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Evaluation of the Anti-Diabetic Activity of a Novel Formulation of Functional Foods in Streptozotocin-Induced Balb/c Diabetic Mice Model

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Abstract: ***Diabetes mellitus is a predominant public health concern, causing substantial morbidity, mortality, and long-term complications. Many of the conventional drugs used for the management of this disease are not only expensive but also have numerous side effects. Functional foods are highly compatible with the human body and it's functioning, cheap, and quite available. They serve significant roles in the management of diabetes. This study aimed at evaluating a new formulation of functional foods for reduction of the levels of plasma glucose in Balb/c mice, avoiding major adverse effects. Diabetes was induced in Balb/c mice by injection of streptozotocin at a dose of 160 mg/kg. The mice were orally treated with the researcher's proposed functional food formula for diabetes (FFFD) at the doses of 200, 400, and 600 mg/kg body weight (BW). The glucose levels were measured after 2 hr, 3 hr, and 2 weeks from the administration of the FFFD. Metformin was injected into a number of mice for them to serve as a positive control. The formulation at the dose of 200, 400 and 600 mg/kg reduced plasma glucose levels significantly in all the dose in STZ-induced diabetic mice on 1st, 2nd, 5th day and 2 weeks of administration including metformin group. However, in case of non-treated diabetic mice, the changes in blood glucose levels at 2 weeks of experimental periods has increased the blood glucose level in the experimental mouse. Results shows that FFFD treatment help in reducing blood glucose level and body weight in most of the mice in all groups at different durations. Comparisons of blood glucose level and body weight of all 18 mice compared with control group (untreated mice) were done using the one way Annova and Student's t-test. Most of the results found to be statistically significant compared to the control (untreated seedlings) at P < 0.05. This novel formula of functional foods significantly reduced the levels of the plasma glucose in STZ-induced diabetes in the Balb/c mice. This formula is recommended for the prevention and treatment of diabetes.***

Keywords: Anti-diabetic, functional food, diabetes, streptozotocin, Balb/c mice

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is classified as type 1 diabetes, type 2 diabetes (Kharroubi and Darwish, 2015). Persistent hyperglycemia in diabetes mellitus leads to the development of secondary complications including neuropathy, nephropathy, and retinopathy (Sheikh, et al, 2015). The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar, et al 2008). Traditional treatment only focuses on pancreatic islet function recovery and blood glucose regulation. Additionally, some oral antihyperglycemic agents display various adverse effects including hypoglycemia, edema, gastrointestinal disturbances, and insulin resistance (Scheen, 2007). The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Hence, they play an important role as alternative medicine due to less side effects and low cost (Welihinda, et al, 2008). Hyperglycemia is involved in the etiology of development of diabetic complications. Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver (Hongxiang, et al, 2009). Functional Food is a Natural or processed food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases such as diabetic without side effect. Recently functional foods have attracted the attention of the pharmaceutical and medical scientists due to its benefits for the prevention and long-term treatment of chronic diseases without or minimum side-effects. Now-a-days, functional foods are considered as the medicines which are so friendly to the patients avoiding remarkable side-effects that are very common for the Allopathic medicines. Many functional foods have proven benefits to reduce high blood glucose levels in diabetes. For example, aloe Vera, bitter guard, ginger, broccoli, fenugreek, cinnamon, coriander and lettuce have scientifically proven benefits for diabetes (Fallah, et al, 2012; Hiba, et al, 2009). This research investigates potential of some medicinal plants possessing anti-diabetic activity for treatment of diabetes and elucidates their mechanisms of action. The study performed experiments on animals and examined the therapeutic efficiency of the plant extracts. We hypothesize that the formula of FFFD will be significantly reduce fasting plasma glucose (FPG) and post-prandial blood glucose (PPG) levels in treated diabetic mice compared to untreated mice, significantly increase the plasma insulin levels due to the enhance secretion of insulin by the pancreatic beta-cells, and reduce the lipid profile in treated diabetic mice compared to untreated diabetic mice.

2. Objective

The aim of this study was to develop a new formula of functional foods for treatment of diabetes (FFFD) and to evaluate its therapeutic potential for effective management of Type-1 diabetes without side effects

3. Methods

3.1. List of the Materials that were used for Preparation of the Functional Food Formula:

3.1.1.. Dark brown, natural, unprocessed, Yemeni, pure honey (500 mL).
3.1.2.. Ginger rhizomes (Zingier officinal) 200g- washed, cut into small bits, dried, and crushed to get it in powder form and then weighed.
3.1.3..Fresh Bitter Gourd (momordica charantia) 200g - washed, cut into small bits, dried, and crushed to get it in powder form and then weighed.
3.1.4.. Black seed (Nigella sativa) 200g- crushed to get in the powder form and then weighed.
3.1.5.. Cinnamon sticks (Cinnamomum zeylanicum) 300g- crushed to get in the powder form and then weighed.
3.1.6.. Fenugreek seed (Trigonella foenum-graecum) 200g- crushed to get in the powder form and then weighed.
3.1.7.. Daun Insulin (500g) - crushed to get in the powder form and then weighed.
3.1.8.. Apple Cider Vinegar (500 mL).

3.2 Preparation of the Powders of the Materials that were used for Functional Food Formulation.

The researcher employed six plant materials for preparation of the suggested FFFD: Cinnamon, Bitter guard, Fenugreek, Ginger, Dauan insulin, and Black seeds. The fresh materials were dried and crushed using an electric blender until a fine powder was obtained. Extracts of the materials composing the suggested FFFD were obtained by mixing specific weight of the powder of each material powder with specific volume of (80%) ethanol and concuss the jar twice daily to mix well the ingredients. The amount of alcohol was changed every day for two weeks, and keep them in dark place. All the used materials for preparation FFFD have been processed by the same method.

3.3 List of Chemicals.

The present study mainly used the following chemicals: Ethanol, Citric acid, and Sodium Citrate where it was their source of Dhe tech enterprise Taman Mayang, PJ Malaysia. Streptozotocin, its source was Sigma aldrch USA, and Metformin its source was santa Crutz. Ethanol was used in preparation of the extract of the materials used in the suggested FFFD while STZ was employed to induce T1D in the experimental Balb/c mice. On the other hand, Metformin was used to determine the levels of the blood glucose in the mice of the third experimental group (Group-3). Citric acid and Sodium Citrate were used in combination to prepare the buffer solution.

3.4 Experimental Animals.

The experimental mice were 18 male Balb/c mice at the age of 6-9 weeks weighing approximately 20–25 g each that were purchased from (Pets Wonderland Paradigm Mall in Malaysia). Their source was Envigo (USA). These mice were maintained under standard environmental conditions in the animal house of Lincoln University College. They were fed on normal mice diet, which was too purchased from (Pets Wonderland Paradigm Mall in Malaysia), and watered with mineral water for two weeks before starting the experiments.

3.4.1. Experimental Groups and Protocol

The mice were allocated to six experimental groups consisting of 3 mice, each, in the beginning of the study.
The groups were as follows:
Group-1: Non-diabetic Mice (negative control-1) no treatment.
Group-2: Diabetic Mice without treatment (positive control-2) no treatment.
Group-3: Diabetic mice with metformin (std. antidiabetic drug) 200 mg/kg, BW.
Group-4: Diabetic mice treated with FFFD that divided into 3 groups based on different dose of FFFD:
Dose 1: functional food formulation (200 mg/kg, BW).
Dose 2: functional food formulation (400 mg/kg, BW).
Dose 3: functional food formulation (600 mg/kg, BW).
3.3.3. Extraction of Sesame.

3.5 Alcohol Preparation

The 80% alcohol solution was prepared by mixing absolute ethanol with distilled water at the alcohol: water volumetric ratio of 80:20.

3.6. The amounts of the materials under study and the volumes of the 80% alcohol solution used in extracting the active ingredients from them were as follows:.

3.6.1. Fenugreek extraction: 200 g of fenugreek powder added to 600 mL of 80% ethanol.
3.6.2. Ginger extraction: 200 g of ginger powder added to 500 mL of 80% ethanol.
3.6.3. Bitter Guard extraction: 200 g of Bitter Guard powder added to 500 mL of 80% ethanol.3.4.4.Sesame indicum: 500 g after drying become 20.36 g, 3 g was taken for making formulation.
3.6.4. Black Seed extraction: Black Seed powder added to 500 mL of 80% ethanol.
3.6.5. Cinnamon extraction: 300g of cinnamon added to 500 mL of 80% ethanol.
3.6.6. Dauan Insulin extraction: 59.07 g of dauan insulin powder added to 200 mL of 80% ethanol.

3.7 Filtration of the Extracts

The jars containing the mixture of material powders in ethanol were kept in a dark place for two weeks. Then, the extracts were filtered user filter paper, and the filtrates were transferred to glass jars and, again, kept in a dark place. A heating mantle at 40 ºC and oven at 50 ºC were used to evaporate the alcohol and dried the extract to get the solid mass of the active ingredients from each material, which was

collected in bottles and kept them in fridge until used. The amount of final material extract after filtration of materials are (Bitter guard 32.14 g, Black seed 18.31 g, Ginger12.05 g, Cinnamon 55.25 g, Dawn Insulin 52.43 g, Fenugreek 103.05).

3.8 Preparation of Novel Formulation of Functional Foods for Diabetes.

A laboratory electric balance was used to weigh the specific mass of the retrieved active ingredients so as to prepare the FFFD. The specified masses of the active ingredients of all study materials were added together and mixed well to get the suggested novel FFFD. The formulation of function food should keep in fridge. The amounts of materials extract that used to prepare the final FFFD (Bitter Guard 4g, Black Seed 2g, Ginger 3g, Cinnamon 2g, Fenugreek 5g, Daun Insulin 1g, Pure Honey 15ml, Apple Cider Vinegar 1ml).

3.9. Induction of T1 Diabetes in Mice by Injection with Streptozotocin:

3.9.1. Preparation of Citrate Buffer Solution.
A citrate buffer solution with a pH of 4.5 was prepared in three steps: (i) mixing 1.92 g of citric acid with 100 mL of distilled water, (ii) mixing 2.94 g of sodium citrate with 100 mL of distilled water, and (iii) mixing 44.5 mL of the citric acid solution with 55.5 mL of the sodium citrate solution.

3.10 Induction of Diabetes in Mice.

Diabetes was induced in the mice by a single intra-peritoneal injection with 160 mg/kg of freshly-prepared STZ. The relevant doses were determined according to the body weights of the mice. Streptozotocin was freshly prepared for immediate use within 5 min by dissolving STZ in 100 mL of citrate buffer (pH = 4 - 4.5).

3.11 Treatment of the Diabetic Mice with the Functional Food Formulation

After preparation of final FFFD then Balb/c mice divided into 6 Experimental Groups Each group contained 3 mice, and group number (4) contained (3) doses each dose contained (3) mice:-
Group1: Non-diabetic mice without treatment (negative control)
Group 2: diabetic mice without treatment (positive control)
Mice at (1 and 2 Groups) were administrated distilled water daily using oral gap injection.
Group 3 (metformin) 200mg/kg: Dissolved 0.045g of metformin in 4ml of distilled water and mixed well by pipette, then given 200 microliters of solution to the mice in group- 3 by oral gavage.
Group 4: diabetic mice treated with FFFD
(Dose-1) 200mg/kg: Dissolved 0,040 g of formulation in 4ml of distilled water a mixed well by pipette then given 200 microliters of solution to the mice.
(Dose-2) 400 mg/kg: Dissolved 0,080g of formulation in 4ml of distilled water and mixed well by pipette pipet then given 200 microliters of solution to the mice.
(Dose-3) 600 mg/kg: Dissolved 0,160g of formulation in 4ml of distilled water and mixed well by pipette then given 200 microliters of solution to the mice.

Metformin and functional food formulation treatment given to the mice for 3 Consecutive days and after one week.

3.12 Measurement of blood glucose level:

Blood samples were obtained from the tail vein of the mice by aseptic prick of the tail tip. The tail was nibbled by use of a sterilized needle and a drop of blood was squeezed into a strip of Glucometer. After collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in absolute ethanol to protect the animal from infection and to arrest further bleeding. Then, the levels of glucose in the blood of the mice were assessed before and after treatment with the proposed FFFD, using one-touch glucometer. The glucose levels were measured as follows:

On the first day, before the FFFD, and metformin treatment and three hours after treatment, on the second day, before the treatment and two hours after the treatment, on the third day, before the treatment and two hours after the treatment, and after two weeks of the treatment.

Also, blood glucose levels of the mice in the group 1 and group 2 were measured in the same method and at the same time of other groups.

3.13 Measurement of mice body weight

Measurement of body weight before beginning the experiment, and after finishing the experiment with treatment by FFFD (after 2 weeks) was done by using Electric balance as shown below. All 6 groups reading were compared together.

3.14 Data analysis

Statistical Analysis was done using IBM SPSS 22 version software. The results for blood glucose level were presented as mean ± S.D (standard deviation). One way analysis of variance (ANOVA) test was used in this study with P values < 0.05 being considered as significant.

4. Results

The present research work was done in lab environment at Lincoln University. Total 18 mice were used in this study, which were equally divided into 6 different groups (3 mice each group).

Diabetes was induced in Balb/c mice was treated with FFFD 200, 400 and 600 mg/kg dose. Group 3 diabetic mice were treated with metformin at dose of 200 mg/kg and use as a standard. Non-diabetic Mice (group-1) used as a negative control and diabetic mice without treatment was used as a positive control (group-2). The blood glucose level and body weight was measured at different durations.

4.1 Effect of Formulation Extract on the Body Weight of Mice before and After the Treatment.

The body weight of mice was measured before starting the experiment and after completion the experiment, and it was observed that the body weight of mice that treated with FFFD in end the experiment decreased compared to its weight before starting experience as shown in Figure 1.

The changes between the body weight of mice between the first and last day of experiment for 2 weeks have been shown in Table 1. The average body weight of diabetic mice treated with 200, mg/kg of FFFD were significantly reduced whereas at other doses and duration it was not significantly reduced. This shown that the FFFD formula may have effect on lipid profile, and it causes reduce weight of body mice.

Table 1: Effect of Formulation Extract on the Body Weight of Mice Before and After the Treatment

Mice	Non-Diabetic Control Group-Before	Non-Diabetic Control Group-After	Diabetic Control Group-Before	Diabetic Control Group-After	Diabetic mice +Metformin (200mg/kg)-Before	Diabetic mice +Metformin (200mg/kg)-After	FFFD (200 mg/kg)-Before	FFFD (200 mg/kg)-After	FFFD (400 mg/kg)-Before	FFFD (400 mg/kg)-After	FFFD (600 mg/kg)-Before	FFFD (600 mg/kg)-After
1	13.63±0.18*	12.88±1.24	20.63±9.72	19.27±7.79	14.88±1.58	13.74±0.02*	16.97±4.54	14.38±0.88*	18.44±6.62	17.38±5.11	18.08±6.1	17.16±4.8
2	17.99±0.0*	15.39±0.08	18.74±0.36	15.54±0.14	18.99±0.0	16.44±0.14*	20.13±0.04	18.27±0.04*	22.84±0.04	20.86±0.08	21.93±0.09	19.3±0.02
3	13.97±0.03*	11.21±0.02	19.64±0.05	16.2±0.14	16.34±0.02	13.21±0.03*	20.01±0.01	17.86±0.05*	22.78±0.02	18.98±0.02	24.8±0.14	20.61±0.09

An asterisk signifies a statistically significant difference change in body weight in FFFD Balb/c mice before and after 2 weeks of administration at $P < 0.05$.).

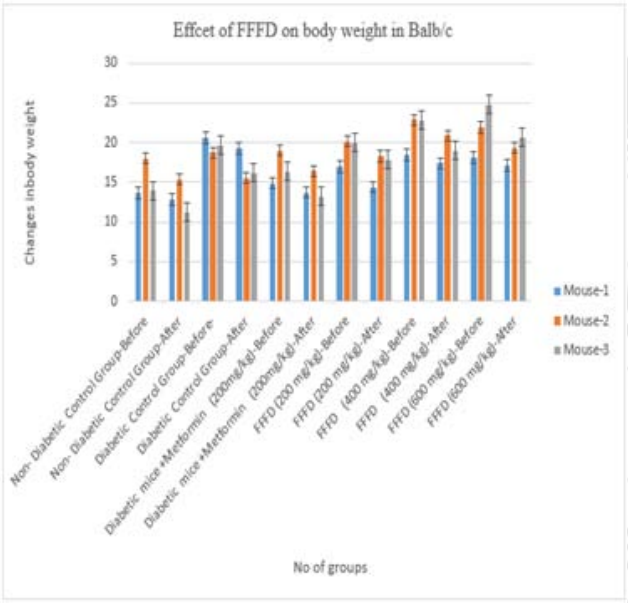

Figure 1: Effect of Formulation Extract on the Body Weight of Mice Before and After the Treatment

4.2. Effect of FFFD on change in glucose level in streptozotocin induced diabetic Balb/c mice 3 hours after treatment (first day)

Balb/c diabetic mice were treated with different doses of (FFFD) (200, 400 and 600 mg/kg B.W.) and blood glucose levels were measured from the tail vein 3 hours after the treatment. Metformin was used as a standard antidiabetic drug to compare the effect of the sample. In non-diabetic (group 1) and diabetic (group 2) the blood glucose level was found to be increase. The formulation at the dose of 200, 400 and 600 mg/kg in mice plasma glucose levels found to be significantly reduced by t-test method (Figure 2). In metformin treated mice also significantly reduced plasma glucose levels was observed. Changes in blood glucose levels by 3 hours experimental periods have increased the blood glucose levels in all experimental mice (Table 4.2).

Table 2: Effect of Feed on change in glucose level in streptozotocin induced diabetic Balb/c mice 3 hours after treatment (first day)

Mice	Non- Diabetic Control Group-Before	Non- Diabetic Control Group-After	Control Diabetic Group-Before-	Diabetic Control Group-After	Diabetic mice +Metformin (200mg/kg)-Before	Diabetic mice +Metformin (200mg/kg)-After	FFFD (200 mg/kg)-Before	FFFD (200 mg/kg)-After	FFFD (400 mg/kg)-Before	FFFD (400 mg/kg)-After	FFFD (600 mg/kg)-Before	FFFD (600 mg/kg)-After
1	5.18±0.02*	5.36±0.05*	8.3±0.25*	11.1±0.14	23.66±0.05*	15.13±0.09*	10.34±0.07*	8.95±0.07*	10.4±0.14*	9.05±0.07*	11.95±0.07*	9.95±0.07*
2	4.78±0.02*	5.8±0*	10.45±0.06*	12.04±0.05	12.56±0.04*	8.11±0.12*	7.9±0.14*	5.67±0.04*	13.9±0.14*	7.1±0.14*	14.03±0.04*	8.64±0.06*
3	6.3±0.14*	7.1±0.14*	8.07±0.09*	11.65±0.21	15.7±0.42*	15.15±0.21*	18.63±0.09*	8.25±0.06*	18.46±0.04*	11±0.12*	31.58±0.58*	26.55±0.21*

An asterisk signifies a statistically significant difference change in blood glucose level in FFFD Balb/c mice before and after 3 hours of administration at $P < 0.05$.

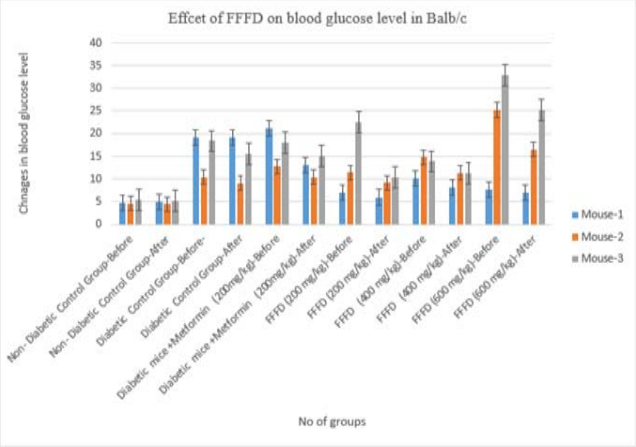

Figure 2: Effect of FFFD on change in glucose level in streptozotocin induced diabetic Balb/c mice 3 hours after treatment (first day)

4.3. Effect of functional food formula on change in glucose level in streptozotocin induced diabetic Balb/c mice 2 hours after treatment (second day)

Balb/c diabetic mice were treated with different doses of PMFD (200, 400 and 600 mg/kg B.W.) and blood glucose levels were measured from the tail vein 2 hours before and after the treatment. Metformin was used as a standard antidiabetic drug to compare the effect of the sample. The result has been presented in the Figure 3 and 4.

The formulation at the dose of 200, 400 and 600 mg/kg in balb/c mice shows significantly reduction in plasma glucose levels at all the duration in both observations (1st observation and 2nd observation). However, in case of non-treated diabetic mice, the changes in blood glucose levels by 2 hours experimental periods has increased the blood glucose level in all experimental mice (1st observation and 2nd observation). An asterisk in tables signifies a statistically significant difference change in blood glucose level in FFFD Balb/c mice before and after 2 hours of administration at $P < 0.05$ (Table 3 and 4).

Table 3: Effect of FFFD on blood glucose levels in STZ- induced balb/c mice 2 hours after treatment of diabetic mice (1st observation)

Mice	Non- Diabetic Control Group-Before	Non- Diabetic Control Group-After	Group- Control Diabetic Before-	Diabetic Control Group-After	Diabetic mice +Metformin (200mg/kg)-Before	Diabetic mice +Metformin (200mg/kg)-After	FFFD (200 mg/kg)-Before	FFFD (200 mg/kg)-After	FFFD (400 mg/kg)-Before	FFFD (400 mg/kg)-After	FFFD (600 mg/kg)-Before	FFFD (600 mg/kg)-After
1	4.8±0.0*	4.95±0.07*	19.14±0.05*	19.07±0.07*	21.14±0.06*	13.05±0.07*	7.05±0.07*	5.95±0.07*	10.1±1.41*	8.05±0.07*	7.65±0.07*	6.95±0.07*
2	4.55±0.07*	4.47±0.03*	10.45±0.06*	9.05±0.07*	12.76±0.05*	10.45±0.06*	11.46±0.05*	9.14±0.07*	14.8±0*	11.35±0.06*	25.21±0.05*	16.46±0.05*
3	5.35±0.07*	5.16±0.05*	18.36±0.05*	15.46±0.04*	18.05±0.07*	15.020.02*	22.46±0.05*	10.37±0.03*	13.85±0.07*	11.28±0.02*	32.85±0.05*	25.27±0.04*

An asterisk signifies a statistically significant difference change in blood glucose level in FFFD Balb/c mice before and after 3 hours of administration at $P < 0.05$.

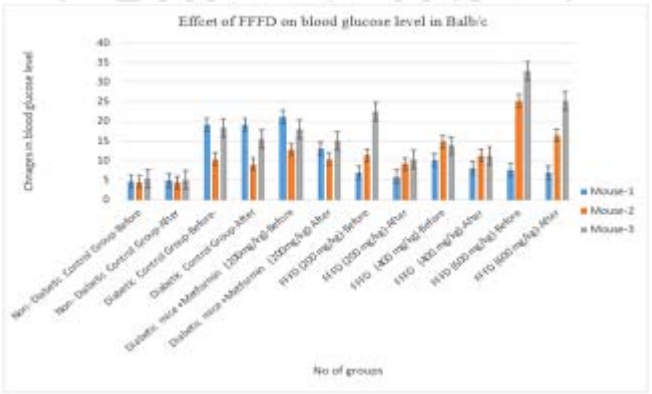

Figure 3: Effect of FFFD on blood glucose levels in STZ- induced balb/c mice 2 hours after treatment of diabetic mice (1st observation)

Table 4: Effect of (FFFD) on blood glucose levels in STZ- induced mice 2 hours after treatment of diabetic mice (2nd observation)

Mice	Non- Diabetic Control Group-Before	Non- Diabetic Control Group-After	Group-Control Diabetic Before-	Diabetic Control Group-After	Diabetic mice +Metformin (200mg/kg)-Before	Diabetic mice +Metformin (200mg/kg)-After	FFFD (200 mg/kg)-Before	FFFD (200 mg/kg)-After	FFFD (400 mg/kg)-Before	FFFD (400 mg/kg)-After	FFFD (600 mg/kg)-Before	FFFD (600 mg/kg)-After
1	5.27±0.03*	4.81±0.02*	6.27±0.03*	9.95±0.07*	16.04±0.05*	15.65±0.07*	4.65±0.07*	3.45±0.07*	19.38±0.02*	17.25±0.07*	9.05±0.07*	7.45±0.05*
2	4.58±0.02*	4.17±0.03*	13.57±0.04*	10.05±0.07*	19.18±0.02*	17.91±0.01*	4.41±0.02*	4.06±0.03*	21.92±0.02*	14.81±0.02*	26.03±0.04*	19.58±0.02*
3	4.26±0.04*	4.17±0.03*	12.06±0.05*	15.72±0.02*	16.82±0.02*	14.03±0.04*	25.38±0.02*	7.42±0.05*	12.95±0.07*	8.28±0.02*	30.46±0.05*	28.28±0.02*

An asterisk signifies a statistically significant difference change in blood glucose level in FFFD Balb/c mice before and after 2 hours of administration at $P < 0.05$.

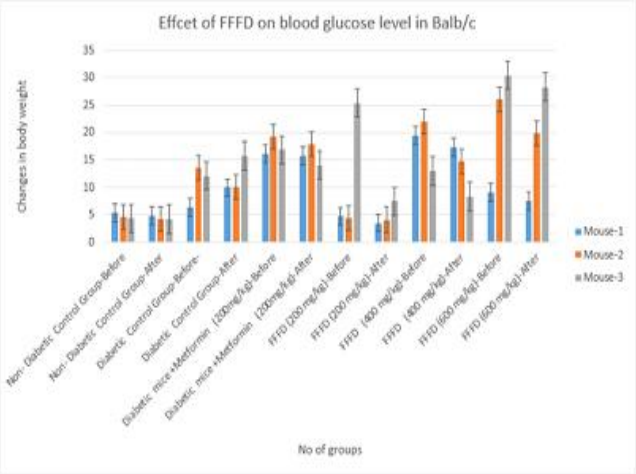

Figure 4: Effect of (FFFD) on blood glucose levels in STZ-induced mice 2 hours after treatment of diabetic mice (2nd observation)

4.4. Effect of functional food formula on change in glucose level in streptozotocin induced diabetic Balb/c mice after 2 weeks

Balb/c diabetic mice were treated with different doses of (FFFD) (200, 400 and 600 mg/kg B.W.) and blood glucose levels were measured from the tail vein 2 weeks after the treatment. Metformin was used as a standard antidiabetic drug to compare the effect of the sample (Table 5). The formulation at the dose of 200, 400 and 600 mg/kg reduced plasma glucose levels significantly in the entire dose in STZ-induced diabetic mice after 2 weeks of administration including metformin group. However, in case of non-treated diabetic mice, the changes in blood glucose levels at 2 weeks of experimental periods has increased the blood glucose level in the experimental mouse.Overall results prove that FFFD treatment help in reducing blood glucose level and body weight in most of the mice in all groups. Comparisons of blood glucose level and body weight of all 18 mice compared with control group (untreated mice) were done using the one way Annova and Student's t-test. Most of the results found to be statistically significant compared to the control (untreated seedlings) at $P < 0.05$.

Table 5: Effect of functional food formula on change in glucose level in streptozotocin induced diabetic Balb/c mice after 2 weeks

Mice	Non- Diabetic Control Group-Before	Non- Diabetic Control Group-After	Group-Control Diabetic Before-	Diabetic Control Group-After	Diabetic mice +Metformin (200mg/kg)-Before	Diabetic mice +Metformin (200mg/kg)-After	FFFD (200 mg/kg)-Before	FFFD (200 mg/kg)-After	FFFD (400 mg/kg)-Before	FFFD (400 mg/kg)-After	FFFD (600 mg/kg)-Before	FFFD (600 mg/kg)-After
1	5.18±0.02*	4.81±0.14	8.48±0.02*	10.27±0.04*	23.67±0.03*	15.15±0.07*	10.36±0.05*	6.92±0.03*	10.48±0.02*	6.07±0.04*	11.93±0.04*	6.16±0.04*
2	4.82±0.02*	3.95±0.07	10.46±0.05*	12.34±0.05*	12.57±0.03*	10.47±0.03*	7.83±0.04*	6.07±0.03*	7.17±0.03*	4.62±0.03*	14.04±0.06*	8.05±0.07*
3	4.57±0.04*	4.15±0.21	8.05±0.07*	15.65±0.07*	15.65±0.07*	14.76±0.05*	18.66±0.05*	6.46±0.04*	18.05±0.07*	6.58±0.02*	32.05±0.07*	7.27±0.04*

An asterisk signifies a statistically significant difference change in blood glucose level in FFFD Balb/c mice before and after 2 hours of administration at $P < 0.05$.

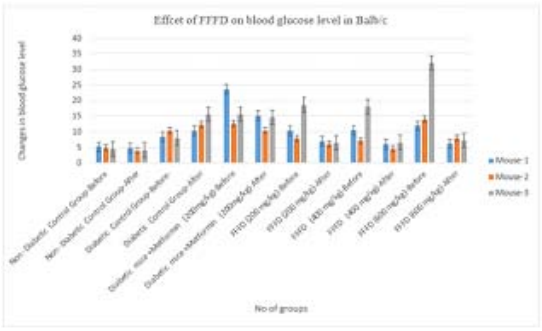

Figure 5: Effect of functional food formula on change in glucose level in streptozotocin induced diabetic Balb/c mice after 2 week

5. Discussion

The epidemic of diabetes threatens the health of a large number of individuals in the developed and developing countries alike (Cheema et al., 2014).Some studies have reported that in around a year approximately 5-10% of the pre-diabetic population will suffer from diabetes and problems associated with it such as heart problems, imbalance in glucose and lipid metabolism, and vascular disorders. Timely interventions in this population can preserve the pancreatic beta cells and improve their performance (Chen et al., 2010). Current medications to control blood glucose and lipid profile may have dangerous side effects over time such as increased risk of weight gain, liver toxicity, and cardiovascular diseases (American Diabetes Association, 2008). Thus, there is a pressing need for use of stronger alternatives with fewer side effects than the medications in common use currently. In this regard, nutritional interventions, change in lifestyle, and behavioral therapy are on the rise. However, these interventions alone may not be effective in preventing the development of T2D (Cao et al., 2010). Traditional (i.e., herbal) medicine is used for treatment of diabetes in developing countries where the cost of conventional medicines is a massive burden on the population (Saravanan and Par, 2008). Traditional plant medicines are used throughout the world as alternative therapies to control diabetes. So far, many herbs have been reported to possess some degree of anti-diabetic activity (Patel et al., 2012). Functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition. It must be stated here that foods are natural sources of nutrients and that a diversified diet can be a rich source of all the necessary bioactive substances (Zeng et al., 2012). These foods can have a higher potential for the prevention or cure of diabetes than plant species. It is indeed the time to give more attention to the functional food ingredients as target medicinal foods in order to prevent or slow down the development of diabetes. Many foods (e.g., cinnamon, fenugreek, and ginger) are used to reduce the blood glucose levels and are used as alternative medicines because diabetic medicines are very expensive and some of them have side effects and may cause complications in the body. In vitro and in vivo animal studies have reported strong insulin-like or insulin-potentiating effects after cinnamon administration (Baker et al, 2008). Extracts of cinnamon activate glycogen synthase and insulin receptor kinase, increases glucose uptake, and inhibit glycogen synthase kinase-3 and dephosphorylation of the insulin receptor, leading to maximal phosphorylation of the insulin receptor (Blevins et al., 2007). Various reports (e.g., Raghuram, Sharma, and Sivakumar (1994), and Puri, Prabhu, and Murthy (2002) have demonstrated that fenugreek seed extracts and powder, and the gum of the seeds and leaves lowered the blood glucose and cholesterol levels in human and experimental diabetic animals. Incorporating just around 25 g fenugreek seeds in the daily diet can serve as an effective supportive therapy in the management of diabetes. The biochemistry and bioactivities associated with the antidiabetic effect of the extracts of bitter melon and M. charantia as a whole have been extensively studied. One in vitro study showed that bitter melon could increase insulin secretion from B cells. Moreover, immunostaining data indicated that the juice of the bitter melon increased B cells in the pancreas of STZ-treated rats. Modes of action of bitter melon and M. charantia include insulin secretion, inhibition of glucose reabsorption in guts, preservation of islet B cells and their functions, increase of peripheral glucose utilization, and suppression of gluconeogenic enzymes. Of note, momorcharin and momordicin, isolated from M. charantia and its fruit, act to lower blood glucose likely because they possess insulin-like chemical structures (Singh, et al, 2011). More recently, Li and colleagues reported that ginger extract enhanced insulin release and reduced insulin resistance (Li, et al, 2012). One clinical study reported that consumption of ginger powder, 3 g per day for 30 days, significantly reduced blood glucose and lipids in T2D patients (Andallu, et al. 2003). The result of this study showed that these material that used as treatment formula have effective compounds that effect on blood glucose level in mice, and may be act as the role of insulin to reduce the blood glucose or enhanced the pancreas to increase insulin secretion in blood. Scientific evaluation of the functional foods recommended for the management of diabetes is lacking. The researcher performed this study to evaluate a formulation of functional foods for the reduction of blood glucose levels. This study tasted the effect of a mixture of six functional foods (includes Ginger, Cinnamon, Apple Cider vinegar, Black seed, Bitter Gourd, Fenugreek, and Daun Insulin) on the levels of glucose in the blood of mice having T1D affected by STZ. The six functional foods were mixed to develop a novel formula for treatment of diabetes in mice. The study results have shown that the proposed formula has anti-diabetic activity. Outcomes of this investigation demonstrated that this novel formula of functional foods did effectively reduce the blood glucose levels. They showed that the mixture of the six materials used in this study for treatment of T1D in mice has profound effect on the level of glucose in the blood of the Balb/c mice and that this suggested FFFD may assume the role of insulin in reducing the blood glucose or enhance functioning of the pancreas to increase insulin secretion in the blood. This data proved that this novel formulation of functional foods effectively reduced blood glucose levels. From our investigation, we found that diabetic mice treated with FFFD at the doses of 200, 400 and 600 mg/kg, shows the reduced plasma glucose levels at 3 hours (Figure 4.2) and 2 hours after treatment (Figure 4.3 and 4.4). Treatment of Metformin in diabetic mice also shows reduced plasma glucose levels. This indicates that in case of normal diabetic mice, the condition of diabetes was deteriorated with the passing of days, with the increasing of glucose levels without treatment. We found that diabetic mice treated with FFFD at the doses of 200, 400 and 600 mg/kg also reduced body weight (Figure 4.1) after 2 weeks treatment. The body weight reduction by FFFD was strongly comparable with diabetic mice untreated groups. This results supports the effectiveness of FFFD as it could effectively control the diabetic and obesity disease status with effective control of blood glucose levels and weight loss in the treated groups.

6. Conclusion

Outcomes of this study lead the researcher to the conclusion that foods such as Ginger, Bitter Gourd, Fenugreek, Black Seed, Cinnamon, Daun Insulin, Apple Cider Vinegar, and Pure Honey possess an anti-diabetic activity. A mixture of these foods producing the proposed FFFD reduced the levels of the plasma glucose in mice with T1D induced by STZ without major side-effects. This proposed FFFD also reduced the lipids in the treated diabetic mice.

The researcher concludes that while the suggested FFFD has comparable anti-diabetic potential and capacity to those of Metformin, an FFFD dose of 400 mg/kg yields better anti-diabetic capacity than Metformin. It gives the best anti-diabetic results. The researcher concludes that the optimum FFFD dose for management of diabetes in the Balb/c mice is 400 mg/kg BW. This provides evidence on that in general the treatment of the T1D induced in the mice by STZ using the suggested FFFD is much more effective that treatment with Metformin. In consequence, this formula is recommended for the prevention and treatment of diabetes.

7. Recommendations

Based on the findings of this study, the researcher gives the following recommendations:

7.1. Additional in-vivo studies and clinical trials would be needed to justify and further evaluate the potential activity of the functional food formulation.

7.2. The development of this formulation to be used for the treatment of diabetes in humans, because of its effect on reducing the level of sugar in the blood.

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