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Phytochemical screening and evaluation of antihyperglycemic effects of a local variety of Cacao (*Theobroma cacao* L.) seed extract in streptozotocin-induced hyperglycemic mice

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Abstract

This study aimed to screen phytochemical constituents and evaluate the antihyperglycemic effects of a locally-grown cacao plant, *Theobroma cacao,* in streptozotocin (STZ) induced hyperglycemic mice. Cacao seeds are commonly used in the preparation of cocoa powder and chocolate production. Fresh (FR) cacao seed extracts and fermented (FE) cacao seed extracts were tested. Cacao seeds were collected, washed, sun dried and roasted. One preparation was fermented before sun drying and roasting. Seeds were defatted in petroleum ether and extracted in aqueous ethanol. When solvent extracts of FR and FE seeds at a dose of 100mg/kg bw, 250mg/kg bw and 500mg/kg bw were given to hyperglycemic mice significant ($P<0.0001$) antihyperglycemic activity was observed. Phytochemical screening of FR and FE seed extracts revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins, terpenoids, cuomarins and xanthoproteins. Total flavonoid content (mg quercetin/g) and total steroid content (mg cholesterol/g) were higher in FE seed extracts, while total phenolic content (mg gallic/g) was higher in FR seed extracts. The antihyperglycemic activity was also exhibited during the oral glucose tolerance test (OGTT).

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Introduction

One of the leading causes of death among Filipinos is diabetes. Philippine Statistics Authority (2021) data showed that deaths due to diabetes mellitus ranked fourth in 2020 at 37,265 and had an annual increase of 7.8 percent (Mapa, 2021). Most importantly, data also revealed that diabetes mellitus already exceeded their averages in the last five years (2015-2019). Diabetes is a chronic condition marked by high glucose content in the blood, a condition also known as hyperglycemia. People with diabetes either do not produce enough insulin, the hormone that converts sugar, starches and other food into energy, or cannot use the insulin that their bodies produce. As a result, glucose builds up in the bloodstream and if left untreated, diabetes can lead to blindness, kidney disease, nerve disease, heart disease, and stroke (Khan *et al.*, 2017).

Diabetes is a metabolic lifestyle-related disease, the risk of which is increased by physical inactivity and unhealthy dietary habits. What is interesting is that Type 2 diabetes mellitus, which usually develops in adulthood, can be improved or reversed by lifestyle changes, such as adopting a healthy diet, becoming more active, and losing excess weight. Lifestyle modifications are cost-effective in preventing or delaying the onset of diabetes, with approximately 58% reduction in risk in 3 years (Chaudhury *et al.*, 2017). Nutrition advice is recommended for patients who are already diagnosed with diabetes. Cacao (*Theobroma cacao L.*) is a high value tree crop and is grown widely in the Philippines mainly for its beans and processed into cacao powder, cake and cocoa. Cacao is one of the well-known "foods of the gods" (Lima *et al.*, 2011). It is also recognized as a food for pleasure and has remained to be a main part of diet among Filipinos. The traditional uses of cacao include the preparation of chocolate and by-product beverages from dried fermented cacao beans (Magat and Secretaria, 2007). Cocoa powder and chocolates represent the products of cacao that are most commonly consumed. The potential health benefits derived from the consumption of chocolates have been attributed to the presence of the polyphenols, one of the most numerous and widely distributed groups of substances in the plant kingdom.

This present study explores the potential of cacao in addressing nutritional requirements to reduce the risk of diabetes by evaluating the anti-hyperglycemic property of cacao seed extracts in STZ-induced hyperglycemic mice in vivo. Specifically, it determined the active phytochemical components of fresh and fermented cacao seeds qualitatively and quantitatively. In addition, this study investigated the effect fresh cacao seeds extract and fermented cacao seed extract on glucose levels using *in-vivo* methods.

Materials and methods

Preparation and Extraction of Samples

One variety of cacao plant commonly grown in Cagayan was utilized in this study. Two preparations of raw (fresh and fermented) cacao beans consisting of 2000g each was tested. The first set was immediately washed then sun-dried for 3-4 days. The second set was allowed to ferment for 5 days then sun-dried. The dried seeds were then roasted in an oven for 20 min at 140°C (Ruzaidi *et al.*, 2008). Aqueous extracts were then subjected to phytochemical screening.

For the preparation of extracts for the animal study, the dried seed samples were ground using a blender then defatted with petroleum ether (b.p. 40-60°C) in a Soxhlet apparatus for 20 min at 70°C. These were air-dried to remove the solvent residue. The defatted powder residue was treated with 80% (v/v) aqueous ethanol for 2 hours, then subjected to rotary evaporator for 20 min at 70°C to remove alcohol residue. The resulting extracts were then stored in the freezer until it was used.

Phytochemical Analysis

Standard methods (Sorescu *et al.*, 2018, Ragesh *et al.*, 2014) were used to measure qualitatively and quantitatively the aqueous extract prepared from cacao seeds. Qualitative assay determined the presence and degree of colorimetric signal of eleven (11) secondary metabolites in cacao seeds, namely: 1) tannins; 2) saponins; 3) flavonoids; 4) anthraquinones; 5) alkaloids; 6) terpenoids; 7) carotenoids; 8) quinones; 9) steroids; 10) coumarins; and 11) xanthoproteins.

Test for Tannins

2mL of 5% ferric chloride was added to 1mL aqueous extract of cacao seeds. The appearance of a dark-blue or greenish-black color indicates the presence of tannins.

Test for Saponins

2mL of distilled water was added to 2mL of aqueous extract of cacao seeds and shaken for 15 min in a graduated cylinder. The presence of a foam layer indicates a positive response to the presence of saponins.

Tests for Flavonoids

1mL of 2N sodium hydroxide was mixed with 2mL aqueous extract. A concentrated yellow color indicates the presence of flavonoids.

Test for Anthroquinones

1mL aqueous extract of cacao seeds was added with a few drops of 10% ammonia solution, shaken vigorously for 30 seconds. The formation of a pink precipitate indicates the presence of anthroquinones.

Test for Alkaloids

1mL of aqueous extract of cacao seeds was mixed with 1mL of Wagner's reagent (iodine in potassium iodine solution). A reddish-brown precipitate formed indicated the presence of alkaloids.

Test for Terpenoids

2mL of chloroform was reacted to 1mL aqueous extract with and then, slowly, few drops of concentrated sulfuric acid. An interface with a reddish-brown coloration indicated the presence of terpenoids.

Test for Steroids

10mL of chloroform was mixed with 1mL aqueous extract of cacao seed, then slowly added by drips 10mL concentrated sulfuric acid. Upper layer turns red and sulfuric acid layer turns yellow-green. A red color indicated the presence of steroids

Test for Quinones

1mL of concentrated sulphuric acid was added to 1mL of the cacao seed extract. The formation of red color indicated the presence of quinones.

Test for Coumarins

1mL of cacao seed extract was placed in a small test tube, covered with filter paper soaked in 1 N NaOH, then tube was placed in boiling water for a few min. The filter paper was then examined under UV light to detect for yellow fluorescence which indicates the presence of coumarins.

Test for Xanthoproteins

1mL of extract is taken and to this few drops of nitric acid and ammonia are added. Reddish brown precipitate indicates the presence of xanthoproteins

Test for Carotenoids

1mL of extract was reacted with 85% sulphuric acid. A blue color at the interface of the mixture shows the presence of carotenoids.

Total Phenolic Content (TPC)

The Folin-CIocalteu method was used to determine the total phenolic contents of the Cacao seed extracts. Different concentrations of extracts were prepared. From each concentration 100 µL was mixed with 0.5mL of Folin–Ciocalteau reagent (1/10 dilution) and 1.5mL of Na_2CO_3 2% (w/v), then incubated for 15 min at room temperature. The absorbance of blue-colored solution of all samples was measured at 765 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/mL. Total phenolic content was expressed as mg gallic acid equivalent (mg GAE/g extract).

Total Flavonoid Content (TFC)

The total flavonoid content of the cacao seed extracts were determined by Dowd method described by Senguttuvan *et al.* (2014). Aliquot (1mL/tube) of cacao seed extracts was diluted with 200µL of distilled water followed by the addition of 150µL of sodium nitrite (5%). This was mixed with 150µL of 10% $AlCl_3$ then incubated for 10 min at room temperature. After that, 2mL of 4% sodium hydroxide solution and distilled water were added up until 10mL before incubated in dark for 2 hours at room temperature. UV-Vis spectrophotometer was used in determining the TFC at 510 nm of absorbance wavelength.

To obtain the TFC amount, the concentration of the sample was referred to as a calibration curve of quercetin solution as a standard by comparing the absorbances. The results of TFC were in the unit of mg quercetin equivalents per 1g of a dry sample.

Total Sterol Content

Estimation of total sterols was performed by Liebermann Burchard reaction. LB reagent was prepared by mixing 0.5mL of concentrated sulfuric acid and 10mL of acetic anhydride. To 1mL of each of the cacao seed extract (1mg/mL), chloroform was added to make up the volume to 5mL in a test tube. Two mL of LB reagent was added and the resulting mixture was vortexed for 10 seconds, placed in a 37°C water bath for 10 min, then allowed to cool down. These tubes were then covered with black paper and kept in the dark for 15 min to avoid any exposure to light. UV-Vis spectrophotometer was used in determining the TSC at 640 nm of absorbance wavelength. Beta-sitosterol was used as the standard to prepare a calibration curve. Total sterol content was expressed asmg cholesterol/g sample.

Experimental Treatments of Hyperglycemic Mice

Approval from the Institutional Animal Care and Use Committee (IACCUC) and DA-BAI was secured prior to the conduct of the study. ICR male or female mice (8 to 10-week old), weighing 17-35g, were obtained from an accredited company in Laguna. Mice were housed in a modified version of mouse cages fabricated out of thick plastic which were regularly cleaned. These were placed under a 12/12 hour normal light/dark cycle. The animals were fed with standard diet and water ad libitum. Mice were acclimatized in laboratory condition for one week prior to experimental procedure.

A total of 54 mice were randomly divided into 9 groups, each group consisting of 6 mice. Group I served as baseline control and received regular mice food and drinking water *ad libitum*. Group II served as diabetic control and received streptozotocin, (STZ), 130mg/kg (Deeds *et al.,* 2011) ± nicotinamide, (NIC), 210mg/kg (Novelli *et al.*, 2010) metformin, 500mg/kg. Group III served as the standard (positive control) and received NIC + STZ. Group IV to IX served as preventive/curative regimen. Specifically, Group IV-VI received NIC + STZ and cacao fresh seed extracts, 100, 250, 500mg/kg, respectively. Group VII-IX received NIC + STZ and fermented cacao seed extracts, 100, 250, 500mg/kg, respectively. All extracts were given once daily by oral route using gastric tube.

Group	Treatment
I	Baseline Control
II	Standard (Positive Control I)
III	Hyperglycemic Control
IV	Hyperglycemic + 100mg/kg FRESH (FR) cacao seed extracts
V	Hyperglycemic + 250mg/kg FR
VI	Hyperglycemic + 500mg/kg FR
VII	Hyperglycemic + 100mg/kg FERMENTED (FE) cacao seed extracts
VIII	Hyperglycemic + 250mg/kg FE
IX	Hyperglycemic + 500mg/kg FE

Evaluation of Hyperglycemic Effect of Cacao Seed Extracts in Streptozotocin-Induced Hyperglycemic mice

Induction of Hyperglycemia

ICR male/female mice, 8 to 18-week-old mice, weighing 17-30g, and fasted overnight received intraperitoneally-administered nicotinamide (210mg/kg body weight) dissolved in sterile saline solution 5 min (Novelli *et al.*, 2010) before intraperitoneal administration of 130mg/kg of streptozotocin, STZ, (Deeds *et al.,* 2011), dissolved in citrate buffer immediately before use.

Determination of Blood Sugar Level

The blood glucose level of mice was monitored before NIC and STZ induction and after induction as basis for diagnosis of insulin resistance or beta cell failure. Blood samples were collected sequentially from the tail vein 30 min and a week after induction. Blood glucose level was measured by using a glucometer. Normal mice fasted for 16 hr usually have blood glucose between 50 and 100mg/dL (2.8-5.6nM). Mice with type 2 diabetes have fasting blood glucose levels of around 150-300mg/dL (8.3-1.6 nM) (King, 2012).

Determination of Body Weight

The body weight of the mice was determined in all groups at 0, 7th, 14th and 35th day of pre and post-treatment after overnight fasting.

Glucose Tolerance Test (GTT)

A week after STZ induction, mice were subjected to glucose tolerance test (GTT) to determine impairment of glucose tolerance. The glucose load (500mg/kg) were given orally using a gavage. Glucose level was monitored after 1 hr and 2 hr. Levels between 7.8mmol/L (140mg/dl) and 11.1mmol/L (200mg/dl) indicate "impaired glucose tolerance", and any level above 11.1mmol/L (200mg/dl) confirms a diagnosis of diabetes (Zhang, 2011).

Administration of Extracts to Experimental Animals

Three concentrations (100mg/kg, 250mg/kg and 500mg/kg) of fresh (FR) cacao seed extracts and fermented (FE) cacao seed extracts were prepared by suspending extracts in 100µL saline. These were administered daily to the experimental mice by gastric intubation using a plastic tubing followed by 100µL saline flushing. Cacao extract supplementation was given after STZ and NIC induction. Blood glucose level was monitored 1 hr, 2 hr and 24 hr after cacao extract administration.

Statistical Analysis

Data are expressed as means ± SD. t-test was used to compare body weight of mice before and after cacao treatment and blood glucose level before and after STZ induction. One-way ANOVA was used to determine effect of cacao seed extracts. LSD test was used to determine any significant differences between the means at 5% level of probability.

Result and discussion

Phytochemical Qualitative Analysis

Table 1 shows the qualitative analysis of phytochemicals present in fresh and fermented cacao seeds. Alkaloids, flavonoids, steroids, tannins, saponins, terpenoids, coumarins and xanthoproteins were present in both fresh (FR) and fermented (FE) samples, except the notable absence of flavonoids (-) in FE samples. However, greater amounts of steroids, tannins and xanthoproteins were present in FE samples compared to the FR samples. In both samples, terpenoids were observed to be present in highest amounts (+++) while alkaloids, saponins and cuomarins in moderate (++) amounts. On the other hand, anthraquinones, carotenoids and quinones were also absent (-) in both fresh and fermented samples.

Quantitative Phytochemical Analysis

The results of the total phenolic content, total flavonoid content and total steroid content analysis of cacao seeds are shown in Table 2.

Generally, FE seeds have higher flavonoid content (22.4061mg quercetin/g) and steroid content (mg cholesterol/g) than FR seeds, which were 3.9152mg quercetin/g and 15.463mg cholesterol/g, respectively.

On the other hand, total phenolic content is higher in FR seeds (6.3879mg gallic acid/g) compared to FE seeds (1.3091mg gallic acid/g).

Fermentation, drying, and cocoa powder and chocolate production chemically modify most polyphenols contained in the seeds where a significant reduction of polyphenol content and antioxidant capacity during the first 2 days of fermentation have been observed (Albertini *et al.*, 2015). Althoµgh, fermentation and drying are necessary to obtain cocoa flavor and palatability, these are also responsible for greatly compromising polyphenolic content.

Table 1. Qualitative phytochemical analysis of cacao seeds (n=2).

Phytochemicals	Fresh (FR)	Fermented (FE)
Alkaloids	++	++
Flavonoids	+	-
Steroids	+	++
Tannins	++	+++
Saponins	++	++
Terpenoids	+++	+++
Cuomarins	++	++
Xanthoproteins	++	+++
Carotenoids	-	-
Quinones	-	-
Anthraquinones	-	-

+++, high; ++, moderate; +, low; -, absent

Table 2. Quantitative phytochemical analysis of cacao seeds.

Cacao Seed Extract	Total Phenolic Content (mg gallic acid/gram sample)	Total Flavonoid Content (mg quercetin/gram sample)	Total Steroid Content (mg cholesterol/gram sample)
Fresh (FR)	6.3879	3.9152	15.4636
Fermented (FE)	1.3091	22.4061	17.6091

Values represent mean ± standard deviation of triplicate sample

In Vivo Anti-hyperglycemic Effect of Theobroma cacao L. Seeds in STZ-induced Mice

Effect on Body Weight

Table 3 shows the effect of cacao seed extracts on body weight of mice during the 6-week experiment. There was an observable difference in body weight between the normal and STZ-induced mice. There was an increase in body weight of mice in Group I (untreated, baseline) and Group II (Standard, Positive control) whereas those in the STZ-induced groups (Group III-IX) decreased in body weight. Moreover, there was a significant mortality in the STZ-induced groups which can be attributed to extreme hyperglycemia as a result of STZ induction. Generally, body weights of mice increased at the end of the experiment (week 6) but there was no significant differences in body-weight changes. On the other hand, there was significant reduction ($p < 0.05$) in body weight of hyperglycemic mice in GIII (Hyperglycemic control) and GVIII (250mg/kg FE). The body weight of hyperglycemic in Group III at the end of the experiment decreased from 29.968g to 23.633g. On the other hand, the body weight of mice in Group VIII (250mg/kg FE) decreased from 29.947g to 23.233g. Results suggest that the FR and FE cacao seed extracts were able to maintain the body weight of hyperglycemic mice.

Table 3. Effect of cacao seed extract on body weight of STZ-induced hyperglycemic mice.

Group	Treatment	Number	Body Weight (g)		t-value
			Week 0	Week 5	
I	Baseline control	4	23.62333	27.60000	-1.62191ns
II	Standard control (STZ+NIC+met)	4	26.01600	29.53333	-1.73943ns
III	Hyperglycemic control (STZ+NIC)	3	29.96833	23.63333	3.66574*
IV	Hyperglycemic + 100mg/kg FR	3	28.30167	35.80000	-2.04197ns
V	Hyperglycemic + 250mg/kg FR	3	29.29333	23.00000	1.97986ns
VI	Hyperglycemic + 500mg/kg FR	3	29.72833	28.03333	0.40125ns
VII	Hyperglycemic + 100mg/kg FE	3	28.82667	21.16667	2.29331ns
VIII	Hyperglycemic + 250mg/kg FE	3	29.94667	23.23333	2.46590*
IX	Hyperglycemic + 500mg/kg FE	3	30.19000	27.73333	1.15647ns

ns = not significant; * - significant at α = .05

Body weight was monitored in this study as weight loss has been associated with mechanisms of lowering blood glucose levels (Knudsen, 2010 as cited by King 2012). In this study, no significant decrease in body weight was observed in the FR and FE groups sμggesting that both fresh and fermented cacao extracts are able to normalize the weight loss caused by STZ as observed in Hyperglycemic control mice (GIII).

Effect of SZT induction and Glucose Tolerance Test (GTT) on Blood Glucose Level.

Table 4 showed the blood glucose level of mice before and after STZ induction. Initial plasma glucose level of mice before STZ induction was in the range of 91.74mg/dL – 116.93mg/dL. After induction, the mean plasma glucose level in normal (GI), 103.1667mg/dL and positive control (GII), 130.8333mg/dL were not significantly different from the initial plasma glucose.

However, in the STZ-induced groups, glucose level increased significantly within the range of 148.1667mg/dL – 193.3333mg/dL after 2 hrs of induction and mice remained hyperglycemic for 5 weeks. After GTT at week 6, glucose levels in hyperglycemic mice remained within the range indicating insulin resistance and/or beta cell failure. Glucose tolerance test (GTT) was used to associate

beta cell function of the experimental mice after STZ induction. Results of this study clearly demonstrated that oral administration of 100mg/kg, 250mg/kg and 500mg/kg of FR and FE cacao seed extracts exhibited a significant decrease ($p < 0.05$) in plasma glucose levels in STZ-induced hyperglycemic mice compared to the positive control group but showed comparable potential with the Hyperglycemic control group.

Table 4. Blood Glucose Level Before and After STZ Induction.

Group	Treatment	Number	Mean Glucose Level mg/dL		t-value
			Before STZ Induction	1 hr after Induction	
I	Baseline control	6	91.74	103.1667	-0.70747**ns**
II	Standard control (STZ+NIC+met)	6	113.16	130.8333	-1.66875**ns**
III	Hyperglycemic control (STZ+NIC)	6	91.26	148.1667	-4.26857*
IV	Hyperglycemic + 100mg/kg FR	6	111.66	192.8333	-3.34764*
V	Hyperglycemic + 250mg/kg FR	6	96.3	193.3333	-5.06544*
VI	Hyperglycemic + 500mg/kg FR	6	116.13	193.1667	-4.44946*
VII	Hyperglycemic + 100mg/kg FE	6	103.8	164.5	-5.08945*
VIII	Hyperglycemic + 250mg/kg FE	6	94.89	165.3333	-3.03902*
IX	Hyperglycemic + 500mg/kg FE	6	105.84	177	-3.7843*

ns = not significant; * - significant at α = .05

In this study, mice with a fasting blood glucose level of 150mg/dL and above was considered hyperglycemic. As cited by King (2012), mice tend to have higher blood glucose concentrations than humans, and it has been suggested that a non-fasting blood glucose concentration over 250mg/dL^{-1} (13.8mm) or preferably a chronic elevation over 300mg/dL^{-1} (16.7mm) is appropriate to consider a mouse hyperglycemic. Normal mice fasted for 16 hr during the entire dark period when they usually feed and usually have blood glucose of between 50 and 100mg/dL^{-1} (2.8–5.6mm), whereas mice with type 2 diabetes will have fasting blood glucose levels of around 150–300mg/dL^{-1} (8.3–16.7mm).

In Vivo Anti-hyperglycemic Effect of Cacao Extracts

Administration of cacao extracts was conducted in week 6 and results are shown in Table 5. Results showed that blood glucose level are still significantly high after GTT among the hyperglycemic groups compared to the normal group (GI). Mice in Group III (GIII, hyperglycemic control) had significantly decreased blood glucose level (78mg/dL) after GTT indicating a developing hypoglycemia, hence, were excluded in the subsequent cacao treatment.

Administration of cacao seed extracts significantly decreased the blood glucose level of hyperglycemic mice within 24 hr of cacao extract feeding. However, the decrease in glucose level was immediately apparent at 1 hr after the mice were fed with cacao extract. In normal mice (GI), no significant differences in glucose levels were observed between week 0 and 5 (Table 4). For hyperglycemic mice fed with FR cacao seed extract, plasma glucose levels decreased significantly ($p < 0.05$) after feeding with 100mg/kg FR (GIV) at 38.628% at the end of experiment (Table 5).

Table 5. Effect of Cacao Seed Extracts on Blood Glucose Level of Hyperglycemic Mice.

Group	Treatment	Blood Glucose Level mg/dL	Percentage of decrease of Blood Glucose Level mg/dL after Cacao Extract Treatment		
		After GTT	1 hr	2 hr	24 hr
I	baseline control	105.667	-	-	-
II	Standard control (STZ+NIC+met)	155.667	39.238^{a}	52.939^{a}	69.065^{a}
III	Hyperglycemic control (STZ+NIC)	78.000	-	-	-
IV	Hyperglycemic + 100mg/kg FR	144.000	38.628^{a}	25.246^{b}	33.577^{a}
V	Hyperglycemic + 250mg/kg FR	147.333	0.000	0.000	26.44^{a}
VI	Hyperglycemic + 500mg/kg FR	164.000	0.000	3.608^{b}	21.320^{a}
VII	Hyperglycemic + 100mg/kg FE	120.000	22.655^{b}	12.977^{b}	-
VIII	Hyperglycemic + 250mg/kg FE	149.667	0.000	7.924^{b}	-
IX	Hyperglycemic + 500mg/kg FE	158.000	17.475^{bc}	20.198^{b}	22.282^{a}

Results are expressed as Means ± SD for three mice per group. Values followed by the same superscript are not statistically different (P = 0.05; Analysed by ANOVA followed by LSD test).

This is comparable to the effect of metformin given to the positive control group (GII). In hyperglycemic mice fed with FE cacao seed extract, the percentage of decrease in blood glucose level is generally lower than those fed with FR cacao seed extract. In hyperglycemic mice fed with 100mg/kg FE (GVII) and 500mg/kg FE (GIX), the percentage reduction of blood glucose level was 22.655% and 17.457%, respectively. The effect of FE groups tends to be lower compared to FR, and it was significantly different from each other, indicating that at 1hr after cacao feeding, FR cacao seed extracts are more effective than FE cacao seed extracts.

There was further reduction in blood glucose level at 2 hr after cacao feeding in most treatments with hyperglycemic mice in Group VI and Group VIII responding to 500mg/kg FR and 250mg/kg FE with percent decrease of 3.608% and 7.924%, respectively. However, at the end of 24 hrs, the decrease in blood glucose levels in most treatments is not significantly different from each other. This indicates that the effect of FE seed extracts regardless of the concentration were comparable with each other and with the Hyperglycemic control (Group II) at 24 hr. On the other hand, only Group IX (100mg/kg) among those fed with FE seed extracts, was effective in lowering blood glucose level.

The observed effectiveness of cacao seed extracts in lowering blood glucose level could be related to the polyphenols in which the cacao seeds are known to be rich. Polyphenols were also reported to be the potential bioactive component for hypoglycemic properties (Ivora *et al*., 1989; Manickan *et al*., 1997 as cited by Ruzaidi *et al*., 2008). These components were reported to demonstrate marked antioxidant activity. In the present study, a significant reduction of plasma glucose levels in the group treated with FR or FE cacao seed extracts could be attributed to the antioxidant properties of the cacao seed extracts. Antioxidant compounds are well known to possess free radical scavenging activity. Thus, it is suggested that the glucose-lowering activity by FR or FE could be due to polyphenols that inhibit or suppress the generation of free radical by STZ in diabetic mice.

In addition, flavonoids have also been reported to regenerate damaged β-cells in alloxan diabetic rats (Agwaya *et al*, 2016). This might also be the same mechanism by which the FR or FE cacao seed extracts could also regenerate the damaged of β-cells cause by STZ.

Flavonoids are potent water-soluble oxidant and free-radical scavenger, which prevent oxidative stress, and hence, have strong anticancer activity. It has also been found to help in managing diabetes-induced oxidative stress. Saponins possess the unique property of precipitating and coagulating red blood cells and steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response. Terpenoids are also known to possess anti-hyperglycemic properties.

In the current study, the presence of these secondary metabolites demonstrated that cacao has several medicinal potential as anti-diabetes, anti-cancer, anti-immunomodulatory, anti-inflammatory, and cholesterol-reducing properties. It also suggests that both fresh and fermented cacao seeds provide the same potential in terms of their terpenoid content, alkaloid content, saponin content and cuomarin content. Fermented seeds could provide better potential in terms of steroid and tannin contents while fresh seeds have flavonoids but not fermented seeds.

Conclusion

The results of this study demonstrated that *Theobroma cacao* seed extracts had the ability to decrease level of blood sugar in STZ-induced hyperglycemic mice. The best results were obtained at a concentration of 100mg/kg FR after a short time treatment. These actions were exhibited probably due to cumulative effect of phytochemicals present in the extract including phenols, anthraquinones, alkaloids, tannins, terpenoids, flavonoids, saponins and sterols. These phytochemicals reduced blood glucose levels probably through enhancing secretion in partially damaged pancreas.

Recommendations

Further investigation should be done in order to isolate the constituents responsible for the blood sugar lowering effect of this plant through bioassay guided fractionation. Other parameters such as determination of insulin levels in plasma of hyperglycemic mice may be conducted to provide more support to the finding.

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