitro antioxidant activity showed that DCM fraction exhibited scavenging activity against hydroxyl radical and hydrogen peroxide with an IC50 value of 0.275 mg/mL and 4.954 mg/mL, respectively. The results indicate that DCM fraction from the leaves of Ficus odorata has potential hypoglycemic and antioxidant properties. It is non-toxic up to 2000 mg/Kg.

Keywords: Ficus odorata, diabetes mellitus, antioxidant, quercetin, streptozotocin.

INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing all over the world[1]. According to the World Health Organization (2012), there were approximately 347 million people globally who suffer from this disease and it is predicted to become the seventh leading cause of death in 2030[2]. In the same year, it was projected that the Philippines will rank as the 9th country with the highest number of people with diabetes reaching up to 7.8 million people[1]. Among these cases worldwide, 90 percent comprises type 2 diabetes[2]. Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both[3]. It is classified on the basis of the pathogenic process that leads to hyperglycemia. The most common type of diabetes is type 2 diabetes which is a heterogeneous group of disorders

characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production[4]. Chronic hyperglycemia may induce oxidative stess that may lead to long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels[5]. Oxidative stress, a serious imbalance between production of reactive species and antioxidant defence[6], plays a major role in the progression of β-cell dysfunction found in diabetes[7]. This has impelled researchers to investigate the use of antioxidants as a complementary therapy for diabetes.

Antioxidant activity has been known as a significant property of plant medicines associated with diabetes[8]. Plants from the genus Ficus are known to have hypoglycemic and antioxidant properties. These have been attributed to the presence of phenolic compounds such as flavonoids [9,10]. In the Philippines, *Ficus odorata* locally known as "pakiling" is used in the treatment of diabetes[11]; however, there are no scientific data regarding such use.

In this research, the leaves of *Ficus odorata* were evaluated for their hypoglycemic and *in vitro* antioxidant activity in relation to the presence of flavonoids, specifically, quercetin. The result of this research contributes to the body of knowledge on the use of *Ficus odorata* as a hypoglycemic agent and be a good starting point in the development of new and effective therapy in the management of diabetes and its complications.

Material and Methods

2.1. Standards, Reagents and Chemicals

The reagents were purchased from Belman laboratories and Office of the Laboratory Equipment and Supply (OLES) of the University of Santo Tomas. The ascorbic acid was purchased from Sigma Chemicals. Rutin and quercetin were purchased from Chemline Scientific Corporation. Streptozotocin and glyburide were procured from Merck Biosciences, Philippines.

2.2. Plant Preparation and Extraction

Fresh mature leaves of *Ficus odorata* were collected from Barangay San Ramon, Buhi, Camarines Sur. It was authenticated at the Botany Division, National Museum. The leaves were air-dried for two weeks and ground into powder using Wiley mill.

A total of 1500 grams of ground *Ficus odorata* leaves were utilized in the study. It was soaked in 70 % methanol (MeOH) with a ratio of 1:10 (w/v) for 24 hours[12]. Extraction was repeated for four times and methanolic crude extract was filtered and collected. The residue was discarded and filtrate was placed in a rotary evaporator (Büchi Rotavapor R-200) until syrupy consistency was obtained. Then it was evaporated to dryness in a water bath maintained at 40°C. The crude extract was then suspended in water and was subjected to fractionation using hexane, dichloromethane (DCM), and ethyl acetate as solvents with increasing polarity as previously described[13]. A ratio of 1:1 (v/v) was used in the liquid-liquid partition. The fractions obtained were hexane, DCM, ethyl acetate, and water.

2.3. Quantitative Test for Flavonoids

Confirmatory test for flavonoids was performed using Wilstatter "cyanidin" test, which detects compounds having Ɣ-benzopyrone nucleus generally present in flavonoids[14].

A pinch amount of the extract was defatted with 9 mL hexane and water (2:1). Hexane layer was discarded and the defatted aqueous layer was subsequently combined with 10 mL of 80 percent ethyl alcohol. The mixture was filtered and the filtrate was divided into two test tubes. One portion served as the control. In the other test tube, the mixture was treated with 0.5 mL conc. 12M HCl. Three to four pieces of magnesium turnings were added. Color changes within 10 minutes were recorded and compared with the control tube. The mixtures with definite coloration were diluted with an equal volume of water and 1 mL octyl alcohol. The mixture was shaken and allowed to stand. The color in each layer was noted. Colors ranging from orange to red, to crimson and magenta and occasionally to green or blue give positive result for flavonoids.

2.4. Determination of Total Flavonoid Content

The total flavonoid content (TFC) of hexane, DCM, ethyl acetate and water fractions were estimated using the method described[15] with Quercetin (Q) as the standard. To 0.5 mL of each fraction, 0.5 mL of 2 percent $AlCl_3$ ethanol solution was added. The mixture was incubated at 30°C for one hour. The absorbance was read at 420 nm. The results were expressed as mg quercetin equivalent (QE) / g of sample.

The standard curve was prepared using quercetin solution of different concentrations and TFC was computed using the equation below which was taken from the graph of the standard curve for total flavonoid content.

2.5. Thin Layer Chromatography

The constituents of the DCM fraction from *F. odorata* leaves were separated using pre-coated plate (3 cm x 5 cm) of chromatographic silica gel. One milligram per milliliter of DCM fraction was used as a sample. The plate was marked with a pencil at a distance of 1 cm both from top and bottom of the plate. The plate was first activated by pre-drying at 100 °C in an oven. With the use of hematocrit tubes, solutions of DCM fraction, quercetin and rutin were applied on the marked line on the chromatoplate. The size of the spot of application was limited to 4 mm in diameter[14]. The spot was allowed to dry before the chromatoplate was placed inside the developing chamber. The developing solvent was prepared by mixing 5:1 of chloroform and methanol respectively. About 12 mL of the prepared solution was placed in a closed chamber. Saturation of the atmosphere inside the chamber with the developing solvent was achieved by storing the chamber at room temperature for 15 minutes. When the solvent reached the top line, the plate was removed from the chamber and allowed to dry. The developed chromatograms were observed visually and with the aid of a spray reagent, potassium ferricyanide and $FeCl_3$.

2.6. High Performance Liquid Chromatography

The DCM fraction was analyzed at the Chemistry Department of De La Salle University using Reverse Phase HPLC system (1200 series; Agilent Technologies) with Supelcosil-plc 18 column, 25cm × 4.6 mm, 12 µm. The mobile phase was a mixture of 0.5 percent phosphoric acid and methanol with a ratio of 50:50 (v/v) at a flow rate of 0.9 mL/minute. Aliquots of 25 µL of each sample were injected into the column and eluate was monitored at 285 nm. All operations were carried out at ambient temperature. Quercetin and rutin were used as standards.

Linear regression analysis was used to determine the quantitative estimation of quercetin present in the DCM fraction. Different concentrations of quercetin in parts per million was used to make the standard curve quercetin.

2.7. In vitro Antioxidant Activity

Hydrogen peroxide scavenging activity

The H_2O_2 scavenging ability of DCM fraction was determined according to the method described[16]. Different concentrations at 1, 2.5, 5, 7.5 and 10 mg/mL of DCM fraction dissolved in ethanol and standard ascorbic acid dissolved in distilled water were added to 0.6 mL solution of 40 mM H_2O_2 in phosphate buffer with a pH of 7.4. After 10 minutes, absorbance of H_2O_2 was recorded at 230 nm against blank solution without H_2O_2.

Hydroxyl radical scavenging activity

Hydroxyl radical (•OH) scavenging activity of DCM fraction was determined[17]. Ferric-ascorbate–EDTA–H_2O_2 (Fenton reaction) generates •OH, which react with deoxyribose to produce thiobarbituric acid reactive substances (TBARS) and formation of pink chromogen with the aid of heat. The hydroxyl quenchers reduced TBARS production and formation of pink chromogen by competing with deoxyribose for •OH. The Fenton reaction mixture which contains 3 mM deoxyribose, 0.1 mM ferric chloride, 0.1 mM EDTA, and 0.1 mM ascorbic acid and 2 mM H_2O_2 in 20 mM phosphate buffer pH 7.4 were added to various concentrations at 0.063, 0.125, 0.25, 0.5, 1.0 mg/mL of DCM fraction and ascorbic acid. The reaction mixture was incubated for 30 minutes at 37°C. One (1) mL of 5% trichloroacetic acid (TCA) and 1 mL of 1% thiobarbituric acid (TBA) were added to yield a final volume of 3 mL. The reaction mixture was kept in boiling water bath for 30 minutes and cooled. The absorbance was measured at 532 nm against an appropriate blank solution.

2.8. Acute Oral Toxicity Test and Hypoglycemic Study

A total of 35 healthy female Sprague-Dawley rats, 6-8 weeks old weighing 100-200 grams was procured at the Food and Drug Administration, Philippines. They were housed at the Research Center for the Natural and Applied Sciences (RCNAS) Animal Facility. They were acclimatized for seven days and fed with normal pellet diet or as specified in the treatment and water *ad libitum*. The protocol for proper handling and care of animals in the study was approved by the Institutional Animal Care and Use Committee of the University of Santo Tomas.

The acute oral toxicity was based on the main test of Organization for Economic Cooperation and Development (OECD) test for chemicals guideline no. 425[18]. Five healthy

female Sprague-Dawley rats were used in the study. The rats were nulliparous and non-pregnant. They were fed with normal pellet diet and water *ad libitum*. They were kept in an individual cage in the RCNS animal house facility. The animals were randomly selected, and labeled properly with identification marks.

Prior to the administration of the DCM fraction, the rats were fasted overnight and the fasted weight of each animal were measured. The dose was calculated according to the body weight. Initial dose of 175 mg/kg was employed in compliance to the default progression factor of the main test. Animals were dosed one at a time in sequence at 48 hours interval. The extract was administered in a single dose by oral gavage. The first rat survived, hence, the succeeding animal received a higher dose. The dosing continued on a 48 hour interval with doses of 175, 550 and 2000 mg/kg bodyweight. The testing stopped since three consecutive animals survived at the upper bound of 2000 mg/kg bodyweight. Animals were observed individually, once on the first 30 minutes after dosing, then during the first 24 hours and daily thereafter for 14 days. At the end of the test, surviving animals were weighed and humanely killed by the use of carbon dioxide chamber. Gross necropsy and histopathological examination of the liver and kidneys were performed.

Induction of diabetes using combination of low dose streptozotocin and high fat diet (HFD)

The protocol was based on the methods described[19]. Thirty female Sprague-Dawley rats, 6-8 weeks old, weighing 100-150g were used in the study. They were acclimatized for seven days at the Thomas Aquinas Research Complex animal house facility. Induction of diabetes by administration of streptozotocin 30 mg/kg bodyweight intraperitoneally (IP) and high fat diet was conducted for 28 days. Blood collection was done through tail tipping to get the initial fasting blood sugar. Initial weight was also noted. They were grouped randomly into five groups consisting of six rats each. Group 1 serves as negative control group and received 0.25mL/kg sodium Citrate buffer IP and normal pellet diet (NPD). Groups 2-5 received single administration of 30 mg/kg streptozotocin IP and high fat diet (HFD). HFD was prepared by mixing powdered normal pellet diet (365 g), lard (310 g), casein (250 g), cholesterol (10 g), vitamin and mineral mix (60 g), dL-methionine (3 g), yeast powder (1 g), and sodium chloride (1 g), expressed in g/kg body weight[20].

After one week, fasting blood sugar (FBS) was measured. The rats with fasting blood sugar of <140mg/dL received another dose of streptozotocin. Every week, FBS was measured. After 28 days of STZ injection and high fat diet, the rats with FBS of ≥140 mg/dL were considered diabetic.

Experimental Treatment

After induction of diabetes, there were 5 treatment groups assigned:

Group 1	Non-diabetic (negative control) Treated orally with 5% Tween 80
Group 2	Diabetic control Treated orally with 5% Tween 80
Group 3	Low Dose DCM fraction Treated orally with 50 mg/kg DCM fraction
Group 4	High dose DCM fraction Treated orally with 200 mg/kg DCM fraction
Group 5	Glyburide (positive control) Treated orally with 10 mg/kg Glyburide

Daily treatment was done for 14 days. Weekly FBS and weight were noted. At the end of the treatment, all rats were sacrificed through cervical dislocation, liver and pancreas were harvested and submitted for histopathological analysis at the Department of Anatomic Pathology, UST Hospital.

Fasting blood sugar determination by glucose oxidase method

All FBS was measured by United Diagnostics Laboratory using Prestige 24i automated analyzer through glucose-oxidase method. The collected blood was centrifuged at 5000 rpm for 15 minutes to separate the plasma from the blood. Plasma is the specimen of choice for glucose estimation.Glucose present in the plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured at 515 nm[21].

Histopathological analyses

Liver and pancreas samples for histopathological examinations were sent to the Philippine Kidney Dialysis Foundation where the samples were fixed in 10% buffered formalin for 48 hours and dehydrated in ascending grades

of alcohol and then embedded in a paraffin wax. For the analysis, approximately seven micrometer thick section of the specimen was stained with hematoxylin and eosin for the observation of the general morphology[22]. Afterwards, each slide was read and evaluated by an anatomical pathologist at the UST Hospital-Pathology Department.

2.9. Data Analyses and Interpretation

Statistical analyses were done using SPSS software version 17.0. Mean and standard error (SEM) were used to summarize all the data. Single factor – Analysis of Variance (ANOVA) was used in the total flavonoid content to determine if there is a significant difference between the mean QE/g samples of each fraction. Also, it was used to compare the mean fasting blood sugar and weight at day 28. Repeated Measures Analysis of Variance (RMANOVA) was used to determine if there is a significant change in the mean fasting blood sugar and weight of each group before and after treatment. Kruskal-Wallis Analysis of Variance a non-parametric method was used to analyze the results of the histopathological analyses of the pancreas. All p-values less than 0.05 indicate significant difference. In the *in vitro* antioxidant activity, IC_{50} values were estimated using the four parameter logistic (4PL) non-linear regression model.

RESULTS

3.1. Plant material preparation

DCM fraction produced dark green semi-solid extract. The extraction procedure used 1.5kg of powdered *Ficus odorata* leaves. Percolation obtained 316.2 grams of crude methanolic extract and subsequent fractionation yielded 10.04 grams of DCM fraction. The computed percentage yield for methanolic crude extract of *Ficus odorata* leaves was found to be 21.08% and for DCM fraction, it was found to be 3.17%.

3.2. Test for Flavonoids and Total Flavonoid Content

The crude methanolic extract of *Ficus odorata* leaves was tested for the presence of flavonoids using Wilstatter Cyanidin Test. Results produced dark orange colored solution, indicating the presence of Y-benzopyrone nucleus generally found in Flavonoids.

The crude methanolic extract was fractionated by liquid-liquid partition using organic solvents with increasing polarity. Four fractions were obtained namely the hexane, DCM, ethyl acetate and water fraction. Each fraction was evaluated for quantitative estimation of total flavonoid content using quercetin as the standard.

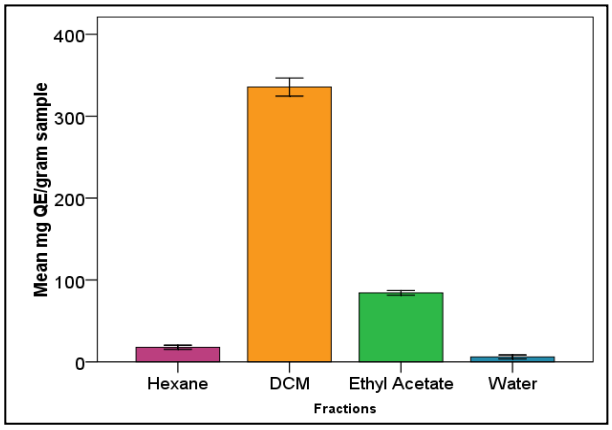

Figure 1. Total flavonoid content of each fraction in mg quercetin equivalent (QE)/ g sample

DCM fraction yielded the highest amount of flavonoid content with 335.63 mg QE/g sample, followed by ethyl acetate with 84.27 mg QE/g sample, hexane with 17.20 mg QE/g sample and water with 6.00 mg QE/g sample (see Figure 1). Results showed significant difference in the mean mg QE/gram sample of the four fractions with a p value of <0.001.

3.3. Identification and Detection of the Bioactive Compound

There were eight spots separated using chloroform and methanol (5:1) solvent system (see Figure 2). One spot corresponded to quercetin with the same Rf value of 0.38 ± 0.02. Addition of a spray reagent potassium ferricyanide and ferric chloride produced blue spots in the presence of flavonoids.

In the HPLC chromatogram shown in Figure 16, DCM fraction produced 4 peaks; further dilution of the fraction revealed 2 peaks. One peak corresponded to quercetin at 3.532/min. This indicates that quercetin may be present in the DCM fraction of *Ficus odorata* leaves (see Figure 3).

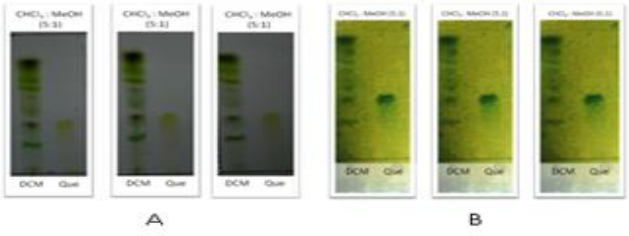

Figure 2. Thin Layer Chromatography plates of DCM fraction using chloroform: methanol (5:1) solvent. (a) visual inspection (b) with spray reagent: 1% potassium ferricyanide and 1% ferric chloride

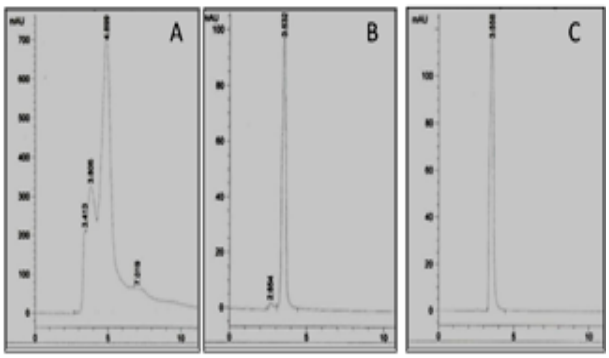

Figure 3. HPLC chromatogram of DCM fraction (A) showing quercetin at 3.412/min, DCM diluted fraction (B) showing quercetin at 3.532/min and quercetin (C) at 3.558/min

Quantitative estimation of quercetin present in the DCM fraction was computed using linear regression analysis of the standard curve of quercetin in parts per million (ppm). Computed results revealed that quercetin is present at a concentration of 69.66 ppm in the DCM fraction.

3.4. In vitro Antioxidant Activity

Hydrogen Peroxide Scavenging Activity

The H_2O_2 scavenging activity of DCM fraction was found to inhibit 81% at a concentration of 10 mg/mL compared to the 86 percent inhibition of ascorbic acid with the same concentration. Percentage inhibition on hydrogen H_2O_2 was found increasing in a dose dependent manner (see Figure 4). The IC_{50} value was found to be 4.954 mg/mL and 3.594 mg/mL for DCM fraction and ascorbic acid, respectively. The ability to inhibit H_2O_2 was comparable with the ascorbic acid indicating that DCM fraction can be considered as a potent scavenger of H_2O_2.

Hydroxyl Radical Scavenging Activity

The •OH scavenging activity was measured by the competition between deoxyribose and the sample for •OH generated with Fe^{3+}/ ascorbate / EDTA / H_2O_2 system. The •OH attacks deoxyribose, that eventually result in TBARS formation, which can be quantified spectrophotometrically. The •OH scavenging activity of DCM fraction was found to inhibit 81% at 1 mg/mL compared to the 93% inhibition of ascorbic acid with the same concentration (see Figure 5). The IC_{50} values of DCM fraction and ascorbic acid in this assay were 0.160 mg/mL and 0.275 mg/mL, respectively. Thus, the ability to inhibit •OH is comparable with the ascorbic acid indicating that DCM fraction can also be considered as a good scavenger of •OH.

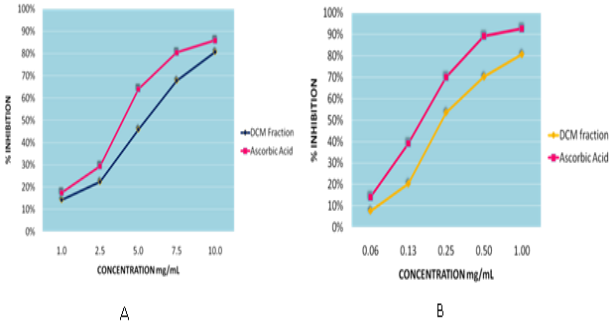

Figure 4. Percent inhibition of hydrogen peroxide scavenging activity (A) and hydroxyl radical scavenging activity (B) of DCM fraction and ascorbic acid with values represent the mean of three determinations

3.5. Acute Oral Toxicity

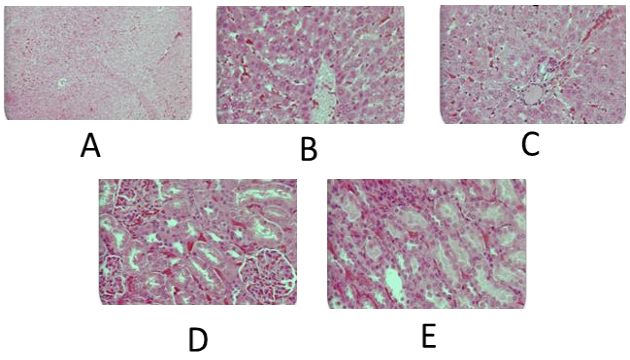

Figure 5. Micrographs of liver tissues under LPO (A), portal tract in the liver under HPO (B), centrilobular vein in the liver under HPO (C), glomeruli and tubules of kidney under HPO (D) and proximal convoluted tubule long section under HPO (E).

Five rats utilized in the main test survived for 14 days. Doses used were 175, 550, 2000 mg/kg bodyweight. Zero mortality rate was observed. Gross necropsies were all normal.

Microsections from specimens labeled as "Liver 1-5", all disclosed unremarkable liver tissues (see Figure 5A-C). The hepatic lobular architecture was intact and uninterrupted. The portal tracts and portal vessels were unremarkable. The hepatocytes were arranged in a radial formation converging from the centrilobular veins and show no cytologic atypia. No abnormal accumulation of bile pigment was observed. The hepatic sinusoids were not congested. No area of hepatic zonal necrosis and tumour formation was identified.

Microsections of the kidneys showed both unremarkable cortical and medullary regions (see Figure 5D-E). The renal cortex showed glomerular unites spaced by renal tubular structures (predominantly proximal convoluted tubes),

showing no evidence of acute and irreversible tubular injury (coagulation necrosis).

Histopathological analysis of the liver and kidneys were unremarkable. Thus, DCM fraction is non-toxic up to 2000 mg/kg bodyweight and is therefore safe for consumption.

3.6. Hypoglycemic Study

Mean Weight and Fasting Blood Sugar upon Induction

while the mean FBS of the other 4 groups are all significantly higher or equal to 140 mg/dL. This means successful induction of diabetes was done in groups 2-5 at day 28 (see Figure 6A).

Mean Weight and Fasting Blood Sugar post induction

The mean weight post induction of the non-diabetic group, (p=0.007) and diabetic control group (p<0.001)

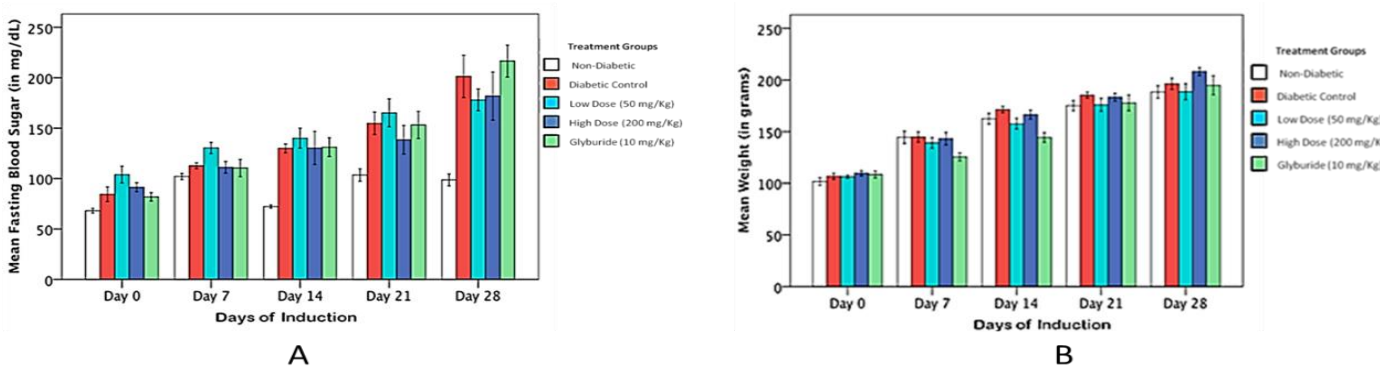

Figure 6. Mean FBS (in mg/dL) (A) and weight (in grams) (B) from Day 0 to Day 28 upon induction of diabetes. N = 30 rats (Repeated Measures of Analysis of Variance test)

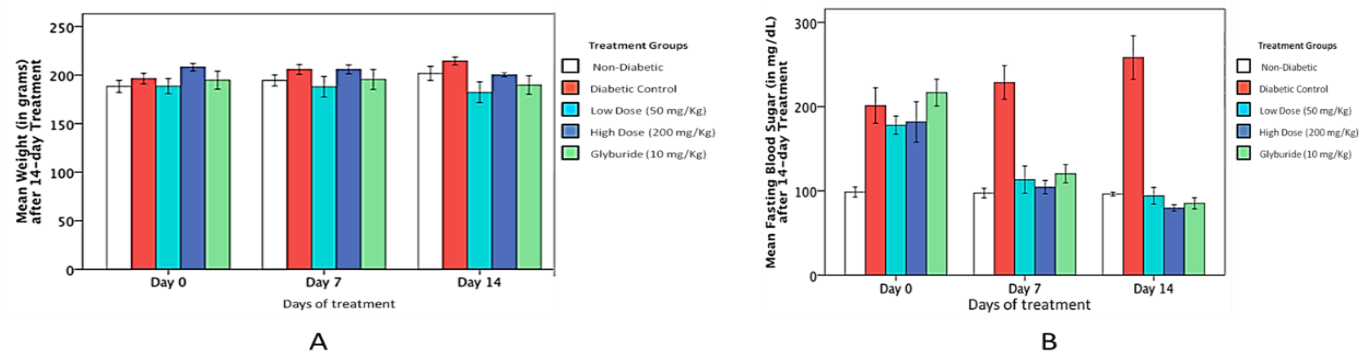

Figure 7. Mean weight (in grams) (A) and FBS (in mg/dL) (B) of groups 1-5 from Day 0 to Day 14 post induction. N = 5 groups (Repeated Measures of Analysis of Variance test)

There was a significant increase (p<0.001) in the mean weights (in grams) of the five groups upon induction of diabetes. However, there was no significant difference (p=0.255) in the mean weight of the five groups at day 28 (see Figure 6B). Results showed that the increases in the weight of all rats were normal and it was not affected by manipulation in diet or administration of streptozotocin.

Upon induction of diabetes, all groups showed significant increase in the mean FBS from day 0 to day 28. However at day 28, there is a significant difference with p value of <0.001 in the mean FBS of the five groups. Post hoc analysis indicates that the mean FBS of the non-diabetic group is significantly less than the four groups. The mean FBS of the non-diabetic group is significantly less than 140 mg/dL, significantly increased after the succeeding 14 days. On the other hand, the mean weights of the low dose group (p=0.148) and glyburide group (p=0.218) did not differ after the 14-day treatment. Only the high dose group (p=0.026) had significant decrease in their mean weight after the 14-day treatment (see Figure 7A). The reduction in weight at 200 mg/kg bodyweight of DCM fraction may be a good factor in preventing weight gain associated in type 2 diabetes.

The mean FBS in the post induction showed that the non-diabetic group did not differ (p=0.882) after the succeeding 14 days. The diabetic control group had significant increase in the mean fasting blood sugar (p=0.031). On the contrary, the low dose group (p<0.001), high dose group (p=0.002)

and glyburide group ($p<0.001$) had significant decrease in their mean blood sugar after the 14-day treatment indicating that DCM fraction exerted hypoglycemic (glucose lowering) effect even at a low dose of 50 mg/kg (see Figure 7B).

Post hoc analysis further indicates that the mean FBS percentage decrease of the low dose, high dose and glyburide group did not differ suggesting that the hypoglycemic effect of DCM fraction at 50 and 200 mg/kg is comparable with glyburide at 10mg/kg.

3.7. Histopathological Analyses of the Liver and Pancreas

Microsections from specimens labeled as liver all disclose unremarkable liver tissues. The hepatic lobular architecture was intact and uninterrupted. The portal tracts and portal vessels were unremarkable. The hepatocytes were arranged in a radial formation converging from the centrilobular veins and showed no cytologicatypia. No abnormal accumulation of bile pigment was observed. The hepatic sinusoids were not congested. No area of hepatic zonal necrosis and tumour formation was identified. All liver from the rats were normal.

Microsections from the pancreas taken from the rats disclosed pancreatic tissue showing both the exocrine and endocrine components. The exocrine pancreas consisted of sheets of acinar and centroacinar cells clustered into lobular units. The endocrine pancreas consisted of islets of Langerhans showed variable degrees of atrophic changes in the four diabetic groups as compared to the pancreas of the normal rats. These changes were based on (1) number of islet cells per low power objective field (LPO) and/or (2) average size per 10 LPO fields. No reactive peri- islet inflammatory infiltrates were identified. These observable changes had defined as a grading scheme based on such atrophic changes, as follows (see Figure 8):

Score of 1+ = 3 – 5 islets identified / 10 LPO
Score of 2+ = 2 – 3 islets identified / 10 LPO
Score of 3+ = 0 – 2 islets identified / 10 LPO

The histomorphologic results of each group are summarized and tabulated as follows:

Table 1. Degree of atrophic changes found in the pancreas of the rats

LANGERHANS ATROPHY					
Rat no.	ND	DC	LD	HD	G
1	2+	2+	3+	1+	3+
2	2+	1+	2+	2+	3+
3	2+	1+	3+	1+	1+
4	1+	2+	2+	1+	3+
5	1+	3+	1+	2+	3+
6	2+	2+	1+	1+	3+

Statistical analysis showed that there was no significant difference ($p=0.0734$) between the number of islets present in the pancreas of each group. However, the size of the islets observed in the normal group was bigger compared with the 4 groups which were smaller in size under LPO.

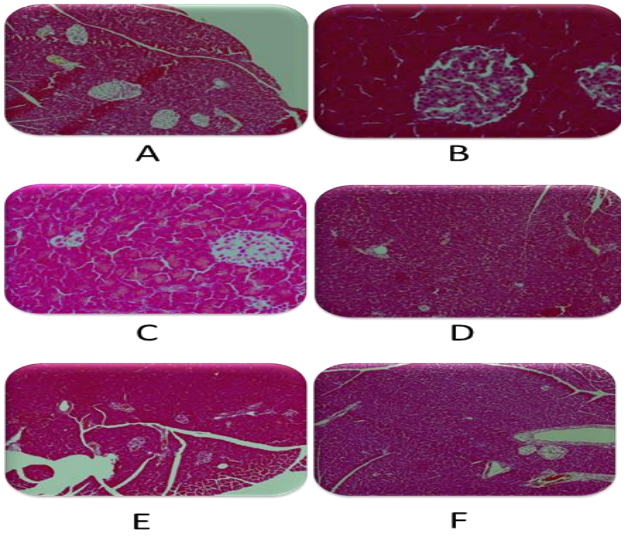

Figure 8. Micrograph of a normal pancreas from the non-diabetic group under (A) LPO and (B) HPO; pancreas with a 3+ score showing with (C) atrophic form under HPO and (D) scanty islets under LPO; pancreas showing a score of (E) 1+ and (F) 2+ under LPO.

DISCUSSION

This study was intended to establish the hypoglycemic and *in vitro* antioxidant activity of the leaves of *F. odorata*. This was based on the local use of this plant as a treatment for diabetes and in addition to the previous studies conducted on various *Ficus* species having the same activity. Evaluation of the hypoglycemic activity was done using combination of low dose streptozotocin and high fat diet and determination of

antioxidant activity was conducted *in vitro* using •OH and H_2O_2.

Several studies presented that insulin resistance may develop upon high fat diet feeding[19,23,24] and subsequently administration of low dose streptozotocin is known to induce mild impairment of insulin secretion[20]. Successful induction of type 2 diabetes was achieved after 28 days. Fasting blood sugar of the groups 2-5 were $\geq$ 140 mg/dL and the negative control had an increase in fasting blood sugar, however, it did not exceed 140 mg/dL. On the other hand, change in body weight of rats between the control group and the other four groups induced with type 2 diabetes was not statistically different indicating that manipulation in diet or administration of streptozotocin does not affect the change in weight. These are consistent with the result of some study that higher induction rate was achieved by twice injection of 30 mg/kg of streptozotocin combined with high fat diet and the change in the the body weight gain during their study was not statistically different between control group and diabetic group[19].

Combination of low dose streptozotocin and high fat diet significantly induced hyperglycemia mimicking type 2 diabetes. As previously described, type 2 diabetes is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction)[20]. In the early stages of the disorder, the pancreatic beta cells compensate insulin resistance by increasing insulin secretion. As the insulin resistance and compensatory hyperinsulinemia progresses, beta cell function gets damaged leading to deterioration of glucose homeostasis and consequently causing impaired glucose tolerance and hyperglycemia[4].

In the present study, daily oral administration of DCM fraction from the leaves of *F. odorata* at a dose of 50 mg/kg and 200 mg/kg for 14 days produced a significant decrease in the mean fasting blood sugar. Post hoc analysis indicates that the hypoglycemic activity of DCM fraction was comparable with that of glyburide at 10 mg/kg. Glyburide, is a sulfonylurea that causes hypoglycemia by stimulating insulin release from pancreatic beta cells[25]. Based from the results, it may be understood that the effects of DCM fraction may be due to the stimulation of β-cells of islets of langerhans to release more insulin similar to Glyburide. However, this proposed mechanism has to be tested in a molecular level in a further study.

In the progression of diabetes, it is established that tissue damage may be due to free radicals by attacking membranes through peroxidation of unsaturated fatty acids leading to extensive damage and dysfunction of membranes[26]. An improved antioxidative defense mechanism may be a good management to prevent diabetic complications[27]. In this study, DCM fraction exhibited free radical scavenging activity against •OH and H_2O_2 , two of the most deleterious reactive oxygen species produced in the mitochondria that may cause oxidative damage. DCM fraction from the leaves of *F. odorata* has a potential antioxidant activity due to abundance of phenolic compounds like flavonoids.

The presence of quercetin in the DCM fraction as identified in TLC and HPLC chromatogram is likely the bioactive compound responsible for being the hypoglycemic and antioxidant activity of *Ficus odorata*. Quercetin, a known natural antioxidant is found to be one of the major flavonoids present in various *Ficus* species[28]. Flavonoids can improve altered glucose and oxidative metabolisms of diabetic states[29]. This is supported by the study where quercetin supplementation is evidently effective in reducing blood glucose concentration, promoting regeneration of the pancreatic islets and increasing insulin release in streptozotocin-induced diabetic rats[30]. Furthermore, it was reported that upon exposure of isolated rat islets to certain flavonoids such as quercetin, it enhanced insulin release by 44–70% via alteration in Ca^{2+} fluxes and in cyclic nucleotide metabolism as explained[31]. In addition to its hypoglycemic property, quercetin is also known to scavenge oxygen radicals, chelate metal ions and protect against lipid peroxidation, thus, preventing oxidant injury and cell death[32].

CONCLUSION

This study establishes the effectiveness of DCM fraction from the leaves of *Ficus odorata* in lowering the blood glucose level of streptozotocin- and high fat diet-induced type 2 diabetic rats at a dose of 50 and 200 mg/kg. The effect of the two doses of DCM fraction is comparable with that of glyburide. DCM fraction has a potential antioxidant activity

against •OH and H_2O_2. It is non-toxic up to 2000 mg/kg bodyweight. Overall, *Ficus odorata* leaves possess hypoglycemic and antioxidant activities due to their flavonoid content and may be a good herbal supplement in the prevention and management of diabetes.

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REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 27: 2568-2569.
2. Diabetes (2012). Retrieved from WHO, Media Center Fact Sheets website, http://www.who.int/diabetes/action_online/basics/en/index1.html
3. Sharma B, Balomajumder C, Roy P. (2008). Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin-induced diabetic rats. Food Chem Toxicol. 46: 2376-2383.
4. Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL, Loscalzo J. (2008). Harrison's Principles of Internal Medicine (17th edition). USA: McGraw-Hill.
5. Huang T, Peng G, Kota B, Li GQ, Yamahara J, Roufogalis BD, Li Y. (2005). Anti-diabetic action of *Punica granatum* flower extract: activation of ppar-u and identification of an active component. Toxicol App Pharmacol. 207: 160-169.
6. Halliwell Barry (2001). Free radicals and other reactive species in disease. Encyclopedia of Life Sciences. 1-7.
7. Kaneto H, Katakami N, Matsuhisa M., Matsuoka T. (2009). Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Med Inflam. 2010. doi:10.1155/2010/453892
8. McCune L, Johns T. (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J Ethnopharmacol. 82: 197-205.
9. Abdel-Hameed ESS. (2009). Total phenolic contents and free radical scavenging activity of certain egyptian *Ficus* species leaf samples. Food Chem. 114: 1271-1277.
10. Vessal M, Hemmati M, Vasei M. (2003). Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. Comp Biochem Physiol Part C: Toxicol Pharmacol. 3: 357-364.
11. Herrera AA, Ramos JB, Ipulan L.A. (2010). Teratogenic activity of *Ficus odorata* (Blanco) Merr.and *Baccaureate tandra* (Baill.)Müll in mice embryos. Phil Agri Sci. 93: 255-262
12. El-Sayed MM, El-Hashash MM, El-Wakil EA, Ghareeb MA. (2009). Total phenolic contents and antioxidant activities of *Ficus sycomorus* and *Azadirachta indica*. Pharmacol Online. 3: 590-602.
13. Pagno T, Blind LZ, Biavatti MW, Kreuger MRO. (2006). Cytotoxic activity of the dichloromethane fraction from *Vernonia scorpioides* (Lam.) Pers. (Asteraceae) against Ehrlich's tumor cells in mice. Brazilian J Med Bio Res. 39: 1483-1491.
14. Guevara BQ. (2005). A Guidebook to Plant Screening: Phytochemical and Biological, Research Center for the Natural Sciences, University of Santo Tomas, Manila.
15. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika P. (2008). Antibacterial and antioxidant properties of the methanolic extract of the leaves and stems of C*alpurnia aurea*. BMC Comp Altern Med. 8:53.
16. Ebrahimzadeh MA, Nabavi SY, Nabavi SF, Bahramian F, Bekharadnia AR. (2010). Antioxidant and free radical scavenging activity of *H. officinalis l. var. angustifolius, v. odorata, b. hyrcana and c. speciosu*. Paki J Pharma Sci. 23: 29-34.
17. Samak G, Shenoy RP, Manjunatha SM, Vinayak KS. (2009). Superoxide and hydroxyl radical scavenging actions of botanical extracts of *Wagatea spicata*. Food Chem. *115:* 631-634.
18. OECD/OCDE Guideline for the Testing of Chemicals. Revised Draft Guideline 425: Acute Oral Toxicity. October, 2008.
19. Zhang M, Xiao-Yan L, Li J, Xu ZG, Chen L. (2008).The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res, doi:10.1155/2008/704045.
20. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res. 52: 313–320.
21. Kangasabapathy AS, Kumari S. (2000). Guidelines on Standard Operating Procedures for Clinical Chemistry. 15-16.
22. Sener G, Sehril AO, Ipci Y, Centinel S, Cikler E, Gedik N., 2005. Chronic nicotine toxicity prevented by aqueous garlic extract. Plant Food Hum Nutri (60) 77-86.
23. Flanagan AM, Brown JL, Santiago CA, Aad PY, Spicer LJ, Spicer MT. (2008). High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. J Nutri Biochem. 19 (8): 505–513.
24. Tanaka S, Hayashi T, Toyoda T. (2007). High-fat diet impairs the effects of a single bout of endurance exercise on glucose transport and insulin sensitivity in rat skeletalmuscle. Metabolism. 56: 1719–1728.

25. Davis S. (2006). Goodman and Gilman's The Pharmacological Basis of Therapeutics (11th edition). New York: McGraw-Hill.
26. Pandit R, Phadke A, Jagtap A. (2010). Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. J Ethnopharmacol. 128: 462-466.
27. Prince PSM, Kamalakkannan N. (2006). Rutin improves homeostasis in streptozotocindiabetic tissues by altering glycolitic and gluconeogenic enzymes. J Biochem Mol Toxicol. 20: 96–102.
28. Vaya J, Mahmood S. (2006). Flavonoid content in leaf extracts of the Fig (*Ficus carica* L.), Carob (*Ceratonia siliqua* L.) and Pistachio (*Pistacia lentiscus* L.). Biofactors. 28: 169-175.
29. Pinent M, Blay M, Bladé MC, Salvadó Arola L, Ardévol A. (2004). Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. Endocrinology. 145: 4985–4990.
30. Hii CS, Howell S.. (1984). Effects of epicatechin on rat islets of Langerhans. Diabetes. 33: 291–296.
31. Coskun O, Kanter M, Korkmaz A, Oter S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacol Res. 51: 117-123.

