Potency of polyherbal formulation in improving adiponectin level of streptozotocin induced-diabetic rats.

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ABSTRACT: In this research investigation, hydro alcoholic extracts of polyherbal formulation comprising *Nigella sativa* (black seed), *Trigonella foenum graecum (fenugreek,), Trachyspermum ammi (Ajwain*)has a role in improving in Adiponectin level of Streptozotocin induced diabetic rat. **Materials and method:** A 30 Male Albino Wistar rats are selected were in each group consists of 6 rats. Group1- normal, Group2-diabetic control (Streptozotocin 40mg/kg ip), Group3-positive control (Glibenclamide 600µ/kg p. o), Group4-lower dose of polyherbal formulation (320mg/kg p. o), Group5-High dose of poly herbal formulation (640mg/kg p. o) treatments were given orally for 21 days. Polyherbal formulation groups were compared to diabetic control **Results:** The animals treated with streptozotocin (diabetic control), showed significant($p<0.01$) decrease in adiponectin levels, when compared with normal control group animals. The animals groups treated with polyherbal formulation extract at different two dose level (lower dose; 320mg/dl po and higher dose;640mg/dl po) as showed significant ($p<0.01$ and $p<0.001$) increase in plasma adiponectin levels as compared with diabetic control groups**. Conclusion:** Plasma Adiponectin study showed the Polyherbal formulation extract have significant effect in improving the Adiponectin and overall health. polyherbal formulation of high dose (640mg/kg p. o) exhibited increased adiponectin level compared to lower dose (320mg/kg p. o).

Keywords: Streptozotocin, adiponectin, polyherbal formulation.

I. INTRODUCTION

Diabetes mellitus is a serious, noncommunicable, long-term (or "chronic") condition, is a class of metabolic disorder which is caused by deficiency in insulin secretion, insulin action or both[(1)]. Diabetes mellitus (DM) is a chronic metabolic disorder where in human body involves deficiency of insulin, a hormone which is used to convert sugar, carbohydrates and other food into energy. Absence or decreased insulin in turn raised to persistent abnormal hyperglycemic and impaired fasting glucose. It is commonly known as oldest diseases in man. It is also reported as black-death from the 14th century[(2)].

Diabetes mellitus is a group of metabolic diseases leading to hyperglycemia due to lack of insulin secretion, decrease in function or the complete destruction of β-cells (these cells are not put back, as the human pancreas seems not capable of regenerated β-cells following the age of 30 years) which are caused by several mechanisms' insulin action or various pathogenic processes are involved in the diabetes. This leads to autoimmune destruction of beta cells of the pancreas with consequences of insulin deficiency to deformity that results in insulin resistance[(3)(4)].

World Health Organization (WHO) and American Diabetes Association (ADA) standard which include both clinical and laboratory assessment for Screening and Diagnosis in diabetes mellitus.[(1)]

The burden of diabetes is high and increasing in worldwide, and in developing economies like India, mainly caused by increasing prevalence of overweight/obesity and unhealthy lifestyles. The estimated values in 2019 reported that 77 million individuals had diabetes in India, further it is expected to increase over 134 million people by 2045. Approximately 57% of these individuals remain undiagnosed[(5)].
It is estimated that 439 million people have reported type 2 DM by the year 2030.Estimation of type 2 DM considerably different from one geographical region to the other as a result of environmental condition and civilization factor.[(1)].

Based on the need insulin, diabetes is classified in to two types like Type-I insulin dependent (IDDM) &Type-II Non-insulin dependent diabetes mellitus (NDDM). Based on the onset of Diabetes its classified as Juvenile -onset Diabetes mellitus & Adult-onset diabetes mellitus. Based on the other circumstances its classified as Gestational diabetes & Pre diabetes.
Type2(NIDDM) it is mainly by insulin insensitivity as a result of insulin resistance, declining insulin production and eventual pancreatic beta-cell failure. This leads to a decrease in glucose transport into liver, muscle cell, and fat cells. There is an increase glucose breakdown been recognized in the pathophysiology of type 2 DM. As a result of this dysfunction glucagon and hepatic glucose levels that rise during fasting are not inhibit with a meal given insufficient level of insulin and increased insulin resistance , results in hyperglycemic[(4)(3)(1)].

Adiponectin is a fat derived hormone they play major role in insulin sensitivity provides on glucose uptake and β-oxidation through AMPK pathway activation. Adipose tissue that forms the storage of fat in the form of Triglyceride. Adipose tissue is distributed through the body beneath the skin, forming subcutaneous fat and visceral organ[(6)]. Adiponectin is storage of energy. Utilization of storage fat is regulated by hormone, particularly insulin, depending upon the blood glucose level. If the blood glucose level increases, insulin stimulates synthesis and storage of fat. On the other hand, if the blood glucose level gets reduced insulin causes releases of fat from adipose tissue[(7)].

Many plants medications are explored for antidiabetic activity using different in vivo models as well as in-vitro models. Since from the literature survey it is relatively evident that Nigella sativa[8], Trigonella foenum-graecum[9], Trachyspermum ammi[10],individually are reported to have an effective anti-oxidant and anti-diabetic activity, significantly lowered the blood glucose levels and contributes as a highly significant bio resources of antioxidants to be used in our day-to-day life in food and pharmaceutical industry ,which can serve as an alternative treatment of diabetes mellitus, besides many other pharmacological properties are exhibited by these herbs and they are also proved to be a safe herbs.

II. Research methodology

Materials and methodology

Plant material:

The raw materials *(Nigella sativa, Trigonella foenum- graecum, Trachyspermum ammi*) used in the herbal formulation were procured locally from Amruta kesari herbal drug vendor, Bengaluru. The raw materials were identified and authenticated by Prof. Sathish. Department of Pharmacognosy, Nargund college of Pharmacy, Hosakerehalli, Bengaluru, 560085.

Chemicals and Reagents:

All the chemicals and reagents used in the study were of analytical grade and molecular biology grade. Streptozotocin ($C_8H_{15}N_3O_7$) extra pure was purchased from Sigma Research Laboratories Pvt Ltd., Mumbai, India. Glibenclamide was purchased from medical store Bengaluru, India.

Preparation of polyherbal formulation by Infusion method:

The coarsely powdered crude drug *Nigella sativa* (15 gm), *Trigonella foenum graecum* (20 gm), *Trachyspermum ammi* (15 gm) (50 g) is moistened, in a suitable vessel with a cover, 240 ml boiling water is added and 60ml of ethanol (hydro-alcoholic 80:20), and the vessel is covered tightly and allowed to stand for 30 min. The mixture is strained and the infusion measure 250 ml [11].

Animal laboratory:

Male Albino Wistar rats weighing 180 g-250 gm were procured from Adita Biosys Pvt Ltd, Bangalore. Animals were housed in polypropylene cages with paddy husk as bedding material. They were provided with standard pellet rodent diet (Amrut Laboratory animal feed, Sangli) and free access to water. Institutional Animal Ethics Committee has approved the experimental protocol (IAEC/NCP/111/2021). All the procedures were performed in accordance with the Committee for the Purpose of Control and supervision of Experimental Animals (*CPCSEA).*

Experimental design:

Male Albino Wistar rats of 180-250 gm were randomly distributed to 5 experimental groups, each group consists of 6 animals each. STZ (40mg/kg i.p.) were injected at a single dose to all the group animals except normal group. Streptozotocin was freshly prepared by dissolving in 0.01M ice cold citrate buffer PH-4.5 and kept on ice prior to practice, and was administrated at a dose of 40mg/kg with a single intraperitoneal injection to overnight fasted rats [12]. The first phase is hyperglycemic phase (increases in blood glucose concentration), 1 hr after administration of the STZ, there is decrease in plasma insulin, which usually lasts 2–4 hr, is caused by inhibition of plasma insulin secretion leading to hypoinsulinaemia. The second phase hypoglycemic phase, typically occurs 4–8 h after the injection of the STZ and lasts several hours. It may be also leads to convulsions, and may even be fatal without glucose administration, after 4hrs STZ administration 5% glucose was administered orally given to overcome the early stage of hypoglycemic phase. Rats were allowed to stabilize for three days. On the third day (72hrs) blood samples were withdrawn to estimate the blood glucose concentration to confirm the diabetes. STZ disrupts Partially destruction of β cells in the pancreas leading to the damage of DNA. The rats having the blood glucose above250mg/dl were considered diabetic [13][14]. group1-serves as a normal control group saline p.o. group-2 diabetic control (STZ induced) provided a regular chew pellets and water. group-3 (positive control group) Glibenclamide 600µ/kg p. glibenclamide were dissolved 0.5%carboxymethyl cellulose were administered orally for 21 days. group-4 lower dose of polyherbal formulation (320mg/kg p. o), group-5 High dose of poly herbal formulation (640mg/kg p.o.) were administered orally for 21 days.

Blood sampling:

Blood samples withdrawn from Retro orbital puncturing each rat were collected in separate Eppendorf tubes on 21st day in and streptozotocin induced diabetic model. They were allowed to clot and centrifuged immediately to obtain clear serums that were stored at -20ºC or used immediately to carry out the estimations. Adiponectin levels were estimated by using ELISA kit.

Statistical analysis:

The data were expressed as mean ± SEM. Statistical comparisons were performed by one-way analysis variance (ANOVA) followed by student t test using Graph Pad Prism version 5.3 USA. The p value less than 0.001 were considered as statistically significant when compared to control.

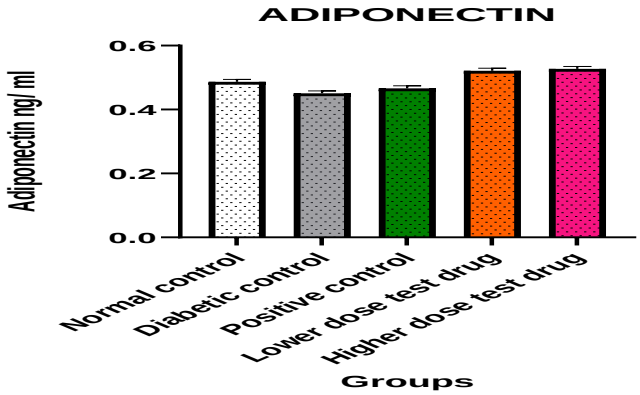
III. RESULTS AND DISCUSSION

Table no 3.1: Plasma Adiponectin levels:

Groups	Mean ± SEM Adiponectin in ng/mL
Normal control	0.48 ± 0.007
Diabetic control	0.45 ± 0.007 **a
Positive control	0.47 ± 0.007 ns
Lower dose(320mg/kg)	0.52 ± 0.007 **b
Higher dose (640mg/kg)	0.53 ± 0.007 ***b

Data was analyzed using one way ANOVA and Student's t test.
*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001
[a] When compared with normal control group.
[b] When compared with diabetic control group.
[ns] not significant.

Figure 3.2: plasma adiponectin graph



The animals treated with streptozotocin (diabetic control), showed significant($p<0.01$) decrease in adiponectin levels, when compared with normal control group animals.
The animals treated with Standard drug- Glibenclamide 600µ/kg (Positive control) in animal groups with diabetic condition showed not significant increase in plasma adiponectin levels as compared with diabetic control group animals
The animals treated with polyherbal formulation extract at two dose level (lower dose; 320mg/dl po and higher dose;640mg/dl po) in animal groups with diabetic condition showed significant ($p<0.01$ and $p<0.001$) increase in plasma adiponectin levels as compared with diabetic control group animals.

Discussion

The adiponectin is a cytokine f (cell to cell signaling kinase) or fat tissue acts through the AMPK pathway. The 5'- AMP- activated protein kinase or AMPK or 5'- adenosine monophosphate – activated protein kinase is an enzyme that plays a role in cellular energy level[(15)]. Adiponectin expressed in a number of tissues, including the liver, brain, and skeletal muscle[(16)]. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta cells. Two particular adipokines, adiponectin and leptin, have even been demonstrated to regulate AMPK[(17)].
Adiponectin is an insulin sensitivity and inflammation[(18)].Adiponectin is fat derived hormone does not depends on the body weight it depends upon the weight of the fat. Adipose tissues are fat storage cells which helps in regulation of hormones when blood glucose level is decreased synthesis and release the fat in adipose[(19)].

IV. CONCLUSION

Since the polyherbal formulation were found to increase the plasma Adiponectin concentration in the experimental animals, they may exert effects through AMPK pathway, hence improve carbohydrate metabolism in total experimental animals.

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vi. Reference

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