



EFFECT OF POLYHERBAL FORMULATION IN OBESITY ASSOCIATED DIABETES

PATIL MANOJ N^{*1}, KAGATHARA VIRENDRA G², HARLE UDAY N³, PUJARI ROHINI R⁴, INGAWALE DEEPA K⁵

¹Junior Clinical Data Analyst, Cognizant Technology Solutions, Mumbai, India, ²Dept. of Pharmacology, Dharamsinh Desai Institute of Pharmacy, Nadiad-387001, Gujarat, India, ³Dept. of Pharmacology, MIT Institute of Pharmacy, Pune, Maharashtra, India, ⁴Dept. of Pharmacology, Modern College of Pharmacy, Borhadewadi, Dehu-Alandi Road, Moshi, Pune, Maharashtra, India, ⁵National AIDS Research Institute (NARI), Bhosari, Pune, Maharashtra, India. Email: mani9622@gmail.com; viru_maitri24@yahoo.co.in.

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ABSTRACT

Despite many herbs having both antidiabetic as well as antiobesity activity; there is no marketed polyherbal formulation for obesity associated diabetes. In light of this, polyherbal preparation for obesity associated diabetes containing *Gymnema sylvestre* R., *Garcinia cambogia*, *Lagerstromia speciosa* L., was investigated in normal and obese streptozotocin induced diabetic rats. Effect of oral administration of polyherbal formulation (412, 825 and 1625 mg/kg body weight) for 21 days on the level of serum glucose, total cholesterol, triglycerides, serum LDL, serum HDL, serum VLDL levels and body weight in obese diabetic rats were evaluated. Administration of the formulation for 21 days significantly decreased serum glucose, total cholesterol, triglycerides, LDL, VLDL levels and body weight and increased the HDL level. A comparison was made between the action of polyherbal formulation and glibenclamide (4 mg/kg), the standard antidiabetic drug, sibutramine (5mg/kg), the standard antiobesity drug. The antidiabetic and antiobesity effect of the formulation was found to be nearly similar to that observed for glibenclamide and sibutramine respectively. It can be concluded that, the formulation should be considered as an excellent candidate for future studies of obesity associated diabetes.

Keywords: *Gymnema sylvestre* R, *Garcinia cambogi*, *Lagerstroemia speciosa* L, *Streptozotocin*.

INTRODUCTION

Obesity is a condition in which excess body fat is accumulated to an extent that health may be negatively affected. Obesity is commonly defined as a body mass index (BMI) of 30 kg/m² or higher. This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m² but less than 30 kg/m². The excessive storage that creates obesity eventually leads to the release of excessive fatty acids from enhanced lipolysis, which is stimulated by the enhanced sympathetic state existing in obesity. The release of these excessive free fatty acids then incites lipotoxicity, as lipids and their metabolites create oxidative stress to the endoplasmic reticulum and mitochondria. This affects adipose as well as nonadipose tissue. Obesity increases the risk of type 2 diabetes, cardiovascular disease, cancer, and premature death. More than 1.1 billion people are estimated to be overweight of which around 320 million are calculated to be obese. More than 2.5 million deaths each year are attributed to higher BMI, a figure that is expected to double by 2030. Incidence rate of obesity is about 300 million adults worldwide. At an individual level, a combination of excessive caloric intake, lack of physical activity, and genetic susceptibility is thought to explain most cases of obesity, with a limited number of cases due solely to genetics, medical reasons, or psychiatric illness.

Type 2 diabetes mellitus is characterized differently and is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute. The defective responsiveness of body tissues to insulin almost certainly involves the insulin receptor in cell membranes. The predominant abnormality is reduced insulin sensitivity, characterized by elevated levels of insulin in the blood. The prevalence of diabetes worldwide was estimated to be 2.8% in 2003 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The international diabetes federation (IDF) estimates total number of diabetics to be around 40.9 million in India and this is further set to rise 69.9 million by year 2025. Insulin production is more or less constant within the beta cells, irrespective of blood glucose levels. It is stored within vacuoles pending release, via exocytosis, which is primarily triggered by food, chiefly food containing absorbable glucose. The chief trigger is a rise in blood glucose levels after eating. Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Most of the carbohydrates in food are converted within a few hours to the monosaccharide glucose, the

principal carbohydrate found in blood and used by the body as fuel. Insulin is released into the blood by beta cells (β -cells), found in the Islets of Langerhans of pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for the storage.

Obesity and diabetes are closely related to each other as about 80% diabetics are obese. Obesity is a common finding in Type-II diabetes. There is impaired insulin sensitivity of peripheral tissues such as muscle and fat cells to the action of insulin in obese individuals (insulin resistance). Weight reduction in such obese patients produces improvement in the diabetic state. Obesity increases the risk of type 2 diabetes, cardiovascular disease, cancer, and premature death. Pharmacological factor involved in obesity and diabetes includes lipoprotein lipase (LL), having a central role in the metabolism of both triglyceride-rich particles and high density lipoproteins (HDL). LL is determinant of serum triglyceride and HDL concentrations. Many traditional plant remedies for obesity and diabetes are used throughout the world. Plant drugs and herbal formulation are frequently considered being less toxic and devoid of any side effects than synthetic one. Few of the traditional plant treatments for diabetes have received scientific scrutiny and the World Health Organization (WHO) has recommended that this area warrants attention.

Despite many herbs having both activities, there is no marketed polyherbal formulation for obesity associated diabetes. In light of this, polyherbal preparation for obesity associated diabetes containing *Gymnema sylvestre* R., *Garcinia cambogia*, *Lagerstromia speciosa* L., was investigated in normal and obese streptozotocin induced diabetic rats and was found to produced significant reduction in glucose level, lipid profile and body weight.

EXPERIMENTAL

Plant material

The hydroalcoholic extracts of leaves of *Gymnema sylvestre* R. and *Lagerstromia speciosa* L. and aqueous fruit rind extract of *Garcinia cambogia* were received as gift sample from Chemiloids Ltd, Vijaywada (India).

Animals

Sixty male wistar rats of body weights ranging from 150-200 g were obtained from Agharkar research institute, Pune, India. The animals were fed with high fat diet (Amrut Feeds, Chakan, India) and water

ad libitum. They were maintained in a controlled environment (12:12 h light/dark cycle) and temperature (30 ± 2 °C). The experimental protocol was approved by the Institutional Animal Ethical Committee.

Drugs and chemicals

Streptozotocin was purchased from Hi-Media, Mumbai, India. Sibutramine and Glibenclamide were received as a gift sample from Glenn mark Pharmaceuticals Ltd, Mumbai, India. All other chemicals used for the experiments were purchased from the local market.

Acute toxicity studies

Healthy adult male wistar albino rats (180-250 gm), starved overnight were divided into four groups (n=6) and were orally fed with the extracts in increasing dose levels of 100, 500, 1000 and 3000 mg/kg body weight¹⁶. The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after 24 and 72 for any lethality¹⁷.

Dose selection

The polyherbal formulation of the three herbs (*viz. Gymnema sylvestre* R, *Garcinia cambogia*, *Lagerstroemia speciosa* L.) was prepared according to their effective doses ED₁₀₀. They were well mixed in a mortar and pestle along with addition of 1% acacia (Suspending agent) till the stable and homogeneous suspension formed. Polyherbal formulation was quantitatively evaluated for any incompatibility by visible observation of precipitation and changes in spectrophotometric analysis.

Experimental induction of obesity in rats

For induction of obesity, male wistar rats (160- 180 gm) were fed with high fat diet for 2 weeks. After 2 weeks, rats with BMI > 35 were selected for further study. Control rats were fed with normal pellet diet¹⁸.

Experimental induction of diabetes in rats

For the induction of diabetes, streptozotocin was administered at a dose of 35 mg/kg intraperitoneally using a 5% solution of freshly prepared 0.1 M citrate buffer (pH 4.5). Control rats received citrate buffer only. Blood sugar estimation was done on Day 4. Rats with a blood sugar level >250 mg were selected for the study.

Experimental design

The rats were divided into ten groups of six animals each and treated orally for 21 days as follows.

- | | |
|----------|--|
| Group 1 | Normal rats administered with vehicle |
| Group 2 | Diabetic control rats |
| Group 3 | Diabetic rats administered with <i>Gymnema sylvestre</i> (400 mg/kg body weight) |
| Group 4 | Diabetic rats administered with <i>Garcinia cambogia</i> (400 mg/kg body weight) |
| Group 5 | Diabetic rats administered with <i>Lagerstromia speciosa</i> (400 mg/kg body weight) |
| Group 6 | Diabetic rats administered with Polyherbal formulation (412 mg/kg body weight) |
| Group 7 | Diabetic rats administered with Polyherbal formulation (825 mg/kg body weight) |
| Group 8 | Diabetic rats administered with Polyherbal formulation (1650 mg/kg body weight) |
| Group 9 | Diabetic rats administered with Glibenclamide (400 mg/kg body weight) |
| Group 10 | Diabetic rats administered with Sibutramine (400 mg/kg body weight) |

After confirmation of diabetes on 4th day by glucose estimation,

Doses were given from next day as day 1 up to 21 days. Blood glucose level (BGL), serum cholesterol level (CH), triglycerides level (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL) and body weight were estimated on 7th, 14th and 21st day.

Biochemical analysis

Blood glucose level was estimated by glucose oxidase method (GOD). Triglycerides level was estimated by glycerol phosphatase oxidase (GPO) method, cholesterol level (CH) was estimated by Enzymatic endpoint CHOD- PAP method (Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase). VLDL composition and LDL-cholesterol can be calculated with reasonable accuracy by the Friedewald formula and the concentrations of the total cholesterol, HDL-cholesterol and triglycerides were known^{19, 20}. The HDL-Cholesterol (HDL-C) was estimated by Homogenous Enzymatic Direct Assay²¹.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) followed by student's 't' test. Values with p < 0.05 were considered statistically significant

RESULTS AND DISCUSSION

In the acute toxicity study, the administration of the Polyherbal formulation at various doses did not elicit any mortality up to 3000 mg/kg body weight in rat. Even at this high dose there were no gross behavioral changes or any clinical symptoms observed.

Our Polyherbal formulation containing *Gymnema sylvestre* R, *Garcinia cambogia*, *Lagerstroemia speciosa* L., possesses antidiabetic and antiobesity activity through varied mechanisms of action. *Gymnema sylvestre* containing gymnemic acid and gymnemosides, has been reported to show antihyperglycemic activity in different models of diabetes²². Gymnemic acid isolated from the leaves of *Gymnema sylvestre* R. has been reported to inhibit the intestinal absorption of glucose and oleic acid and reported to exert beneficial effect in diabetes and obesity^{23, 24}. The weight reducing effect may be attributed to *Garcinia cambogia* which contains hydroxy citric acid which is reported to inhibit lipogenesis²⁵.

The potential antidiabetic and antiobesity activity of the leaves of *Lagerstroemia speciosa* L. (LSL) can be attributed to triterpenes (oleanolic acid, arjunolic acid, asiatic acid, maslinic acid and corosolic acid). Corosolic acid, which shows best bioactivity against -glucosidase (IC₅₀ = 3.53 µg/mL), contributes most to the -glucosidase inhibitory activity of ethanolic extract²⁶. High fat diet given to rats for 2 weeks produced obesity (BMI > 30). Due to increase in body weight rats become obese and developed mild hyperglycemia¹⁸. Rats fed with high fat diet develop hyperlipidemia, insulin resistance, hyperinsulinemia and hyperglycemia, which are the main features associated with the obesity-associated diabetes²⁷. Streptozotocin induces diabetes in rats by β cell destruction, through the generation of free radicals, causing alkylation of DNA and ultimately inducing hyperglycemia. Hexokinase is the regulatory enzyme of glycolytic pathway, which is decreased in STZ, induced diabetes^{28, 29}.

In obesity associated diabetes, the serum levels of blood glucose (Figure 2), cholesterol (Figure 3), triglycerides (Figure 4), LDL (Figure 6), VLDL (Figure 7) and body weight (Figure 1) are considerably elevated, while there was marked decrease in the levels of HDL (Figure 5). During the administration of polyherbal formulation (PF) for 21 days, biochemical estimation and body weight determination was done at 0th, 7th, 14th, 21th days. The polyherbal formulation showed reduction in body weight in dose dependent manner which was more significant than individual plant (Figure 1). Polyherbal formulation was found to possess nearly same activity as that of standard drugs used. This effect may be due to inhibition of lipogenesis in rats²⁵.

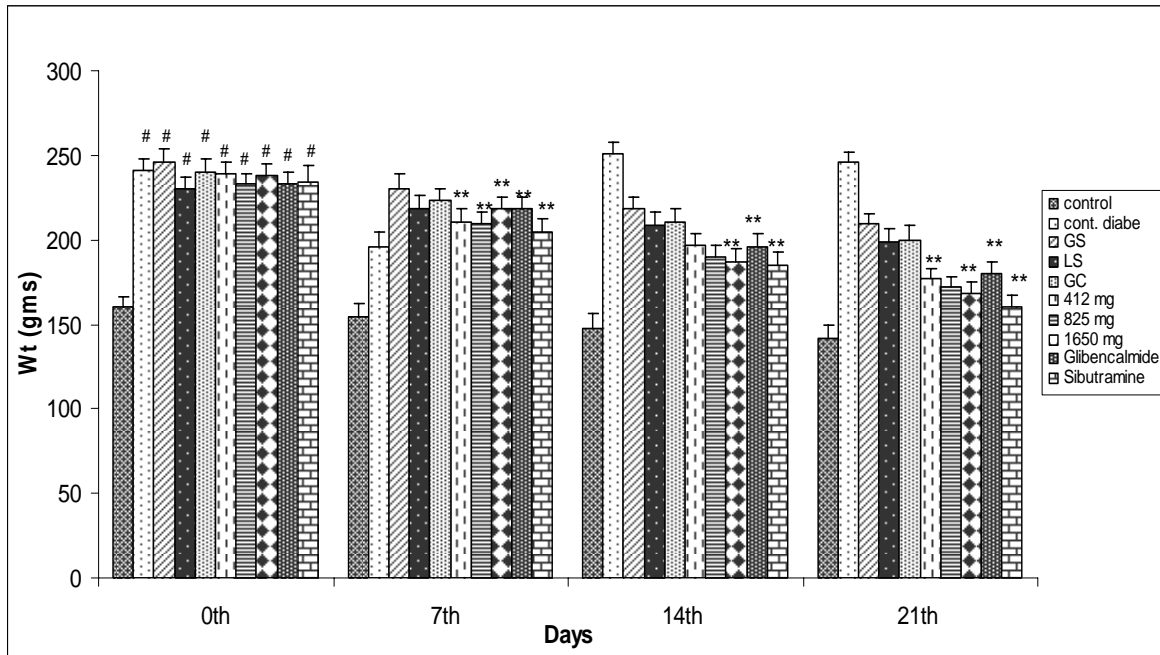


Fig. 1: It shows effect of Polyherbal formulation on body weight of HFD induced obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean ± S.E.M., n=6, #P<0.01, *P < 0.05; **P < 0.01.

The decrease in glucose level shown by polyherbal formulation (PF) was almost same as that of the standard drugs used. Also, polyherbal showed better activity than individual plants. Formulation seems to

improve insulin resistance through enhanced insulin sensitivity in peripheral tissues, as evident from the decreased serum glucose levels in rats (Figure 2).

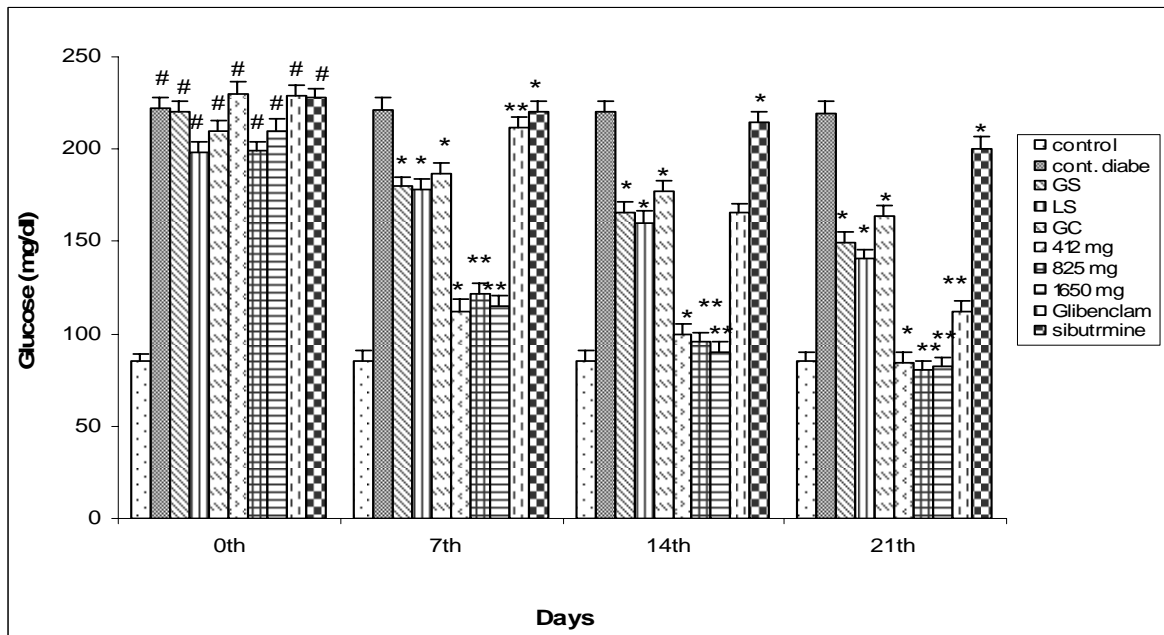


Fig. 2: It shows effect of Polyherbal formulation on glucose level of obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean ± S.E.M., n=6; *P < 0.05, **P < 0.01, #P<0.01

PF decreased the cholesterol level more significantly as compared to individual herb (Figure 3) and almost same as that of standards glibenclamide and sibutramine. The reduction of cholesterol level may be due to its decreased synthesis or increased excretion through intestinal tract by HMG CoA reductase, which is the

important enzyme in the formation of cholesterol. One of the possible mechanism may be due to inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract³⁰.

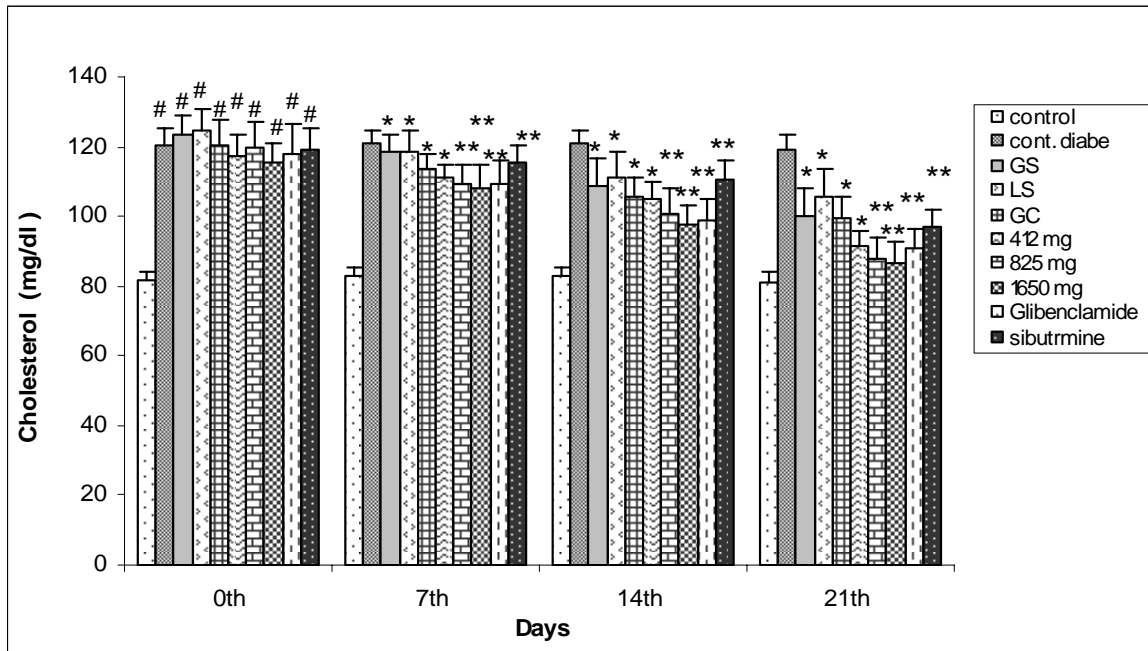


Fig. 3: It shows effect of Poly herbal formulation on Serum cholesterol level of obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean ± S.E.M., n=6; *P < 0.05; **P < 0.01, #P < 0.01.

Also, there was significant decrease in triglyceride level of rats by PF as compared to diabetic control (Figure 4). PF showed superior activity than individual herbs and significant activity as that of standard antidiabetic and antiobesity drugs. The possible mechanism of action may be due to the hydrolysis of triglycerides and activation of insulin by the enzyme lipoprotein lipase ³¹.

Pretreatment of PF for 21 days exhibited significant increase in HDL levels (Figure 5). Results of Polyherbal formulation showed nearly same activity as that of standard antidiabetic and antiobesity drugs i.e. glibenclamide and sibutramine respectively. It also showed more significant activity than that of individual herbs. The possible mechanism of action of polyherbal formulation for increased HDL may be due to increase in lecithin activity ³².

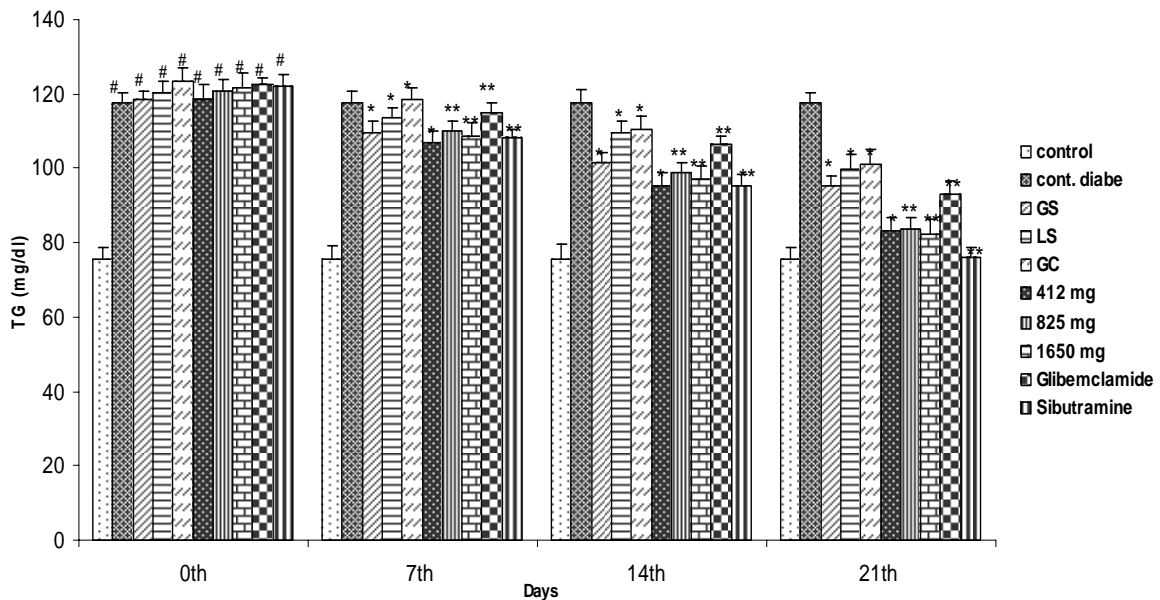


Fig. 4: It shows effect of Polyherbal formulation on serum triglycerides level of obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean ± S.E.M., n=6; *P < 0.05; **P < 0.01, #P < 0.01.

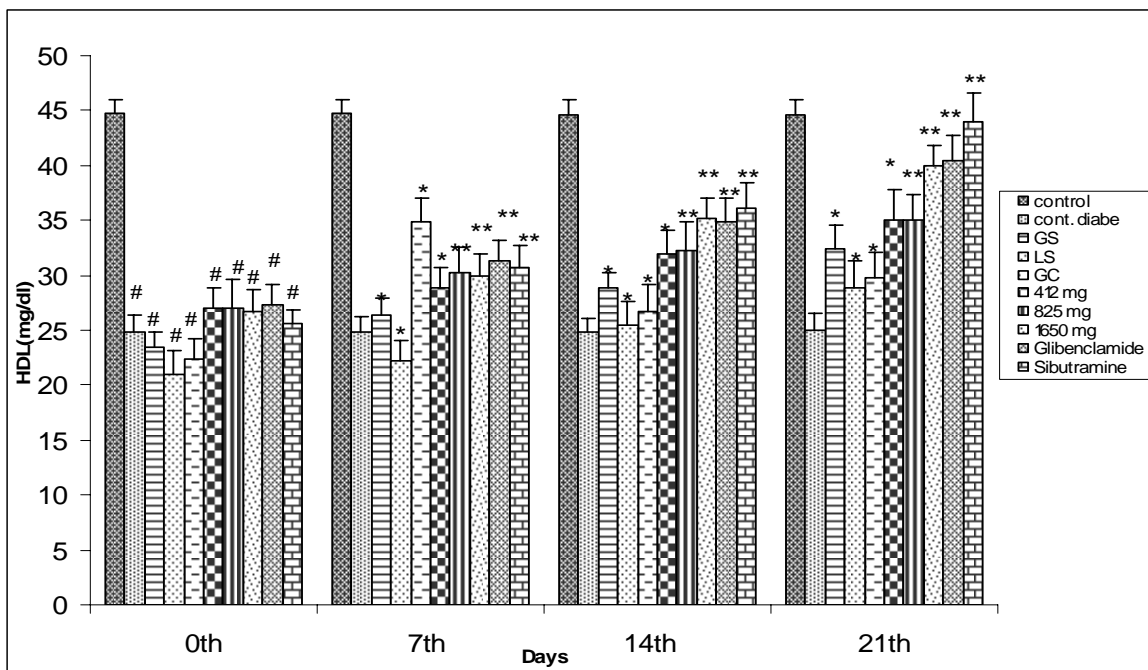


Fig. 5: It shows effect of Polyherbal formulation on serum HDL level of obese diabetic rats. Data are expressed as mean \pm S.E.M., n=6; *P < 0.05, **P < 0.01, #P<0.01

All the doses of PF showed significant reduction in LDL levels as compared to control diabetic rats (Figure 6). The individual herb

didn't show comparatively significant activity as that of PF. The possible mechanism of action may be oxidation of LDL by enzymes.

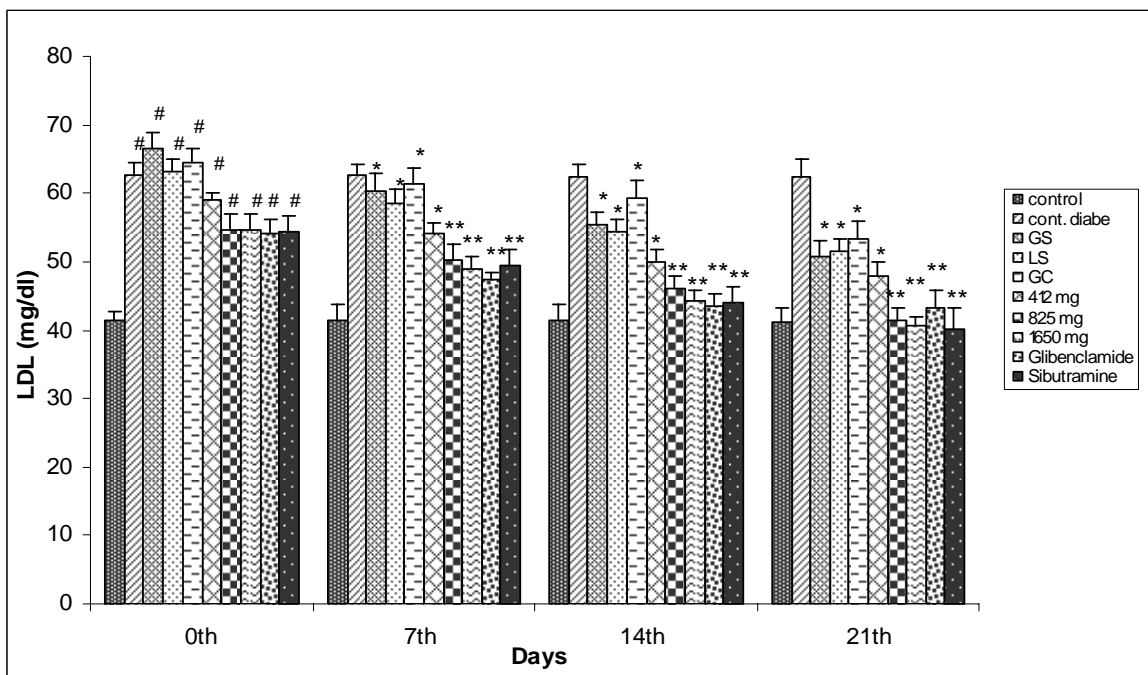


Fig. 6: It shows effect of Polyherbal formulation on serum LDL level of obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean \pm S.E.M., n=6; *P < 0.05, **P < 0.01, #P<0.01.

**P < 0.01, #P<0.01.

Also, VLDL level decreases significantly by administration of PF irrespective of the dose (Figure 7). But polyherbal formulation was found to possess much better activity as compared to that of single

herbs. However, study needs further investigation to know the exact mechanism of polyherbal formulation for its use in obesity associated diabetes.

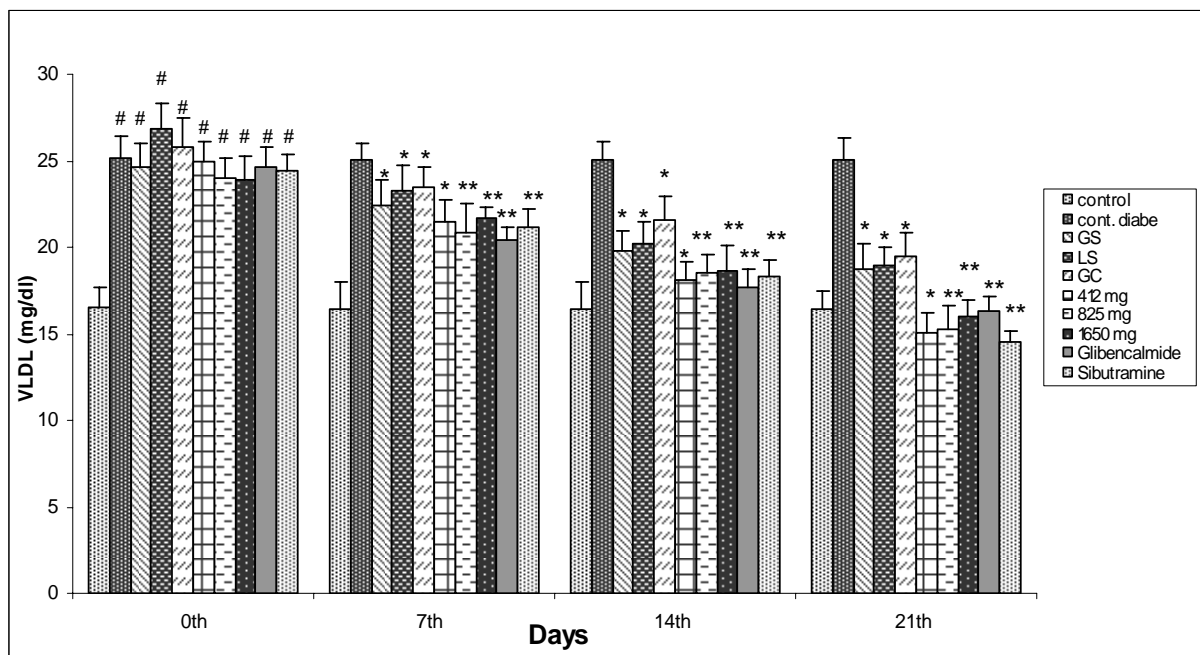


Fig. 7: It shows effect of Polyherbal formulation on serum VLDL level of obese diabetic rats at 0th, 7th, 14th and 21th day. Data are expressed as mean \pm S.E.M., n=6; *P < 0.05, **P < 0.01, #P < 0.01

CONCLUSION

To conclude, the polyherbal formulation showed significant improvement in various alterations produced in obesity associated diabetes and it could be effectively used as an herbal substitute to treat the patients suffering from obesity associated diabetes.

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