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Evaluation of A Novel Formulation from Functional Foods for Hypoglycemic and Weight Loss Activities in High-Fat Balb/C Mice

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Abstract: ***Diabetes is a metabolic disorder in which the body does not produce enough insulin to regulate blood glucose levels or where the insulin produced is unable to work efficiently. There are two main types of diabetes: Type 1 and Type 2 diabetes. Obesity is only associated with type 2 diabetes. Diabetes mellitus and obesity has the relationship since many years and it is most prevalent despite intense research. Functional foods have a potentially positive effect and help reduce the risk of diseases on health beyond basic nutrition. Our present study aimed to develop a new formulation from functional foods (FFF) and to evaluate its therapeutic potential for the effective management of diabetes and obesity avoiding major adverse effects in animal model. Obesity was induced to Balb /c mice with high fat diet for four weeks. The mice were treated with the formulation of functional foods (FFF) at the doses of 200, 400 and 600 mg/kg and metformin (200 mg/kg) orally. The glucose levels were measured 2 hours, 3 hours, 2 weeks and body weight was measured after 2 weeks of treatment with the formulation. Treatment of 200 mg/kg Metformin shows significant reduction at 3 hours, 2 hours and 2 weeks, FFF treatment of 200 mg/kg shows promising results at 3 hours, 2 hours on 2nd day, FFF treatment of 400 mg/kg signify decrease in glucose level at 2 hours of 3rd day and 2 weeks and FFF treatment of 600 mg/kg produce significant reduction at 3 hours and 2nd week. The body weight results of at all the 4 treatment was found to be expressively reduced. All the data compared to that of the positive and negative control (untreated) were done using the Student's t-test at P < 0.05. The formulation of function foods significantly reduced the post-prandial plasma glucose and enhanced weight loss in HFD Balb/c mice. Taken together, this study determined the present formulation of functional foods, which might be recommended for the prevention and treatment of obesity and diabetes.***

Keywords: Diabetes Multiuse, Hypoglycemia, Functional foods, High fat diet, Obesity, Nutraceuticals

1. Introduction

Globally, more than 250 million people are facing diabetes and it is predicted to be double in over 20 years (Matthews et al., 2010; Wild et al., 2004; Stefánsson et al., 2015). Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action or both (ICMR 2005). The world is facing an epidemic of diabetes, which clearly reflects the imbalance in the pancreas and the secretion of the hormone insulin in the blood. Diabetes is a crucial, chronic illness, which requires continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. (Kaufman, 2012). The level of seriousness of diabetes in general are vary between person to person, but can be controlled if the disease has been detected at early stage and diagnosed with good care. Obesity is a major serious disease condition in today's world. It can happen when the body keep accumulating more fat that normal body condition that cause cardiovascular diseases and type 2 diabetes mellitus. Diabetes and obesity are two main risk factors for most of the acute and chronic diseases including renal impairment, cardiovascular diseases, stroke, and so on. Almost all the obesity and diabetes synthetic drugs exist as an allopathic medicine, which are associated with several other potential adverse effects. Thus, depending only with the allopathic medicine for controlling lipid level and diabetes is not very safe as they increase the additional risk of several other diseases. Therefore, searching for novel drug or formulation of drugs from natural sources, especially from functional foods is important to solve this problem (Kai et al., 2015). The consumption of functional foods and its formulated products (nutraceuticals) have greatest safety over the conventional allopathic medicines, its less expensive and provide long-term therapeutic benefit and improve quality of life for patients with the improvement of change in glucose level and weight loss.Towards this goal, we analyzed a few group of mice administered with the desired doses of the formulation of functional foods for weight loss and hypoglycemia before and after the mice feed with high fat. Another group of Metformin, a standard glucose lowering drug, used to compare the effectiveness of the formulation of functional food (FFF). We hypothesize that this FFF was strongly control the production of glucose level in blood and weight loss in mice feeding with high fat. The expected result may significantly contribute for the management of blood glucose level and obesity with no or minimizing the side-effects of conventional diabetes and obesity. This animal study result can be a use for further study of the effectiveness of new formulation of function food as the effective management of obesity and diabetes, which will help to improve the quality of life.

2. Objective

Study aimed to develop a new formulation from functional foods (FFF) and to evaluate its therapeutic potential for the effective management of diabetes and obesity avoiding major adverse effects in animal model.

3. Methods

3.1 Collection of functional food.

Used for experiment below listed is the function food that used in experiment, including the quantity as follow:

3.1.1. Cinnamon sticks (500g) - crushed to get in the powder form and then weighed.
3.1.2. Apple Cider Vinegar (500 ml).
3.1.3. Fresh Avocado fruit (1kg) - washed, cut into small bits, dried, and crushed to get it in powder form and then weighed.
3.1.4. Lemon juice (500ml)
3.1.5. Extra virgin olive oil (500ml)
3.1.6. Sesame indium (500g) - crushed to get it in powder form and then weighed.

3.2 Experimental Animal

The mice (18) were distributed to six experimental groups. Each group contained 3 mice, first group (1st), Mice fed by normal diet (negative control), While second group (2ed) was mice fed by high fat diet without treatment (positive control), and was third group (3th) Contained mice fed with HFD and treated with metformin (200 mg/kg, B.W.), The forth group (4th) Contained mice fed with HFD and treated with FFF (200 mg/kg, B.W.), In addition to the fifth group (5th) contain mice fed with HFD and treated with FFF (400 mg/kg, B.W.), finally last group was (6th) and Contained mice fed with HFD and treated with FFF (600 mg/kg, B.W.)

3.3 Extraction of function foods materials.

Preparation the extraction of material by mixing specific weight of material powder *(cinnamon powder, Avocado powder and Sesame powder)* with (80%) ethanol and concuss the jar twice daily for the ingredients to mix well and change the amount of alcohol every day for 2 weeks. All extraction formulation of function food should keep in fridge.

3.3.1. Extraction of Cinnamon.
The cinnamon powder (300 g) added to 500 ml of Ethanol 80% and concuss the jar twice daily for mixing well and change the amount of alcohol every day for 2 weeks and keep it in dark place. Finally, all Cinnamon extraction was filtering user filter paper. The collected extract was dried by using heating mantle at 40 ºC temperature and oven at 50 ºC.

3.3.2. Extraction of Avocado.
The Avocado powder (141.28 g) added to 400 ml of Ethanol 80% and concuss the jar twice daily for mixing well and change the amount of alcohol every day for 2 weeks and keep it in dark place. Finally, all Avocado extraction was filtering user filter paper. The collected extract was dried by using heating mantle at 40 ºC temperature and oven at 50 ºC.

3.3.3. Extraction of Sesame.
The Sesame powder (200 g) added to 400 ml of Ethanol 80% and concuss the jar twice daily for mixing well and change the amount of alcohol every day for 2 weeks and keep it in dark place. Finally, all Sesame extraction was filtering user filter paper. The collected extract was dried by using heating mantle at 40 ºC temperature and oven at 50 ºC.

3.3.4. Extraction of lemon juice.
Lemon juice (500ml) was dried by using heating mantle at 40ºC temperature and oven at 50ºC.

3.4 Formulation of functional food

3.4.1.Cinnamon: (500 g) after drying become 61.34 g, 10 g was taken for making formulation.
3.4.2.Lemon juice: (500 mL) after drying become 28.1 g, 6 g was taken for making formulation.
3.4.3.Avocado fruit: 1 kg after drying become 11.34 g, 3 g was taken for making formulation.
3.4.4.Sesame indicum: 500 g after drying become 20.36 g, 3 g was taken for making formulation.
3.4.5.Apple Cider Vinegar (500 ml) was taken 1 ml for making formulation.
3.4.6.Extra virgin olive oil (500ml) was taken 15ml for making formulation.
Formulation of functional foods that include Cinnamon (10 g), Avocado (3 g), Sesame (3 g), Apple Cider Vinegar (1 ml) and Extra Virgin olive oil (15 ml) collected in bottle and kept it in fridge until used.

3.5 Preparation of high fat diet (HFD)

Composition of High Fat Diet for Balb/c mice are Protein 20%, Carbohydrate 20%, Fat 58%, Calcium carbonate complex with Vitamin 2%, which is Total = 100%.

High fat diet (HFD), preparated by mixing all ingredients together "Red Lentils (Masoor dal) (protein), Casein (protein), Cornstarch (carbohydrate), Sugar (carbohydrate), Soybean oil and Calcium carbonate complex with Vitamin" and squeezed all ingredients very well, after that formed as tablets to feed Balb/c mice daily for two weeks before proceed in the experiments and after two week proceed in the experiments.

3.6 Treatment of mice with formulation of functional food (FFF)

After preparation of final functional food formulation FFF then Balb/c mice divided into 6 experimental groups:

3.6.1 Group1-Non-HFD without treatment (negative control)

3.6.2 Group 2 - HFD without treatment (positive control)

3.6.3 Group 3 (metformin): 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- 6 by oral gavage.

3.6.4 Group 4 (Dose-1): Dissolved 0,080g of formulation in 4ml of distilled water and mixed well by pipette then given 200 microliters of solution to the mice.

3.6.5 Group 5 (Dose-2): Dissolved 0,160g of formulation in 4ml of distilled water and mixed well by pipette pipet then given 200 microliters of solution to the mice.

3.6.6 Group 6 (Dose-3): Dissolved 0,040g 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 one week.

3.7 Measurement of blood glucose level

Blood samples were obtained by aseptic prick from the tip of the tail. The tail was nibbled by use of a sterilized needle; a drop of blood was squeezed into some strips 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. Measured blood glucose levels on first day before and after 3h of treatment with formulation. Glucose levels were measured on second and third days before and after 2h of treatment with formulation to mice. Glucose levels were measured before and after 2 week of treatment.

3.8 Measurement of body weight

Measurement of body weight before and after feeding to the mice with high fat diet (HFD) on the first day of experiment with treatment by FFF and at 2 weeks was done by using Electric balance. All 6 groups reading were compared together.

3.9 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 study was carried out in controlled laboratory environment. Total 18 Balb/c mice were used in this study, which were equally divided in 6 groups that is 3 mice each group. The administration of high fat diet and measured their blood glucose levels on 1st day before and after treatment of 3 hours, 2nd and 3rd day before and after treatment of 2 hours, and after 2 weeks. Body weight was measured on 2nd week before and after treatment with FFF.

The mice with normal fed diet was considered as negative control (Group-1) and the mice fed with HFD without treatment was considered as a positive control (Group-2). Other groups experiment on treated mice as listed below.

Group-3: Mice fed with HFD and treated with metformin (200 mg/kg, B.W.)

Group-4: Mice fed with HFD and treated with FFF (200 mg/kg, B.W.)
Group-5: Mice fed with HFD and treated with FFF (400 mg/kg, B.W.)

Group-6: Mice fed with HFD and treated with FFF (600 mg/kg, B.W.)

From the results (Figure 4.1), the effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice before and after 3 hours of administration (1st day) shows that the glucose level was reduced after the treatment of functional food formula in group 3, 4, 5 and 6 compared to before treated mice. However, the changes in glucose level was not significantly changes in group 5 regardless of the amount of 400 mg/kg treatment and group 3, 4 and 6 shows statistically significant results (Table.1). Figure 2 reading shows the effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice after 2 hours of administration (2nd day) was reduced in group 3, 4, 5 and 6 compared to before the treatment. But, only group 4 indicates the statistically significant result whereas group 3, 5 and 6 shows large standard deviation rendered the increase statistically insignificant (Table 2). Blood glucose level in HFD Balb/c mice after 2 hours of administration of functional food formula (FFF) on 3rd day shows significant reduction in group 3 and group 5, but group 4 and 6 did not appear statistical significant (Table 3 and Figure 3). The effect of functional food formula (FFF) on high fat (HFD) Balb/c mice after 2 weeks of administration shows reduction in blood glucose level and body weight respectively in all the group (Figure 4 and Figure 5). Whereas, the glucose was significantly decreased in group 3, 5 and 6, only group 4 was not significantly change from before treated mice. The body weight results of all the group in all the mice was found to be significantly correlated with untreated mice (Table 4 and Table 5). Overall results confirmed the changes in blood glucose level and body weight of the mice was found to be reduced. Comparisons of the changes in glucose levels and body weight of all the groups and treated mice compared to that of the control (untreated) were done using the Student's t-test. Most of the results shows to be statistically significant difference compared to the control (untreated) at $P < 0.05$). Considered as significant.

Table 1: Effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice before and after 3 hours of administration (1st day)

mice	Group-1		Group-2		Group-3		Group-4		Group-5		Group-6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	5.10±1.41	5.20±0.28	2.95±0.70	4.20±0.28*	16.10±1.14*	15.40±0.28*	8.10±1.14	6.25±0.35*	8.20±0.28	3.20±0.28	7.15±0.21*	2.25±0.35*
2	6.40±0.56	5.55±0.35	5.25±0.35	7.25±0.35*	12.30±0.42*	9.10±1.41*	7.50±0.42	3.25±0.35*	7.25±0.35	4.25±0.35	8.30±0.42*	4.25±0.35*
3	7.20±0.28	7.10±0.14	4.25±0.35	6.25±0.35*	16.30±0.42*	15.20±0.28*	5.45±0.49	3.60±0.42*	6.40±0.56	4.30±0.42	3.95±0.07*	2.10±0.14*

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

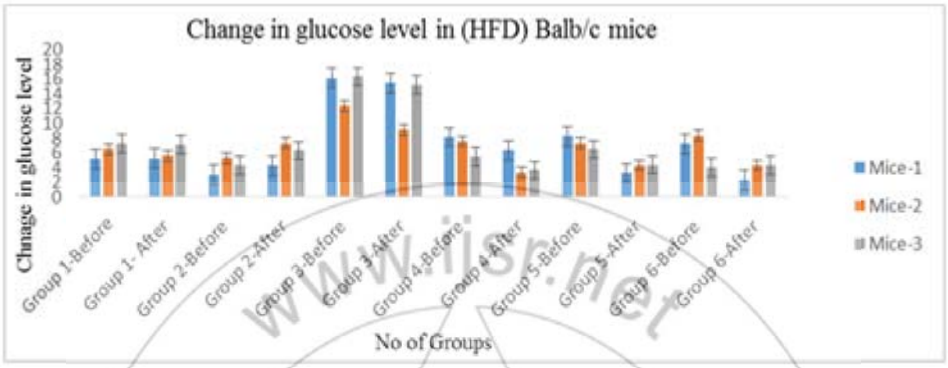

Figure 1: The error bar indicates the statistically significant difference compared to the control at $P < .05$.

Table 2: Effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice before and after 2 hours of administration (2nd day)

	Group-1		Group-2		Group-3		Group-4		Group-5		Group-6	
mice	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	4.5±0.42	4.7±0.28	3.95±0.07*	5.95±0.07*	16.35±0.21*	13.15±0.21	4.9±0.14	2.1±0.14*	6.05±0.07*	2.9±0.14	7.1±0.14*	3±0.14
2	4.3±0.42	4.25±0.35	6.65±0.21*	8.15±0.21*	10.25±0.35*	10.25±0.35	5.7±0.35	3.1±0.14*	4.15±0.21*	2.15±0.21	4.9±0.14*	2.3±0.28
3	3.2±0.28	4.15±0.21	4.9±0.35*	6.25±0.35*	9.9±0.14*	9.9±0.35	6.65±0.21	3.4±0.14*	5.2±0.28*	2.85±0.21	5.15±0.21*	2.15±0.21

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

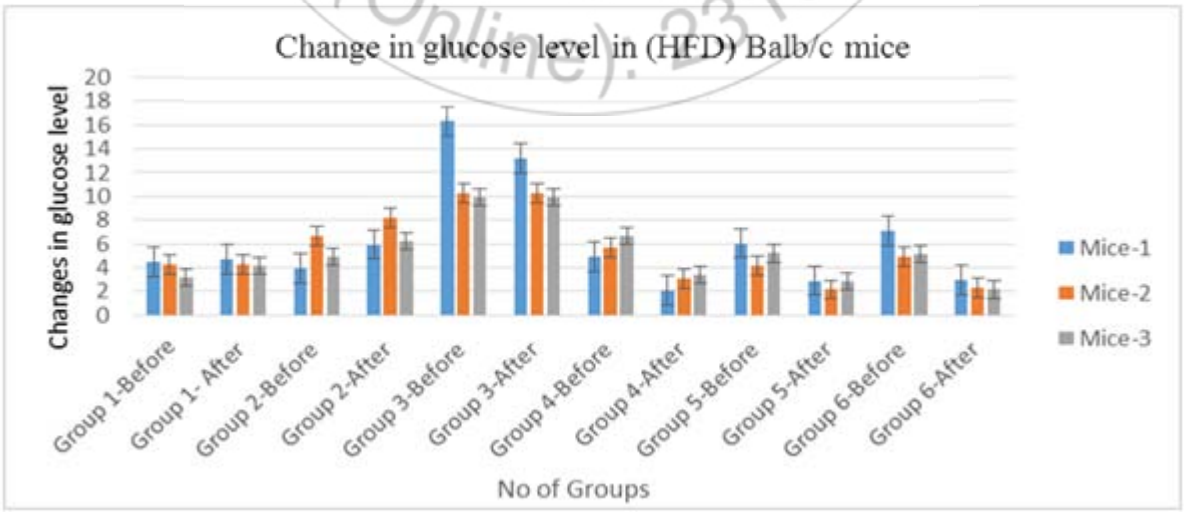

Figure 2: The error bar indicates the statistically significant difference compared to the control at $P < 0.05$.

Table 3: Effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice before and after 2 hours of administration (3rd day)

mice	Group-1		Group-2		Group-3		Group-4		Group-5		Group-6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	5.15±0.21*	4.9±0.14	4.25±0.35*	6.2±0.28*	16.16±0.22*	15.44±0.22*	6.13±0.18*	5±0.21	7.25±0.21*	0.7±0.14*	6.36±0.19*	2.67±0.24
2	3.62±0.03*	4.51±0.12	7.11±0.16*	8.56±0.08*	10.25±0.06*	10.2±0.28*	6.43±0.09*	5.7±0.12	7.61±0.12*	5.85±0.21*	4.97±0.24*	3.52±0.1
3	4.27±0.04*	4.14±0.08	5.15±0.21*	6.95±0.07*	9.11±0.15*	8.9±0.20*	4.78±0.02*	4.13±0.19	5.54±0.06*	4.43±0.09*	3.96±0.08*	3.08±0.11

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

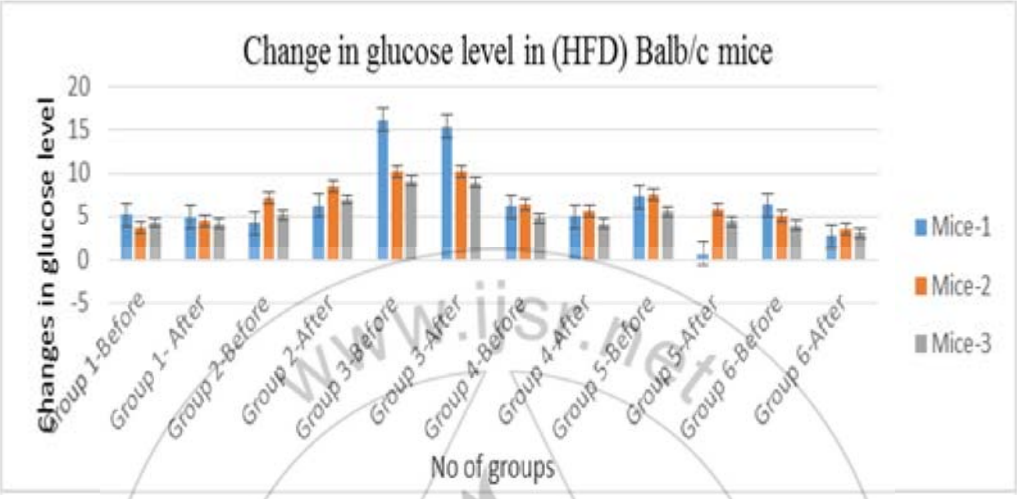

Figure 3: The error bar indicates the statistically significant difference compared to the control at $P < 0.05$

Table 4: Effect of functional food formula (FFF) on change in glucose level in HFD Balb/c mice before and after 2 weeks of administration

Mice	Group-1		Group-2		Group-3		Group-4		Group-5		Group-6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	5.25±0.07	4.9±0.14*	2.95±0.07*	6.15±0.21*	16.1±0.14*	12.1±0.14*	8.25±0.07*	3.85±0.21	8.15±0.21	4.15±0.21*	7.15±0.21*	2.75±0.21*
2	4.85±0.07	3.95±0.07*	5.48±0.39*	7.53±0.09*	12.3±0.42*	10.25±0.35*	7.79±0.0*	4.46±0.04	7.61±0.43	2.26±0.08*	8.5±0.14*	5.68±0.11*
3	4.9±0.14	4.37±0.04*	4.6±0.14*	6.25±0.06*	16.25±0.06*	4.96±0.04*	5.85±0.07*	3.15±0.21	6.9±0.14	5.99±0.01*	3.95±0.07*	2.05±0.07*

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

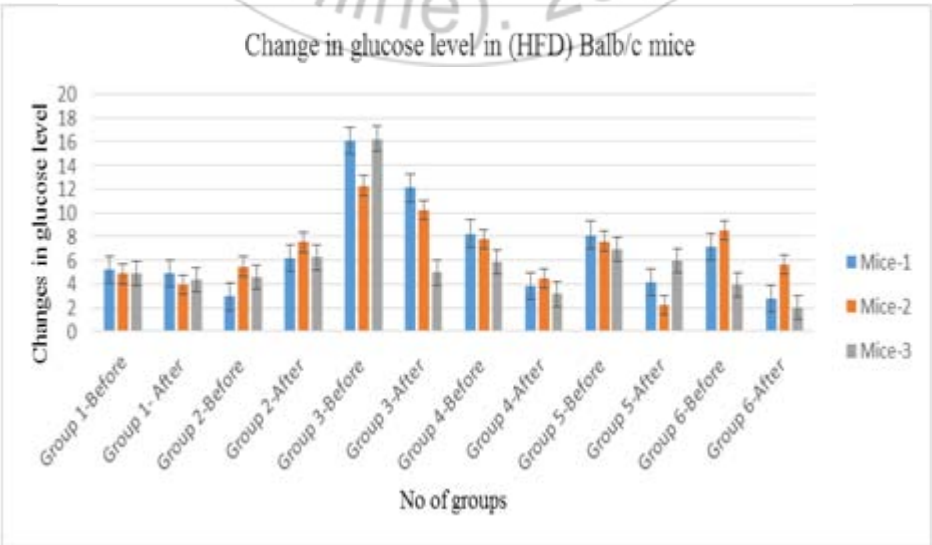

Figure 4: The error bar indicates the statistically significant difference compared to the control at $P < 0.05$

Table 5: Effect of functional food formula (FFF) on change in body weight in HFD Balb/c mice before and after 2 weeks of administration

mice	Group-1		Group-2		Group-3		Group-4		Group-5		Group-6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	22.56±0.04*	23.63±0.09*	24.58±0.02*	30.56±0.05*	23.8±0.13*	24.55±0.06*	19.42±0.11*	15.58±0.02*	22.04±0.06*	18.09±0.13*	30.64±0.08*	24.18±0.26*
2	20.95±0.07*	22.7±0.28*	20.1±0.14*	25.16±0.22*	18.82±0.1*	20.04±0.06*	20.15±0.21*	17.95±0.07*	27.24±0.21*	21.4±0.28*	28.4±0.13*	24.14±0.12*
3	18.95±0.07*	21.9±0.14*	24.15±0.07*	25.16±0.23*	26.18±0.16*	26.35±0.21*	22.5±0.14*	17.35±0.21*	20.1±0.14*	17.35±0.21*	25.45±0.07*	22.44±0.08*

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

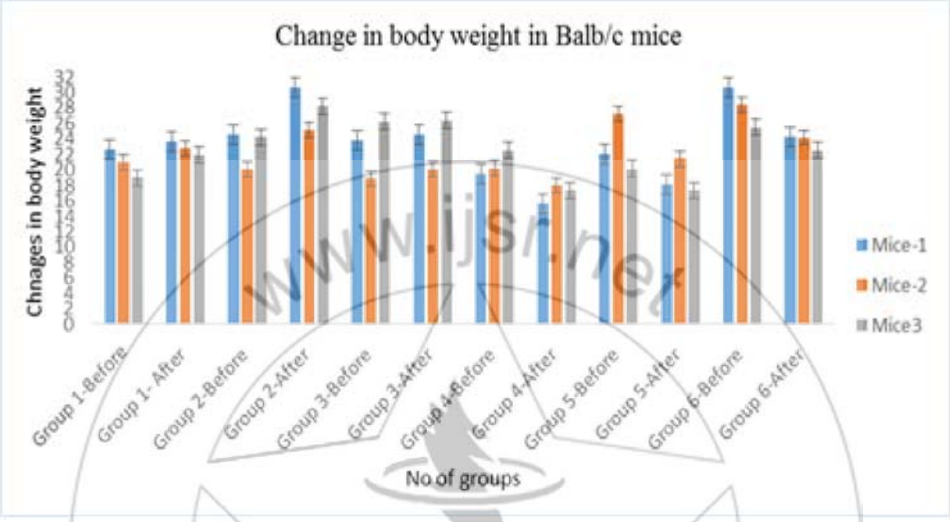

Figure 5: The error bar indicates the statistically significant difference compared to the control at $P < 0.05$

5. Discussion

Formulation of functional food has been used since many years in pharmaceuticals, medicine and natural therapies. Functional food includes *Avocado, Cinnamon, Apple Cider vinegar, Sesame, olive oil and lemon juice* have scientifically proven beneficial for hyperglycemia activity and obesity (Al-Dosari et al., 2011; Beheshti et al., 2012; Ibrahim et al., 2014; Thirumalai1 et al., 2014). Extra virgin olive oil (EVOO) has been traditionally used for wide range of clinical diseases without unveiling underling mechanism. These included diseased conditions like neurodegenerative diseases, diabetes, obesity (Nassar et al., 2009). Consuming olive oil may help in preventing type 2 diabetes (T2D) (Guasch-Ferre, 2015; Storniolo et al., 2015; Salas Salvado et al., 2014). Providing extra-virgin olive oil to adults at high risk for cardiovascular disease reduced the risk of T2D by 40% in only 4 years (Storniolo et al., 2015). A population study in Spain showed that those who consumed olive oil compared to sunflower oil had less risk of impaired glucose regulation (Soriguer et al., 2013), a condition which often leads to the development of T2D. Cinnamon has been shown to be generally safe when ingested and to have many pharmacological properties, such as effects of cinnamon on glucose and lipids levels(Jellin, 2006; Lopez et al., 2005). Many nutraceuticals properties of sesame seeds have been established, including hypocholesterolemia, hyperglycemia (Shenoy et al., 2011). Study showed that ingestion of 40 g/d of sesame seeds for 60 days caused significant decreases in plasma TC and LDL- C (Alipoor, 2012). Also, reported that daily ingestion of 32 g sesame seeds for 4 weeks decreased LDL-C concentrations by 16 % in hyper cholesterol emic patients (Hirata,1996). Vinegar apple is thought to affect glucose levels by delaying the gastric emptying rating (Khan et al., 2003; Soltan et al., 2012). The study has been done to investigates the hypoglycemic effects of apple cider vinegar and its combination effects with antihyperglycemic agents. It is thought to be beneficial for decreasing uses of antihyperglycemic agents and their side effects. In other research, it was proved that after two weeks of streptozosin injection, blood glucose levels were measured and those with fasting glucose levels above 11.1 mmol/L were included in the study (Esposito et al., 2008; Graham et al., 2011). This work showed that, all the selected functional food includes *Avocado, Cinnamon, Apple Cider vinegar, Sesame, olive oil and lemon juice* inhibited obesity in high fat diet Balb/c mice and hyperglycemic in all formulation groups. Although different concentration of formulation of functional food that was given to each group, most of the result was shown to be statistically significant from decreasing blood glucose level and weight loss in all mice. Lamount in 2016 found that such a diet in prediabetic mice was associated with reduced glycemic excursion after a meal but caused increased weight gain and adipose tissue mass. This research was designed to evaluate a formulation of functional foods for weight loss and reduction of blood glucose levels to the significantly lower levels. This data proved that this novel formulation of functional foods effectively reduced blood glucose levels

(post prandial and random blood glucose levels as well). From our investigation, we found that HFD mice treated with FFF at the doses of 200, 400 and 600 mg/kg, shows the reduced plasma glucose levels at 3 hours (Figure 4.1) and 2 hours after treatment (Figure 4.2 and 4.3). Treatment of Metformin in HFD 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 HFD mice treated with FFF at the doses of 200, 400 and 600 mg/kg also reduced body weight (Figure 4.5) after 2 weeks treatment. The body weight reduction by FFF was strongly comparable with HFD mice untreated group. This result supports the effectiveness of FFF as it could effectively control the diabetic and obesity disease status with effective control of blood glucose levels and weight loss in the treated group.

6. Conclusion

From this study, it is concluded that the formulation a novel functional food from *Cinnamon*, *Sesame, Avocado, Apple Cider Vinegar, Lemon juice and olive oil* have common effect on mice to redu`ced plasma glucose levels and weight loss in high fat die mice. The high fat diet (HFD) mice with essential formulation of functional food showed decrease diabetes and weight loss activity, even in low concentration of formulation. These results also concluded that the ability of formulation as a drug for diabetes and obesity avoiding major adverse effects. Additional *in-vivo* studies and clinical trials would be needed to justify and further evaluate the potential of these formulations as drug to obesity and diabetic. Thus, this novel formulation of functional foods can be used for the management of diabetes and obesity as well. Results in this thesis will contribute to the understanding the role of functional food in reducing blood glucose level and body weight. Thus, our work is expected to contribute to the aim of development of new drugs.

7. Recommendations

7.1. The novel formulation of functional foods may be beneficial for the obesity and hyperglycemic patient to control weight loss and blood glucose level as well as to prevent the onset of obesity and diabetes.

7.2. Detailed animal studies and clinical trials on obesity and hyperglycemia patients are recommended.

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