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phosphate metabolism[31]. X-ray crystallographic studies revealed that both globular adiponectin and osmotin consist of antiparallel β-strands arranged in the shape of a β-barrel. The domain I (lectin-like domain) of osmotin can be overlapped with adiponectin, suggesting that the two proteins share the lectin-like domain[28]. Osmotin can induce AMP kinase phosphorylation in mammalian C2C12 myocytes via adiponectin receptors[28]. This strongly suggests that further research examining similarities between adiponectin and osmotin function may facilitate efficient screening of potential adiponectin receptor agonists.

Thus, enhancing or mimicking adiponectin action through modulation of expression and/or function of the adiponectin receptors may be a novel and promising therapeutic strategy for insulin resistance, type 2 diabetes, obesity and the metabolic syndrome. The present study was undertaken to examine the above hypothesis that osmotin may be effective as adiponectin type II diabetes produced using high fat diet and low dose streptozotocin.

MATERIALS AND METHODS

Isolation and purification of osmotin

Osmotin was isolated from salt-adapted tobacco cell suspensions (Nicotiana tabacum L) as described by Singh et al.[29]. Briefly, the two isoforms of osmotin (osmotin-I and osmotin-II) were isolated after homogenization of salt adapted tobacco cells in phosphate buffer followed by ammonium sulfate fractionation, CM Sephadex cation-exchange chromatography and cationic preparative HPLC separation. Purified fractions of both osmotin-I and osmotin-II were labeled and homogeneity of osmotin preparations was confirmed using SDS-PAGE (12%). For pharmacological studies, both fractions of osmotin were dissolved in normal saline. Optimum dose of osmotin was determined to be 5 mg/kg from the pilot studies carried out in our laboratory

Chemicals

Streptozotocin (STZ) was purchased from Sigma, USA. The feed ingredients such as casein, dl-Methionine, yeast extract, cholesterol, lard and vitamin and mineral were procured from commercial sources.

Animals

Sprague Dawley (SD) rats (150–170 g) were procured from the central animal facility of the Institute. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$) with 12:12 h light and dark cycle. All the rats were provided with commercially available rat normal pellet diet (NPD) (Pranav Agro, Baroda) and water ad libitum, prior to the dietary manipulation. The study was approved by the institutional animal ethics committee and procedures were followed according to the guidelines of committee

for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India.

Study protocol and Treatments

The rats were allocated into two dietary regimens consisting of 6 and 54 rats by feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks[32]. The composition[33] and preparation of HFD were as described by Srinivasan et al., 2004[34]. After the 2 weeks of dietary manipulation, a subset of HFD fed rats were divided into five groups of 6 animals each. Group II received only HFD till the completion of the study. On day 15, groups III, IV, V and VI animals were injected intraperitoneally (i.p.) with single dose of STZ (35 mg kg^{-1}) dissolved in 0.05M citrate buffer (pH-4.5) while group IV, V and VI received osmotin-I (5 mgkg^{-1}, i.v.), osmotin-II (5 mgkg^{-1}, i.v.) and pioglitazone (10 mgkg^{-1}, p.o.) respectively from day 15 to day 28. NPD fed rats were given only citrate buffer as vehicle. The body weight and biochemical estimations (total serum cholesterol, serum triglyceride and oral glucose tolerance test[34] (serum glucose and serum insulin) were carried out just before and at day 14 (before animal allotment). The feed and water intake of the animals were also measured. The rats were allowed to continue to feed on their respective diets until the end of the study.

Collection of blood and analytical methods

Blood was collected from retro-orbital plexus of the rats under light ether. The serum was separated and was analyzed for glucose (GOD-POD), triglycerides (GPO-POD) and total cholesterol (CHOD-POD) levels using commercially available colorimetric diagnostic kits (Span Diagnostics, Surat, India).

Statistical Analysis

The results are expressed as mean±S.E.M. The unpaired Student's t-test was used for analyzing the data between two groups where as one-way ANOVA followed by multiple comparison tests (Tukey's test) was employed if there were more than two groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Features of HFD-fed insulin-resistant rats

Table1 illustrates that that the feeding of HFD for 2 weeks resulted in significant increase ($p < 0.05$) in body weight as well as triglycerides and total cholesterol levels in rats as compared to NPD-fed rats.

Effect of STZ and test drugs on HFD-fed insulin-resistant rats

STZ administration results into significant changes in various parameters (Table 2) as measured on day 28. The body weight was significantly reduced when STZ was administered to HFD fed rats, which was still considerably higher than NPD fed rats. Treatment with osmotin-I,

osmotin-II and pioglitazone also showed similar decrease in body weight. In addition, there was a significant (p<0.05) increase in basal STG and STC in HFD+STZ rats when compared to HFD fed and NPD fed rats alone.

Serum glucose and insulin responses to oral glucose loading

Feeding of high fat diet for 2 weeks produces a condition of insulin resistance similar to human. Injection of STZ (35 mg kg^{-1}, i.p.) after 2 weeks of dietary manipulation significantly (p<0.05) increased serum glucose in HFD rats, thus producing the frank hyperglycemia. Oral glucose tolerance test was carried out on day 0, day 14 and day

28 to determine the effect of STZ and various treatments on HFD fed rats. After 14 days, there was significantly higher serum glucose (fig. 1A) and serum insulin (fig. 1B) in HFD group as compared to NPD fed rats (p<0.05).

Treatment with osmotin-I, osmotin-II and pioglitazone was started from day 15 and continued till day 28. There was significant (p<0.05) decrease in serum glucose (fig. 2A) and serum insulin (fig. 2B) in both osmotin treatment groups as compared to HFD-STZ rats, which was still higher than pioglitazone treated rats. It was observed that both osmotin-I and osmotin-II showed similar effects on all biochemical parameters as well as body weight.

**Table 1:** Effect of HFD on body weight and biochemical parameters in rats after two weeks

Parameters	NPD fed	HFD Fed
Body weight (g)	175 ± 1.77	214.1 ± 2.17*
Serum triglyceride (STG) (mg dl^{-1})	97.98 ± 2.92	211.95 ± 2.93*
Serum total cholesterol (STC) (mg dl^{-1})	99.62 ± 6.33	190.93 ± 2.71*

Values are mean ± SEM. *p<0.05 vs. NPD group (n=6)

Table 2: Effect of STZ and Osmotin on body weight and biochemical parameters in rats

Parameters	NPD fed	HFD Fed	HFD + STZ	Osmotin-I	Osmotin-II	Pioglitazone
Body weight (g)	169.17 ± 2.94	293.67 ± 4.37*	207.00 ± 3.46#	194.17 ± 3.16	202.83 ± 2.98	191.17 ± 3.40
STG (mg dl^{-1})	116.49 ± 4.01	270.94 ± 5.30*	309.97 ± 6.14#	252.62 ± 5.20^	260.46 ± 6.19^	213.64 ± 4.98^
STC (mg dl^{-1})	97.66 ± 3.47	222.93 ± 3.45*	240.69 ± 6.52#	174.56 ± 4.54^	183.61 ± 4.99^	154.30 ± 3.66^

Values are mean ± SEM. *#p<0.05 vs. NPD group (n=6); ^p<0.05 vs. HFD+STZ group (n=6)
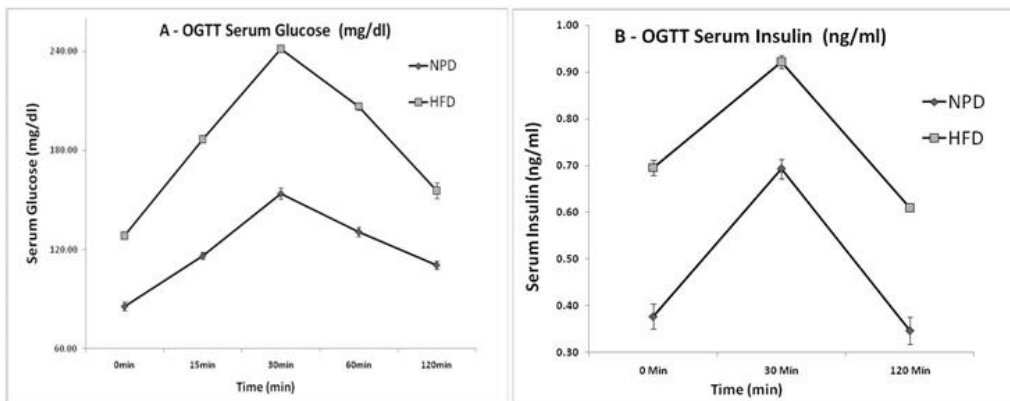
Figure 1: Effect of HFD on serum glucose and serum insulin after oral glucose load. Values are mean ± SEM.
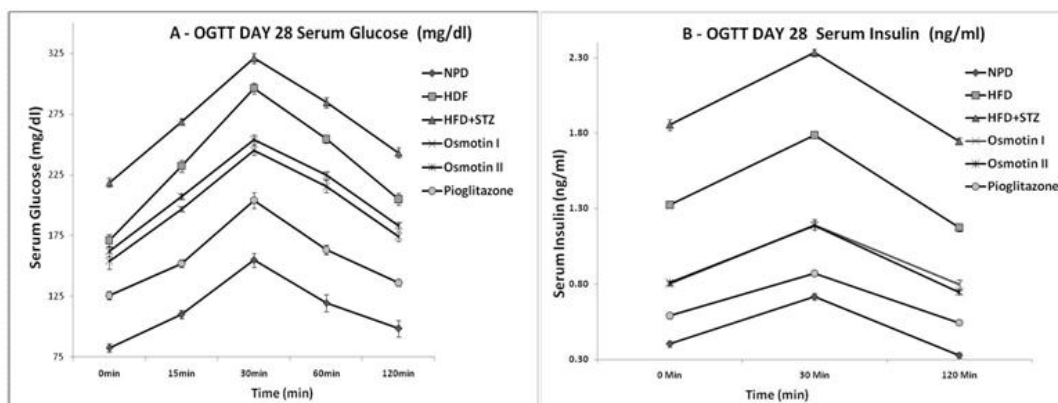
Figure 2: Effect of test drugs on serum glucose and serum insulin after oral glucose load. Values are mean ± SEM.

DISCUSSION

Obese subjects had significantly lower plasma adiponectin concentrations than non-obese subjects, although adiponectin derives exclusively from adipose tissue[35]. Adiponectin increases insulin sensitivity in the liver and skeletal muscle and reduces atherosclerosis. In addition to these effects, adiponectin also seems to have pleiotropic effects, particularly in relation to metabolic syndrome[5] [15]. High fat diet induced insulin resistance associated with obesity is a major risk factor for diabetes and cardiovascular diseases, the prevalence of which is increasing sharply[36]. There are no currently available therapeutic strategies that have been shown to mimic the actions of adiponectin in activating its receptors. Narasimhan et al., (2005) has identified a novel protein, Osmotin, a member of the PR-5 (pathogenesis related-5) family of plant defence proteins, as a potential adiponectin receptor agonist[28].

Osmotin is a member of a large PR-5 protein family, which is both ubiquitous (fruits and vegetables, etc.) and diverse. PR-5 proteins are also extremely stable and may remain active even when in contact with the human digestive or respiratory systems[5]. It is reported that osmotin activates AMPK via adiponectin receptor in mammalian C2C12 myocytes and as well as three dimensional structure of osmotin and adiponectin are similar and overlap each other[28]. Thus it can be suggested that similarities between osmotin and adiponectin may facilitate development of potential adiponectin receptor agonists and may be used as novel therapeutic strategy for insulin resistance and Type II diabetes. In the present study, we have isolated osmotin from salt adapted cultured tobacco cell and have made an effort to evaluate its efficacy in Type II diabetes and insulin resistance induced by HFD and low dose streptozotocin.

The HFD was prepared (58% calories as fat), such that it causes insulin resistance in rats over a short period of time. Thus, the feeding of HFD for a period of 2 weeks produced rats with insulin resistance syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia as shown in table 1 and fig. 1. It was observed that administration of STZ in HFD fed rats has increased the basal levels of serum glucose as consequence of insulin resistance. HFD has been shown to increase level of triglycerides due to excess fat intake and thus constitute a source of increased fatty acid availability and oxidation[37]. This preferential use of increased fatty acids for oxidation blunts the insulin-mediated reduction of hepatic glucose output and reduces the glucose uptake or utilization in skeletal muscle leading to compensatory hyperinsulinemia, a common feature of insulin resistance[38] [39]. Adiponectin increases expression of molecules involved in fatty-acid transport such as CD36, in combustion of fatty-acid such as acylcoenzyme A oxidase, and in energy dissipation such

as uncoupling protein 2. These changes leads to decreased tissue TG content in skeletal muscle[40]. Osmotin, acting as adiponectin agonist, may also cause increased fatty acid combustion and decreased TG content in muscle, and thus improving insulin signal transduction. In our results, we found that treatment with both osmotin fractions has resulted in decreased in serum triglyceride and total cholesterol level. Also it was observed that due to decreased TG content, there was decrease in serum insulin levels which in turn caused decrease in serum glucose level as shown in fig.2A and 2B. Thus our results are consistent with above mentioned hypothesis. However, it was also observed that both osmotin-I and osmotin-II shows similar efficacy.

Adiponectin increases the expression level of PPARα in vivo[40]. Thus osmotin, like adiponectin, presumably via PPARα activation at least in part, leads to decreased TG content in the liver and skeletal muscle and increases insulin sensitivity. Other studies have demonstrated that adiponectin modulates insulin sensitivity by stimulating glucose utilization and fatty acid oxidation via phosphorylation and activation of AMPK in muscle and liver[41] [42]. Osmotin is also reported to activate AMPK in mammalian C2C12 myocytes. It can be thus said that osmotin may stimulate β-oxidation and glucose uptake via AMPK and increase fatty acid combustion and insulin sensitivity.

CONCLUSION

In conclusion, osmotin-I and osmotin-II both shows promising therapeutic results in treatment of Type-II diabetes mediated by adiponectin pathway. Although both osmotin fractions are effective, they show similar therapeutic effects. Thus further experimental and clinical investigations are required to ascertain these results.

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