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RESEARCH ARTICLE

Anti-hyperglycemic effect of *Swertia chirata* root extract on indinavir treated rats

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ABSTRACT

Background: Indinavir belongs to class of protease inhibitors which is widely used for the treatment of AIDS. Treatment with Indinavir may induce diabetic like symptoms and prolonged treatment leads to diabetes mellitus. **Aim and Objectives:** To study the anti-hyperglycemic effect of *Swertia chirata* root extract on indinavir treated rats. **Materials and Methods:** Swiss albino Wister rats were divided into seven groups of seven animals each. Group I (control) received normal saline (oral), Group II received Indinavir 216 mg/kg (oral), Group III received *S. chirata* root extract 500 mg/kg (oral), Group IV received pioglitazone 4 mg/kg (oral), Group V received metformin 180 mg/kg (oral), Group VI received pioglitazone 4 mg/kg (oral) along with *S. chirata* root extract 500 mg/kg (oral), and Group VII received metformin 180 mg/kg (oral) along with *S. chirata* root extract 500 mg/kg (oral). All the groups (Except control) were treated with indinavir 216 mg/kg (oral) for 15 days. Moreover, the treatment with extract and standard drug is carried out from day 8 to day 15. The biochemical estimations - such as serum glucose, Insulin, and lipid levels - were carried on day 15. Statistical analysis is performed using one-way analysis of variance followed by Bonferroni's multiple comparison test. **Results:** The group treated with indinavir (216 mg/kg) showed significant ($P < 0.05$) increase in biochemical parameters compared to the other groups. This shows that indinavir is capable of producing diabetic like symptoms in rats. The group treated with *S. chirata* root extract (500 mg/kg) decreases glucose and insulin levels and also improves lipid levels which are almost similar to the effect produced by the standard drug metformin and pioglitazone. **Conclusion:** The treatment with indinavir produces elevated glucose, insulin, and lipid levels. The groups treated with *S. chirata* root extract showed improved glucose, insulin and lipid profile in Indinavir treated rats.

KEY WORDS: Protease Inhibitors; Indinavir; Insulin Resistance; Diabetes Mellitus; Hyperglycemia; *Swertia chirata*

INTRODUCTION

The drugs belong to class of protease inhibitors (PIs) prevents the progression of the disease caused by HIV infection. Since three drug combinations are the minimum standard of care of this infection, constitute at least 1000 possible combined regimens. The treatment of HIV infection requires lifelong administration to control virus replication and avoid rapid emergence of irreversible drug resistance if they are not used properly. The treatment of HIV infection by using highly active antiretroviral therapy (HAART) which includes PIs, which saved many patients life over last decades. On long-term treatment with PIs may cause complications such as insulin resistance (IR), hyperglycemia and type 2 diabetes mellitus (DM), and other complications. It has also been found to be associated with some other complications such as

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hyperglycemia, hypercholesterolemia, hypertriglyceridemia, and lipodystrophy. It is estimated around 40 million people found to be suffering from HIV infection around the worldwide, the number is more in resource poor countries. Indinavir is the drug belongs to class of PIs which suppress HIV infection, increases CD4 lymphocyte count, reduced disease progress. The benefit of drug must be balanced against short and long-term toxicity in HIV-1 infected subjects receiving HAART, especially with protease PIs.[1,2] Much of concern is due to the recognition of the long-term use of protease PIs has been associated with hyperglycemia, hypercholesterolemia, hypertriglyceridemia, and lipidystrophy.[3]

Some of research studies pointed out the incidence of DM and cardiovascular diseases in patients with HIV infection on HAART which leads to death. Further, patients on HIV-infection receiving antiretroviral therapy (ARVT) are at a 4-fold increased risk of developing diabetes in comparison to HIV-seronegative men.[4] The availability of HAART/ combined ARVT has resulted in marked reduction in morbidity and mortality in HIV infected patients. Moreover, the lifespan of an HIV patient has steadily increased.[5,6]

The HAART therapy started in 1990's, it is reported that abnormal glucose homeostasis has been observed in patients with HIV infection who is exposed to HAART.[7,8] The public health advisory warning was issued by Food and Drug Administration about the adverse events.[9] By the year1997, a total of 83 reports was received for causing exacerbation of diabetes/hyperglycemia or development of diabetes in patients taking the drugs among these 83 patients, 27 was admitted to hospital (6 patients found to be having life threatening risk).[10] IR was found in 41 (61%) of 67 Protease PIs treated patients with HIV infection.[11] and impaired glucose tolerance was observed in 25 (35%) out of 71 HIV-infected patients using HAART.[12] Prominent effects are observed with Indinavir and ritonavir, diabetes began to appear when the drugs are in use in HIV-infected patients. According to some hypothesis that indinavir found to cause acute IR, insulin levels observed glucose tolerance tests were nearly two-fold higher compared to indinavir-treated animals.[13]

The plant *Swertia chirata* belongs to the family Gentianaceae. The plant usually found in Himalayas. *S. chirata*, commonly known as chirata, chirayata, nelaveppa, chireita, Nelavemu or kirata-tikta in Sanskrit and it is mentioned in Charaka Samhita for its multifarious therapeutic value. The medicinal extracts of plant are widely used in Ayurvedic, Unani, and Siddha systems of Medicines. At Diabetic Conference in Glasgow March 2009, researchers based on their research experience announced that *S. chirata* may now be considered as a potential antidiabetic agent.[14] Hypoglycemic and Anti-diabetic effect of *S. chirata* with ethanolic extract along with few other fractions of *S. chirata* were studied to observe the effect on blood sugar in rats. Moreover, hexane fraction was also found to be having blood sugar lowering activity in albino rats. Mangiferin is present in *S. chirata* also responsible for its hypoglycemic action.[15] The *S. chirata* possees antidiabetic activity, hence we have selected it for this study, effect of *S. chirata* root extract with metformin and pioglitazone on indinavir induced diabetic like symptoms in albino rats.

Pioglitazone is one of the standard antidiabetic drugs, which causes increase in insulin sensitivity in peripheral tissue, and which will also increase glucose transport to the muscle and adipose tissue. It will activate the genes which are known to regulate fatty acid metabolism in peripheral tissue.[16] Metformin a antidiabetic drug which is known as insulin sensitizer, which causes increase in glucose uptake and utilization by target tissues, also known to cause decrease IR. It requires insulin to produce therapeutic effect but do not promote insulin secretion.[17]

MATERIALS AND METHODS

Raring of Animals

Male albino Wister rats are used for this study, most of them weighing between 220 and 300 g. Rats were obtained from central animal house of institution. The experiment protocol was approved by the Institutional Animal Ethical Committee. They were maintained under standard laboratory condition at temperature 23 ± 2°C, humidity 50 ± 10% with 12 h light/12 h 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 was obtained from Mahalakshmi Chemicals, Hyderabad. Ketamine injection was obtained from Neom Laboratories Limited, Mumbai.

Reagents and Kits

The biochemicals 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 was obtained from Gen X Bio Health Sciences Private Limited, New Delhi.

Preparation of *S. chirata* Root Extract

Root extract of *S. chirata* was used for this study; roots were shade dried at room temperature. Dried roots are taken into a soxhlet apparatus; ethanol is used for the process of extraction.

Experimental Design

For this study, rats were divided into seven groups, six rats in each group. The study was conducted for 15days. Initially,

pilot study was conducted to determine dose of indianvir which causes hyperglycemia or diabetic like symptoms. Same time antidiabetic dose of Metformin and Pioglitazone was determined.

Group I (control) received normal saline (oral), Group II received Indinavir 216 mg/kg (oral), Group III received *S. chirata* root extract 500 mg/kg (oral), Group IV received pioglitazone 4 mg/kg (oral), Group V received metformin 180 mg/kg (oral), Group VI received pioglitazone 4 mg/kg (oral) along with *S. chirata* root extract 500 mg/kg (oral), and Group VII received metformin 180 mg/kg (oral) along with *S. chirata* root extract 500 mg/kg (oral). All the groups (Except control) were treated with indinavir 216 mg/kg (oral) for 15 days. Moreover, the treatment with extract and standard drug is carried out from day 8 to day 15.

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

The blood samples were subjected to centrifuge at 2000 rpm and serum was separated. The separated serum was used for the biochemical investigations. Serum glucose levels were measured using glucose oxidase-peroxidase (POD) method, triglycerides (TG's) measured by glycerol phosphate oxidase-POD, total cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL)-cholesterol by oxidase phenol-aminophenazone methods. Very LDLs (VLDL) cholesterol was estimated using the formulas, VLDL = TG/5. The estimation of serum insulin levels is done by ELISA method with the help of ELISA reader.

Oral Glucose Tolerance Test (OGTT)

OGTT was performed on the last day of experiment on all groups. The rats were fasted for 12 h and then they are treated with oral glucose solution at a dose of 2 g/kg body weight by oral route, followed by collection of blood sample for biochemical 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

Biochemical Parameters

There is a significant increase in insulin, CH, TG's, LDL, VLDL levels in the Group II rats which is treated with Indinavir 216 mg/kg (oral)/day when compared to control group, without causing much effect on HDL levels. This shows that Indinavir induces diabetic like state in rats. The Group III treated with ethanolic extract of *S. chirata* root extract showed significant reduction in the insulin, CH, TG's, LDL, VLDL levels compared to Group II. There was no significant difference on insulin and lipid profile between the groups treated with standard drugs Metformin, pioglitazone and the combination standard drug with *S. chirata* root extract on rats, as shown in Figure 1 and Table 1.

Random serum glucose concentration was measured which shows significant difference compared to control and treatment groups (Figure 2).

OGTT

OGTT is done and it is observed that after a 2 h glucose load there was a significant increase in serum glucose levels in Group II compared to normal. There was a marked reduction in glucose levels in treatment groups when compared to Group II (Figure 3).

DISCUSSION

Indinavir belongs to class of protease PIs are known to induce IR along with that it may also cause hyperglycemia and hyperlipidemia. Many studies were conducted to estimate IR by homeostasis model assessment of IR (HOMA). Estimated score higher than 4 using HOMA-IR indicates probability of IR; the clamp studies indicated the increased IR. Indinavir can cause decrease in insulin stimulated glucose transport in fat cells.[18] Before beginning of HAART therapy patients suffering from HIV infection must be screened, even during the treatment. Professionals suggest that fasting blood glucose is a screening tool, but few others recommend OGTT should be performed as a part of screening procedures.[19]

The mechanism by which IR develops is not well known. When normal insulin levels are not sufficient to stimulate glucose uptake in the insulin signaling pathway in insulin sensitive tissues such as muscle, liver and subcutaneous adipose tissue IR occurs.[20]

PIs are known to cause partial lipodystrophies in some reports and also found to cause DM or IR along with higher TG and cholesterol levels. Due to suppression of hormone sensitive lipase and insulin stimulated activation of lipoprotein lipase would result in net increase of fat. Free fatty acids levels are increased due to inhibition of lipogenesis and stimulation of lipolysis. Increase hepatic TG synthesis and increases hepatic glucose output due to increase in availability of free fatty acids, which causes compensatory increase in insulin secretion to maintain glucose uptake homeostasis. Many theories suggests that PIs may therapy induce DM, along with that there is increasing evidence that there is increase in insulin levels which suggests emergence of IR.[21,22]

Table 1: Effect of indinavir, *S. 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	72.6±2.7	96.5±2.1	40.1±2.3	14.6±0.5	42.2±3.8
Group-II	219.4±13.4*	144.7±7.1*	30.7±2.2	43.9±2.6*	69.8±8.2*
Group-III	80.4±4.6†	96.7±2.2†	41.4±2	16.2±0.9	39.2±3.3
Group-IV	86.2±3.9†	98.2±4.6†	37.1±1.5	17.3±0.7†	43.8±5.0†
Group-V	145.7±22.1†	109±6.1†	31.5±1.7	29.2±4.4†	48.3±7.9†
Group-VI	88.8±4.6†	87.8±3.6†	35.1±2.7	17.7±0.8†	34.9±2.7†
Group-VI	86.3±9.6†	107.1±4.2†	36±2.4	17.2±1.9†	53.9±4.9†

Values are expressed in mean±SEM, *P<0.05 versus normal control, †P<0.05 versus diabetic control, TG: Triglyceride, TC: Total cholesterol, HDL: High density lipoproteins, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, SEM: Standard error of the mean, *S. chirata: Swertia chirata*

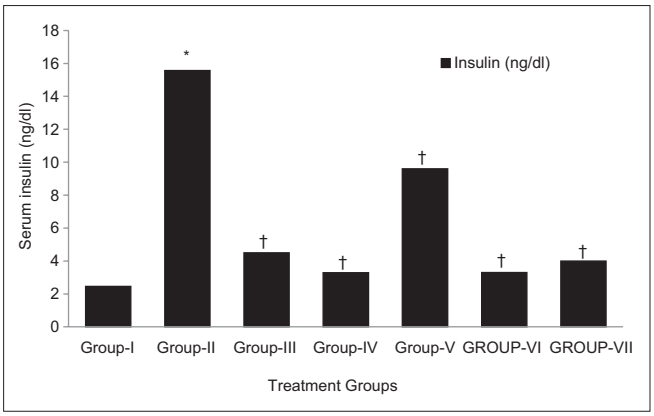

Figure 1: Effect of Indinavir, *Swertia chirata* extract and standard drugs on rat serum insulin levels. Values are expressed in mean ± standard error of the mean,*P<0.05 versus normal control, †P<0.05 versus diabetic control

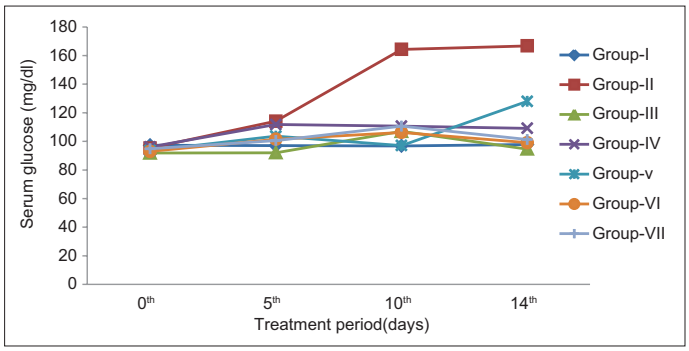

Figure 2: Effect of indinavir, *Swertia chirata* extract and standard drugs on rat serum glucose levels

As per the research studies PIs are known to selectively inhibit Glucose Transporter4 (GLUT4) activity *in vitro*, which is known to cause IR in patients with HIV infection. GLUT4 is present in tissues are responsible for glucose disposal (skeletal/cardiac muscle and fat), it is a principal transporter for mediating insulin-stimulated glucose uptake at these sites. PIs inhibit GLUT4 and which is reversible once the treatment is stopped. PIs cause IR which is also

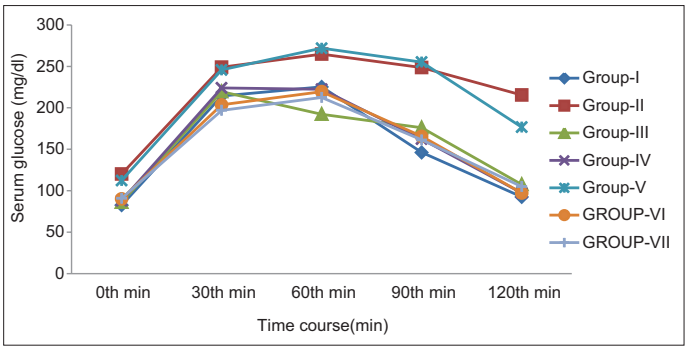

Figure 3: Effect of Indinavir, *Swertia chirata* extract and standard drugs on rat serum glucose levels

associated with lipodystrophy syndrome. Prolonged therapy with indinavir causes increase in free fatty acid levels which secondarily contribute to IR, may cause redistribution of fat and hypertriglyceridemia. Hyperinsulinism caused by GLUT4 inhibition contributes to fat distribution.[23]

The plant *S. chirata* was known to posse medicinal properties. It is used as an antidiabetic agent since long time. For this study to observe the hypoglycemic and antidiabetic role of *S. chirata* 95% ethanolic root extract were used, the antidiabetic effect was significant and which decreased blood sugar level.[15] *S. chirata* root extract significantly decreased the cholesterol and lipid levels. The *S. chirata* is also known to contain some phytochemicals such as amarogentin, mangiferin and swertia merin so on. Mangiferin is known to have blood glucose lowering property and also decreases lipid levels.[24,25]

CONCLUSION

In this study, it is proved that *S. chirata* is effective against Indinavir induced hyperglycemia or IR and hyperlipidemia. *S. chirata* root extract known to possess multiple medicinal effect which can be used as an adjuvant in combination therapy for patients having HIV infection AIDS along with other standard drugs such as Indinavir (protease inhibitor).

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