EFFECTS OF SOLANUM NIGRUM FRUITS ON LIPID LEVELS AND ANTIOXIDANT DEFENSES IN RATS WITH FRUCTOSE INDUCED HYPERLIPIDEMIA AND HYPERINSULINAEMIA

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Summary

Increased Fructose supplementation produced significant elevations in serum cholesterol, triglycerides, glucose and insulin and also in body weight. Treatment with ethanolic extract of *solanum nigrum* fruit (SNE) (100mg/kg, 250 mg/kg *p.o* daily) for 3 weeks on fructose fed rats produced a significant decrease ($P<0.05$) in elevated levels of glucose, cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein, atherogenic index and increased high density lipoprotein cholesterol levels and HDL-ratio without affecting serum insulin levels in fructose-fed rats. Furthermore, SNE treatment also reduced lipid peroxidation and increased antioxidants such as superoxide dismutase, catalase and glutathione in the liver homogenates of the treated animals. The results of present study indicate that SNE has significant beneficial effects against fructose induced hyperlipidemia and shows good antioxidant defense.

Key words: *solanum nigrum*, fructose, hyperlipidemia, antioxidant defense

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Introduction

Increased fructose consumption in humans is believed to be one of the important factors for impaired glucose tolerance in humans leading to diabetes (1). Epidemiologic and clinical evidences document a close association among hypertension, insulin resistance and dyslipidemia (hypertriglyceridemia and lower levels of high-density lipoprotein cholesterol) and they also are the predominant features of metabolic syndrome (2).

Hyperinsulinemia / insulin resistance, hypertriglyceridemia have been documented in the fructose-induced hypertensive rat model (3). High fructose fed diet induce well characterized metabolic dysfunction, typically resulting in a rapid elevation of serum triglycerides with a corresponding increase in blood pressure within two weeks. Animals maintained on this diet for longer periods of time develop elevated free fatty acids and hyperinsulinemia at the expense of glycemic control. Fructose has also been shown to have pro-oxidant effects. Increased formation of lipid peroxidation end products and defects in free radical defense system have been documented in high fructose-fed rats (4). Thus this animal model exhibits many of the hallmarks of an early stage of the Metabolic Syndrome (or "Syndrome X"). This cluster of changes appears to be major risk factor in the pathogenesis of coronary artery disease.

Solanum nigrum L. (Solanaceae) commonly called as 'Makoi'in Hindi and 'Piludi'in Gujarati is indigenous to India. It is erect, angular, branching stem grows 1 to 2 feet hight and may be glabrous or covered with inward- bent hairs. The leaves are alternate, dark green, ovate, and wavy-toothed or nearly entire. The fruit is a many-seeded, pea-sized, purple or black berry. It grows as a weed all over dry parts of India. The ripe fruit are bitter, pungent, heating, laxative, aphrodisiac, tonic, improve appetite and taste.

Leaves, fruits stem and roots of *solanum nigrum* have been used in ethnomedicine for several medicinal properties: diseases of heart and eye, in pains, piles, inflammation, leucoderma, itch, worms in the ear, dysentery, vomiting,

cough, asthma, bronchitis, fever, urinary discharges, favor conception, facilitate delivery laxative, liver disease, psoriasis and ringworm. *Solanum nigrum* plant is also known as herbal medicine for the treatment of diabetes (5)**.** The plant contains glycoalkaloids, steroidal glycosides, steroidal saponins, steroidal genin, tannin and polyphenolic compounds. So it was worthwhile to undertake such investigation using the extract of mature fruits of *Solanum nigrum L.* (SNE).

The intake of fructose is raised with an increased consumption of soft drinks and many beverages containing high fructose in recent years. Studies in rats have been demonstrated that high intake of fructose produced a decline of insulin sensitivity in the liver and peripheral tissues (1).

Thus fructose has been implicated as the useful tool to induce insulin resistance and hyperlipidemia in animals. In the present study, we employed rats with insulin resistance and hyperlipidemia induced by fructose fed diet to investigate the effects of *Solanum nigrum L.* on hyperlipidemia and insulin resistance.

Materials and Methods

Plant Material

The plant material used in the present study was collected during month of October from region of Anand, Gujarat. The plant was authenticated by comparison with voucher specimen No. VSM502 and ARM 2174 at the Prof. G.L.Shah Herbarium of S.P.University, Vallabh Vidyanagar,Anand,Gujarat,India. The extract was prepared by soxhlet extraction of the crushed fresh fruits with ethanol, and extract evaporated to dryness (7.9 % yield w/w).

Experimental animals and Treatment protocol

Male Sprague Dawley rats weighing 200-250 g, (Zydus Research Center, Ahmedabad, India) housed under well controlled conditions of temperature (22±2°C), humidity (55±5%) and 12/12-h light dark cycle were given access to food and water *ad libitum*.

The protocol of the experiment was approved by the Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Twenty four male Sprague Dawley rats were divided into four groups of six each.
Group 1: Normal diet and water ad libitum and received vehicle solvent.
Group 2: Normal diet and water with 10% fructose and received vehicle solvent (Control)
Group 3: Normal diet and water with 10 % fructose and received ethanolic extract of SNE (100 mg/kg)
Group 4: Normal diet and water with 10 % fructose and received ethanolic extract of SNE (250 mg/kg)
Each group was administered vehicle or drugs daily for 3 weeks by gavage using oral feeding needle. Feeding of animals by fructose and extract were done simultaneous from the beginning. At the end of 3 weeks period, animals were kept for overnight fasting and the blood samples were collected from the retroorbital plexus in the centrifuge tube. The blood samples were allowed to clot for 30 miutes at room temperature, and then centrifuged at 5000 rpm for 15 minutes. Serum samples thus obtained were stored at -4°C until biochemical estimation were carried out. After blood collection animals were killed using deep ether anesthesia and the liver was excised, subjected to antioxidant estimation.

Biochemical analysis
The serum parameters were analyzed spectrophotometrically by using double beam UV-Visible spectrophotometer (Shimadzu UV- UV-Visible spectrophotometer, model 1700). Estimation of serum glucose (GOD-POD method), cholesterol (enzymatic method), triglyceride (enzymatic method) and HDL-cholesterol (phosphotungstate method) were carried out using respective diagnostic kits (Bayer Diagnostic Ltd. India). Serum insulin was estimated by a radioimmunoassay method from Bhabha Atomic Research Centre, Mumbai, India. In addition to the above parameters,

body weight, food intake, and water intake of animals were recorded. VLDL-cholesterol and LDL-cholesterol were calculated as per Friedewald's equation (6).

VLDL= Serum triglyceride/ 5

LDL= Total serum Cholesterol - Total serum triglycerides/5- Total serum HDL-C

HDL ratio = HDL–Cholesterol × 100 /Total serum Cholesterol - HDL-C

Atherogenic Index (7) = Total serum triglycerides/Total serum HDL-C

Estimation of antioxidants

After 3 weeks, animals were sacrificed; the liver was quickly removed and washed in ice-cold saline. One hundred milligrams of liver tissue was homogenized in ice-cold tri hydrochloride buffer (pH 7.2). The homogenate was centrifuged at 800 rpm for 10 min, followed by centrifugation of the supernatant at 12,000 rpm for 15 min. The supernatant obtained was used for the estimation of reactive oxygen metabolites in terms of lipid peroxidation (8), superoxide dismutase (SOD) (9), catalase(10), reduced glutathione (GSH) (11), and total protein estimation (12).

Statistical analysis

Results were analyzed statistically using one way analysis of variance- (ANOVA) followed Dunnet's test. Data were considered statistically significant at $p<0.05$.

Results

Effects on body weight, glucose, insulin and lipid Profile

Fructose fed rats exhibited significant increase in body weight as compared to normal control rats ($P<0.05$). Treatment with extracts in fructose fed rats reversed this increase in body weight ($P<0.05$). Fructose fed rats were hyperglycemic and hyperinsulinemic as compared to normal control animals ($P<0.05$). Treatment with extract in fructose fed rats reduced glucose level without affecting insulin levels ($p<0.05$). (Table1). Fructose fed animals exhibited significantly higher serum cholesterol, triglyceride, VLDL-cholesterol and LDL-cholesterol levels whereas there was a decrease in HDL-cholesterol and HDL ratio as compared to

normal control animals. Extract treatment in fructose fed rats produced a significant decrease in serum cholesterol, triglycerides, VLDL-cholesterol, LDL-cholesterol levels, with an increase in HDL--cholesterol and HDL-ratio (Table 1). Furthermore, extract treatment to fructose fed rats exhibited significant improvement in atherogenic index (Table 1).

Effects on antioxidant defenses

Fructose fed animals showed significant increase in lipid peroxidation in terms of amount of malondialdehyde and super oxide dismutase (SOD) in liver tissue homogenates when compared to normal control animals. Treatment with extracts in fructose fed rats significantly decreased lipid peroxidation and increase SOD in liver tissue homogenates ($P<0.05$) (Table-2). Fructose fed rats showed significant decrease in catalase and glutathione levels in liver tissue homogenate as compared to normal control animals. Treatment with extract significantly increased catalase and glutathione levels in liver tissue homogenate ($P< 0.05$) (Table-2).

Discussion

The prevalence of insulin resistance and associated diseases has risen seriously around the world. The general view of insulin action places this hormone at the point of multiple organ adaptations to the ingested nutrients, in particular, dietary carbohydrates. It has been established that insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension and hyperlipidemia are associated with fructose intake in animal models. Increasing consumption of dietary fructose might be one of the factors responsible for the development of obesity and the accompanying insulin resistance syndrome (13). Thus, rats received 3 weeks of fructose-rich drinking water could be served as a reliable model for the investigation of insulin resistance (14).

Table 1: Effects of Treatment of *solanum nigrum L.*fruit extract on General Characteristics of the fructose induced hyperlipidemia and hyperinsulinemia

	Normal	**Fructose fed control**	**Fructose fed treated with extract (100 mg/kg)**	**Fructose fed treated with extract (250 mg/kg)**
Body weight (g/rat)	228 ± 9.564	334 ± 12.453	292 ± 13.483	273 ± 8.561**
Water intake (ml/rat/day)	43 ± 7.325	46 ± 5.687	41 ± 7.230	47 ± 4.624
Food intake (g/rat/day)	15.8 ± 2.781	18.7 ± 1.569	15.9 ± 1.989	16.5 ± 1.349
Serum Glucose (mg/dl)	75.4 ± 7.620	117.3 ± 4.945*	86.0 ± 5.060*	76.4 ± 6.514**
Serum Insulin (µU/ml)	17.1 ± 4.385	42.5 ± 4.660*	35.6 ± 6.898	31.8 ± 2.358
Serum Cholesterol (mg/dl)	83.1 ± 7.556	135 ±9.125*	103.5 ± 11.105	94.33 ± 6.647**
Serum HDL (mg/dl)	33.8 ± 2.548	14.7 ± 2.088*	22.6 ± 2.765**	29.8 ± 1.797**
Serum Triglyceride (mg/dl)	99.1±11.649	166.8±8.920*	99.8±8.495**	90.8±9.793**
Serum VLDL (mg/dl)	19.8±2.330	33.3±1.784*	19.9±1.699**	18.1±1.959**
Serum LDL(mg/dl)	42.6±8.710	87.4±10.538*	53.7±11.858**	40.5±15.157
HDL Ratio	37.7±3.903	12.2±2.504*	45.1±7.622**	58.8±3.956**
Athrogenic index:	4.7 ± 0.878	11.5 ± 1.112*	3.4 ±0.438**	2.7 ± 0.369**

n=6, *significantly different from normal control, $p<0.05$

**significantly different from fructose fed control, $p<0.05$

Table 2: Effects of Treatment of *solanum nigrum L.* fruit extract on antioxidant parameters in fructose induced hyperlipidemia and hyperinsulinemia

	Normal	Fructose fed control	Fructose fed treated with extract (100 mg/kg)	Fructose fed treated with extract (250 mg/kg)
Lipid peroxidation(µg/mg)	1.1 ± 0.101	4.9 ± 0.340*	3.1 ± 0.244**	1.8 ± 0.280**
Superoxide dismutase (U/mg of protein)	0.22 ± 0.0850	0.08 ± 0.011*	0.175 ± 0.010**	0.20 ± 0.009**
Catalyst (U/mg of protein)	24.34 ±1.847	12.46 ±3.100*	17.77 ± 1.983**	29.25 ± 1.440**
Glutathione (µ mole/mg of protein)	5.047 ± 0.680	1.952 ± 0.539*	2.847 ± 0.365**	4.485 ± 0.700**

n=6, *significantly different from normal control, $p<0.05$
**significantly different from fructose fed control, $p<0.05$

In the present study, an oral administration of ethanolic fruit extract was found to decrease the plasma glucose concentration of fructose fed rats in a dose-dependent manner, showing the beneficial action of *Solanum nigrum* in rats with insulin resistance. The mechanism of glucose lowering action might involve actions other than pancreatic β cells insulin secretion because in our study we did not observe any improvement in insulin resistance. Furthermore, it is likely that it might reduce blood glucose level by inhibiting the glucose absorption from the intestine by suppressing α- glucosidase enzymes.

In our study, Fructose fed rats exhibited clear cut abnormalities in lipid metabolism as evidence from the significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C levels. Treatment with ethanolic extract of Solanum nigrum fruit extract for 21 days significantly reduced serum total cholesterol, triglycerides and LDL-C associated with

concomitant significant increase in HDL-C levels and decrease in atherogenic index in hyperlipidemic rats indicating its potent antihyperlipidemic and antiatherogenic activity. The glucose lowering action of extract can be due to the consequence of an improved lipid metabolism apart from the direct interaction with glucose homeostasis. The triglycerides lowering property of extract could indirectly contribute to the overall antihyperglycemic activity through a mechanism called glucose-fatty acid cycle (15). According to the Randle's glucose-fatty acid cycle, increased supply of plasma triglycerides per se could constitute a source of increased free fatty acid (FFA) availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycemia.

It has been postulated that fructose can accelerate free radical production similar to glucose. For example, (16) have observed an increased production of H_2O_2 and formation of hydroxyl radicals by hamster pancreatic cells incubated with fructose in the presence of a metal ion catalyst. Furthermore, due to hyperglycaemia, increase in non enzymic glycosylation occurs, accompanied with glucose oxidation and these reactions are catalysed by Cu^{+2} and Fe^{+2}, resulting in formation of O_2^- and OH^- radicals which further accelerates the risk of cardiac diseases in dyslipidemia (17). Lipid peroxidation is one of the characteristic features of chronic fructose consumption. In this context, a marked increase in the concentration of TBARS was observed in liver of fructose fed rats. Increased lipid peroxide concentration in the liver of fructose fed animals has already been reported (4, 18). Administration of the extract significantly decreased the levels of TBARS in fructose fed rats (Table 2).

Glutathione (GSH), a tripeptide present in all the cells is an important antioxidant. Decreased glutathione levels in fructose fed animals have been considered to be an indicator of increased oxidative stress. GSH also functions as free radical scavenger in the repair of radical caused biological damage. A decrease was observed in GSH in liver during fructose fed animals (4). Administration of the extract increased the content of GSH in liver of fructose fed rats (Table 2).

The cellular radical scavenging systems include the enzymes such as superoxide dismutase (SOD), which scavenges the

superoxide ions by catalysing its dismutation and catalase (CAT), a haeme enzyme which removes hydrogen peroxide.

Therefore, reduction in the activity of these enzymes (SOD, CAT) results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. Administration of ethanolic extract increased the activity of SOD and catalase in fructose fed rats. Since the extract showed *in vivo* antioxidative activity in fructose fed rats, improvement of the liver functions and the subsequent increase in the uptake and utilization of blood glucose might be the mechanism of action of this extract as glucose lowering agent and hypolipidemic agent.

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