THE SIGNIFICANCE OF *TRIGONELLA FOENUM-GRACEUM* ON DIABETES MELLITUS: EFFECT ON LIPID CONCENTRATION IN BLOOD OF MALE SWISS ALBINO MICE

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Abstract - The past two decades have seen an explosive increase in the number of people diagnosed with diabetes mellitus worldwide. This is mainly due to increasing sedentary lifestyle and unhealthy habits like smoking and drinking alcohol or we can say poor life style. According to World health organization, the global prevalence of diabetes has grown from 4.7% in 1980 to 8.5% in 2014. The objective of the present study was to investigate the hypolipidemic effects of *Trigonella foneum-graceum* whole seed powder in lipid profile of alloxan induced murine model system. This profile includes total-cholestrol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), very low density lipoproteins (VLDL). Our study evaluated the effect of different doses (5, 10 and 15 mg/kg) of *Trigonella foenum-graceum* whole seed powder in different groups of experimental rats. All doses show improvement but when compared to normal group. All doses show improvement but when compared, it was found that in control rats, the value of cholesterol was 96.31 mg/dl where as in diabetic rats it was 156.14 mg/dl. In low dose treated the cholesterol level was 130.24 mg/dl, medium dose treated group cholesterol value was 126.28 mg/dl and high dose treated group shows cholesterol value 119.14 mg/dl. The value of HDL-Cholestrol in normal control was 37.15 mg/dl where as in diabetic control it was 27.05 mg/dl , low dose treated the HDL-cholesterol level was 33.61 mg/dl and in medium dose treated group HDL-cholesterol value was 35.13 mg/dl and high dose treated group shows HDL-cholesterol value was 37.95 mg/dl. The value of LDL-Cholestrol in normal control was 45.87mg/dl where as in Diabetic control it was 105.79 mg/dl.

Keywords: Diabetes mellitus, sedentary, diabetic rats, HDL,Cholestrol

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1. Introduction:

Diabetes is the condition in which the body does not properly process food for use as energy. Most of the food we eat is turned into glucose, or sugar, for our bodies to use for energy. The pancreas, an organ that lies near the stomach, makes a hormone called insulin to help glucose get into the cells of our bodies. When we have diabetes, our body either doesn't make enough insulin or can't use its own insulin as well as it should. This causes sugars to build up in your blood. This is why many people refer to diabetes as "sugar." Diabetes can cause serious health complications including heart disease, blindness, kidney failure, and lower-extremity amputations1. Too much glucose in the blood is a condition called hyperglycemia. It is responsible for many complications affecting various organs in the body. Several studies have demonstrated that diabetes and pre-diabetes do not develop until the β-cell fails to compensate appropriately to the peripheral insulin resistance state2. Nature consists of diverse natural anti-oxidants that hold assurance to be a novel approaches for the management of insulin resistance diabetes mellitus. As plants produces large amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity and thus Trigonella foenum is one of them3-5.

1.1. Types of Diabetes mellitus:

1.1.1. Type 1 Diabetes mellitus – Diabetes develops when the pancreas fails to produce enough insulin. Insulin treatment is very effective in reducing the plasma glucose level in this case. Type 1diabetes is used to be known as juvenile-onset diabetes or insulin-dependent diabetes mellitus (IDDM), because a majority of these diabetes cases were in children. The majority of type 1 diabetes occurs when a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus leaves you with little or no insulin. Hence termed as autoimmune disease where there is lymphocytic infiltration and destruction of the pancreatic islets led to the the onset of the disease, which is rapid and may occur over a few days to weeks.

1.1.2. Type 2 Diabetes mellitus – Type 2 diabetes used to be known as Non-Insulin-Dependent Diabetes Mellitus (NIDDM) or adult-onset diabetes. Insulin resistance refers to when cells of the body such as the muscle, liver and fat cells fail to respond to insulin, even when levels are high. Thus type2 diabetes is often accompanied by other conditions also, including hypertension, high serum Low-Density Lipoprotein (LDL) cholesterol concentrations, and low serum high-density lipoprotein (HDL) cholesterol concentrations.

1.1.3. Gestational diabetes – Another common condition linked to an increased risk of type 2 diabetes is gestational diabetes. Gestational diabetes occurs during pregnancy and usually resolves after the baby is born. People who have experienced gestational diabetes do, however, have an increased risk of developing type 2 Diabetes after pregnancy. In women with gestational diabetes, the chances of having problems with the pregnancy can be reduced by controlling blood sugar levels.

2. Symptoms of Diabetes:

• Frequent urination • Excessive thirst • Unexplained weight loss • Extreme hunger 1 • Sudden vision changes • Tingling or numbness in hands or feet • Feeling very tired much of the time • Very dry skin • Sores that are slow to heal • More infections than usual

3. Material and Methodology:

3.1. Test Animals:

All animals used in this work were male Swiss albino mice weighing 200 to 220 grams approx. They were housed individually in special clear sided cages at controlled temperature (20-25º C) with a 12:12-h light**:** dark cycle and had free access to water and chow diet.

3.2. Chemicals and Reagents:

Assay kits were purchased from Sigma Aldrich Chemicals Pvt. Ltd. All reagents and chemicals that were used in this work were of analytical grade.

3.3. Induction of Diabetes:

The Diabetes was induced in the rats, after 12h fasting the rats were weighed and a solution of alloxan at 2% diluted in saline at 0.9% was administered to the animals in a single dose corresponding to 40 mg of alloxan per kg of animal weight injected into their penial vein. The same volume of 0.9% NaCl injectable solution was injected to the control rats. After 72 hours of alloxan injection; the diabetic rats (glucose level > 150 mg/dl) were separated and used for the study. In the present experimental study, the mice were divided into five groups with six animals in each group. Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment6-11.

3.4. **Experimental setup:**

In the present experimental study, the mice were divided into five groups with six animals in each group. Group1 consist of normal control treated with normal diet, group2 consisted of untreated diabetic contol, group 3, 4 and 5 consisted of diabetic animals that were treated with *Trigonella foenum-graceum* whole seed powder at a dose of 5 mg/kg bodyweight(b.w.), 10 mg/kgb.w. and 15 mg/kgb.w. of animal respectively.

4. Biochemical Estimation:

4.1. Estimation of Cholesterol:

Total Cholesterol (TC) was determined by cholesterol oxidase/peroxidase method (Trinder, 1969) Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm.

4.2. Estimation of Triglycerides:

Triglyceride (TG) was determined using enzymatic method (Trinder, 1969). Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of 4-Chlorophenol and (4-AAP) 4-aminophenazone in the presence of Peroxidase (POD) and hydrogen peroxide produces a rose colored dye which is measured at 550 nm.

4.3. **High Density Lipoprotein Cholesterol (HDL-C):**

Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was

allowed to stand for 10 minutes at room temperature centrifuged for 10 minutes at 4000rpm. The supernatant represented the HDLC fraction. The cholesterol concentration in the HDL fraction, which remains in the supernatant, was determined.

LDL-cholesterol and VLDL-cholesterol values were calculated according to Friedewald's formula (Friedewald et al. 1972).

4.4. Low Density Lipoprotein (LDL -C):

The concentration of LDL cholesterol was calculated using Friedewald's equation as stated below:

$$LDLC = TC - (HDLC + TGL/5)$$

Where TC = Total Cholesterol , TGL = Triglycerides

4.5. Very Low Density Lipoprotein (VLDL-C):

The concentration of VLDL cholesterol was calculated using Friedewald's equation as stated below:

$$VLDL = TGL\ (mg/dl)\ /5$$

Where TGL = Triglycerides

5. Statistical Analysis:

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value. $(p<0.0001)^a$, $(p<0.001)^b$, $(p<0.01)^c$, $(p < 0.05)^d$ represents significant changes against normal control.

6. Results and discussion:

The results show that the alloxan administration, significantly rose serum level of cholesterol, triglyceride, LDL, VLDL in group2 when compared to group1 except HDL which decreases significantly. This increase level of serum lipids is because of uninhibited actions of lipolytic hormones on the fat deposits due to the action of insulin. All parameters of lipid profile show improvement after treating with *Trigonella foenum* whole seed powder but when compared to normal control group, it was found that in group2 1.62 fold increase in cholesterol was observed but group3 show 1.35 fold increase, group4 show 1.31 fold increase but in high dose treated by *Trigonella foenum* whole seed powder increase were only 1.24 folds in cholesterol. When compared to normal control triglycerides in diabetic control rat's show1.83 fold increase, low dose treated group3 show 1.27 fold increases in triglycerides, medium dose show 1.23 fold increase but high dose treated group5 show 1.22 fold increases in Triglycerides. Serum HDL-cholesterol value in group2 show 0.73 fold increase when compared to normal control while low dose show 0.90 fold increase in group3,medium dose in group4 show 0.95 increase and 1.02 folds increase was seen in high dose treated group5 animals. In Diabetic control rats 2.28 fold increase of LDL-cholesterol was observed compared to normal control of group1. In low dose treated group3 animals LDL increase was 1.74 fold. In medium dose

treated group4 increase was 1.64 fold while high dose in group5 show increase of 1.42 fold. In diabetic rats of group2 1.83 fold increases in VLDL-cholesterol was observed when compared to normal control of group1. In low dose treated group3 1.27 fold increases in VLDL value was seen, medium dose show increase of 1.22 fold while high dose treated group5 animals also show increase of 1.22 fold. High dose was found better in comparison of medium and low doses treatment as shown in figure1.

Table 1: Lipid profile test

S.N.	Group	TOTAL CHOLESTROL (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VDL-C (mg/dl)	TRIGLYCERIDES (mg/dl)
1	Group1	96.31±3.43 100	37.15±4.17 100	45.87±3.23 100	13.29±4.94 100	66.43±4.82 100
2	Group2	156.14±4.17[a] 162.5	27.05±4.82[c] 72.81	104.79±1.64[a] 228.44	24.292±4.16[c] 182.784	121.46±3.76[a] 182.83
3	Group3	130.24±3.24[a] 135.22	33.61±5.94 90.47	79.768±2.16[a] 173.90	16.862±3.92 126.87	84.31±5.78[a] 126.91
4	Group4	126.28±4.94[a] 131.12	35.13±4.34 94.56	75.046±1.82[a] 163.60	16.234±4.72 122.152	81.17±6.07[a] 122.19
5	Group5	119.14±4.82[a] 123.70	37.95±4.92 102.15	65.026±1.92[a] 141.76	16.164±4.31 121.625	80.82±3.82[b] 121.66

Values are expressed as mean ± SD of 6-8 animals. Values in parentheses represent relative change in parameters assessed. $(p<0.0001)^a$, $(p<0.001)^b$, $(p<0.01)^c$, $(p < 0.05)^d$ represent significant changes against normal control.

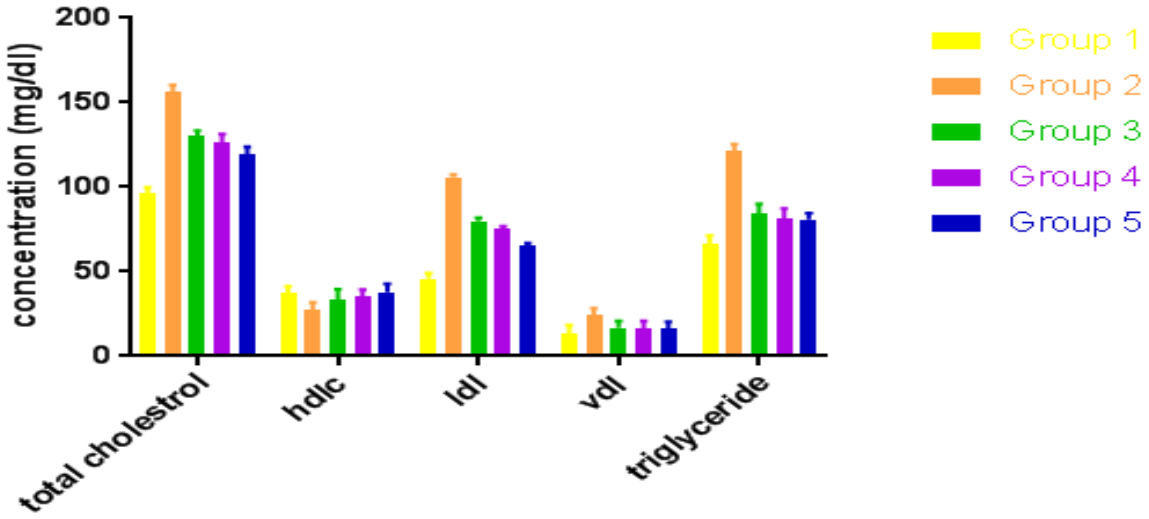

Figure 1: Represents comparision of different lipid profile parameters among experimental groups.

Conclusion:

This study evaluated that *Trigonella foenum-gracem* whole seed powder was effective in hypolipidemic activity associated with diabetes. Thus its beneficial role could be utilized by adding in diet for managing diabetes. At the same time it should be kept in mind to avoid oxidant sources (cigarette, alcohol, bad food, stress, etc) must be considered as necessary as taking diet rich in antioxidants. Indeed, our health also depends on our lifestyle choice.

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