Metabolic effects of *Sapindus trifoliatus* in animal models

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Summary

We have evaluated the potential metabolic effects of the aqueous extract of *Sapindus trifoliatus* [(ST), (family: Sapindaceae)], a traditional phytomedicine used in the treatment of migraine and other CNS disorders. ST (at 100 mg/kg, i.p. dose) was evaluated for its effect on glucose, triglycerides and total cholesterol in mice and rats. The effect on glucose disposal also carried out in male Wistar rats. ST, exhibited a moderate elevation of 18.26% in plasma glucose levels of mice. However, no difference was found in the triglyceride (TG) and total cholesterol (TC) levels in ST and vehicle treated animals. In rats a significant increase ($P<0.05$) in the glucose levels were observed (95.62±4.56 vs 157.80±13.55, mg/dl in vehicle and ST treated group respectively), and no difference was observed in TG and TC levels. Further the hyperglycemic responses during oral glucose tolerance test (OGTT) were significantly greater in ST treated group than the vehicle treated animals. The standard hypoglycemic agent glibenclamide at the dose of 10 mg/kg, ip significantly reduced the glucose levels during GTT. The results of the metabolic studies of ST indicate that, ST has a diabetogenic potential in normal animals.

Keywords: *Sapindus trifoliatus*; diabetes; serotonin; antipsychotics ; dopamine

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Introduction

Atypical antipsychotic medications have become more common in primary care practice for the treatment of schizophrenia. These drugs have superior clinical efficacy and a better safety profile regarding extrapyramidal symptoms and prolactin levels compared with conventional antipsychtics. Despite this overall superior safety profile, however, a metabolic syndrome has recently been attributed to atypical antipsychotic use. Interestingly both the older drugs (chlorpromazine and fluphenazine) and the newer atypical medications (clozapine, olanzapine and quitiapine) have been reported to elevate blood glucose levels in patients (Arneson, 1964; Kamran et al., 1994; Kostakoglu, 1996). Recently, Henderson et al (2004) reported that more than one third of schizophrenics treated with clozapine for five years developed diabetes. Recent data on clinical studies revealed that clozapine a second generation antipsychotic carries a higher risk of diabetes than other drugs in the class. The use of clozapine is associated with dyslipdemia which is a known risk factor in the development of diabetes. Various researchers have attempted to identify the mechanisms underlying these clozpaine induced adverse metabolic effects; however no general conclusion could be reached. Apart from weight gain, antagonism of 5-HT receptors, histamine H_1 receptors, muscarinic acetylcholine receptors, acute pancreatitis, and elevated leptin levels, glucose uptake inhibition were also reported to be a causative factor for antipsychotic-induced hyperglycemia (for review see Lean and Pajonk, 2005).

It has been reported that chlorpromazine and clozapine induce hyperglycemia in mice (Norman et al., 1995; Ryall, 1956; Dwyer and Donohoe, 2003) after acute administration. A recent study described the subchronic administration of clozapine on some metabolic parameters (Cheng et al., 2005).

Sapindus trifoliatus (ST), a phytomedicine used for the treatment of hemicrania, exhibited dopamine-2 and serotonin-2 antagonism by inhibiting the behavioral effects induced by dopamine and serotonin agonists in animal models also in receptor radioligand binding studies ST exhibited affinity towards 5-HT_{2A} receptors and dopamine D_2 receptors in the ligand binding studies (Arulmozhi et al., 2005a)

Since ST has been reported to have affinities towards dopamine D2 and serotonin-2 receptors in receptor radio-ligand binding and functional in vivo studies the present study has been designed to investigate the acute effect of ST on various metabolic parameters viz. plasma glucose, triglycerides and total cholesterol with the belief that the present data may shed some light on the mechanisms underlying these adverse effects of antipsychotics.

Material and Methods

Plant material and extraction procedure: The dried pericarps of fruits of *Sapindus trifoliatus* Linn, family Sapindaceae were collected from the local market and was authenticated by Dr. A. M. Mujumdar, Agharkar Research Institute, Pune, India. Aqueous extract of ST was prepared as reported in our previous papers (Arulmozhi et al., 2004 & 2005b).

Animals: Adult male Swiss albino mice (22-26g) (5 animals per group per treatment) and male Wistar rats (5 animals per group per treatment) were obtained from National Toxicology Center, Pune, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 $^{\circ}$C and relative humidity of 30-70%. A 12:12 light:dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet.

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Poona College of Pharmacy, Pune, India.

***Per se* effect of ST on plasma metabolic parameters in mice and rats**

Male Swiss albino mice and Wistar rats were used at 7-8 weeks of age. Overnight fasted animals were administered ST (100 mg/kg, i.p) or saline vehicle (10 ml/kg, i.p). Thirty minutes after the administration of ST or vehicle, blood was withdrawn into heparinized (10 IU/ml) microcentrifuge tubes by retro-orbital bleeding under mild ether anesthesia. Plasma was separated by centrifuging the blood at 4000 rpm for 10 minutes.

Determination of plasma metabolic parameters

Plasma obtained from the mice was used to estimate the metabolic parameters. Glucose and triglyceride levels were measured spectrophotometrically using commercially available kits (Bayer Diagnostics, India).

Oral glucose tolerance test

Oral glucose tolerance test was performed in overnight fasted male Wistar rats. One hour after the intraperitoneal administration of either ST (100 mg/kg) or glibenclamide (10 mg/kg) a glucose challenge of 2 g/kg body weight given orally by gastric intubation. Blood glucose was measured at 30, 60 and 120-min intervals. Control animals received 10 ml/kg, body weight of saline intraperitoneally. Blood glucose was measured using Lifescan® one touch glucometer by nipping the tail with a sterile scalpel.

Statistical analysis

Results are expressed as mean ± S.E.M. Comparisons between groups were made by Student *t*-test or analysis of variance (ANOVA) and Dunnetts' post test as per suitability. A *P* value of < 0.05 was considered as significant.

Results

Effect of ST on plasma glucose, triglyceride levels in normal Swiss albino mice and Wistar rats

ST, administered intraperitoneally at the dose of 100 mg exhibited a moderate elevation of 18.26% in plasma glucose levels (127.49 mg/dl in vehicle group vs 155.98 mg/dl in ST treated group) in overnight fasted male Swiss albino mice. However, no difference was found in the triglyceride (TG) and total cholesterol (TC) levels in ST and vehicle treated animals (Fig.1). When male Wistar rats were treated with ST (100 mg/kg, i.p) a significant increase ($P<0.05$) in the glucose levels were observed (95.62±4.56 vs 157.80±13.55, mg/dl in vehicle and ST treated group respectively), and no difference was observed in TG and TC levels (Fig.2)

Glucose tolerance test in male Wistar rats

Figure 3 illustrates changes in the blood glucose levels during OGTT in Wistar rats. Hyperglycemic responses during OGTT were significantly greater in ST treated group than the vehicle treated animals. The standard hypoglycemic agent glibenclamide at the dose of 10 mg/kg, ip significantly reduced the glucose levels during GTT.

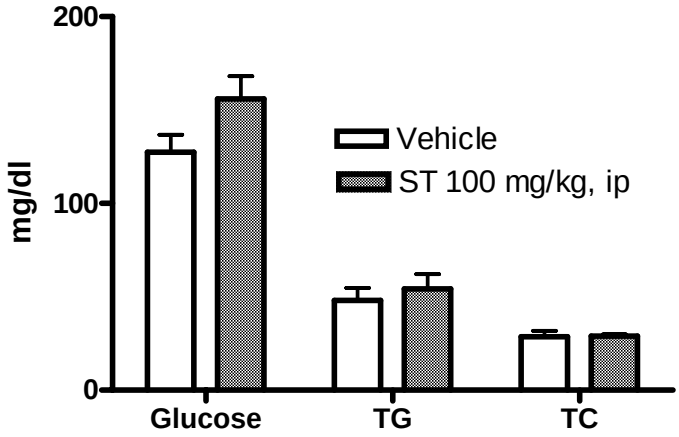

Fig. 1. The effect of Sapindus *trifoliatus* (100 mg/kg, i.p.), on plasma glucose, triglyceride and cholesterol levels in male Swiss albino mice after one hour of treatment. Each column represents mean ± S. E. M. from n=5-6.

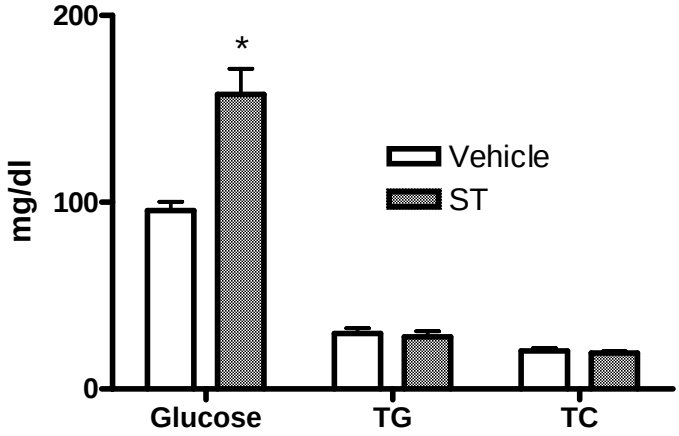

Fig. 2. The effect of Sapindus *trifoliatus* (100 mg/kg, i.p.), on plasma glucose, triglyceride and cholesterol levels in male Wistar rats after one hour of treatment. Each column represents mean ± S. E. M. from n=5-6. * $P < 0.05$, compared to vehicle treated animals

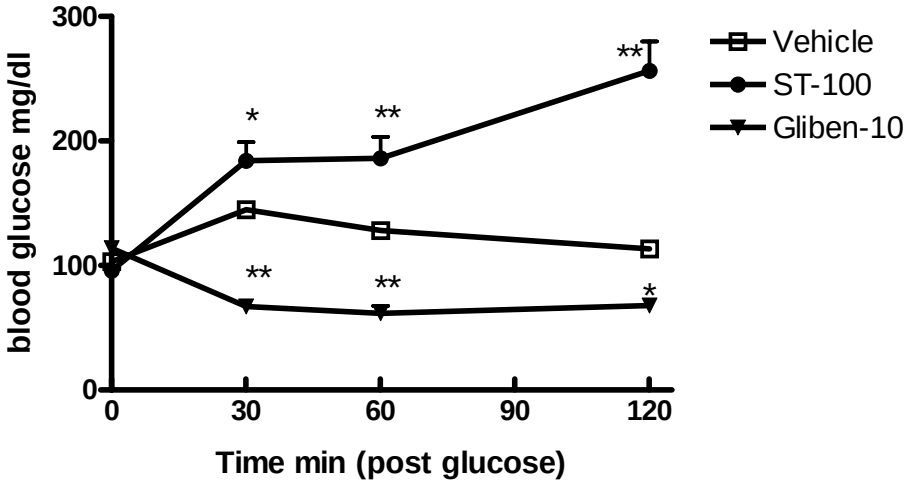

Fig. 3. The effect of Sapindus *trifoliatus* (100 mg/kg, i.p.), on blood glucose, levels in male Wistar rats during oral glucose tolerance test. Each point represents mean ± S. E. M. from n=5-6. * * $P < 0.05$, ** $P < 0.01$ compared to vehicle treated animals.

Discussion

ST, a phytomedicine used for the treatment of hemicrania, exhibited dopamine D_2 and 5-HT_2 antagonism by inhibiting the behavioral effects induced by dopamine and serotonin agonists in various animal models and suggested to have antipsychotic potential. ST exhibited affinity towards 5-HT_{2A} receptors and dopamine D_2 receptors in the ligand binding studies (Arulmozhi et al., 2005a).

The mechanisms responsible for the increased diabetes risk of the second-generation antipsychotics are not clearly known. The diverse receptor binding nature of the antipsychotics (Table. 1) may be indicative for their potential to elevate glucose and lipid levels. Antagonism at 5-HT_{2C} receptors has recently been debated recently for the weight gain induced by clozapine and olanzapine because; 5-HT_{2C} receptors are probably involved in the regulation of food intake.

However, some selective 5-HT_{2C} receptors did not exhibit hypoglycemia rather they caused hyperglycemia in rats (Chaouloff et al., 1990).

The antagonism at histamine H_1 (Tecott et al., 1995) and muscarinic acetylcholine receptors is also suggested for the metabolic abnormalities with antipsychotics. It has been reported that, antimuscarinic activities of clozapine might interfere with glucose utilization and in liver release of acetylcholine from parasympathetic nerve endings has been shown to increase hepatic glucose uptake in preclinical models (Wirshing, 2001). In contrast, activation of adrenergic receptors via the sympathetic nerves increases glucose output from the liver (Xie and Lautt, 1995).

Table 1. Potency of clozapine and ziprasidone at different receptors*

	Clozapine (200-450 mg/day)		Ziprasidone (20-160 mg/day)	
	*K*i (mol/l)	Relative potency vs D_2 receptors	*K*i (mol/l)	Relative potency vs D_2 receptors
D_2	177.82	1	6.76	1
H_1	1.07	166	64.56	0.11
5-HT_{1A}	190.55	0.93	5.50	1.23
5-HT_{2A}	6.31	28.2	2.09	3.24
5-HT_{2C}	12.59	14.1	6.46	1.05
ACh_m	33.11	5.37	2,454.71	0.0028
α_1-Adrenoceptors	22.39	7.94	13.18	0.51
α_{2A}-Adrenoceptors	53.70	3.31	186.21	0.036

* From Lean and Pajonk (5)

The presence of high affinity antimuscarinic agent could block the parasympathetic transmission, which has been suggested to contribute to increased hepatic glucose production (Nonogaki, 2000). Recently, clozapine and olanzapine have been studied for their inhibitory effects on carbachol - enhanced insulin secretion in perfused rat islets (Gottstater et al., 1999).

It has been demonstrated that glucose uptake into skeletal muscle, which is responsible for a large portion of glucose clearance, can be enhanced through a recently described pathway involving agonist activation of 5-HT_{2A} receptors (Johnson et al., 2005). Since most antipsychotics are high affinity antagonists to 5-HT_{2A} receptor, it could one of the mechanism along with other mechanisms like inhibition of glucose uptake (Dwyer and Donohoe, 2003) which has been suggested as the mechanism of acute hyperglycemia observed in mice treated with antipsychotics.

The role of α_1-adrenceptors in antipsychotic-induced glucose dysregulation may also be suggested as prazosin, a potent α_1-adrenceptor antagonist is associated with hyperglycemia in animals (Hajduch et al., 1999).

Receptor radioligand studies with ST (Table. 2) also revealed that, ST exhibited affinity towards various receptors purportedly involved in inducing hyperglycemia indicating that, the elevated levels of glucose observed with the administration of ST in the present models could be due to any one or combination of receptors as observed with other typical and atypical antipsychotics.

As per our knowledge this is first report on the metabolic effects of an antipsychotic plant extract. The present study paves the way for the further investigations into the diabetic potential of ST and also other reported antipsychotic plants.

Table 2 Selected receptor radioligand binding data of ST*#

Receptor	Receptor Source	Ligand	Reference compound	% inhibition at 250 μg/ml
Adrenergic, alpha-1, Non-selective	Rat Forebrain	(^{3}H)7-MeOxy-Prazosin	Phentolamine	58.96
Adrenergic, alpha-2, Non-selective	Rat Cortex	(^{3}H)RX 821002	Phentolamine	49.38
Dopamine, D1	Rat Striata	(^{3}H)SCH 23390	R(+)-SCH 23390 HCl	20.78
Dopamine, D2	Rat Striata	(^{3}H)Sulpiride	(+/-)Sulpiride	104.54
Dopamine, D3 recombinant	Rat cDNA/SF9	(^{3}H)7-OH-DPAT	(+/-)-7-OH-DPAT HBr	55.18
Histamine, H1	Bovine Cerebellum	(^{3}H)Pyrilamine	Triprolidine HCl	0.25
Histamine, H2	Guinea pig Spleen	(^{125}I)-Aminopotentidine	Tiotidine	27.52
Histamine, H3	Rat Forebrain	(^{3}H)N-a-MeHistamine	N-a-Methylhistamine	7.14
Serotonin, 5HT1A	Bovine Striata	(^{3}H)-8-OH-PAT	(+/-)-8-OH-DPAT HBr	13.41
Serotonin, 5HT2A	Rat Cortex	(^{3}H)Ketanserin	Methysergide maleate	47.53
Serotonin ,5HT2C	Porcine Choroid Plexus	(^{3}H)Mesulergine	Mianserin HCl	17.40
Muscarinic, M3	Guinea pig ileum	^{3}H)Scopolamine, N-Methyl	4-DAMP methiodide	66.49

*The receptor radio-ligand binding studies were carried out by NovaScreen Biosciences Corporation, USA . #Data from Arulmozhi et al (10)

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References

Arneson, G.A., 1964. Phenothiazine derivatives and glucose metabolism. Journal of Neuropsychiatry 5, 181-185.

Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., Arora, S.K., 2005a. Investigations of Sapindus trifoliatus in dopaminergic and serotonergic systems: Putative antimigraine mechanisms. Indian Journal of Pharmacology 37, 120-125.

Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., Arora, S.K., 2005b. Pharmacological studies of the aqueous extract of *Sapindus trifoliatus* on central nervous system: possible antimigraine mechanisms. Journal of Ethnopharmacology 97, 491-496.

Arulmozhi, D.K., Sridhar, N., Bodhankar, S.L., Veeranjaneyulu, A., Arora, S.K., 2004. In vitro pharmacological investigations of *Sapindus trifoliatus* in various migraine targets. Journal of Ethnopharmacology 95, 239-245.

Chaouloff, F., Laude, D., Baudrie, V., 1990. Effects of the 5-HT1C/5-HT2 receptor agonist DOI and α-methyl-5-HT on plasma glucose and insulin levels in the rat. European Journal of Pharmacology 187, 435-443.

Cheng, C.Y., Hong, C.J., Tsai, S.J., 2005. Effects of subchronic clozapine administration on serum glucose, cholesterol and triglyceride levels, and body weight in male BALB/c mice. Life Sciences 76, 2269-2273.

Dwyer, D.S., Donohoe, D., 2003. Induction of hyperglycemia in mice with atypcical antipsychotic drugs that inhibit glucose uptake. Pharmacology, Biochemistry and Behavior 75, 255-260.

Gottstater, A., Ahmed, M., Fernlund, P., Sundkvist, G., 1999. Autonomic neuropathy in type 2 diabetic patients is associated with hyperinsulinemia and hypertriglyceridemia. Diabetes Medicine 16, 49-54.

Hajduch, E., Rencurel, F., Balendran, A., Batty, I.H., Downes, C.P., Hundal, H.S., 1999. Serotonin (5-Hydroxytryptamine), a novel regulator of glucose transport in rat skeletal muscle. Journal of Biological Chemistry 274, 13563-13568.

Henderson, D.C., Cagliero, E., Gray, C., 2000. Clozapine, diabetes mellitus, weight gain and lipid abnormalites: a five year naturalistic study. American Journal of Psychiatry 157, 975-981.

Johnson, D.E., Yamazaki, H., Ward, K.M., 2005. Inhibitory effects of antipsychotics on carbachol-enhanced insulin secretion from prsused rat islets: role of muscarinic antagonism in antipsychotic induced diabetes and hyperglycemia. Diabetes 54, 1552-1558.

Kamran, A., Doraiswamy, P.M., Jane, J.L., 1994. Severe hyperglycemia associated with high doses of clozapine. American Journal of Psychiatry 151, 1395.

Kostakoglu, A.E., Yazici, K.M., Erbas, T., 1996. Ketoacidosis as a side effect of clozapine: a case study. Acta Psychiatria Scandinavia 93, 217-218.

Lean, M.E.J., Pajonk, F.G., 2003. Patients with antipsychotic drugs: another high risk group for type 2 diabetes. Diabetes Care 26, 1597-1605.

Nonogaki, K., 2000. New insights into sympathetic regulation of glucose and fat metabolism. Diabetologia 43, 533-549

Norman, D., Hiestand, W.A., 1995. Glycemic effects of chlorpromazine in the mouse, hamster and rat. Proceeding of Society of Experimental Biology and Medicine 90, 89-91.

Ryall, R.W., 1956. Some actions of chlorpromazine. British Journal of Pharmacology 1, 339-345.

Tecott, L.H., Sun, L.M., Akana, S.F., 1995. Eating disorders and epilepsy in mice lacking 5-HT2C serotonin receptors. Nature 374, 542-546.

Wirshing, D.A., 2001. Adverse effects of atypical antipsychotics. Journal of Clinical Psychiatry 62, 7-10.

Xie, H., Lautt, W.W., 1995. Induction of insulin resistance by cholinergic blockade with atropine in the cat. J Autonomic Pharmacology 15, 361-369