Curative effects of the ethanolic extract of leaves of *Solanum* torvum on obese, diabetic - induced albino rats.

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ABSTRACT

The present study dealt with the investigation of anti-obesity and anti-diabetic effects of ethanolic extract of leaves of S*olanum* torvum (STE) using obese, diabetic -induced albino rats. The animals received either normal diet, high-fat diet or high-fat diet with additional STEfor 12 weeks. After the end of administration, body weight, plasma glucose, insulin, and liver triglyceride**,** Cholesterol Content and antioxidant activity were measured. The result revealed that , compared to the high-fat diet group, increases in body weight, plasma glucose and insulin were significantly suppressed for STE groups.STE also proved to possess good antioxidant activity.These results suggest that STE is expected to be an useful plant extract for alleviating the adversse effect of obesity associated with diabetes mellitus.

1. Introduction

Solanum torvum a member of the family Solanaceae and its fruits are commonly called as" night berries"which are used as a common vegetable by south Indian people. This plant is used for its haemostatic properties [1].Recent investigations demonstrated that *Solanum torvum* has antimicrobial activity. There have been several reports regarding the chemical constituent of this plant which include [2] ocumental steroidal compounds [3].

Leaves have been reported to contain the steroidal gluco-alkaloid, solasonine. In addition, they contain steroidal sapogenins, neochlorogenin, neosolaspigein and solaspigenine. They have also been found to contain triacontanol, tetratriacontanic acid, z-tritriacontanone, sitosterol, stigmasterol and campesterol. Fruits also contain the gluco-alkaloid, solasonine, sterolin (sitesterol-Dglucoside), protein, fat and minerals (4).

In the present study, to investigate the anti-obesity and anti-diabetic actions of STE, a high-fat diet was administered to albino rats, an obese type-II diabetes model, to induce severe obesity and diabetes. The effects of STE on obesity and diabetes were then studied.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

The leaves of *Solanum torvum* were collected and authenticated by Dr. Sankaranarayanan, Assistant Director, Dept of Research and Development, Sairam siddha Medical College and research centre, Chennai. India. The voucher specimen is also available in herbarium file of the same centre.

2.2. Materials

All routine chemicals were obtained from SD Fine Chemicals Mumbai. Standard glibenclamide & nicotinamide were purchased from Ranbaxy (India) Ltd, New Delhi. All the chemicals used were of analytical grade. Streptozotocin was obtained from, Sigma Aldrich, Germany.

2.3. Preparation of extract

The leaves of *Solanum torvum* was washed thoroughly in tap water, shade dried and powdered. The powder was passed through 40-mesh sieve and exhaustively extracted with 90% (v/v) ethanol in Soxhlet apparatus at 60oC. The extract was evaporated under pressure till all the solvent had been removed and further removal of water was carried out by freeze drying to give an extract sample with yield of 24.56% (w/w).The extract was stored in refrigerator and a weighed amount of the extract was dissolved in 2% (v/v) aqueous Tween- 80 and used for the present investigation.

2.4. Animals

Adult albino male rats of Wister strain weighing 150 - 175 g were used in the pharmacological and toxicological studies.The inbred animals were taken from animal house in Central Leather Research Institute, Adyar,Chennai, India.The animals were maintained in well ventilated room temperature with natural 12 ± 1 h day–night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station Nandam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

2.5. Acute Toxicity studies

Acute toxicity studies were conducted with the plant extract in Wister albino rats by staircase method [5].First group served as normal control. The animals were subjected to overnight fasting prior to the experiment and were administered with single dose of the extract dissolved in 2% aqueous Tween 80 and observed for mortality for 48 hours. Based on the short-term toxicity, the dose administered to the next animal was determined as per OECD guideline 423.All the animals were also observed for further 14 days.LD50 doses were selected for the evaluation of hepatoprotective activity. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment

2.6. Induction of non- insulin dependent diabetes mellitus (NIDDM)

NIDDM was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin (Sigma Aldrich, Germany) followed by nicotinamide (Ranbaxy Chemicals Ltd, Mumbai, India) 120 mg/kg, i.p, 15 min afterwards. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The threshold value of fasting plasma glucose to diagnose diabetes had been taken as >126 mg/dl. Only those rats that have permanent NIDDM were used for the study.

Rats that had been acclimatized were divided into five groups (n=10). Each group were provided with ad libitum access to LF, HF, HF containing 2.5% (w/w) STE (HF/STE 2.5%), HF containing 5.0% (w/w) STE (HF / STE 5.0%) and diabetic rats administered the standard drug glibenclamide (0.25 mg/kg) along with HF for 7 weeks. When fed a normal diet, the rats developed obesity and type-II diabetes by 12-weeks. Food intake was measured once weekly. Following this feeding period, after 17 h of fasting, blood sample was collected using heparin from the abdominal vena cava under diethyl ether-induced anesthesia. The liver, white adipose tissue (around the testes, retro peritoneum and kidney) and skeletal muscle was also be removed, weighed and placed in either neutral-buffered formalin or liquid nitrogen.

2.7. Blood Analysis

Blood samples were centrifuged (1000*g* for 15 min at 4°C), and plasma stored at –80°C until assay. Plasma glucose concentrations were enzymatically quantified using Glucose Test. Plasma insulin concentrations were measured according to the protocol. Plasma glutamate oxalacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)[6] alkaline phosphatase (ALP) (7) concentrations were quantified using Transaminase Test kit. Plasma triglyceride, cholesterol content, VLDL (8], and HDL (9) levels were estimated.

All the enzymatic and biochemical assays were read at specific wavelength using Shimadzu spectrophotometer, UV-1601 model.

Statistical Analysis

Values reported are mean ± S.E. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's't' test. P values <0.05 were considered as significant[10].

Homeostasis Model Assessment of Insulin Resistance Index

Homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was calculated according to the following equation using fasting insulin and glucose concentrations.

$$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U ml}^{-1}) \times \text{fasting glucose (mmol l}^{-1})/22.5.$$

2.8. Measurement of Liver Triglyceride/Cholesterol Content and antioxidant activity

Liver triglyceride and cholesterol content was measured as described. Briefly, a portion (100 mg) of liver tissue was homogenized in phosphate buffer saline (pH 7.4, 1 ml). The homogenate (0.2 ml) was extracted with isopropyl alcohol (1 ml), and the extract was analyzed to determine liver triglyceride content. The homogenate (0.2 ml) was extracted with chloroform-methanol (2: 1, 1 ml), and the extract was concentrated under a nitrogen stream. The residue was dissolved in isopropyl alcohol and analyzed to determine the Cholesterol content.

Liver lipid peroxidation was measured through Malondealdehyde (MDA) levels, liver glutathione content and catalase (CAT) activity ware also determined.

2.9. Preparation of Slide for Histopathology

2.9.1. Hematoxylin– Eosin Staining

Liver and epididymal fat was fixed in 10% neutral-buffered formalin. After trimming the tissues, liver and epididymal fat were embedded in paraffin using a tissue processor. Sections were taken in 3–4 µm thicknesses, and was stained with HE solution for microscopy.

2.9.2. Oil red O Staining

After frozen liver sections about 7 µm thick was prepared using a cryostat apparatus, oil red O staining was conducted for microscopy.

3. RESULTS

3.1. Acute toxicity studies

In the acute toxicity studies death was recorded during the treatment period in treated groups receiving 2000mg/kg po of plant extract orally. The animals showed changes in general behavior and other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, and finally convulsion. From the above toxicity studies the ED50 dose of the STE was calculated and it was fixed as 200 mg/kg body weight.

3.2. Body Weight and Visceral Fat Accumulation.

During the 7-week experimental period, food intake was measured once weekly. Mean energy intake was significantly higher for the HF group than for the LF group. No significant difference existed in energy intake between the HF, HF/STE 2.5% and HF/STE 5.0% groups (Table 2). At the end of administration, body weight was significantly higher for the HF group than for the LF group. STE administration significantly suppressed HF-induced body weight increases. In particular, the degree of body weight increase was comparable between the HF/STE 5.0% and LF groups.

Furthermore, visceral white adipose tissue weight was significantly higher for the HF group than for the LF group.STE significantly suppressed this increase in a dose-dependent manner (Table 2). HE staining of epididymal white adipose tissue showed that mature adipocytes for the HF group were hypertrophied, with numerous immature adipocytes in the stroma.

Conversely, HF-induced hypertrophy of mature adipocytes was not seen for the HF/STE 5.0% group, and the size of adipocytes and number of immature adipocytes were comparable to those for the LF group (Figure 1).

3.3. Liver Lipid Accumulation.

Table 2 shows liver weight and liver lipid content. At the end of administration, liver weight was about 1.8 times greater for the HF group than for the LF group. In addition, liver triglyceride and cholesterol levels were significantly higher for the HF group when compared to the LF group. For both HF/STE 2.5% and HF/STE 5.0% groups, increased liver weight and triglyceride and cholesterol accumulation were significantly suppressed.

The liver was subjected to HE staining and oil red O staining, and the HF group displayed large fat droplets throughout liver lobules. However, HF/STE 2.5% and HF/STE 5.0% groups showed smaller fat droplets in individual hepatocyte and fewer fat droplets throughout liver lobules, thus indicating suppression of fatty liver (Figure 2).

3.4. Plasma Glucose, Insulin, GOT and GPT Concentration.

Table 2 shows fasting plasma glucose, plasma insulin, GOT and GPT. Plasma glucose and plasma insulin levels were significantly higher for the HF group than for the LF group.
Conversely, plasma glucose and insulin levels for the AP groups decreased to levels similar to those for the LF group, and HOMA-IR index in the HF/STE 2.5% and HF/STE 5.0%
groups was significantly decreased compared to the HF group. Plasma GOT and GPT, indicators of hepatopathy, were significantly higher for the HF group than for the LF group. AP administration significantly suppressed increases in GOT and GPT.

4. Results & Discussion

The anti-obesity effects of STE were investigated using obese diabetic induced albino rats. In the present study, lard was administered to albino wister rats for 7 weeks to induce severe obesity and diabetes and the effects of STE Furthermore, visceral white adipose tissue weight was significantly higher for the HF group than for the LF group.STE significantly suppressed this increase in a dose-dependent manner (Table 2). HE staining of epididymal white adipose tissue showed that mature adipocytes for the HF group were hypertrophied, with numerous immature adipocytes in the stroma.
Conversely, HF-induced hypertrophy of mature adipocytes was not seen for the HF/STE 5.0% group, and the size of adipocytes and number of immature adipocytes were comparable to those for the LF group (Figure 1) was evaluated. Lard is widely used in studies on obesity and diabetes. STE was found to significantly suppress increases in body weight and white adipose tissue weight, showing anti-obesity actions (Table 2, Figure 1).
HF also increased liver fat accumulation and induced fatty liver, but STE administration lowered
fat accumulation, clarifying that STE suppresses fatty liver (Table 2, Figure 2). Furthermore, measurements of plasma GOT and GPT clarified that STE suppressed fatty liver induced hepatopathy (Table 2).
Plasma glucose and insulin levels were significantly higher for the HF group than for the LF group, and severe type II diabetes was induced. STE suppressed these increases in plasma glucose and insulin levels. The HOMA-IR index, a simpler method to measure insulin sensitivity usually used in clinical and animal studies [13, 14], was significantly decreased in STE-treated groups compared to the HF group, indicating that STE reduced hyperglycemia and hyperinsulinemia (Table 2). These findings clarify that STE suppresses obesity and diabetes caused by a high-fat diet. Obesity is caused by low energy expenditure and increased fatty acid synthesis from carbohydrates and fat intake by organs. STE used in the present study acted on skeletalmuscle, liver and white adipose tissue and was shown to possess anti-obesity and anti-diabetic actions (Figure 7).
The mechanism of most of the herbals used to treat diabetes has not been defined[15]. Many traditional treatments have been recommended in the complementary and alternative system of medicine for treatment of diabetes mellitus[16]. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important[17]. Plant drugs[18] and herbal formulation[19] are frequently considered to be less toxic and more free from side effects than synthetic one. The liver and kidney exhibits numerous morphological and functional alterations during diabetes[20]. It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent with minimal adverse effects, which can be taken for long durations (21,22).

5. Conclusion

On the basis of the results obtained in the present investigation it can be concluded that the ethanolic extract of *S.torvum* (STE) proved to possess both antidiabetic as well as anti obesity activity when fed to high-fat diet-induced obesity and type –II diabetic male albino rats. In the future, STE seems to offer a potential plant extract that may serve as a useful adjuvant in several clinical conditions associated for alleviating obesity with diabetes mellitus.

Table 1: Food, Energy intake and Body weight gain, on the experimental diets for 12 weeks.

Data represent means ± SDs of 6 mice per group. Tukey's test: [a]P .05 versus LF; [b]P .01 versus LF; [c]P .05 versus HF; [d]P .01 versus HF.

Groups	Food intake (g/mouse/day)	Energy intake (kcal/mouse/day)	Pre-diet body Pre-diet body weight (g)	Post-diet body Post-diet body weight (g)	Body weight Body Weight gain (g)
I (LF)	25.2 ± 3.8	104.5±1 4.6	165.4±12.5	171.5±15.4	13.1±2.3
II (HF)	20.4 ± 2.7	106.6± 13.6a	170.3±11.5b	198.6±14.2bc	23.3±3.2b
III(HF/2.5%STE)	20.5 ± 2.3	104.7± 13.8a	171.5±16.3b	185.7±16.1c	10.2±2.5b
IV(HF/5%STE)	20.4 ± 2.5	106.2± 13.5a	166.3±15.2b	170.5±13.4c	6.2±1.8b
V (HF/0.25mg Std	20.4± 2.5	109.6±13.2 a	161.5±14.8b	185.6±14.2bc	14.1±2.1

Table 2: White adipose tissue and liverweight of mice fed on the experimental diets for 12 weeks.

Groups	Whole WAT (g)	Epididymal WAT (g)	Retroperitoneal WAT (g)	Perirenal WAT (g)	Liver weight (g)
I (LF)	9.13±1.05	2.64±0.33	2.55±0.22	0.96±0.12	1.96±0.33
II (HF)	13.05±1.91	2.98±0.31a	3.95±0.36b	1.54±0.18c	3.88±0.56d
III(HF/2.5%STE)	10.56±1.08	2.79±0.33a	3.05±0.41b	1.12±0.11c	1.95±0.72d
IV(HF/5%STE)	9.57±1.37	2.70±0.29a	2.74±0.45b	1.05±0.13c	1.85±0.45d
V (HF/0.25mg Std	11.05±1.09	2.75±0.35a	2.86±0.35	1.43±0.12	2.45±0.23

Table 3: Plasma and hepatic biochemistry of mice fed on the experimental diets for 12 weeks.

Groups	Glucose (mmol)	Insulin (U m)	HOMA-IR	Liver TGL (mg)	Liver cholesterol (mg)	GOT (U)	GPT (U)
I (LF)	9.2±3.2	32.8±4.4	14.2±3.4	90.2±12.5	3.5±0.7	45.7±12.9	18.4±4.2
II (HF)	30.2±4.5	192.7±20.9	189.4±30.4	186.4±13.7	4.6±0.5c	187.4±22.5	65.3±12.3
III (HF/2.5% STE)	15.4±4.2	63.4±13.2	33.3±9.5b	95.2±12.2	2.5±0.3c	95.3±20.7	21.3±5.4
IV (HF/5% STE)	7.2±3.1	39.5±11.5	13.6±3.4b	65.5±10.5	1.2±0.2c	65.8±25.1	19.4±3.3
V (HF/0.25 mg Std	8.5±3.5	35.4±12.4	14.3±4.5b	112.6±11.6	3.1±0.4c	87.2±19.3	25.7±6.5

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