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Effectiveness of different extracts of cocoa powder on plasma glucose and lipid profile in alloxan-induced diabetes mellitus rats.

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ABSTRACT

The use of different solvents to extract plant materials is to obtain component of utmost activity for the management of various disease conditions. Cocoa powder was subjected to different menstruum (aqueous, ethanol, n-hexane and chloroform) to remove inert materials and obtain pure active components whose effect was determined. Diabetes was induced in forty female Albino rats divided randomly into five groups, each consisting of eight rats, using 120mg/kg body weight alloxan. Different extracts of the powder were administered orally for twenty one days at a dose of 200mg/kg each except in the diabetic untreated group. Fasting blood sample was collected from the tail vein daily to determine the blood glucose concentration. On 22nd day, fasting blood samples were collected from all the rats for lipid profile estimation. Results showed a significant decrease ($P<0.05$) in the blood glucose level in ethanol extract-treated diabetic group compared with the others and a decrease in plasma TC, TG, and LDL-C in the extract-treated groups when compared with the diabetic untreated group. The reduction in lipid profile was significant ($p<0.05$) in ethanol extract when compared to other extracts. Conclusively ethanol extract of cocoa powder exhibited significant antihyperglycemia, hypolipodemia, and reduction in LDL-C.

Key words: Cocoa powder, diabetes mellitus, blood glucose, lipid profile, atherogenic index.

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INTRODUCTION

Cocoa processing involves harvesting, fermentation and roasting. Fermentation could either be real fermentation or under-fermentation (sun drying without fermentation or fermented for only 1-2 days) processes [1]. The antioxidant activity and phenolic content of cocoa is dependent on the variety[2] and method of fermentation, as fermentation was reported to degrade about 6-17% of epicatechin[3]. During fermentation process the polyphenol content will reduce through oxidation and exudation[4]. While epicatechin and catechin were reported to constitute 60% of the total phenolic in raw unfermented cocoa beans[5], epicatechin alone comprises approximately 35% of the total phenolic content[6].

Most communities in Africa obtain medicinally effective herbal preparations by soaking various plant parts (leaves, stem bark, roots, fruits and seeds) in alcohol for the purpose of extracting high activities of such materials. Different variations in biochemical parameters, using different solvents and means of extraction of plant materials, had been reported by researchers.

While Sun and Ho [7] reported that extracting solvents influences the degree of antioxidant capacity and phenolic contents obtainable from plant materials; Azizah et al.[3] only corroborated the effect of different extracting solvents on the different antioxidant activities obtainable from cocoa beans.

The antihyperglycemic effect of aqueous cocoa powder had earlier been reported[8] but in the course of obtaining phytochemicals of high pharmacologic effects to improve biochemical abnormalities in chronic conditions such as diabetes mellitus, further processing is needed, hence the basis of this research.

MATERIALS AND METHOD

Experimental design

The research was a longitudinal study involving 40 Wistar strain female Albino rats randomly selected and divided into five different groups, each consist of eight rats. The different extracts (aqueous, ethanol, n-hexane and chloroform) were prepared and administered orally in a single dose using oral cannula once daily for 21 days. The extracts were administered in a constant volume for the duration of the experiment. The rats were fed on normal rat chow and also water was given *ad libitum*.

At the expiration of the 21 days treatment period, the rats were fasted overnight and blood collected by cardiac puncture using diethyl ether as anesthetic agent. Blood was collected into heparinized bottle, centrifuged at 5000 rpm for five minutes to obtain plasma which was stored at -

2^0C till assayed. The care of the animals was done in accordance with the U.S. Public Health Service Guidelines[9].

Extraction/partitioning procedure

Cold maceration extraction methods was used in case the active constituents are thermolabile and efforts were made to prevent incomplete extraction due to insufficient time and longer extraction timing to avoid extraction of unwanted constituents. Maceration was done by placing five hundred grams (500 g) each of cocoa powder in a stoppered container with 1.5 L (x3) each of the different solvents and allowed to stand at room temperature for 72hr with frequent shaking until the soluble matters have dissolved. On the fourth day the mixture was decanted and the solution separated and filtered (using filter paper). The solid fractions were removed while liquid fractions were transferred into rotary evaporator chamber and concentrated. Evaporation was carried out at temperature of 60°C for 3.5 hr. The concentrates were subsequently dried at room temperature to obtain solid particles which were reconstituted and administered orally to the rats at a dose of 200mg/kg body weight each for 21 days.

Constitution of pure extract

About 6g each of the pure extracts was dissolved in 100mls distilled water and a dose of 200mg/ kg body weight of each of the extract was administered orally on daily basis over the period of the experiment.

Induction of diabetes

Diabetes was induced by a single dose of alloxan monohydrate dissolved in sterile injection water at a dose of 120mg/kg body weight intraperitoneally (I. P.). Diabetes was confirmed 48hr after induction as evidenced by fasting blood glucose levels of ≥140mg/dl[10].

Blood glucose estimation

Fasting blood glucose estimation was done using one touch accu-chek glucometer[11]. One drop of fasting tail vein blood sample was placed directly on to the strips (as specified by the manufacturer) to determine fasting blood glucose level in all the extract-treated and diabetic untreated rats (control).

Plasma lipid profile estimation

Fasting plasma lipid profiles (total cholesterol, HDL-C and triglyceride) was determined enzymatically using commercial test kits obtained from Randox Laboratories, Crumlin, England, based on standard universally acceptable methods described by Friedwald et al [12], Schettler and Nussel[13], and Nagele et al [14]. The Sniderman et al. [15] formula was used to calculate LDL-C, while

the formula of Abolt et al. [16] was used to calculate atherogenic (LDL-C/HDL-C ratio) and coronary risk (total cholesterol/HDL-C ratio) indices for all samples.

Data Analysis

Obtained data was analyzed using SPSS version 21 statistical package. The variables were expressed as Mean and standard error of mean (Mean ± SEM) and levels of statistical significance was set at $p<0.05$. Bar chart was used to describe variables and mean differences amongst the groups were compared using one way ANOVA.

RESULTS AND DISCUSSION

Glucose level

Figure 1 and Table 1 shows the effect of the various extracts of cocoa powder on blood glucose. There was a significant decrease ($P<0.05$) in the blood glucose level in ethanol extract-treated diabetic groups compared to the other extract-treated groups (aqueous, n-hexane and chloroform) and diabetic untreated groups. Similar anti-hyperglycemic effect was observed in aqueous extract but not as significant as ethanol extract. The improvement in blood glucose was in the order of ethanol extract > aqueous extract > chloroform > n-hexane while the change observed in the diabetic untreated group is an increase in blood glucose concentration from the value at diagnosis level.

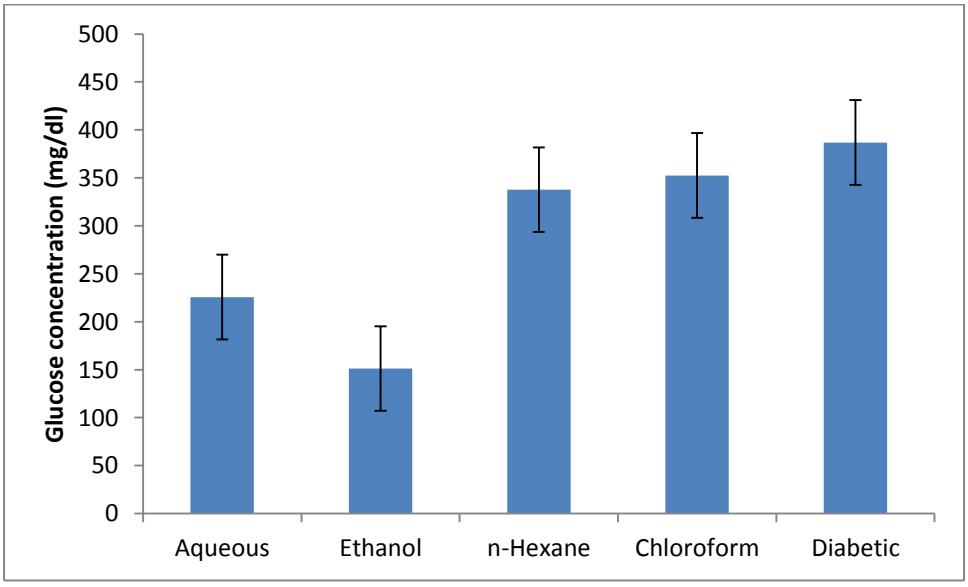

Figure 1: Hypoglycemic effect of 200mg/kg concentration of various extracts of cocoa powder in Alloxan diabetics

Table 1: Changes in plasma glucose by different extracts of cocoa powder

Parameters	Initial Glucose(mg/dl)	Final Glucose(mg/dl)	Difference (mg/dl)	% Glucose change (%)
Aqueous Extract (200mg/kg)	329.3±13.1	225.7±10.1	103.6	31.5
Ethanol Extract (200mg/kg)	330.6 ±13.2	151.2± 8.2	179.4	54.3
n-Hexane Extract (200mg/kg)	436.4±15.1	337.7±14.2	98.7	22.6
Chloroform Extract (200mg/kg)	463.4±15.6	352.5±14.5	110.9	23.9
Diabetic untreated	333.1±13.2	386.9±15.0	53.8	16.2

Values are Mean ± Standard error of mean (SEM), level of statistical significance was set at $p<0.05$ as indicated by *

Lipid profile:

Table 2 summarizes the results of the plasma total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). There was a decrease in the levels of plasma TC, TG, and LDL-C in the extract treated groups when compared with the diabetic untreated group. Meanwhile, the reduction in lipid profile was significant ($p<0.05$) in ethanol extract-treated group when compared to other extract- treated groups (i.e aqueous, chloroform and n-hexane). Also, a non-significant change ($p>0.05$) was noted in the plasma AI, CRI, and HDL-C in all groups as compared with diabetic untreated group.

Table 2: Effect of different extracts of cocoa powder on plasma lipid profile

Extraction /Parameters	Aqueous	Ethanol	n-Hexane	Chloroform	Diabetic	F-value	p-value
Cholesterol (mg/dl)	183.5±3.0*	108.8±4.8*	196.1±3.3	193.4±1.4	203.6±5.5	100.7	0.000
HDL-C(mg/dl)	46.6±1.2	27.3±5.3*	41.8±3.0	49.6±1.1	42.4±3.3	7.24	0.000
Triglyceride (mg/dl)	136.9±3.2*	30.1±4.5*	156.5±3.7	136.4±4.3*	157.8±3.4	191.3	0.000
LDL-C(mg/dl)	109.5±2.9*	75.5±8.9*	123.1±5.2	116.5±1.9	129.7±6.9	13.5	0.000
CRI	3.9±0.09	4.8±0.67	4.9±0.42	3.9±0.09	5.0±0.44	1.8	0.150
AI	2.4±0.08	3.5±0.64	3.1±0.35	2.4±0.09	3.3±0.37	2.1	0.090

Values are Mean ± Standard error of mean (SEM), level of statistical significance was set at $p<0.05$ as indicated by *

The chronicity of diabetes mellitus, various socio-economic impact on the community; complications from the disease, overall cost implication of its management, various side effects from chronic use of the various available drugs, readily availability of various phytochemicals in plant materials, and advancement in technology of phytochemical characterization are the driving forces towards research into plant materials with antihyperglycemic activities all over the world.

To obtain a pure compound with high medicinal activity from cocoa powder, different menstruum (aqueous, ethanol, n-hexane and chloroform) were used. Relatively complex mixture of metabolites (such as flavonoids, alkaloids, terpenoids, and glycosides) were extracted from cocoa

powder[17].The purpose of extraction in polar (water, ethanol) and non-polar (chloroform, n-hexane) solvents is to separate medicinally active portions of the cocoa powder. The extraction techniques yielded mixture of metabolites in liquid, semisolid state, and dry powder form (after removing the solvent) for oral use. This allows for attainment of the therapeutically desired portions and to eliminate unwanted inert materials.

Result from this study showed that cocoa powder have pronounced antihyperglycemic activity in the ethanol extract-treated group in comparison to aqueous, n-hexane and chloroform extract-treated groups (Figure 1). The percentage reduction in the blood glucose were 54.3%, 31.4%, 22.6% and 23.9% respectively for ethanol, aqueous, n-hexane and chloroform extracts, suggesting a better antihyperglycemic activity in the ethanol extract (Table 1). The antihyperglycemic activity observed in alloxan diabetes had been reported to be as a result of high flavonoid content which scavenges the hyperglycemia-induced circulating free radicals, prevents further destruction of the remaining islet β-cells and allows for induction of regenerative activities by other important phytochemicals present in the powder[9].

Plant-based foods such as cocoa are rich in polyphenols (such as flavonoids) and exhibits high antioxidant activities[18]. Because of high OH^- content of polyphenols they are much soluble in polar solvents such as water and ethanol (like dissolves like). The high antihyperglycemic activity observed in aqueous and ethanol extract is as a result of high polyphenol content and exhibition of high antioxidant activity[3]; and high epicathechin and cathechin activity in polar solvents while the relatively non-activity in n-hexane and chloroform extracts is as a result of absence or presence of few epicathechin and cathechin activity in non-polar solvents.

Considering the effect of the different extracts on lipid profile and atherogenic index, ethanol extract exhibited significant hypocholesterolemic and hypotriglyceridemic activities in the plasma and reduces plasma LDL-C (bad cholesterol) levels when compared with other extracts (Table 2).

CONCLUSION

Aqueous and ethanolic extracts of cocoa powder exhibited significant hypocholesterolemia, hypotriglyceridemia and reduction in plasma LDL-C. Thus, the use of such extracts in the management of diabetes mellitus either singly or as supplement will prevent cardiovascular complications. In order to be used as a modern drug, the ethanol extract may be further processed through various techniques of fractionation to isolate individual active chemical entities therein present. Also, further research on human subjects will be of scientific importance.

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