COMPARATIVE STUDY OF EFFECT OF *SWERTIA CHIRATA* LEAF EXTRACT ON INDINAVIR TREATED RATS

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ABSTRACT

Background: Indinavir is widely used for the treatment of human immunodeficiency virus (HIV) infection. It is known to cause hyperglycemia or insulin resistance and hyperlipidemia.

Aim and Objectives: To study the effect of *Swertia chirata* leaf extract with metformin and pioglitazone on indinavir treated rats.

Methods: Swiss albino rats were divided into five Groups of six animals each. All the groups (except control) were treated with indinavir 216 mg/kg (oral) for 15 days. Group I (control) received normal saline (oral) from day 1 to day 15, Group II received indinavir 216 mg/kg (oral), Group III received *S. chirata* plant extract 500 mg/kg (oral) from day 8 to day 15, Group IV received pioglitazone 4 mg/kg (oral) from day 8 to day 15, and Group V received metformin 180 mg/kg (oral) from day 8 to day 15. The biochemical parameters such as serum glucose, insulin, and lipid levels were measured on day 15. Results were analyzed using one-way analysis of variance followed by Bonferroni's multiple comparison test.

Results: Indinavir (216 mg/kg) treated rats showed a significant ($p < 0.05$) increase in glucose and insulin levels and also altered lipid levels. This indicates indinavir produces diabetic-like state in rats. *S. chirata* extract (500 mg/kg) decreases glucose and insulin levels and also improves lipid levels the effect is almost similar to metformin and pioglitazone.

Conclusion: Indinavir causes elevated glucose, insulin and lipid levels, so care must be taken while prescribing indinavir for HIV patients. Treatment with *S. chirata* extract improved the altered glucose, insulin, and lipid profile in indinavir treated rats.

Key words: Indinavir, Insulin resistance, Diabetes dyslipidemia, Glucose intolerance.

INTRODUCTION

Antiretroviral agents prevent the progression of disease which is caused by human immunodeficiency virus (HIV). Therapy includes a combination of minimum three drugs and available regimens forms more than 1100 combinations. The unique property of these drugs is need for lifelong administration to control virus replication and to prevent the development of drug resistance. The contribution of PI's is considered to a great extent in the prevention of HIV-associated morbidity and mortality over many years and remain a cornerstone of HAART. Long-term therapy with PI's is found to cause insulin resistance (IR) and hyperglycemia, epidemic Type-2 diabetes mellitus and other complications such as hyperglycemia, hypercholesterolemia or lipodystrophy. PI's found to produce long-term suppression of viremia, increase in CD4 lymphocyte counts, reduced disease progression, and decrease in mortality, the benefits and toxicity of this drug needs to be balanced [1-4].

This class of drug found to be very effective but they also found to be associated with metabolic changes that greatly increase lifetime risk [5,6]. Among these, one of the important metabolic changes is alteration of normal glucose-insulin homeostasis which remains particularly prevalent and alarming clinical change in affected patients. IR, impaired glucose tolerance, and Type-II diabetes mellitus are the most common in patients receiving HAART, especially with PI's [7-9].

The occurrence of DM is four-fold higher in HIV-infected patients receiving HAART compared to HIV-seronegative men [10]. Lifespan of HIV infected patients is increased due to availability advanced antiretroviral drugs. HIV-infected patients can lead a normal life but there is an increased chance of risk from noninfectious caused such as cardiovascular disease and diabetes [11-13].

Pioglitazone act by increasing insulin sensitivity in peripheral tissue and it increases glucose transport into muscle and adipose tissue. It also activates the genes that regulate fatty acid metabolism in peripheral tissue [14]. Metformin is known as insulin sensitizer; that is, it increases glucose uptake and utilization by target tissues, thereby decreasing IR. It requires insulin for its action but does not promote insulin secretion [15,16].

Swertia chirata belongs to the family of Gentianaceae. It is also known as *Chirata*, *Chirayata* and this is also mentioned in Charaka Samhitha a classical Indian medical text. Based on research, scientists confirmed this plant as a potential antidiabetic agent. Recent decades researchers also revealed its antimalarial, anti-inflammatory, antioxidative, and anticarcinogenic properties of this plant. Hence, we have selected it for this study, the effect of *S. chirata* leaf extract with metformin and pioglitazone on indinavir-treated rats.

METHODS

Raring of animals
For this study male albino Wister rats, weighing between 230 and 300 g were used. Rats were procured from the central animal house of institution. The experiment protocol used for this study was approved by the institutional animal ethical committee [Ref.No: KSHEMA/

IAEC/03/2013]. They were maintained under standard laboratory condition at temperature 23±2°C, humidity 50±10% with 12 hrs light/12 hrs dark cycles. Animals were maintained at polypropylene cages, rat pellets and water were given *ad libitum*.

Drugs and chemicals
Indinavir was obtained from yarrow chem products, Mumbai. Metformin and Pioglitazone were obtained from Mahalakshmi chemicals, Hyderabad. Ketamine injection was obtained from Neon Laboratories Limited, Mumbai.

Reagents and kits
The biochemical parameters such as glucose and lipid profile were measured using commercially available kits (Agappe Diagnostics Ltd, Kochi). To measure insulin levels the Ultra sensitive Rat insulin enzyme-linked immunosorbent assay (ELISA) kit were obtained from Genxbio Health Sciences Private Limited, New Delhi.

Preparation of *S. chirata* leaf extract
Leaves of *S. chirata* were used for this study; leaves were dried at room temperature. Dried leaves are taken into a Soxhlet apparatus; ethanol is used for the process of extraction.

Phytochemical analysis
S. chirata ethanolic extract was subjected to preliminary phytochemical analysis for detection of major chemical constituents such as alkaloids, phenols, resins, tannins, terpenoid, and saponins.

Experimental design
The animals were divided into five Groups (n=6). The study was conducted for 15 days. A pilot study was conducted initially to determine dose of indinavir which causes hyperglycemia or IR and hyperlipidemia. At the same time, dose of antidiabetic drugs such as metformin and pioglitazone was determined.

Group I (control) received normal saline (oral) from day 1 to day 15, Group II received indinavir 216 mg/kg (oral), Group III received *S. chirata* plant extract 500 mg/kg (oral) from day 8 to day 15, Group IV received pioglitazone 4 mg/kg (oral) from day 8 to day 15, and Group V received metformin 180 mg/kg (oral) from day 8 to day 15. All the groups (Except control) were treated with indinavir 216 mg/kg (oral) for 15 days. The 2% Gum acacia were used as a vehicle.

At the end of study period, i.e., on 15th day, rats were anesthetized and fasting blood glucose was collected by retro-orbital sinus puncture for estimation of biochemical parameters.

Biochemical estimation
Blood samples were centrifuged at 2000 RPM and serum was separated. The serum was used for the biochemical investigations. Serum glucose levels were measured by glucose oxidase and peroxidase (GOD-POD) method, triglycerides measured by glycerol phosphate oxidase-POD, total cholesterol (CH), high-density lipoproteins (HDL), and low-density lipoproteins (LDL)-CH by oxidase POD (CHOD-PAP) methods. Very LDL (VLDL) CH was estimated using the formulas, VLDL=TG/5. The serum insulin levels were estimated by ELISA method using ELISA reader.

Oral glucose tolerance test (OGTT)
At the end of experimental period, OGTT was performed in all groups of rats. The 12hr fasted rats were treated with glucose solution at a dose of 2 g/kg body weight by oral route, followed by collection of blood sample for analysis.

Statistical analysis
The data were presented in mean±standard error of the mean. Results were analyzed using one-way analysis of variance followed by Bonferroni's multiple comparison test. $p<0.05$ was considered to be significant.

RESULT

Phytochemical analysis
The preliminary phytochemical analysis of *S. chirata* leaf extract showed the presence of Carbohydrate, Coumarins and Tannin.

Biochemical parameters
In Group II rats treated with indinavir 216 mg/kg (oral) significantly increased Insulin, total CH, TG's, LDL, and VLDL levels without causing much change in HDL levels in comparison to control group. It indicates that indinavir produces diabetic-like state in rats. Treatment of rats in Group III with ethanolic extract of *S. chirata* leaf extract significantly reduced the elevated insulin, total CH, TG's, LDL, and VLDL levels in comparison Group II. There was no significant difference on insulin and lipid profile between the standard drugs Metformin, pioglitazone and with the treatment of *S. chirata* leaf extract on rats, as shown in Fig. 1 and Table 1.

OGTT
It was observed that after a 2 hrs glucose load Group II shows significant high serum glucose levels compared to normal. In Groups III and V, the glucose levels were significantly less compared to Group II (Fig. 2).

Random serum glucose concentration was measured which shows a significant difference compared to control and treatment groups (Fig. 3).

DISCUSSION

Protease inhibitors are known to induce a syndrome of IR along with that it may also cause hyperglycemia, hyperlipidemia, and fat
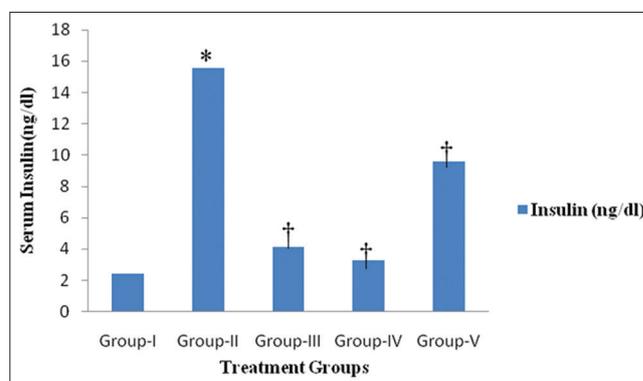

Fig. 1: 1 Effect of indinavir, *Swertia chirata* extract and standard drugs on rat serum insulin levels. Values are expressed in mean±standard error of the mean, *$p<0.05$ vs. Normal control, †$p<0.05$ vs. diabetic control
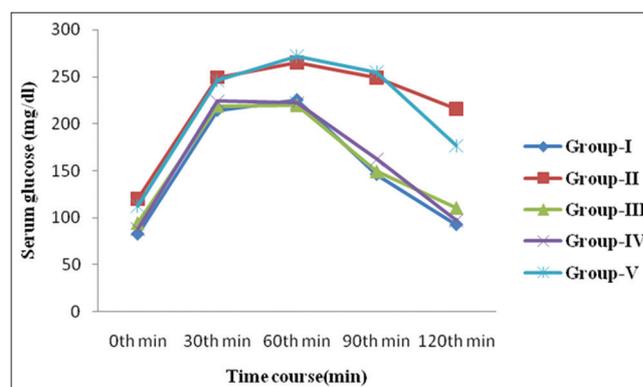

Fig. 2: Effect of indinavir, *Swertia chirata* extract and standard drugs on rat serum glucose levels

Table 1: Effect of indinavir, *Swertia chirata* extract, and standard drugs on rat lipid profile

Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Group-I	72.5±2.74	96.6±2.1	40.0±2.3	14.5±0.5	42.1±3.8
Group-II	219.3±13.4*	144.6±7.1*	30.8±2.2	43.8±2.6*	69.9±8.2*
Group-III	79.6±2.6†	98.5±2.1†	35.3±2.4	15.9±0.5†	47.2±3.9
Group-IV	86.1±3.9†	98.1±4.6†	37.0±1.5	17.2±0.7†	43.9±5.0
Group-V	145.8±22.1†	109.02±6.1†	31.6±1.7	29.1±4.4†	48.2±7.9

Values are expressed in mean±SEM, *p<0.05 vs. Normal control, †p<0.05 vs. diabetic control, HDL: High density lipoproteins, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, *S. chirata*: *Swertia chirata*, SEM: Standard error of the mean
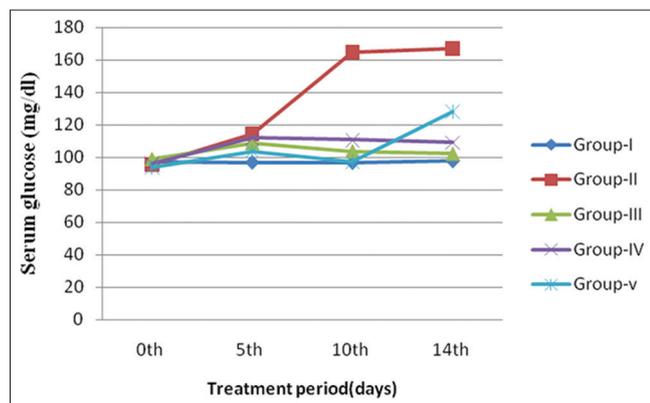

Fig. 3: Effect of indinavir, *Swertia chirata* extract and standard drugs on rat serum glucose levels

redistribution. It resembles the metabolic syndrome with high risk of cardiovascular diseases. Many research studies were done to assess IR using a homeostasis model assessment of IR (HOMA-IR). The calculated score higher than four using HOMA-IR indicates IR. Early studies of insulin profile in the patients infected with HIV infection were no IR or hyperglycemia was observed, but clamp studies indicated the increased IR. Protease inhibitors can cause decrease in insulin-stimulated glucose transport in fat cells [16]. Patients with HIV infection must be screened at the beginning of HAART therapy, and later during the treatment. Certain professionals suggest for fasting blood glucose as a screening tool; others recommend OGTT which should be performed as a part of screening procedures [17].

The actual mechanism of IR is not well understood. IR occurs when normal insulin levels are no sufficient to stimulate glucose uptake in the insulin signaling pathway in insulin-sensitive tissues such as muscle, liver, and subcutaneous adipose tissue. Following manifestations such as hyperinsulinemia, hyperglycemia, and dislipidemia (hypertriglyceridemia) are seen in patients with protease inhibitors [18,19].

The treatment with PIs caused partial lipodystrophies in some reports and also found to cause DM or insulin resistance along with higher triglyceride and CH levels. There is also peripheral fat wasting, accumulation of intra-abdominal fat has been observed in some patients. Inhibition of hormone sensitive lipase and insulin induced stimulation of lipoprotein lipase would result in net increase of fat. Inhibition of lipogenesis and stimulation of lipolysis results in inappropriate increase in free fatty acids. Increased availability of free fatty acids would further increase hepatic triglyceride synthesis and increases hepatic glucose output, which causes compensatory increase in insulin secretion to maintain glucose uptake homeostasis. There are many theories suggests that PIs therapy induces DM, beside that there is increasing evidence that there is an increase in insulin levels suggesting emergence of IR [20,21].

It has been observed that PIs selectively inhibit GLUT4 activity *in vitro*, which provides an important mechanism for the development of IR observed in the patients with HIV infection. Whole body glucose disposal takes place by glucose transport, GLUT4 is expressed in tissue responsible for glucose disposal (skeletal/cardiac muscle and fat), and it is believed to be principal transporter for mediating insulin stimulated glucose uptake at these sites. PIs causes inhibition of GLUT4, and the inhibition is reversible when the treatment is stopped. PIs inhibitors induced IR also associated with lipodystrophy syndrome caused by GLUT4 inhibition. Chronic treatment of indinavir causes elevated free fatty acids secondarily contribute to IR, prolonged treatment may cause redistribution of fat and hypertriglyceridemia may also cause IR. Hyperinsulinism from GLUT4 inhibition may contribute to fat distribution [22,23].

S. chirata was known to possess antidiabetic activity since a long time. To study the hypoglycemic and antidiabetic role of *S. chirata* 95% ethanolic extract and other four fractions were used, and the significant effect was produced by lowering blood sugar level [24]. The *Swertia* extract significantly decreases the CH and lipid levels. Phytochemical analysis was performed and the extract was known to contain carbohydrate, coumarins, and Tannin. The *S. chirata* is also known to contain three main phytochemicals amarogentin, mangiferin, and *Swertia merin*. Mangiferin is known to have blood glucose lowering property and also shows suppressive effect on lipid profile [25,26].

CONCLUSION

This study reveals that *S. chiratais* is effective against indinavir-induced hyperglycemia or IR and hyperlipidemia. *S. chirata* with its multiple beneficial effects would seem to be useful as an adjuvant with indinavir (protease inhibitor) on AIDS patients.

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