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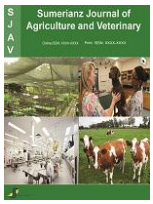

Original Article Open Access

Hypoglycemic Potential of Cocoa Powder in Monosodium Glutamate-Diet Induced Diabetic Mice

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Abstract

Diabetes mellitus is a public health problem, one of the four priority non-communicable diseases (NCDs) targeted for actions by World leader. Historical documents from the 17th through mid-19th centuries contain numerous references to cocoa powder not primarily as a beverage, but as a prophylactic and therapeutic for a variety of ailments. Monosodium glutamate (MSG) enhances appetite and palatability of meals since it is been consumed as additives in most of our meal without caution, there is need to investigate if its overdose consumption can lead to diabetes which is a common ailment in our society nowadays. This study investigates the effect of cocoa powder on mice induced with diabetes using MSG the experiment was categorized into six groups ranging from mice fed with cocoa feed and those fed with MSG diet. The result showed significant increases in blood glucose level amongst those fed with MSG diets alone, thus suggested that MSG has the potential to induce elevation in blood glucose level thereby resulting to diabetes mellitus. Moreover, the result from this study showed that cocoa powder was able to cause reduction in blood glucose level in the MSG-induced diabetic mice. Therefore, it is no doubt that medicinal plants still play important role in the discovery of novel anti-diabetic compounds as in this case cocoa powder.

Keywords: Cocoa powder; MSG; Diabetes mellitus; Antidiabetic; hypoglycaemia.

1. Introduction

Diabetes mellitus (DM) popularly called Diabetes is known as the world most common endocrine disorder [1]. DM is a glucose metabolism disorder resulting from dysfunction of pancreatic beta cells and insulin resistance. It has become a serious problem of modern society due to severe long-term health complications associated with it. DM is associated with reduced life expectancy, significant morbidity due to specific diabetes related condition primarily defined by the rising level of hyperglycemia leading to increased risk of microvascular complications (retinopathy, nephropathy and neuropathy), increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life [2]. Result from epidemiological data reveals that approximately 177 million people worldwide are suffering from this disease and there are postulations that this will be doubled and increase to up to 300 million by the year 2030 [3]. About 14.2 M adults (20-79) years have diabetes in Africa. Nigeria, South Africa, Democratic Republic of Congo and Ethiopia are Africa 's most populous countries with highest diabetic patients with 1.6, 2.3, 1.8 and 1.3 million respectively [4].

DM is not a single disease, it's group of heterogeneous syndromes such as heart attack, obesity, stroke and peripheral vascular disease [5]. Diabetes also resulting in reduced haemoglobin was reported and may as well be accompanied by a fall in the red blood cell count and packed cell volume [6, 7].

Diabetes mellitus is divided into four categories. Type-1 diabetes is also called insulin-dependent DM because this disease is characterized by an absolute deficiency of insulin. Beta cells are destructed due to invasion by virus, action of chemical toxins or due to action of autoimmune antibodies. Patel, *et al.* [8]. Type-2 diabetes is a non-insulin dependent DM or Type-2 and frequently accompanied by target organ insulin resistance that limits responsiveness to both endogenous and exogenous insulin [9]. Type-3 diabetes is a type of diabetes caused by chronic pancreatitis or chronic drug therapy with glucocorticoids, thiazide diuretics, diazoxide, growth hormone and with some protease inhibitors (e.g. saquinavir). Type-4 diabetes is observed in approximately 4-5% of all pregnancies, due to placental hormones that promotes insulin resistance [10]. At present time best and quickest way to induce diabetes is with use of chemicals (alloxan, streptozotocin, dithizone, monosodium glutamate), viruses and genetically diabetic rats [11].

Monosodium glutamate (MSG) is a salt of the amino acid glutamate, [12, 13]. It is reported to enhance flavour in certain dishes and processed foods, MSG is said to invoke a 'fifth taste' a complex, savoury flavour. Yamaguchi

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and Ninoya [14], through stimulation of the oro-sensory receptors. There are assertions too, that MSG is a food additive and as a major constituent of Nigerian diets [15]. It enhances appetite and palatability of meals [14]. MSG remains a source of concern considering the controversies about their risks and benefits. Of a particular interest is the use of MSG, which, according to Eweka and Om'Iniabohs [16] is popularly known in Nigeria as white maggi. Many studies have shown that MSG is toxic to humans and experimental animals [13]. It induces seizures, liver damage [13], brain damage [17], diabetes, obesity and anemia [15].

Pharmacological treatment of Diabetes Mellitus is based on oral hypoglycemic agents and insulin which have many side effects. In diabetes, the causes and sites of intervention in biochemical process are diverse and high serum total triglyceride level, high level of transaminase; creatinine kinase and urea have been implicated [18]. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed, because of the inability of existing therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability for many rural populations in developing countries [2].

The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest. The World Health Organization also recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate [1]. It however emphasized the fact that safety should be the major criteria in the selection of herbal medicine for use in healthcare. Some plants used locally in managing diabetes include Neem, bitter leaf, okro, pawpaw, bitter cola, plantain, ginger among others [19]

In recent investigation, suggestions have been made that polyphenolics components from natural sources may act as antioxidants and also prevent disease process such as nausea, abnormal pain and so on. This is a driving force to intensify the search for alternative medicine from natural source which is relatively cheap with minimal side effects, thus necessitated the use of cocoa powder for this study.

Cocoa beans contain natural compounds such as polyphenols, methylxantines, peptides and minerals. The naturally occurring compounds were reported to have significant effect on certain health symptoms and contributed to various health promoting attributes such as high antioxidant properties, cardioprotective effects [20], hypocholestrolemic property [21], glucose lowering property and to reduce severity of hepatocarcinogenesis [22]. Studies carried out also revealed that consumption of flavanol-rich cocoa powder may extend to the brain and have important implications for learning and memory and also as prophylactic against malaria [23, 24].

This study therefore, determines the effect of chronic ingestion of MSG on blood glucose level using mice as models and also the effect of cocoa powder in ameliorating diabetes in mice

2. Materials and Methods

This experiment made use of mouse model to determine the anti-diabetic property of cocoa powder. Laboratory mice have been the most important non-human models for studying the effectiveness of new drug therapies and efficacies of medicinal plants.

The experiment was conducted at the animal house and at the departmental laboratory of the vetenary medicine, University of Ibadan. Oyo State, Nigeria

Mice: Adult Female Naïve BALB/C mice (N=60) of 14-16 weeks old (28-30g) were used for this study and they were purchased from Animal breeding house, University of Ibadan, Oyo State, Nigeria.

Cocoa: Natural flavanol-rich cocoa powder (non-alkalized) which was produced by an innovative industrial process and packaged by Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Ibadan, Oyo State, Nigeria, was used for this experiment. This is to evaluate its functionality in a short-term study through the use of an experimental rodent model for anti-diabetes.

MSG: Monosodium glutamate was purchased in local Bodija market and were packaged and sold in 3gram satchet. Enough quantity needed for this study was obtained from the market.

Substance of Study: Natural cocoa powder, Diabetes, Monosodiun Glutamate.

Modified experimental feed: The experimental feed was specially formulated on request to be made of the normal rat diet. The modified feed consisted of maize starch, sucrose, soybean oil, fibre (cellulose powder), mineral premix, choline bitartrate, tert-butyl-hydroquinone [24]. This was made into rat feed pellet by Pfizer feed mill, Iwo road, Ibadan. The diet contained (g/kg): maize starch, 397·486; casein, 200·000; dextrinised maize starch, 132·000; sucrose, 100·000; soyabean oil, 70·000; fibre (cellulose powder), 50·000; cocoa powder, 20.000; AIN-93G mineral mix, 35·000; AIN-93 vitamin mix, 10·000; L-cystine, 3·000; choline bitartrate, 2·500; tert-butylhydroquinone, 0·014, some with inclusion of 2% natural cocoa powder and some with 8% MSG for inducing diabetes.

Experimental animals: Mice were housed in polypropylene cages maintained at standard condition (12 hours light/dark cycle 25$\pm$ 3^{o}C, 45-65% humidity). The animals had free access to modified standard mouse feed and water *ad libitum*. All the animals were acclimatized to laboratory condition for 3 days before commencement of the experiment as described.

Experimental design: Experimental mice were grouped into six groups (A to F) randomly containing 10 animals each, according to their weight.

Group A – Normal mice + normal mice feed
Group B – Diabetic mice with normal mice feed
Group C – Normal mice + 2% cocoa feed
Group D – Diabetic mice + 2% cocoa feed
Group E – Diabetic mice + 8% MSG + 2% cocoa feed
Group F – Diabetic mice + 8% MSG feed

Induction of Hyperglycaemia: Diabetes was inducted into the mice after been fed on MSG diet for 12 weeks. At the end of 12 weeks, body weight gain and mice with blood glucose level of above 200mg|dl and signs of polyuria, polydipsia were considered as diabetic and was used for this experiment for another 12 weeks

Sample Collection: The blood sample was collected through the tail vein using Acucheck glucometer weekly.

Determination of Body Weight: The body weight was determined using a standard digital scale. The body weight of mice was monitored and recorded weekly.

2.1. Blood Sample Collection Method for the Determination of Fasting Blood Glucose

The blood sample was collected through the tail vein to determine the fasting blood level glucose using glucometer (Acucheck advantage II). The fasting blood glucose level was monitored weekly.

2.2. Blood Sample Collection Method for the Determination of Haematological Parameters

The experimental mice were fasted overnight, anesthetized with ether, dissected and their blood was collected through cardiac puncture with a 2ml syringe into an Ethylene Diamine Tetra-Acetic Acid (EDTA) sample bottle for the determination of the haematological parameters. Total white blood cell count was determined manually using the improved Neubauer haemocytometer while the differential leucocytes counts were determined by morphological identification and counting of hundred leucocytes in Giemsa stained smears of each blood sample. Monocytes and eosinophil are expressed as percentages of the total white blood cell. Red blood cell (RBC) was counted with haemocytometer, the packed cell volume (PCV) by the microhaematocrit method and the haemoglobin (Hb) concentration by cyanmethaemoglobin method. Platelet count was determined by direct method using diluent solution. The MCV and MCHC were calculated from the values obtained for RBC, PCV and HB.

2.3. Data Analysis

Data was expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was applied to determine differences between the groups while Duncan multiple range test.

3. Results

Table-1. Fasting blood glucose level of mice after 12 weeks of exposure to MSG diet (N=100)

Weeks	GROUP A (NF+8%MSG DIET)	GROUP B (CP +8%MSG)
1-2	74.2 ± 1.5	76.3± 2.5
2-4	111.7 ± 2.5*	80.7± 6.3
4-6	155.4 ± 4.1**	90.1 ± 4.5
6-8	193.3 ± 2.2**	108.2 ± 3.3$^{\#\#a}$
8-10	220.5 ± 3.2***	128.3 ± 2.1$^{\#\#\#a}$
10-12	254.6 ± 4.5****	130.6 ± 6.2$^{\#\#\#a}$

The fasting blood glucose was measured weekly. Values are expressed as mean ± SEM
Cp = Cocoa Powder
NF= Normal feed
MSG = Monosodium glutamate
***P < 0.001 is statistically different from the normal control
###P < 0.001 is statistically different from the diabetic control
[a]P<0.05 is statistically different from initial

Table-2. Effect of cocoa powder on the fasting blood glucose level of mice after induction of diabetes

Groups	Initial fasting blood glucose (mg/dl)	Final fasting blood glucose (mg/dl)
Normal control	74.2 ± 1.5	83 ± 2.3
Diabetic control	252 ± 22.6***	277 ± 32.2^{***a}
Cocoa powder feed control	130.6 ± 6.2**	90 ± 18.5$^{\#\#\#a}$
Diabetic + 2% Cp	254 ± 26.5***	140 ± 32.8$^{\#\#\#a}$
Diabetic + MSG + 2% Cp	254 ± 30.7***	182.5 ± 21.4$^{\#\#\#a}$
Diabetic + MSG	252 ± 20.8***	286 ± 25.6$^{\#\#\#a}$

The fasting blood glucose was measured weekly. Values are expressed as mean ± SEM
Cp = Cocoa Powder
***P < 0.001 is statistically different from the normal control
###P < 0.001 is statistically different from the diabetic control
[a]P<0.05 is statistically different from initial

Table-3. Comparative body weight of the different groups of experimental mice

Groups	Initial weight (g)	Final weight (g)	Body weight gain (g)
Normal control	28±1.6	29±8.5	1.69$^{*\ a}$
Diabetic control	28±8.6	35±7.0$^{\#\#\#a}$	6.84$^{\#\#}$
Cocoa powder feed control	28±4.2	27±3.1	-1.11
Diabetic + 2% Cp	28 ± 5.7	31 ± 1.5$^{\#\#\#**a}$	2.58$^{**\ a}$
Diabetic + MSG + 2% Cp	28 ± 2.3	32 ± 4.8$^{\#\#\#*\ a}$	4.25$^{\#\#}$
Diabetic + MSG	28 ± 1.1	38 ± 1.2$^{\#\#\#}$	10.01$^{\#\#\#}$

The body weight was measured weekly. Values are expressed as mean ± SEM
**P < 0.01 is statistically different from the normal control
***P < 0.001 is statistically different from the normal control
##P < 0.01 is statistically different from the diabetic control
###P < 0.001 is statistically different from the diabetic control
[a]P<0.05 is statistically different from initial

Table-4. Hematological parameters of diabetic mice fed with MSG and cocoa powder feed

Parameters	Normal feed Control	Diabetic Control	2% Cp feed Control	Diabetic + 2% Cp	Diabetic + 8 % MSG
RBC (X 10^2nm)	8.13±0.33	6.45 ± 1.24$^{\#\#\#}$	8.55± 1.02$^{\#\#\#}$	7.57±0.54$^{**\#\#\#}$	4.91±0.22***
MCV (fl)	61.2 ± 0.72	59.2 ± 2.14	69.1 ± 2.44	67.3 ± 4.63	52.5 ± 2.11
PCV (%)	44.2±1.22	40.3 ± 2.16$^{\#\#\#}$	46.3 ±1.25$^{\#\#\#}$	42.5 ± 2.92$^{\#\#\#}$	21.4±1.51***
Hb (g/dl)	15.2±1.32	11.1 ± 2.61$^{\#\#}$	15.4 ± 1.32	11.2 ± 1.25$^{\#\#}$	6.12±1.21***
MCHC (g/dl)	27.5 ± 1.22	30.3 ± 1.96	26.8 ± 2.32	27.1 ± 2.12	31.6 ± 0.12
Neutrophil	32.2 ± 1.15	40.8 ± 3.75$^{\#\#\#}$	17.6± 3.22$^{\#\#\#}$	34.1 ± 1.42$^{\#\#\#}$	48.2±2.22***
Monocyte	2.44 ± 1.23	2.20 ± 1.32	3.50 ± 1.18$^{\#\#}$	1.66 ± 0.52	1.12 ± 0.35*
Platelet	66121±3212	46040±2240$^{\#\#\#}$	74200±1372$^{\#\#\#}$	61500±1243$^{\#\#}$	30140±1145***
WBC (n/µl)	3367±360	4254±233$^{\#\#\#}$	3050±435$^{\#\#\#}$	3533±242$^{\#\#}$	6875±162***
Lymphocyte	59.2±1.33	57.2±5.19	78.4±2.16	62.5±4.27	50.1±2.44
Eosinophil	1.42 ± 0.51	1.52 ± 0.31	1.15 ± 1.42	1.62 ± 1.53	2.40 ± 1.17

All hematology parameters were measured at the end of the experiment. Values are expressed as mean ± SEM
Cp = Cocoa Powder, PCV = Packed Cell Volume, Hb = Hemoglobin, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, MCHC = Mean Corpuscular Hemoglobin Concentration, WBC = White Blood Cell
**P < 0.01 is statistically different from the normal control
***P < 0.001 is statistically different from the normal control
##P < 0.01 is statistically different from the diabetic control
###P < 0.001 is statistically different from the diabetic control

Fig-1. Rate of glucose level formation in mice as related to weeks of exposure to MSG diet

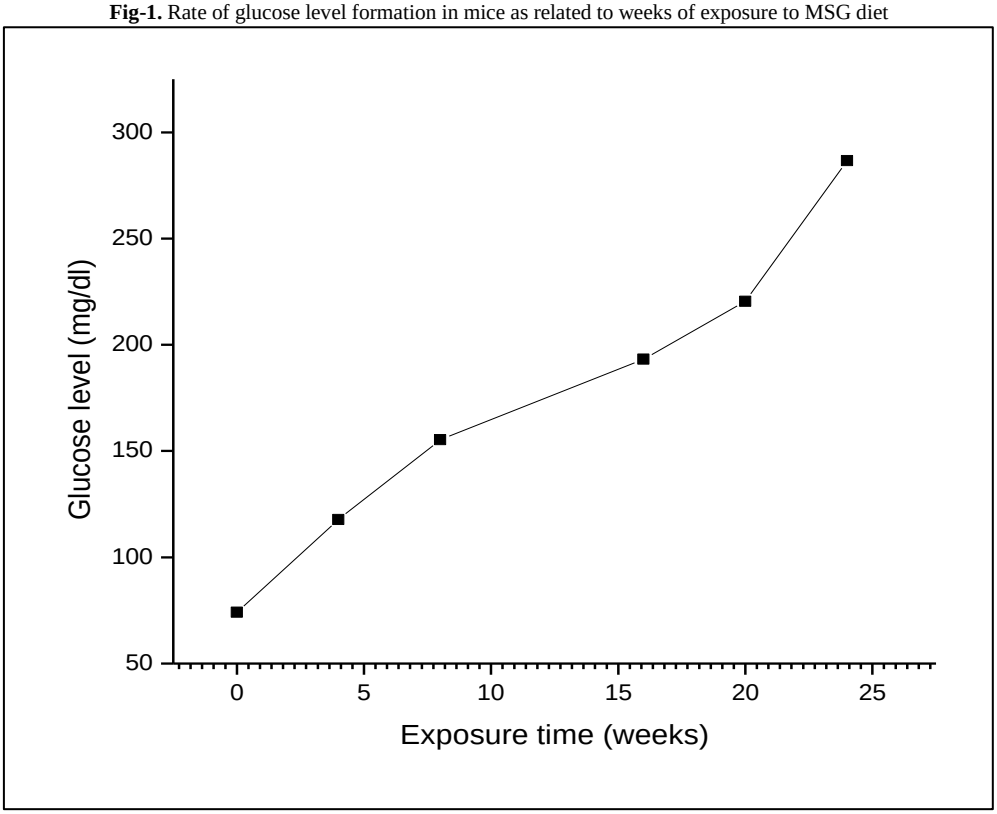
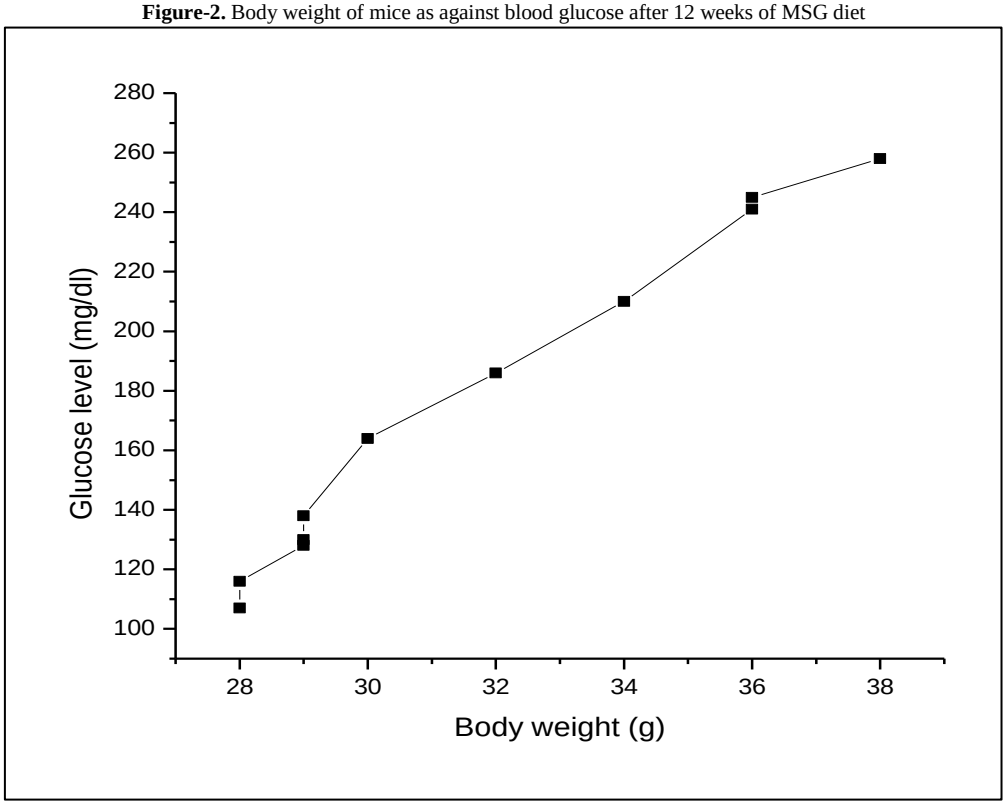


Figure-2. Body weight of mice as against blood glucose after 12 weeks of MSG diet



4. Discussions

Table 1 shows the fasting blood glucose level of mice after 12 weeks of exposure to 8% MSG diet. There was an upward trend in the blood glucose in mice fed with MSG diet as the day progresses. This was in an agreement with the report of Akpamu, *et al.* [15] that MSG consumption can lead to diabetes. According to Kate, *et al.* [25] greater than 30mg/kg is a toxic dose of MSG, this correlate to about 2.1 grams in an average 70g animal. However, many individuals are considered to be allergic to or intolerant of, MSG, for these individuals, much smaller amounts (perhaps even as small as 50 or 100mg) may be considered to be a dangerous dose of MSG. When comparing group A (normal feed compounded with 8% MSG) with group B (cocoa powder diet with 8% MSG) the result indicated that though both showed increase in the blood glucose level but the rate of increase was far more higher in group A (254.6 ± 4.5) resulting to high threshold level. This showed that SG can induce diabetes, at the same time inclusion of cocoa powder in group B indicated that cocoa consumption might slow or prevent the formation of high blood glucose (130.6 ±6.2). The result obtained in group B is still within the normal range of blood glucose due to amelioration effect of cocoa powder in the diet. Table 2 result showed the effect of cocoa powder on the fasting blood glucose level of mice after induction of diabetes. Diet compounded with cocoa powder showed reduction in blood glucose level (90 ± 18.5) and this is an indication that cocoa has the ability to reduce blood glucose level and therefore can be termed as having hypoglycemic and prophylactic effect against diabetes in mice, this could be as a result of the polyphenolic contents present in cocoa powder. Consequently, the diabetic mice that was continued with 8% MSG has the highest incidence of blood glucose level (286 ± 25.6), this is an indication that prolong consumption due to addiction to use will result to diabetes. This report is in agreement with [16] that reported that MSG is toxic when consumed in large quantities. Moreover, result as shown in Table 3 showed the comparative body weight of the different groups of experimental mice and their relative blood glucose levels, and the result indicated that mice with the highest body weight (38 g) has the highest blood glucose level (286 ± 25.6), surprisingly those mice fed with cocoa powder diet showed reduction in weight loosing (-1.11) as against diabetic mice fed with MSD diet with weight gain (10.01)and this showed that cocoa powder exhibit weight reduction property and this is in agreement with the work of Jayeola, *et al.* [26] that reported weight reduction in obese mice fed with cocoa powder incorporated feed.

Anecdotal reports have shown that cocoa powder consumption is effective in boosting imunity but the effect of cocoa powder on haematological parameters such as Red Blood Cells (RBC), Mean Corpuscular Volume, Mean Corpuscular Hemoglobin Concentration (MCHC), Packed Cell Volume(PCV), Hemoglobin(Hg), White Blood cells(WBC) and its differentials like Monocyte, eosinophil and lymphocytes are still very few. The result in Table 4 showed the hematological parameters of diabetic mice fed with MSG and cocoa powder feed. It was observed that cocoa powder fed diabetes mice showed significant increase in RBC, PCV, Hg, monocyte and slight increase in MCV, platelet and lymphocyte. This is in agreement with the report of Olasope, *et al.* [27] that cocoa powder contains some phytochemical which is responsible for the stimulation of the above indices in cocoa powder fed diabetes mice. Moreover, it was observed that there is a significant decrease in white blood cells, eosinophil, MCHC and Neutrophils. This is an indication that cocoa powder exhibit some immune boosting properties that could fight against some infectious diseases as indicated on the table 4 results when comparison were made among cocoa fed diabetic group, Diabetic control group and that of MSG diet group.

The result of Figure 1 showed that as the duration of exposure of mice to MSG diet increases so also the blood glucose level in the mice increases. This is a pointer to the fact that prolong exposure to MSG diet will trigger high blood glucose and invariably diabetic conditions. The result observed in Figure 2 also revealed that there is constant increase in the body weight of the mice as the blood glucose level increases. This attested to the fact that increase in body weight tends to lead to diabetes often times.

The present study on the effect of MSG treatment on FBG, demonstrated that duration and dosage have significant effects on glycemic index. Monosodium glutamate Monosodium glutamate induces Type -2 diabetes without polyphagia.

5. Conclusion

Cocoa powder contains flavanol which are antioxidant that is beneficial to human diets. In this study, cocoa powder acted as prophylactic and therapeutic against diabetes and weight gain. It slows down on the formation of blood glucose level in mice and also against excessive weight gain. Cocoa powder also acted as immune booster through the increase in Red Blood cells count observed in cocoa fed mice and also by normalizing the high count of white blood cells as observed in MSG fed mice.

This study indicated that excessive consumption of Monosodium glutamate (MSG) which is the base for all bouillon condiments that is being used in our day to day cooking at homes and in parties for our daily diets can invariably be the cause of incessant predisposition of many people to diabetic conditions. It is therefore important to cut down the consumption of MSG in our diets in order to live a healthy lifestyle.

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