HYPOGLYCEMIC AND FREE RADICAL SCAVENGING ACTIVITY OF PARTIALLY PURIFIED FRACTION E FROM DCM STEM EXTRACT OF COSINUM FENESTRATUM

NWABUEZE PATRICK OKECHUKWU

Biotechnology program, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Kuala Lumpur, Malaysia, Email: patrickn@ucsi.edu.my

Received: 17 January 2012, Revised and Accepted: 10 April 2012

ABSTRACT

Coscinium fenestratum is from the family Menispermaceae, is a woody plant found in Southeast Asia. Our previous pharmacological investigation reported that the dichloromethane (DCM) stem extracts of this plant possessed antiallergy, anti-diabetic, anti-hypertensive, and anti-histamine and free radical scavenging properties. This study aims to partially purify the crude DCM stem extracts and to investigate the anti-diabetic activity of its individual fractions A-E on streptozotocin (STZ)-induced diabetic rats. Free radical scavenging properties and phytochemical screening of the bioactive fraction E were also determined. Five fractions (A, B, C, D, and E) were obtained from crude DCM extracts and administered orally at a dose of 100 mg/kg on STZ-induced diabetic rats for 15 days. The blood glucose levels and the bodyweights of the diabetic rats were determined on day 1, 5, 10, and 15 of treatment. TPC was measured using 2.5 ml of 10% FCR and 2 ml of Na2 CO3 (2%w/v) was added to 0.5 ml of each sample (3 replicates) of fraction E solution (1mg/ml) and determination of scavenging activity of DPPH free radical. One ml of 0.135 mM DPPH prepare in methanol was mixed with 1.0 ml of fraction E ranging from 0.2-0.8 mg/ml. The data was compared statistically using one-way analysis of variance (ANOVA). Fraction E significantly (p < 0.05) reduced blood glucose levels and increased the bodyweight of the experimental STZ-induced diabetic rats to 8.2 ± 0.7 mmol/L and 422.52 ± 11.1 g compared to negative control 25.0 ± 1.8 mmol/L and 143.47 ± 13.5 g respectively. The phytochemical screening of fraction E revealed the presence of flavonoids. The total phenolic compounds present in fraction E was 256.76 mg GAE/g dry weight and the radical scavenging activity of the fraction E was 21.71%. This fraction E possesses anti-diabetic and free radical scavenging properties. This effect might be as a result of the presence of flavonoids which has been widely reported to have anti-diabetic effects.

Keywords: Coscinium fenestratum; Antidiabetic; Streptozotocin; Antioxidant; Diabetes

INTRODUCTION

Diabetes mellitus is a group of chronic metabolic diseases primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy, and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related macrovascular complications (ischemic heart disease, stroke, and peripheral vascular disease), and diminished quality of life. According to WHO, the prevalence of diabetes for all age groups worldwide is estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.

The prevalence of type 2 diabetes continues to rise inexorably globally and with much of the global burden of this disease that threatens to be devastating is expected to come from the Western-Pacific as well as the South-East Asia regions. The extent to which this risk is expressed is strongly related to the degree of westernisation and urbanisation lifestyle, and its continuing to accelerate in this new millennium. As a result, diabetes has indeed emerged number six (6) as the fourth leading cause of death in the world which now rivals HIV/AIDS in terms of suffering and death.

Diabetes mellitus is also usually associated by increased production of the molecules of reactive oxygen species (ROS) and/or impaired antioxidant defense systems, which result oxidative damage leading to ROS mediated diabetic pathogenesis. Disturbance of antioxidants defense system in diabetes involves enhancement of lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism. When the renal threshold for glucose reabsorption is exceeded glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which, in turn, results in dehydration, thirst, and increased drinking (polydipsia). It has been known that many natural products have effects in controlling diabetes, represented a promising approach to the discovery of new diabetes drugs.

Coscinium fenestratum is a woody climber found in south East Asia and has been widely used as a medicinal plant. The infusion and tincture preparation of stem is widely used in the traditional Ayurvedic system for the treatment of diabetes mellitus. In the siddha system of medicine, the powdered stem is dissolved in milk and given to the diabetic patients. The rural people of Kanyakumari District, Tamilnadu, India use the decoction of the stem for treatment of diabetes. Our previous report showed that DCM crude extract from the stem of CF possesses antidiabetic and free radical scavenging activity (antioxidant). Phytochemical screen revealed the presence of flavonoids, saponins, tannins, alkaloids and terpenes. Therefore this present study aims to partially purify the crude DCM stem extract and to investigate the anti-diabetic activity of its individual fractions A-E on streptozotocin (STZ)-induced diabetic rats. Free radical scavenging properties and phytochemical screening of the bioactive fraction E were also determined.

MATERIALS AND METHODS

Materials

Coscinium fenestratum (20kg) were obtained from Laboratory of Natural Products (NATPRO) in Institute of Bioscience, University Putra Malaysia (UPM), Selangor Darul Ehsan, healthy male adult Wistar strain albino rats weighing between 170-230 g were purchased from Institute of Medical Research (IMR), Kuala Lumpur, Miller from UPM (Hsiang Tai Machinery Industry Co Ltd, Taiwan), rotary evaporator (BÜCHI Rotavapor R-200, Switzerland), quantitative glucose meter, Accutrend® GCT (Roche Diagnostics, Germany), blood glucose test strips, Accutrend® Gluco (Roche Diagnostics, Germany), and disposable syringes (Becton Dickinson & Co., Singapore). Dichloromethane (DCM), hexane, ethyl acetate and methanol (R&M Chemicals, England), tragacanth powder and Tween 40, (MERCK, Germany), sodium chloride and glycerol, (Fisher Scientific, UK). Silica gel 60, 230 – 400 Mesh, 40 – 63 microns (Malindiroad,U.S.A), 20 cm x 20 cm silica gel 60 F254 – coated Thin Layer Chromatography (TLC) aluminum sheets (MERCK Germany), filter paper (Whatman International, England), streptozotocin and tobutamide (Sigma-Alrich, USA).

Collection of plant material

The stems of the plant CF (20kg) were collected from the jungles of Pahang. Mr. Shamsul Khamis a plant taxonomist from the Laboratory of Natural Products (NATPRO) in Institute of Bioscience, University Putra Malaysia (UPM), Selangor Darul Ehsan specifically identified the plant.
Preparation of crude DCM extract

The plant material (stem) was air-dried at 25 °C for a week. The dried stem was cut into thin pieces and ground to coarse powder form using a miller (HsiangTai Machinery Industry Co Ltd, Taiwan) in Institute of Bioscience, UPM. Dried ground stem powder (1 kg) was extracted with DCM (4 L) for 48 hours at room temperature. Then, the extract was filtered through Whatman filter paper and concentrated using rotary evaporator until dry at 54 °C.

Partial purification of DCM extracts

DCM extract was partially purified using column chromatography technique with silica gel as stationary phase and solvents mixture (hexane, ethyl acetate and methanol) as mobile phase. Five fractions namely A, B, C, D and E were eluted. Fractions were eluted as a single spot on thin layer chromatography (TLC) silica-coated aluminum plate (F 254) and examined under UV light of wavelength 365 nm. Eluents were pooled together based on its TLC pattern and similar measured Rf value of each spot in each fraction. The pooled fractions were concentrated with rotary evaporator until dry at 35 °C. These steps were repeated until appropriate amounts were obtained for pharmacological assay.

Phytochemical screening of fraction E

Phytochemical screening of fraction E was performed for the presence of alkaloids, tannins, saponins, flavanoids, cardiac glycosides, and terpenoids.

Animals

Albino Wistar rats of both sexes weighting (180 - 200 g, 11 weeks) were purchased from Institute of Medical Research (IMR), West Malaysia. They were housed in standard metal cages at 26 ± 2 °C and maintained under standard 12 h light/12 h dark cycle throughout the duration of the study. All animals were given access to food and water ad libitum. They were deprived of food but not of water before the commencement of the experiment because the drugs and test substances were administered orally.

Toxicity Test

The toxicity test of fraction A-E was studied according to 1). High concentration fraction A-E (5000mg/rat) prepared in saline was administered orally to group of five rats each for 7 consecutive days.

Table 1: shows the seven treatment group for the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>100mg/kg Tolbutamide</td>
</tr>
<tr>
<td>II</td>
<td>0.9% Saline</td>
</tr>
<tr>
<td>III</td>
<td>100mg/kg fraction A</td>
</tr>
<tr>
<td>IV</td>
<td>100mg/kg fraction B</td>
</tr>
<tr>
<td>V</td>
<td>100mg/kg fraction C</td>
</tr>
<tr>
<td>VI</td>
<td>100mg/kg fraction D</td>
</tr>
<tr>
<td>VII</td>
<td>100mg/kg fraction E</td>
</tr>
</tbody>
</table>

Determination of total phenolic compounds (TPC)

The amount of total phenolic compounds present in the fraction E was determined with Folin-Ciocalteu Reagent (FCR) using the method of Spanos and Wrolstad (1990). The experiment was done by measuring 2.5ml of 10% FCR and 2ml of Na: CO3 (2%w/v) was added to 0.5ml of each sample (3 replicates) of fraction E solution (1mg/ml). The resulting mixture was incubated at 45°C with shaking for 15min. The absorbance of the sample was measured at 765 nm using UV/visible light. Results were expressed as milligrams of Gallic acid (0-0.5mg/ml) dissolved in distilled water.

DPHH Radical Scavenging Activity

The method of Kikuzak and Nakatani (1993) was used for the determination of scavenging activity of DPPH free radical. One ml of 0.135 mM DPPH prepare in methanol was mixed with 1.0ml of fraction E ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30min. The absorbance was measured spectrophotometrically at 517nm.

The scavenging ability of the plant extract was calculated using this equation: DPPH scavenging activity (%) = [(Abs control – Abs sample)] / [(Abs control)] x 100 Where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH Radical + sample (i.e. fraction E or standard).
Statistical analysis

Statistical significance was assessed using one-way analysis of variance (ANOVA) and Dunnett test to compare the data. Probability (P) values of less than 0.05 were considered significant difference between means. All values are presented as means ± standard error (SD).

Table 2: Effects of STZ on the blood glucose level of diabetic rats

<table>
<thead>
<tr>
<th>Treatment Groups (n=5)</th>
<th>Blood Glucose Level (mmol/L)</th>
<th>Before Induction of Diabetes with STZ (Day 0)</th>
<th>72 hours after induction of diabetes with STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>5.5 ± 1.5</td>
<td>17.9 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.1 ± 1.5</td>
<td>20.3 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>5.5 ± 1.5</td>
<td>22.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>4.7 ± 0.5</td>
<td>18.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>5.7 ± 0.7</td>
<td>14.5 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>6.3 ± 0.7</td>
<td>15.6 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Group E</td>
<td>5.3 ± 0.5</td>
<td>12.5 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

RESULT

Effects of STZ on blood glucose level and bodyweight of experimental rats

The fasting blood glucose levels of the experimental rats were determined before induction of diabetes by STZ (day 0) and 72-hours post-STZ induction. It was observed that STZ administration at a dosage of 65 mg/kg showed significant increase in blood glucose levels 72 hours post-STZ induction compared prior to diabetes induction (day 0) (Table 2). On the other hand, the bodyweights of all experimental rats showed significant decrease 72 hours post-STZ induction compared prior to diabetes induction by STZ (day 0) (Table 3).

Effects of extracts (A, B, C, D, and E) on blood glucose level and bodyweight of experimental rats.

Oral administration of 100 mg/kg Tolubutamide, fraction D, and fraction E to the STZ-induced diabetic rats showed significant decrease in blood glucose levels and significant increase in bodyweights throughout the 15 day treatment (Table 4.4). Saline, fractions A, B, C (100mg/kg) did not show significant reduction in blood glucose levels and reversal of bodyweights throughout the 15 day treatment (Table 4 and Table 5).

Phytochemical screening, total phenolic compounds, DPPH radical scavenging activity of fraction E

The phytochemical screening of fraction E revealed the presence of flavonoids. The Total phenolic compounds present in fraction E was 256.67 mg GAE/g dry weight and the radical scavenging activity of the fraction E was 21.71%.

DISCUSSION

The oral toxicity of fraction E was evaluated with concentration five-fold higher than maximum tested antidiabetic concentration (5000 mg/kg, p. o.). At this concentration, no signs and symptoms of acute toxicity were observed in all treated rats. No significant difference was observed in the weight of heart, liver, kidney, or lungs when they were compared with those of control group (saline). None of the treated rats died during the 14 days of observation after the administration of fraction E. The results initially obtained did not indicate any toxicity which confirmed that the extract is safe which motivated us to continue the assays.

All the rats that were induced-diabetes with 65 mg/kg STZ displayed many of the features seen in human subjects with uncontrolled diabetes mellitus, such as elevation of blood glucose levels of above 11.1 mmol/L and slight loss in bodyweight 72-hours post STZ administration.

Table 3: Relative Weight Loss of Diabetic Rats After STZ Induction.

<table>
<thead>
<tr>
<th>Treatment Groups (n=5)</th>
<th>Blood Glucose Level (mmol/L)</th>
<th>Before Induction of Diabetes with STZ (Day 0)</th>
<th>72 hours after induction of diabetes with STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>200.70 ± 17.5</td>
<td>198.04 ± 9.2</td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>180.83 ± 8.7</td>
<td>169.84 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>183.83 ± 11.1</td>
<td>175.92 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>170.77 ± 8.7</td>
<td>164.80 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>179.20 ± 4.4</td>
<td>171.23 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>177.53 ± 6.1</td>
<td>169.47 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Group E</td>
<td>182.69 ± 3.6</td>
<td>173.01 ± 5.8</td>
<td></td>
</tr>
</tbody>
</table>

We also observed symptoms such as: fatigue, slow movement, increased consumption of food (polyphagia) and excessive thirst (polydipsia). STZ is a simple, inexpensive and available method as claimed by researchers globally to induce type 2 diabetes in rodents. It is an antibiotic with the molecular formula C₈H₁₅N₃O₇, produced by an actinomycete, Streptomyces achromogenes.
STZ is actively taken up by pancreatic β-cells via a glucose transporter GLUT-2 and causes alkylation of deoxyribonucleic acid (DNA) 26,27,14,20,29. It was described that the activation of polyadenosine diphosphate ribosylation and nitric oxide release and the production of nitric oxide (NO) and reactive oxygen species may also be involved in DNA fragmentation, destruction of β-cells 26 and other deleterious effects of STZ after being administered to the rodents. NO is a free radical that targets intracellular iron containing enzymes, which results in the loss of their function 31, 32, 33. The pancreatic β-cells are particularly sensitive to damage by NO and free radicals because of their low levels of free radical-scavenging enzymes 34, 35. According to Adeghate and Parvez (2000), rat islet cells exposed to various cytokines have revealed that NO and oxygen free radicals may act together to kill the pancreatic β-cells which is self-explanatory that a single dose administration of STZ at 65 mg/kg produced diabetic effects 72 hours in the Experimental rats.

### Table 4: Effects of treatment on the blood glucose levels of STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment Groups (n=5)</th>
<th>Blood Glucose Level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Positive Control</td>
<td>17.9±5.6</td>
</tr>
<tr>
<td>Negative Control</td>
<td>20.3±3.8</td>
</tr>
<tr>
<td>Group A</td>
<td>22.1±1.6</td>
</tr>
<tr>
<td>Group B</td>
<td>18.5±0.9</td>
</tr>
<tr>
<td>Group C</td>
<td>14.5±2.8</td>
</tr>
<tr>
<td>Group D</td>
<td>15.6±2.0</td>
</tr>
<tr>
<td>Group E</td>
<td>12.5±1.4</td>
</tr>
</tbody>
</table>

Nonetheless the obvious symptoms of the diabetic rodents observed in our study after the induction of STZ such as: weight loss, hyperglycemia, fatigue, slowed movement and increased consumption of food (polyphagia) and excessive thirst (polydipsia) were in line with the common diabetes symptoms. The lack of insulin which is caused by the β-cells destruction causes glucose production to be unregulated, weight loss occurs because muscle protein is broken down into amino acids for the gluconeogenesis process in the liver. Since proteins have to supply enough energy to substitute for carbohydrates, proteins are broken down faster than they are made. When more severe insulin deficiency is present, adipose tissue and fat breakdown occurs, further accentuating weight loss 30.

In an attempt to provide energy for the cells, the body mobilizes its glucose and fat reserves. Polyphagia occurs as a result that the body is trying to compensate for the lack of fluid and sugar 37. As for the rise of blood glucose levels in the diabetic rats, when fasting plasma glucose levels are far higher than normal range which is exceeding 5.6 mmol/L or 100 mg/dl, the excess glucose is spilled over into the urine (glycosuria) and causes an osmotic diuresis with increased loss of water, urine volume and electrolytes (polyuria) 30.

The physiological response to increase fluid loss from the body is increased thirst and drinking called polydipsia 37. The oral administration of saline (1ml), fractions A, B, and C of DCM extract at 100 mg/kg did not reduce the blood glucose level or increase in body weight of STZ-induced diabetic rats after 15 days of treatment. Rather there was an elevation of blood glucose and a reduction of bodyweight. This shows that there were no hypoglycemic effects or reversal in bodyweight of the diabetic rats exerted by the negative control (saline) and fractions A, B, and C of DCM extract at 100 mg/kg. The orally administration of Tolbutamide (positive control) 100 mg/kg to the diabetic rats was able to significantly decrease their blood glucose levels and slightly increased their body weight after the 15th day of treatment. Tolbutamide is an oral anti-diabetic agent of the first generation sulfonylurea. Its principal action is to increase insulin release from the pancreatic β-cells, reduce serum glucagon levels and closure of potassium channels in extrapancreatic tissues to give the combined plasma glucose lowering effect 40. The slight increase in bodyweights of diabetic rats treated with tolbutamide maybe as a result of its antihyperglycemia activity, which may have promoted glucose uptake by enhancing the release of insulin of the cells of the tissues of the diabetic rats. This result is in agreement with that of 41.

### Table 5: Effects of treatment on the bodyweights of STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment Groups (n=5)</th>
<th>Blood Glucose Level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Positive Control</td>
<td>203.04±9.2</td>
</tr>
<tr>
<td>Negative Control</td>
<td>169.84±4.0</td>
</tr>
<tr>
<td>Group A</td>
<td>175.92±5.5</td>
</tr>
<tr>
<td>Group B</td>
<td>164.80±5.2</td>
</tr>
<tr>
<td>Group C</td>
<td>171.23±5.4</td>
</tr>
<tr>
<td>Group D</td>
<td>169.47±6.1</td>
</tr>
<tr>
<td>Group E</td>
<td>173.01±5.8</td>
</tr>
</tbody>
</table>

Nonetheless the obvious symptoms of the diabetic rodents observed in our study after the induction of STZ such as: weight loss, hyperglycemia, fatigue, slowed movement and increased consumption of food (polyphagia) and excessive thirst (polydipsia) were in line with the common diabetes symptoms. The lack of insulin which is caused by the β-cells destruction causes glucose production to be unregulated, weight loss occurs because muscle protein is broken down into amino acids for the gluconeogenesis process in the liver. Since proteins have to supply enough energy to substitute for carbohydrates, proteins are broken down faster than they are made. When more severe insulin deficiency is present, adipose tissue and fat breakdown occurs, further accentuating weight loss 30. In an attempt to provide energy for the cells, the body mobilizes its glucose and fat reserves. Polyphagia occurs as a result that the body is trying to compensate for the lack of fluid and sugar 37. As for the rise of blood glucose levels in the diabetic rats, when fasting plasma glucose levels are far higher than normal range which is exceeding 5.6 mmol/L or 100 mg/dl, the excess glucose is spilled over into the urine (glycosuria) and causes an osmotic diuresis with increased loss of water, urine volume and electrolytes (polyuria) 30.

The physiological response to increase fluid loss from the body is increased thirst and drinking called polydipsia 37. The oral administration of saline (1ml), fractions A, B, and C of DCM extract at 100 mg/kg did not reduce the blood glucose level or increase in body weight of STZ-induced diabetic rats after 15 days of treatment. Rather there was an elevation of blood glucose and a reduction of bodyweight. This shows that there were no hypoglycemic effects or reversal in bodyweight of the diabetic rats exerted by the negative control (saline) and fractions A, B, and C of DCM extract at 100 mg/kg. The orally administration of Tolbutamide (positive control) 100 mg/kg to the diabetic rats was able to significantly decrease their blood glucose levels and slightly increased their body weight after the 15th day of treatment. Tolbutamide is an oral anti-diabetic agent of the first generation sulfonylurea. Its principal action is to increase insulin release from the pancreatic β-cells, reduce serum glucagon levels and closure of potassium channels in extrapancreatic tissues to give the combined plasma glucose lowering effect 40. The slight increase in bodyweights of diabetic rats treated with tolbutamide maybe as a result of its antihyperglycemia activity, which may have promoted glucose uptake by enhancing the release of insulin of the cells of the tissues of the diabetic rats. This result is in agreement with that of 41.

### Table 6: Flavonoids were present in fraction E.

<table>
<thead>
<tr>
<th>Fraction E</th>
<th>Observation / X-Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>X</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>X</td>
</tr>
<tr>
<td>Tannins</td>
<td>X</td>
</tr>
</tbody>
</table>

Similarly the oral administration both fraction D and E extract of DCM at 100mg/kg to the diabetic rats significantly showed plasma glucose lowering effects and increase in their bodyweight after the 15th day treatment. However, fraction E showed the highest effect correlates that of tolbutamide. The reversal of blood glucose level and weight loss in fraction E treated diabetic rats may suggest that...
fraction E has anti-diabetic activity. This is a hypothesis based on the results obtained during the 15 day treatment of the STZ-induced diabetic rats with fraction E that co-relates with the treatment of the STZ-induced diabetic rats with the drug Tolbutamide. Therefore, it can be suggested that fraction E has similar mechanisms to that of Tolbutamide which promoted glucose uptake by enhancing the release of insulin from the pancreatic beta cells of the rats.

Fraction E may be actively protecting muscle wasting through the reversal of gluconeogenesis by repressing the enzymes responsible for this process such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase and glucose-6-phosphatase. Excessive hepatic glycogenolysis and gluconeogenesis is associated with decreased utilization of glucose by the tissues of the diabetic rats which caused its bodyweight to decrease and its reversal may have been the cause for the increase in bodyweights of the diabetic rats 42. Besides, fraction E may be suspected to have also stimulated glucose uptake in adipocytes by activating several insulin-signalling proteins such as insulin receptor substrates (IRS), phosphorylatedinositol 3-kinase (PI3-K), and protein kinase B (PKB) which led to the translocation of insulin-sensitive glucose transporter 4 (GLUT4) vesicles to the plasma membrane and facilitated glucose uptake into adipose tissue 43.

Due to the damage in the pancreatic beta cells caused by STZ-induction, there's a reduction in insulin mediated glucose uptake into skeletal muscle caused by decreasing GLUT-4 protein 44. The decrease in GLUT-4 expression due to the induction of STZ to the rodents may have been reversed during the 15 day treatment with fraction E. An increase of GLUT-4 mRNA expression may contribute to plasma glucose regulation in treated STZ diabetic rats which led to the increase in its bodyweights 45.

Phytochemical tests revealed the presence of flavonoids that was suggested to be involved with the anti-diabetic activity of fraction E on STZ-induced diabetic rats throughout the 15 day treatment. The previous work done by 46 revealed that the total phenolic content of fraction E expressed in gallic acid equivalents (GAE) to be 256.78 ± 0.19 mg GAE/g dry weights and DPPH radical scavenging activity was calculated to be 21.71 ± 0.07%. Flavonoids are a highly diverse group of naturally occurring secondary plant metabolites in the polyphenol family comprising an estimated 9000 identified structures 47. Flavonoids are categorised according to their chemical structure into 7 major subgroups which includes chalcones, flavones, flavonols, flavanones, anthocyanidins and isoflavones 48. The therapeutic effects of many herbal medicines may be related in many cases to the presence of these polyphenols 49. Many studies have reported the anti-diabetic actions of flavonoids. According to 50,48, 51, the bioactivity of flavonoids has been attributed by their powerful antioxidant properties due to the presence of aromatic hydroxylgroups.

They are scavengers of reactive oxygen and nitrogen species and, therefore, inhibit peroxidation reactions which are harmful to human cells daily 52,53. They also protect macrophages from oxidative stress by keeping glutathione in its reduced form while increasing the levels of glutathione in the body cells 54,55. Free radicals that cause oxidative stress are known to be a pathogenic factor in the development of diabetes mellitus 56. Moreover, Ceriello (2003) testified that oxidative stress may injure endothelial cell function which can be related to the development of chronic diabetic complications. What is worse is that abnormally high levels of free radicals may be a leading factor to the depletion of the activity of the antioxidant defence system, damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance 57. Several studies showed good correlation between the total phenols and antioxidant activity of flavonoids is to stabilise the ROS by reacting with the reactive compound of the radical 58. Due to the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive 45.

**CONCLUSION**

The partially-purified fraction E showed a very strong antihyperglycemic property, it may be suggested to have mimicked mechanisms of action of tolbutamide which promoted glucose uptake by enhancing the release of insulin from the pancreatic beta cells of the rats. The antihyperglycemic property maybe as a result of the presence of flavonoids that has antioxidant effects and has been previously reported to possess antihyperglycemic. The free radical scavenging activity of fraction E was 21.71 ± 0.07% which was closely related to the total phenolic content of 256.78 ± 0.19 mg GAE/g dry weight. This result is in consonance with our previous report anti diabetic of DCM crude extract of *Coscinium fenestratum*.

**REFERENCE**

18. Babu V, Gangadevi T, Subramoniam A. Antidiabetic Activity of Ethanol Extract of Cassia Kleini leaf in Streptozotocin-induced


25. Fazio, E.N., and Pin, C.L., Mist1-null mice are resistant to streptozotocin induced β-cell damage. Biochemical and Biophysical Research Communications, 353, 2007;817-823.


50. Coskun, O, Kanter, M, Korkmaz, A, and Oter, S, Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacology Research 51, 2005; 117-123.


56. Ceriello, A, New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care, 26, 2003; 1589-1596.

