



## Glucose-lowering Potential of Cocoa Powder Intake - An Avenue for Positive Management of Diabetes Mellitus

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### Authors' contributions

This work was carried out in collaboration between all authors. Author TMO designed the study, wrote the research protocol as well as the first draft of the manuscript. She also managed the literature searches, the experimental processes as well as the major experimental and statistical analysis of the project. Author GTF supervised the study from the literature searches to the experimental procedure as well as the result and conclusion of the study while authors OOO and COJ sponsored as well as co-supervised the study. All authors read and approved the final manuscript.

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### ABSTRACT

Diabetes mellitus (DM) is a public health problem which is increasing all over the world and various contributions to its prevention and management is crucial. Cocoa powder as a food ingredient has been discovered to have medicinal purposes most especially in the treatment of cardiovascular diseases. This study was conducted to determine the efficacy of cocoa powder on the blood glucose level, body weight, feed and water in-take of experimental normal and albino rats with DM. Sixty matured male and female albino rats with an average weight of 200 g were randomly divided into 10 groups of 6 rats which include the normal and diabetic control and 8 treatment groups. DM was induced intravenously by giving the rats a single dose of Alloxan Monohydrate (100 mg/kg body weight) and the treatments include 1-4% natural cocoa powder mixed-feed.

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The results showed that cocoa powder could normalize increase fasting blood glucose and water in-take as well as body weight loss caused by alloxan. The group with DM fed with 4% cocoa powder feed showed the lowest water intake ( $29.6\pm 8.41$  ml) as well as the lowest final fasting blood glucose level ( $101\pm 3.26$  mg/dl) when compared to the diabetic control group while the normal group fed with 4% cocoa powder had the lowest body weight ( $204\pm 11.6$  g) when compared to the normal control group. There was a significant decrease in the final feed intake of both the diabetic and normal treatment groups when compared to the normal control group. This study suggests that cocoa powder contains glucose-lowering potentials that could yield a positive result in the management of DM.

**Keywords:** Blood glucose; body weight; cocoa powder; diabetes mellitus.

## 1. INTRODUCTION

Diabetes mellitus (DM) is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. If not well managed or treated, diabetes mellitus can result into serious long-term complications such as heart disease, stroke, kidney failure, foot ulcers and damage to the eyes [1].

Worldwide in 2012 and 2013, DM resulted in 1.5 to 5.1 million deaths per year, making it the eighth leading cause of death [1,2] and at least doubles the risk of death [1,3]. According to IDF (2013), the number of people with DM is expected to rise to 592 million by 2035 while the economic costs of diabetes globally as at 2013 was estimated to be \$548 billion [2] and in the United States in 2012 \$245 billion [4] thus making the disease a public health problem. Various contributions to the prevention and management of DM are therefore crucial so as to minimize its effects and promote better health conditions for individual.

Pharmacological treatment of Diabetes mellitus is dependent on the use of oral hypoglycemic agents and insulin which comes with many side effects and is most importantly not cost effective most especially in developing countries.

Cocoa powder, a by-product of Cocoa plant (*Theobroma cacao*) is widely known and has been used in the production of chocolates, cocoa beverages and recently in the formulation of foods such as bread [5]. Studies have however shown that cocoa powder aside from its use as food, it is a rich source of antioxidants such as flavanoids which are said to have a significantly high health promoting attributes such as anti-aging properties [6], anti-malaria activities [7],

cardio-protective effects and blood glucose lowering property [8].

Limited studies have been carried out to know the efficacy of cocoa powder on DM, few of which however concentrate on the use of cocoa extracts which might not be easily accessible by general populace. This study was conducted to determine glucose lowering potential of non-alkalized cocoa powder intake on alloxan induced diabetic and non-diabetic albino rats.

## 2. METHODOLOGY

### 2.1 Experimental Animals

This study was conducted in compliance with ethical guide for care and use of laboratory animals in the University of Ibadan, Ibadan, Nigeria. Ethical approval for this study was obtained from the University of Ibadan/University College Hospital Ethics Committee, Ibadan, Nigeria.

Sixty matured male and female albino rats aged twenty-one weeks with an average weight of 200 g were purchased from the Department of Physiology, University of Ibadan, Nigeria and were housed in metabolic cages and maintained under standard condition (12 hour light/dark cycle  $25\pm 3^\circ\text{C}$ , 45 to 65% humidity). The animals were allowed to have access to standard rat feed and water *ad libitum* for 7 days to acclimatize to laboratory condition after which they were randomly divided into 10 groups of 6 rats each. Baseline and daily assessment of feed and water intake as well as baseline and weekly assessment of body weight and fasting blood glucose were recorded for all the groups of rats for 3 weeks.

Group I: Normal albino rats fed with standard rat ration (Normal control).

Group II: Normal albino rats fed with standard rat ration + 1% cocoa powder.

Group III: Normal albino rats fed with standard rat ration + 2% cocoa powder.

Group IV: Normal albino rats fed with standard rat ration + 3% cocoa powder.

Group V: Normal albino rats fed with standard rat ration + 4% cocoa powder.

Group VI: Diabetic albino rats fed with standard rat ration (Diabetic control).

Group VII: Diabetic albino rats fed with standard rat ration + 1% cocoa powder.

Group VIII: Diabetic albino rats fed with standard rat ration + 2% cocoa powder.

Group IX: Diabetic albino rats fed with standard rat ration + 3% cocoa powder.

Group X: Diabetic albino rats fed with standard rat ration + 4% cocoa powder.

## 2.2 Induction of Diabetes

Diabetes was induced intravenously by giving the rats a single dose of Alloxan Monohydrate (5% W/V) at a concentration of 100 mg/kg body weight [9] after an overnight fast at day 7.

## 2.3 Cocoa Powder Preparation

Already standardized natural cocoa powder (non-alkalized) as packaged by Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Ibadan, Oyo State, Nigeria, was purchased and used for this study.

## 2.4 Modified Cocoa Feed

The treatments were prepared by the incorporation of natural cocoa powder in to the normal rat chow of the experimental albino rats separately at different ratio which included 10:90, 20:80, 30:70 as well as 40:60 separately and labeled as 1, 2, 3 and 4% cocoa-mixed rat feed respectively.

## 2.5 Determination of Water Intake

One hundred ml of water was measured using a standard measuring cup for each of the rats while the remnant was also measured at 8:00 am

every day. The difference between the initial and the final measurement was taken as the water intake and recorded for each day.

## 2.6 Determination of Feed Intake

Twenty grams of the feed (labeled 1%, 2%, 3%, 4% and normal rat chow) was measured using a digital scale for each rats based on their treatment group. The remnant was also measured using the digital scale and the difference between the initial and final measurement was taken as the feed intake and recorded for each day.

## 2.7 Determination of Body Weight

The body weight was determined using a standard digital scale. The body weight of rat was monitored weekly.

## 2.8 Blood Sample Collection Method for the Determination of Fasting Blood Glucose

The blood sample was collected through the tail vein. The tail end was pricked with a needle and pressure was applied to get the blood sample which was dropped on the glucometer strip already placed in the glucometer and then read. The blood glucose was monitored weekly using glucometer (Acucheck advantage II).

## 2.9 Statistical Analysis

Data was expressed as mean  $\pm$  standard error of mean (SEM). One-way analysis of variance (ANOVA) was applied to determine differences between the groups while Duncan multiple range test was used to determine significant difference among means. The results were considered to be significant at the level of  $P < 0.05$ .

## 3. RESULTS

Table 1 revealed an increase ( $P > 0.05$ ) in the final feed intake of the normal control group when compared to their initial. The 2, 3 and 4% normal treatment groups had a significant decrease ( $P < 0.05$ ) in their final feed intake when compared with their initial while the decrease in feed intake observed in 1% treatment group was not significant. There was a significant decrease ( $P < 0.05$ ) in the final feed intake of the diabetic control group as well as in the 1 and 3% treatment groups with DM when compared to their initial.

**Table 1. Effects of cocoa powder on the feed intake of normal and albino rats with diabetes mellitus**

Group (n = 6)	Daily feed intake of rats	
	Initial (g)	Final (g)
Normal control	17.6±1.66	18.7±0.37
N + 1% Cp	19.0±2.04	17.2±0.46
N + 2% Cp	18.2±0.59	16.0±0.33*
N + 3% Cp	16.7±0.83	15.4±0.76*
N + 4% Cp	17.3±1.69	16.1±0.35*
Diabetic control	18.7±0.46	17.4±0.58*
D + 1% Cp	18.2±0.59	16.8±0.44*
D + 2% Cp	17.8±2.62	15.4±1.05
D + 3 % Cp	17.2±0.33	14.7±1.07*
D + 4% Cp	17.7±1.12	15.6±3.13

N = Normal rats; D = Diabetic rats;  
Cp = Cocoa powder; p < 0.05

Cocoa powder induced reduction in feed intake in both normal and albino rats with DM, however reduction in feed intake was more prominent in the diabetic rats.

**Table 2. Effects of cocoa powder on the water intake of normal and albino rats with diabetes mellitus**

Group (n = 6)	Daily water intake of rats	
	Initial (g)	Final (g)
Normal control	24.2±0.91	23.3±0.23
N + 1% Cp	23.3±1.15	24.0±0.47
N + 2% Cp	20.8±2.60	22.2±0.52
N + 3% Cp	18±1.82	22.2±1.11*
N + 4% Cp	23.3±0.55	23.4±0.74
Diabetic control	20.8±2.61	68.5±1.70*
D + 1% Cp	20.0±0.00	50.4±3.35*
D + 2% Cp	22.5±1.22	45.1±3.25*
D + 3 % Cp	24.2±0.91	37.5±3.08*
D + 4% Cp	22.5±1.22	29.6±8.41

N = Normal rats; D = Diabetic rats; Cp = Cocoa powder; p < 0.05

Table 2 showed a decrease (P>0.05) in the final water intake of the normal control rats when compared with their initial. There was an increase (P>0.05) in the final water intake of normal treatment groups which was significant in the 3% normal treatment group when compared to the initial (P<0.05).

The control group with DM as well as 1, 2 and 3% treatment groups with DM had a significant increase (P<0.05) in the final water intake when compared to their initial, however the increase in water intake observed in the 4% treatment group was not significant. All the treatment groups with

DM took less water when compared to the control group with DM.

**Table 3. Effects of cocoa powder on the body weight of normal and albino rats with diabetes mellitus**

Group (n = 6)	Body weight of rats	
	Initial (g)	Final (g)
Normal control	215±6.68	231±15.0
N + 1% Cp	213±9.38	230±6.86*
N + 2% Cp	215±3.98	232±4.90*
N + 3% Cp	217±14.4	233±10.6*
N + 4% Cp	217±11.6	204±11.6*
Diabetic control	214±8.67	122±7.00*
D + 1% Cp	213±17.30	211±13.20*
D + 2% Cp	211±13.20	173±15.30*
D + 3 % Cp	205±6.61	181±13.0*
D + 4% Cp	214±11.90	210±7.40

N = Normal rats; D = Diabetic rats; Cp = Cocoa powder; p < 0.05

In Table 3, there was an increase (P>0.05) in the final body weight of the normal control group when compared to the initial. A significant increase (P<0.05) was however observed in the final body weight 1, 2 and 3% normal treatment groups when compared to their initial while the final body weight of the 4% normal treatment group decreased significantly as compared to the initial.

There was a significant decrease in the final body weight of the control group with DM as well as in the final body weight of 1, 2 and 3% diabetic treatment groups when compared to their initial while the final body weight of the 4% diabetic treatment group also decreased but with no significant difference (P>0.05). A significant increase (P<0.05) was however observed in the final body weight of the treatment groups with DM when compared to the control group with DM.

Cocoa powder diet induced no significant effects on the weight of normal rats but induced significant increase in body weight of the treatment groups with DM (P<0.05). All albino rats with DM had reduced final body weight which indicates that diabetes induced reduced body weight in the rats.

Table 4 showed an increase (P>0.05) in the final fasting blood glucose level of the normal control rats (82.0±4.17 mg/dl) as well as in that of the 3% and 4% normal treatment groups when compared to their initial. There was a however a

significant decrease ( $P < 0.05$ ) in the final fasting blood glucose of the 2% normal treatment group ( $76.0 \pm 4.41$  mg/dl) when compared with the initial while that of the 1% normal treatment group also reduced but with no significant difference. There was a significant decrease ( $P < 0.05$ ) in the final fasting blood glucose of all the groups with DM when compared to their initial as well as when compared with the control group with DM ( $P < 0.05$ ). Cocoa powder diet significantly reduced the fasting blood glucose of the albino rats with DM when compared with the control group with DM.

**Table 4. Effects of cocoa powder on the fasting blood glucose of normal and albino rats with diabetes mellitus**

Group (n = 6)	Fasting blood glucose of rats	
	Initial (mg/dl)	Final (mg/dl)
Normal control	79.5±2.70	82.0±4.17
N + 1% Cp	78.2±3.46	72.5±6.42
N + 2% Cp	77.0±2.39	75.3±4.88*
N + 3% Cp	75.3±4.88	76.0±4.41
N + 4% Cp	74.2±4.99	76.0±4.99
Diabetic control	398±30.9	367±40.2
D + 1% Cp	325±14.7	211±28.1*
D + 2% Cp	418±39.8	144±20.3*
D + 3 % Cp	376±32.4	133±12.8*
D + 4% Cp	392±28.5	101±3.26*

*N = Normal rats; D = Diabetic rats; Cp = Cocoa powder; \* =  $p < 0.05$*

There was however a significant decrease ( $P < 0.05$ ) in the final feed intake on the incorporation of cocoa powder into the diet of normal rats which might be as a result of the slight bitter taste of cocoa powder when added at a high concentration. Study carried out by Olubamiwa et al. [5] revealed that cocoa powder could actually be incorporated into confectioneries such as bread.

#### 4. DISCUSSION

This study revealed an increased in blood glucose level of alloxan-induced albino rats with DM which is consistent with the findings of Olooto et al. [10].

Cocoa powder contains polyphenols which act as antioxidants due to their free their ability to reduce the formation of free radicals and their ability to stabilize membrane by decreasing membrane fluidity [11].

Examples of polyphenolic compounds in cocoa are the flavan-3-ols or flavanols, which include the monomeric forms, (-) - epicatechin and (-) - catechin, and the oligomeric forms of the monomeric units, the procyanidins [12,13].

The incorporation of cocoa powder into the feed of both diabetic and normal rats in this study revealed that cocoa powder may not show a significant effect on feed intake of the diabetic rats when compared to the normal control rats.

There was however a significant decrease ( $P < 0.05$ ) in the final feed intake on the incorporation of cocoa powder into the diet of normal rats which might be as a result of the slight bitter taste of cocoa powder when added at a high concentration. Study carried out by Olubamiwa et al. [5] revealed that cocoa powder could actually be incorporated into confectioneries such as bread.

This study revealed that there was an increase in water in-take of normal treatment groups although this was only significant at 3%. This could suggest that cocoa powder is likely to be able to induce thirst in normal rats. In this study, there was a significant increase ( $p < 0.05$ ) in the volume of water intake of the control group with DM as well as the 1, 2 and 3% treatment groups with DM when compared to the normal control group. No significant difference was observed in the water in-take of albino rats with DM treated with 4% cocoa powder when compared to the normal control group. The water in-take of the groups with DM as observed in this study however decreased with increase in concentration of cocoa powder.

This result could suggest that a good outcome could be obtained from the use of cocoa powder in the management of DM. The effect of dose dependent cocoa powder administration on the feed and water in-take according to this study showed that cocoa powder could normalize polydipsia (i.e. increased water in-take) which is usually associated with DM.

The observation made in this study revealed that cocoa powder at mild concentration had no effect on weight gain of normal groups. However at a higher concentration (4%), it was observed that cocoa powder has a weight reducing effect on the normal growth of rats which can be suggested to be an anti-obesity effect of cocoa powder.

The administration of different proportions of cocoa powder increased the weight of the

alloxan-induced groups with DM when compared to the control group with DM. There was a significant increase ( $P < 0.05$ ) in the body weight of 4% treatment group with DM when compared to its corresponding normal group. This observation is consistent with the report of Ruzaidi et al. [14] and that of Azli et al. [15]. In a study carried out by Marco et al. [16], it was observed that cocoa powder normalizes weight gain in normal mice. Thus, it can be suggested from this study that cocoa powder most especially at 4% normalized the weight loss observed in control group with DM and weight gain in normal group.

The study carried out by Ruzaidi et al. [14] corroborated the findings of this study which revealed that cocoa powder has no significant effect on the fasting blood glucose level of normal treatment group when compared with the normal control group. However, there was a significant decrease ( $P < 0.05$ ) in the final fasting blood glucose level of 2% normal treatment group when compared to its initial value.

There was a significant decrease in the fasting blood glucose level of all treatment groups with DM when compared to the control group with DM. From this study, 4% treatment group with DM had the lowest fasting blood glucose level and this suggests that the management of DM with cocoa powder could be most effective at a higher concentration.

The hypoglycemic effect observed in cocoa powder in alloxan-induced albino rats with DM could be due to the presence of antioxidant polyphenols present in cocoa powder [16]. Polyphenols are reported to be the potential bioactive component for hypoglycemic properties [17]. It could therefore be suggested that the significant reduction of the fasting blood glucose of the albino rats with DM treated with cocoa powder could be due to the antioxidant properties of cocoa powder.

## 5. CONCLUSION

This study has been able to establish that cocoa powder has a beneficial effect on the feed and water in-take, body weight and fasting blood glucose of normal and alloxan-induced DM in experimental albino rats. Alloxan has shown an adverse effect on the body weight and fasting blood glucose of albino rats with DM which can be reversed by concomitant administration of cocoa powder. This study showed that cocoa powder treatment has an anti-diabetic activity

and prevented adverse symptoms in albino rats with DM.

## 6. RECOMMENDATION

Further studies are recommended to see the prolonged effect of cocoa powder on DM and to know if the anti-diabetic effect of cocoa powder as observed in this study could be sustained. This can help to determine its curative effect and can be suggested as an alternative therapy in the treatment of DM.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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