Hypoglycaemic Properties of Malaysian Cocoa (*Theobroma Cacao*) Polyphenols Extract

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ABSTRACT

The objective of the study was to investigate the hypoglycaemic properties of Malaysian cocoa (*Theobroma cacao*) polyphenols extract *in-vivo* and *in-vitro*. Cocoa extract (CE) (containing 190 - 286 mg total polyphenol per gram extract) was prepared from fermented and roasted (140 °C, 20 min) beans by extracting with 80% ethanol in the ratio of 1 to 10. For the *in-vivo* study, the CE was given in three dosages (1%, 2%, and 3%) to groups of normal and diabetic rats for a period of 4 weeks by force-feeding. Results showed that dosages of 1% and 3% CE significantly reduced ($p < 0.05$) the plasma glucose levels in the diabetic rats. BRIN-BD11 cell lines were used to evaluate the effect of CE on insulin secretions. An *in-vitro* study demonstrated that CE at a concentration of 0.1 mg/ml significantly increased ($p < 0.05$) insulin secretion compared to the control. The results of this study showed that Malaysian cocoa polyphenol extract have the potential of being a hypoglycaemic agent. Further studies are needed to elucidate the mechanism action of polyphenols present in CE, which could be utilised to lower the plasma glucose levels and stimulate insulin secretion in patients with Diabetes Mellitus Type 2.

KEYWORDS: · cocoa beans · diabetes . hypoglycaemic . polyphenol-rich extract.

INTRODUCTION

Diabetes mellitus is a non-communicable disease, which is considered one of the five leading causes of death in the world today. Recently, the search for appropriate hypoglycaemic agents has focused on plants used in traditional medicine, partly because of leads provided by traditional medicine to natural products that it may be better treatments than currently used drugs[1, 2, 3]. Drug such as sulphonylureas, lead to higher risk of hypoglycaemia, and metformin brings a higher risk of lactic acidosis[4]. Due to side effects of these drugs, many studies have been conducted to explore natural products derived from plants which have potential hypoglycaemic effect[5,6,7,8].

Besides the traditional medicinal plants, cocoa beans were thought to have fearsome magical powers by the Mayas and were carefully used in rituals, religious ceremonies and healings by priests. The Mayas used cocoa medicinally as a treatment for fever, coughs and to help dispel even discomfort during pregnancy. After the 16th century conquest of Central America by Spain, Cortes introduced cocoa to Europe, where it was typically viewed as a healthy and nutritious beverage[9]. Cocoa beans were recently recognised as a rich source of polyphenols, specifically procyanidins. Zumbe[10] reported that total polyphenol content of the cocoa bean is about 6-8% by weight of the dry bean. Some of the earliest studies on procyanidins also established that the major flavanoids of cocoa bean are catechin, epicatechin, and the dimers epicatechin-(4β→8)-catechin (procyanidin B-l) and epicatechin-(4β→8)-epicatechin (procyanidin B-2) and the trimer [epicatechin-(4β→8)$_2$ epicatechin (procyanidin C-l)[11,12]. On fermentation and roasting, these procyanidins are converted to the largely insoluble red-brown material (tanning) resulting in the characteristic colour and taste of chocolate[13].

Polyphenols have been researched for decades, mostly because of their antioxidant properties[14,15,16]. The consumption of flavonoids, including those found in cocoa and other cocoa products have been associated with reduction in the risk of heart diseases, having potentially beneficial effects on inflammatory activity, and being cancer-protective agents[17]. Recent research showed the ability of cocoa to reduce platelet activation *in vivo*, which can cause atherosclerosis[18]. Oxidative stress is well known to be an important factor leading to chronic disease such as diabetes mellitus and atherosclerosis. Sabu et al.[19] reported that administration of green tea polyphenols was found to reduce oxidative stress and serum glucose tolerance in alloxan-diabetic rats. A similar finding was found by Ceriello et al.[20] for red wine in humans. Our study on oral glucose tolerance test (OGTT) of diabetic rats showed that cocoa extract could reduce the blood glucose levels[21]. However, limited studies have been done on the hypoglycaemic effect of polyphenol-rich cocoa extract. Therefore, this study was designed to assess the effectiveness of polyphenol-rich cocoa extract in reducing hyperglycaemia based on *in-vivo* (animal) and *in-vitro* studies.

MATERIALS AND METHODS

Preparation of Extract from Cocoa Beans

Fermented and dried Malaysian cocoa beans were purchased from KL-Kepong Cocoa Products Sdn. Bhd., Port Klang, Selangor, Malaysia. The beans were roasted using an air-oven for 20 min at 140ºC[22]. After cooling to room temperature, the beans were deshelled using a cocoa breaker (Limprimita, John Gordon & Co., UK). The broken nibs were ground, then defatted with petroleum-ether (b.p 40-60ºC) using a Soxhlet apparatus in order to remove cocoa

butter. The defatted sample was air-dried to remove solvent residue. Extract was prepared by extracting the defatted powder with 80% (v/v) ethanol for 2 hr. The ethanol residue was removed from the extract using a rotary evaporator (Buchi Rotavor R-200, Switzerland) under reduced pressure for 20 min at 70ºC and the sample was then lyophilised.

Determination of Total Phenolic Content

The total phenolic content was determined according to the Folin-Ciocalteu method[23]. Briefly, the cocoa extract was dissolved in 80% (v/v) ethanol and then centrifuged (Rotofix 32, Hettich Zentrifugen, Germany) at 1000 x g for 15 min. A total of 100 µl of the supernatant was mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. Then, 0.75 ml of sodium carbonate (60g/L) solution was added to the mixture. After 90 min standing at room temperature, absorbance was read at 725 nm using a UV-Vis spectrophotometer (Anthelie Advanced 5, Secomam, France). A standard calibration curve was obtained from 0.02 - 0.12 mg/ml of (-)-epicatechin (Sigma Co., St. Louis, USA). Results were expressed as epicatechin equivalents in milligrams per gram extract.

Animal study

Ninety male (n = 90) Sprague-Dawley rats (150-200 g initial weights) were purchased from Syarikat Usaha Cahaya Sdn. Bhd., Batu Caves, Selangor, Malaysia. This study has been approved by the Animal Care and Use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The rats were housed in individual plastic cages with stainless steel covers and kept at room temperature (24-28˚C) under 12 h dark-light cycle. Rats were allowed free access to their respective diets and water. All rats were allowed 1 week to

adapt to the environment before being given the treatment. The experiment was carried out over 4 weeks, and body weights, food intakes, and blood glucose levels were recorded weekly. The rats were divided into nine groups each consisting of ten rats as follows:

Group 1: Normal rats administered saline solution

Group 2: Normal rats administered 1% CE

Group 3: Normal rats administered 2% CE

Group 4: Normal rats administered 3% CE

Group 5: Diabetic rats administered saline solution

Group 6: Diabetic rats administered 1% CE

Group 7: Diabetic rats administered 2% CE

Group 8: Diabetic rats administered 3% CE

Group 9: Diabetic rats administered glybenclamide (100 mg/ml in saline solution)

CE was suspended in 0.9% (w/v) saline solution, then given daily (1 ml/ 100 g body weight) to the experimental rats by gastric intubation, using a force feeding needle. The rats were treated with their respective treatments after induction of diabetic conditions with streptozotocin (STZ).

Induction of Diabetes

The substance used for inducing hyperglycemia in the rats is STZ (Sigma Co., St. Louis, USA). After an overnight fast, rats were injected intravenously with 45 mg/kg body weight of STZ dissolved in 0.05 M citrate buffer, pH 4.5. Normal rats were injected with the same volume of 0.05 M citrate buffer. Three days after STZ injection, blood was collected from the tail vein for checking blood glucose levels using a Blood Glucose Sensor (MediSense Optium, Abbot

Laboratories, USA) to ensure that the rats had diabetes. Only rats with fasting blood glucose of 15 mmol/l and above were included in this study.

Determination of Plasma Glucose Levels

At 0 and 4 weeks of experiment, 5 ml blood was collected from all rats through intracardiac and placed into a vacutainer tube. Then, the samples were centrifuged (Universal 32®, Hettich Zentrifugen, Germany) for 10 min at 1000 x g at room temperature. The supernatant was collected and kept at -20°C for determination of plasma glucose levels using a Chemistry Analyzer (Hitachi 902 Automatic Analyzer, Japan).

Measurement of insulin secretion

For measurement of insulin-release from cell monolayers, BRIN-BD11 cells were seeded into 24-multiwell plates (Nunc, Roskilde, Denmark) at a density of 2.5 x 10^5 cells per well in RPMI-1640 medium and allowed to attach during overnight incubation (37 ºC; 5% CO_2). After washing with PBS buffer, pre-incubations were performed using the Krebs-Ringer bicarbonate buffer pH 7.4 for 40 min (37 ºC; 5% CO_2). Then, the cells were incubated at 37 ºC under 5% CO_2 with KRB buffer (pH 7.4) supplemented with different concentrations of CE. Following 30 min of incubation, the aliquots were removed from each well, then centrifuged at 1000 x g for 1 min at room temperature (Centrifuge 5414, Eppendorf, Germany), and supernatants were kept at -20 ºC before insulin radioimmunoassay.

Statistical analysis

Data were expressed as means ± S.E.M. One-way ANOVA was applied to find the difference between the groups. A Duncan's multiple range test was used to find the significant

difference among the means. Results are considered significantly different at the level of $p < 0.05$.

RESULTS

Table 1 showed the effect of CE on body weights in normal and diabetic rats. Body weights of all normal rats were significantly increased ($p < 0.05$) at the end of the experiment (week 4). No significant difference in body-weight changes were observed among the groups of normal rats. On the other hand, there were significant decreases ($p < 0.05$) in body weights of DC, DCE1, DCE2 and DG groups in diabetic rats. The weight loss in DC, DCE1, DCE2 and DG groups at the end of the experiment were 31%, 17%, 24% and 20% compared to week 0, respectively. The lost in body weights were not significantly different in DCE3 group compared to their initial weights. In addition, the mean body weight in DCE3 group was significantly higher ($p < 0.05$) compared to that of DC, DCE2 and DG groups.

The initial plasma glucose levels of rats were in the range of 3.1 - 8.2 mmol/l. In normal rats, no significant difference in glucose levels were observed between week 0 and 4 (Fig. 1). For diabetic rats, plasma glucose levels had decreased significantly ($p < 0.05$) by feeding with 1% and 3 % CE (DCE1 and DCE3) at the end of experiment (Fig. 2). The reduction in percentages of plasma glucose levels in DCE1 and DCE3 groups were about 47% and 57%, respectively at the end of experiment. Plasma glucose levels in DCE2 and DG groups tend to be lower compared to DC, but it was not significantly different.

Figure 3 showed insulin secretion of BRIN-BD11 rat pancreatic cell-lines at various concentrations of CE. In this study, five concentrations (2.0, 1.0, 0.5, 0.1 and 0.05 mg/ml) of CE were used. CE at concentration of 0.1 mg/ml showed a significant increase ($p < 0.05$) in insulin

secretion compared to the control. BRIN-BD11 cells treated with 2.0 mg/ml CE was significantly lower ($p < 0.05$) compared to the control in insulin secretion.

Results showed that CE, in the concentration range from 0.05–0.1 mg/ml, is capable of enhancing basal insulin secretion (at 2 mmol/l glucose) about 9-15% compared to the untreated BRIN-BD11 cell lines (control). In addition, CE at 0.05 mg/ml showed a slight increase in insulin secretion. However, insulin secretion at concentrations of 0.5, 1.0 and 2.0 mg/ml was lower compared to the control.

DISCUSSION

Very limited published data were available on hypoglycaemic effect of cocoa products based on *in-vitro* or *in-vivo* studies. This study was initiated to investigate the effect of CE on glucose levels using *in-vitro* (cell culture) and *in-vivo* (animal models) methods. For diabetic models, STZ was used to induce diabetic conditions in rats. Elsner et al.[24] suggested that STZ induces hyperglycaemia through damaging DNA in the nuclei of pancreatic β-cells by alkylation, leading to an increase in poly (ADP-ribose) synthase. The increase in this enzyme activity results in a drastic decrease in nicotinamide adenine dinucleotide (NAD) concentrations of the β-cells, then a decrease in the number of β-cells and death of the cells. All these changes may induce dysfunction of the pancreas in insulin secretion. According to Thulesen et al.[25], STZ transported into β-cells through glucose transporter GLUT-2 located on their cell membranes, and this will injure the mitochondria. This will inevitably lead to a reduction of ATP generation through electron transport system and an increase in ADP concentrations. Subsequent degradation of ADP provides hypoxanthine, a substrate of xanthine oxidase (XOD). When XOD reaction takes

place in β-cells, oxygen radicals which are free radicals will be produced, resulting in cell damage and the onset of the diabetic conditions.

In this study, rats were administered orally (force-feeding) with their respective treatment daily. Results revealed that all normal rats showed an increase in body weights. It could be suggested that CE did not affect the growth of normal rats. In diabetic rats, a general decrease in body weights were observed after the injection of STZ. In the DCE3 group, however, no significant decrease was observed, suggesting that CE could normalize the weight loss caused by STZ. Although food and water intakes were not measured in this study, CE seemed to suppress polyphagia and polydypsia in the diabetic rats. This study clearly demonstrated that oral administration of CE at 1% and 3% exhibited a significant decrease ($p < 0.05$) in plasma glucose levels in STZ-induced diabetic rats compared to the control groups.

Hypoglycaemic effects of some plants have been reported due to polyphenols[8,26]. Since cocoa beans is well known rich in polyphenols, it is possible that the hypoglycaemic effect could be related to these components. Polyphenols are reported to be the potential bioactive component for hypoglycaemic properties[27,28]. These components were reported to demonstrate marked antioxidant activity[11]. A significant reduction of plasma glucose levels in the group treated with CE may be due to the antioxidant properties of the CE. Antioxidant compounds are well known to possess free radical scavenging activity. Thus, it is suggested that glucose-lowering activity by CE could due to polyphenols that inhibit or suppress the generation of free radical by STZ in diabetic rats. In addition, flavanoids also have been reported to regenerate damaged β-cells in the alloxan diabetic rats[29]. Therefore, it is postulated that CE could also regenerate or rejuvenate the damaged of β-cells cause by STZ.

Based on the previous studies of hypoglycaemic effect of plants and herbs, several mechanisms of lowering blood glucose were proposed. One of the proposed mechanisms of this

blood glucose lowering activity may be due to stimulation of peripheral glucose utilisation, especially in muscle and adipose tissue. Several medicinal plants have been reported to restore activity of key enzymes of glucose and glycogen metabolism which are strongly disturbed in STZ diabetic rats[30]. Several studies on plants and herbs compounds also revealed that these compounds have the properties of mimicking the insulin action, preferably by interacting with the glycoprotein residues of the insulin receptor and enhanced glucose uptake by cells[31], which is called insulin-like activity.

Insulin is essential for maintaining glucose homeostasis and regulating carbohydrate, lipid, and protein metabolism[32]. After a meal, blood glucose levels increase and insulin plays a major role in keeping blood glucose levels within a narrow range. The β-cells of the pancreas respond to increasing glucose levels by releasing insulin into the blood. Insulin affects glucose turnover in many tissues. In liver, insulin inhibits glycogenolysis and gluconeogenesis[33]. In skeletal muscle, that accounts for approximately 75 % of insulin-mediated glucose disposal after a glucose challenge[34,35], insulin promotes glucose uptake into cells.

It is strongly suggested that CE exhibited significant antihyperglycaemic activities in STZ-induced rats. However, the mechanism of the hypoglycaemic effect of the CE is not clear. Although pancreatic regeneration after partial resection or pancreatic injury has been demonstrated in animal models, whether regeneration occurs or not in human pancreas is still controversial. Studies by Tsiotos et al.[36] revealed that human pancreas does not regenerate after partial anatomical resection. Yanardag et al.[37] reported that plant therapy cannot regenerate β-cells of the endocrine pancreas. Several published reports showed that antihyperglycaemic plants may affect the circulating insulin level[38,39]. This study suggests that the mechanism on hypoglycaemic effect of CE could also be due to the surviving β-cells which are still able to release more insulin.

Studies showed that certain plants (*Agrimony eupatoria* and *Eucalyptus globules*) could improved insulin secretion using BRIN-BD11 cell lines[40,41]. Based on the possibility that CE might stimulate the release of insulin from β-cells, BRIN-BD11 cell lines were used to evaluate the CE effect on insulin secretion. Incubations were performed with glucose-responsive BRIN-BD11 cells[42] to investigate the possible effects of CE on insulin secretion *in vitro*. The results showed that CE at 0.1 mg/ml was able to stimulate the insulin secretion in BRIN-BD11 cell lines. The study indicated that the CE have the potential of reducing high blood glucose levels through enhancing insulin secretion in partially damaged pancrease.

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REFERENCES

1. Lu XJ, Chen XM, Fu DX, Cong W, Ouyang F: Effect of *Amorphophallus Konjac* oligosaccharides on STZ-induced diabetes model of isolated islets. Life Sci 2002; 72(6): 711-719.

2. Jang MH, Chang HK, Shin MC, Lee TH, Kim YP, Kim EH, Kim CJ: Effect of ginseng radix on c-gos expression in the hippocampus of streptozotocin-Induced diabetic rats. J Pharmacol Sci 2003; 91: 149-152.

3. Kar A, Choudhary BK, Bandyopadhyay NG: Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003; 84: 105-108.

4. Shenfield, GM: Drug interactions with oral hypoglycaemic drugs. Aust Prescr 2001; 24: 83-85.

5. Kamtchouing P, Sokeng SD, Moundipa PF, Watcho P, Jatsa HB, Lontsi D: Protective role of *Anacardium occidentale* extract against streptozotocin-induced diabetes in rats. J Ethnopharmacol 1998; 62: 95-99.

6. Jayakar B, Suresh B: Antihyperglycemic and hypoglycemic effect of *Aporosa lindleyana* in normal and alloxan induced diabetic rats. J Ethnopharmacol 2003; 84: 247-249.

7. Ladeji O, Omekarah I, Solomon M: Hypoglycemic properties of aqueous bark extract of *Ceiba pentandra* in streptozotocin-induced diabetic rats. J Ethnopharmacol 2003; 84: 139-142.

8. Maghrani M, Lemhadri A, Jouad H, Michel JB, Eddouks M: Effect of the desert plant *Retama raetam* on glycaemia in normal and streptozotocin-induced diabetic rats. J Ethnopharmacol 2003; 87: 21-25.

9. Dillinger TL, Barriga P, Escarcega S, Jimenez M, Salazar Lowe D, Grivetti LE: Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. J Nutr 2000; 130: 2057S-2072S.

10. Zumbe A: Polyphenols in cocoa: are there health benefits? BNF Nutr Bull 1998; 23: 94-102.

11. Osakabe N, Yamagishi M, Sanbongi C, Natsume M, Takizawa T, Osawa T: The antioxidative substances in cacao liquor. J Nutr Sci Vitaminol 1998; 44: 313–321.

12. Porter LJ, Ma Z, Chan G: Cacao procyanidins: major flavonoids and identification of some minor metabolites. Phytochemistry 1991; 30: 1657–1663.

13. Pettipher GL: An improved method for the extraction and quantification of anthocyanins in cocoa beans and its use as an index of the degree of fermentation. J Sci Food Agri 1986; 37: 289–296.

14. Wiseman SA, Balentine DA, Frei B: Antioxidant in tea. Crit Rev Food Sci Nutr 1997; 37: 705-718.

15. Sanbongi C, Osakabe N, Natsume M, Takizawa T, Gomi S, Osawa T: Antioxidative polyphenols isolated from *Theobroma cacao*. J Agri Food Chem 1998; 46: 452–457.

16. Netzel M, Strass G, Bitsch I, Konitz R, Christmann M, Bitsch R: Effect of grape processing on selected antioxidant phenolics in red wine. J Food Eng 2003; 56: 223-228.

17. Borchers AT, Keen CL, Hannum SM, Gershwin ME: Cocoa and chocolate: composition, bioavailability, and health implications. J Med Food 2000; 3: 77-105.

18. Rein D, Paglieroni TG, Wun T, Pearson DA, Schmitz HH, Gosselin R, Keen CL: Cocoa inhibits platelet activation and function. Am J Clin Nutr 2000; 72: 30-35.

19. Sabu MC, Smitha K, Kuttan R: Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. J Ethnopharmacol 2002; 83: 109-116.

20. Ceriello A, Bortolotti N, Motz E, Lizzio S, Catone B, Assaloni R, Tonutti L, Taboga C: Red wine protects diabetic patients from meal-induced oxidative stress and thrombosis activation: a pleasant approach to the prevention of cardiovascular disease in diabetes. Eur J Clin Invest 2001; 31: 322-328.

21. Amin I, Faizul HA, Ruzaidi A: Effect of cocoa powder extract on plasma glucose levels in hyperglycaemic rats. Nutr Food Sci 2004; 34: 116-121.

22. Jinap S, Wan Rosli WI, Russly AR, Nordin LM: Effect of roasting time and temperature on volatile component profiles during nib roasting of cocoa bean. J Sci Food Agri 1998; 77: 441-448.

23. Velioglu YS, Mazza G, Gao L, Oomah BD: Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agri Food Chem 1998; 46: 4113-4117.

24. Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S: Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia 2000; 43(12): 1528-1533.

25. Thulesen J, Orskov C, Holst JJ, Poulsen SS: 1997. Short-term insulin treatment prevents the diabetogenic action of streptozotocin in rats. Endocrinology 1997; 138(1): 62-68.

26. Sachdewa A, Khemani LD: Effect of *Hibiscus rosa sinensis Linn*. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. J Ethnopharmacol 2003; 89(1): 61-66.

27. Ivora MD, Paya M, Villar A: A review of natural products and plants as potent antidiabetic drugs. J Ethnopharmacol 1989; 27: 243-275.

28. Manickam M, Ramanathan M, Jahromi MA, Chansouria JP, Ray AB: Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat Prod 1997; 60(6): 609-610.

29. Chakravarty BK, Gupta S, Gambhir SS, Gode KD: The prophylactic action of (-)-epicatechin against alloxan induced diabetes in rats. Life Sciences 1982; 29: 2043-2047.

30. Ugochukwu NH, Babady NE: Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Science 2003; 73(15): 1925-1938.

31. Gray AM, Flatt PR: Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant *Coriandrum sativum* (coriander). Br J Nutr 1999; 81(3): 203-209.

32. Saltiel AR, Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414(6865): 799-806.

33. DeFronzo RA, Ferrannini E: Regulation of hepatic glucose metabolism in humans. Diabetes Metab Rev 1987; 3(2): 415-459.

34. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981; 30(12): 1000-1007.

35. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J: Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985; 76(1): 149-55.

36. Tsiotos GG, Barry MK, Johnson CD, Sarr MG: Pancreas regeneration after resection: does it occur in humans? Pancreas 1999; 19(3): 310-313.

37. Yanardag R, Bolkent S, Tabakoglu-Oguz A, Ozsoy-Sacan O: Effects of *Petroselinum crispum* extract on pancreatic B cells and blood glucose of streptozotocin-induced diabetic rats. Biol Pharm Bull 2003; 26(8): 1206-10.

38. Esmaeili MA, Yazdanparast R: Hypoglycaemic effect of *Teucrium polium*: studies with rat pancreatic islets. J Ethnopharmacol 2004; 95(1): 27-30.

39. Gray AM, Flatt PR: Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). J Endocrinol 1998; 157(2): 259-266.

40. Gray AM, Flatt PR: Actions of the traditional anti-diabetic plant, *Agrimony eupatoria* (agrimony): effects on hyperglycaemia, cellular glucose metabolism and insulin secretion. Br J Nutr 1998; 80: 109-114.

41. Gray AM, Flatt PR: Antihyperglycemic actions of *Eucalyptus globulus* (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J Nutr 1998; 128: 2319–2323.

42. McClenaghan NH, Gray AM, Barnett CR, Flatt PR: Hexose recognition by insulin-secreting BRIN-BD11 cells. Biochem Biophys Res Commun 1996; 223(3): 724-728.

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TABLE 1. EFFECT OF COCOA EXTRACT (CE) ON BODY WEIGHT OF RATS

Group	*n*	*Body weight (g)*	
		week 0	*week 4*
Normal control (NC)	8	184.4 ± 5.7^{a}	279.8 ± 8.0^{cd}
Normal + 1% CE (NCE1)	8	182.2 ± 6.2^{a}	282.6 ± 7.3^{d}
Normal + 2% CE (NCE2)	9	184.1 ± 7.3^{a}	277.9 ± 12.0^{cd}
Normal + 3% CE (NCE3)	10	180.4 ± 7.5^{a}	265.1 ± 12.0^{cd}
Diabetes control (DC)	6	256.4 ± 12.1^{cd}	176.4 ± 18.3^{a}
Diabetes + 1% CE (DCE1)	4	254.5 ± 10.6^{cd}	212.5 ± 2.5^{ab}
Diabetes + 2%CE (DCE2)	5	249.0 ± 12.1^{bcd}	189.0 ± 23.7^{a}
Diabetes + 3% CE (DCE3)	5	254.7 ± 10.8^{cd}	241.8 ± 26.1^{bc}
Diabetes + Glibenclamide (DG)	4	248.6 ± 10.2^{bcd}	197.8 ± 7.1^{a}

Values were expressed as means ± S.E.M. Different letters indicated significant difference at the level of $p < 0.05$. Body weights were measured at about 0900 hr every week.

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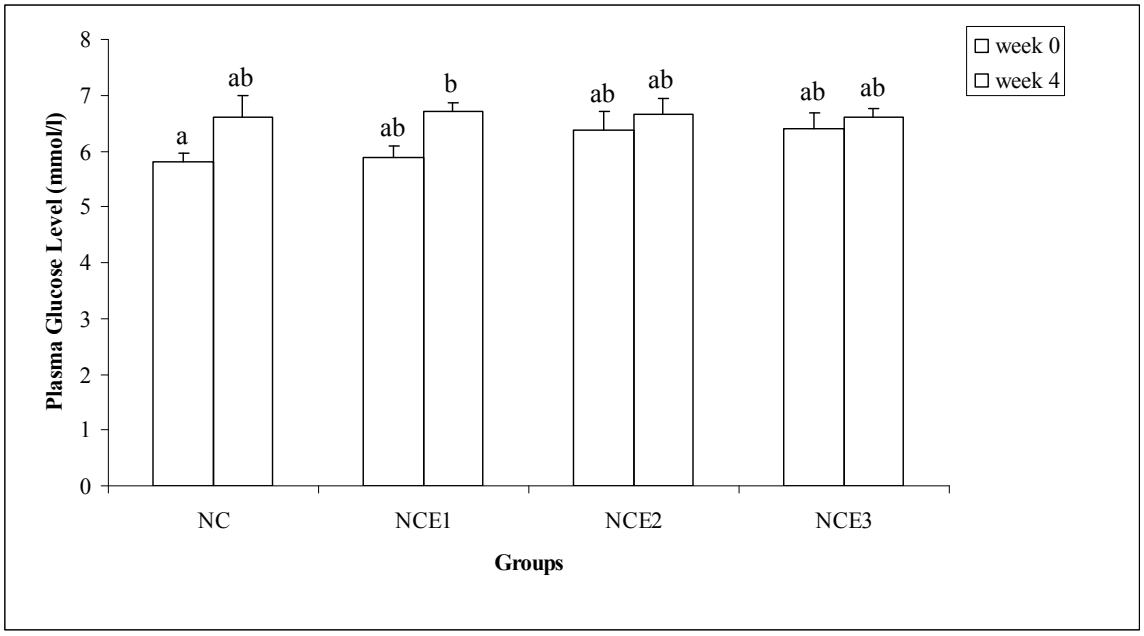
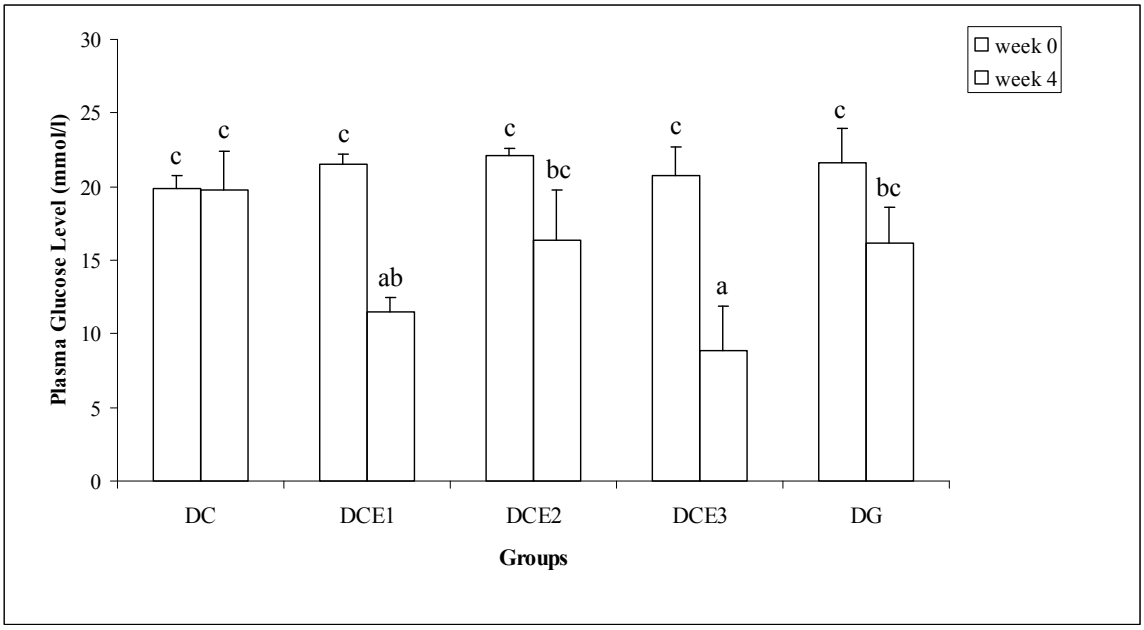


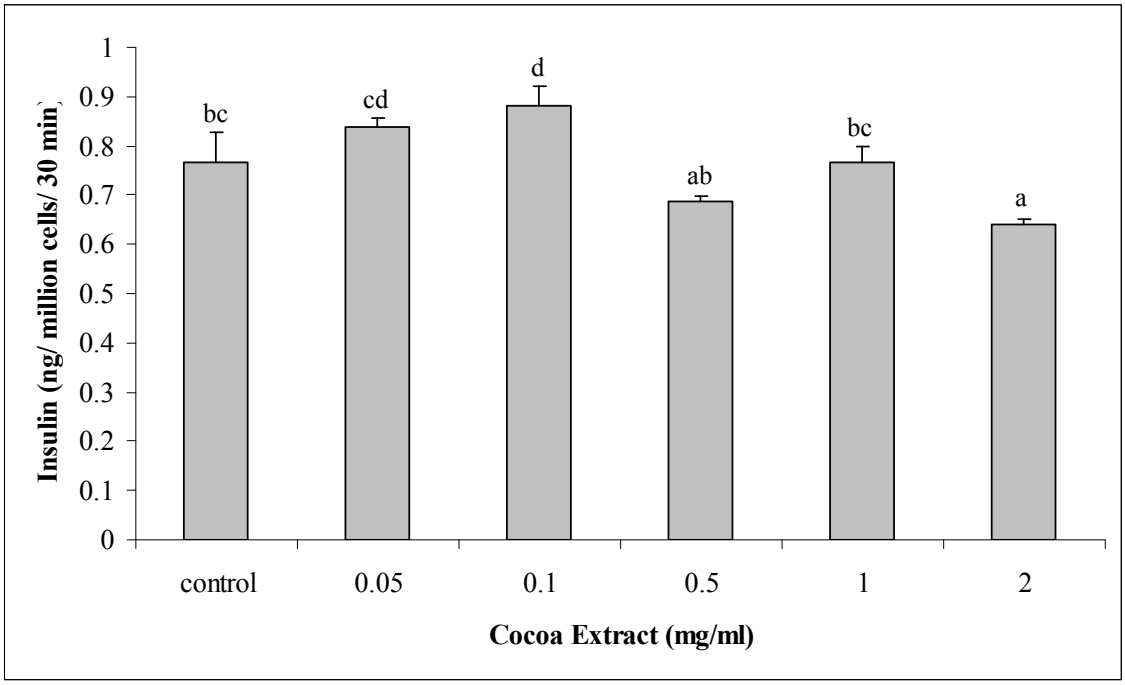
Values with different superscripts were statistically significant at the level of $p < 0.05$. NC: Normal control (n = 8); NCE1: Normal + 1% CE (n = 8); NCE2: Normal + 2% CE (n = 9); NCE3: Normal + 3% CE (n = 10).

FIG. 1. Plasma glucose levels of normal rats fed with cocoa extract (CE)



Values with different superscripts were statistically significant at the level of $p < 0.05$. DC: Diabetes control (n =6); DCE1: Diabetes + 1% CE (n = 4); DCE2: Diabetes + 2% CE (n = 5); DCE3: Diabetes + 3% CE (n = 5); DG: Diabetes + glibenclamide (n = 4).

FIG. 2. Plasma glucose levels of diabetic rats fed with cocoa extract (CE)



The extract was prepared at the concentration of 0.05 to 2.0 mg/ml. Values are expressed as means ± S.E.M. of four replicate determinations. Values with different superscripts were statistically significant at the level of $p < 0.05$.

FIG. 3. The effect of cocoa extract (CE) on *in-vitro* insulin secretion from BRIN-BD11 cell lines

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