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Phytochemical analysis and peripheral glucose utilization activity determination of *Steblus asper*

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ABSTRACT

Objective: To investigate the anti-hyperglycemic and peripheral glucose utilization activity of petroleum ether extract of leafs of *Streblus asper* (SAPE) as well as to find out the hypoglycemic property of Apiol and its presence in the SAPE. **Methods:** The GCMS analysis was conducted to identify the Apiol present in the SAPE. Six groups of animals, containing six animals in each, were selected for this study. The first group served as control and provided only vehicle solution. Group second served as vehicle control and treated with 0.5% CMC (carboxy methyl cellulose) orally. Group third to sixth, were treated with glibemclamide (600 ug/kg bw) and SAPE 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg bw respectively. In the second experiment, three groups of animals containing six animals in each were selected and were made diabetic as described earlier and simultaneously treated with pure Apiol at the dosages of 50 mg/kg bw, 75 mg/bw and 100 mg/kg bw respectively. **Results:** The GCMS analysis reviled the presence of Apiol molecules in the leaves of SAPE. Administration of SAPE and Apiol to the diabetic rats exhibited significant anti diabetic property ($P<0.01$). After 30 days of SAPE treatment, the fasting blood sugar levels of the diabetic rats were significantly reduced ($P<0.001$) along with restoration in their glycolytic and gluconeogenic enzyme activities, glycogen content and insulin level. **Conclusions:** The SAPE exhibited remarkable anti diabetic activity and excellent control over peripheral glucose utilization. Thus, it can be concluded that Apiol may be responsible for its anti-diabetic property.

1. Introduction

In the modern system of medicine, there is hardly any drug available for the complete and safe treatment of diabetes mellitus. Although the different classes of currently available therapeutic agents like sulfonylurea, biguanides, α-glucosidase inhibitors and thiozolidinedione have been used in abundance for the disease management but the long time usage of these drugs can cause insulin resistance diabetes [1,2]. World Health Organization (WHO) has recorded the number of diabetics worldwide to be 171 million in the year 2000 and predicted that the number may increase up to 366 million by the end of 2030 [3] and out of which 90% cases would be suffering from insulin resistance diabetes [4]. The search for effective and safe anti-diabetic medicine from plant origin is thus of great importance as modern medicines possess various side effects [5].

There are many plant derived medicines which can enhance the peripheral glucose utilization during diabetes[6-9]. *Streblus asper* Lour (Moraceae) (*S. asper*) is a commonly found in dry tropical regions, has been well documented in the Ayurvedic Pharmacopoeia of India for its medicinal properties [10-13]. The native people of Assam, India have been using the leaves of this plant for improving the weakness and for wound management. Recently we have reported the anti-diabetic property of petroleum ether extract of leaves of this plant [14].

Based on the above said information of traditional practice and our previous observations about this plant, the present study was planned to investigate the active anti-diabetic phytoconstituent(s) present in the leaves of *S. asper* and also to determine the effect of *S. asper* petroleum ether extract (SAPE) on glycolytic and gluconeogenic enzyme activities with a view to explore its peripheral glucose utilization activity.

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2. Materials and methods

2.1. Plant material and extract preparation

The leaves of *S. asper* were collected in the month of December from Assam, India and shade dried. The plant was identified by the Botanical Survey of India, Southern Circle, Coimbatore, India (BSI/SRC/5/23/09-10/Tech/924). The dried leaves were made into coarse powder and subjected to petroleum ether soxlet extraction for 72 hours. After the recovery of the solvents, the SAPE extract was obtained as 1.6% of the dry weight.

2.2. Chemicals

All the chemicals used for the experiment were purchased from SRL Mumbai whereas; pure Apiol was purchased from LGC Promo Chem. Private India Limited, Bangalore.

2.3. Gas chromatography-Mass spectroscopic (GC MS) Analysis

SAPE was subjected to GC MS analysis and one of the components identified to be presenth was Apiol. Pure Apiol was used as a standard to quantify the amount present in SAPE.The GC MS analysis was performed at SGS India private limited, Chennai using Agilent 5975B inert XL MSD.

2.4. Animal selection and diabetes induction

Male Sprague dawley rats with body weight of 200±20 gms were selected for the present experimentations. The caged animals were subjected to 12 hours of light and dark cycle with a temperature of (22±2) ℃ Celcius and water provided ad libitum. Diabetes was induced to the overnight fasting animals by injecting 45 mg/kg bw streptozotocin (STZ) intraperitoneally [15] and after 72 hours of disease induction, animals with fasting blood sugar > 250 mg/dL were selected for the study. The CPCEA approval for the animal study was obtained (vide no 4/18/IAEC/24/07/07).

2.5. Animal treatment

2.5.1. Experiment 1

Six groups of animals, containing six animals in each, were selected for the present study. The group first served as normal control whereas, group second served as vehicle control and received only 0.5% CMC (Carboxy methyl cellulose) orally. The group III, IV, V and VI animals were made diabetic by intra-peritoneal injection of Streptozotocin (STZ). The III group animals were treated with Glibenclamide (600 μg/kg bw) as a reference drug whereas, group IV, V, VI animals received SAPE at dosages of 100, 250 and 500 mg/kg bw respectively for a period of 30 days.

2.5.2. Experiment 2.

In this experiment, three groups of animals containing six animals in each were selected for the study. All the animals were made diabetic by intra-peritoneal injection of Streptozotocin (STZ) and pure Apiol at the dosages of 50 mg/kg bw, 75 mg/bw and 100 mg/kg bw were administered to each group of animals and their anti- diabetic activities were monitored for two hours.

2.6. Biochemical analysis

Thirty days after the treatment, the first experimental study, animals were sacrificed under mild anesthesia and their blood was collected in two fractions for serum and plasma respectively. The animals were dissected and the liver, muscle (diaphragm) and kidney of the animals' were separated out and washed three times with normal saline. The tissues were homogenized using the buffer solution for different parameters. Hexokinase, glucose 6 phosphatase and glycogen were estimated by the methods of Branstrup *et al.*, King and Carroll *et al.* [16-18] respectively. Plasma insulin and serum lipid profile (cholesterol, triglyceride and HDL C) were measured by kits obtained from Diagnostic Automation, Inc, CA and Span Diagnostics respectively. Blood glucose level of all the animals were estimated from their tail vein by Ascentia glucometer strips.

2.7. Statistical analysis

All quantitative data for statistical analysis were taken in multiples of six and were analyzed by one way Anova and the data was compared by Tukey multiple range test except for drug response curve of Apiol, SAPE where 2- way ANOVA was used. Results were expressed in mean ± SD.

3. Results

3.1. Antidiabetic effect of SAPE

A significant antihyperglycemic activity of SAPE was observed in the present study (Table 1a). Total 32% reduction in the fasting blood sugar levels of the diabetic animals was observed after four hours glibenclamide treatment. Simultaneously, SAPE at the dosages of 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg bw reduced the fasting blood sugar of the diabetic animals by 7%, 9% and 19% respectively. The ED_{50} of SAPE was >500 mg/kg bw however, after 30 days, 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg bw of SAPE treatment reduced the fasting blood sugar of the diabetic animals by 32%, 40% and 41% respectively as compared with glibenclamide treatment, where 51% reduction was noticed(Figure 3).

3.2. Quantification of Apiol in SAPE

Table 1
Effect of *S. asper* petroleum ether extract (SAPE) on blood glucose levels in diabetic rats (Duration of treatment −240 minutes).

Time	Group I	Group II	Group III	Group IV	Group V	Group VI
0 min	84.50 ± 3.78	262.70 ± 14.51	256.30 ± 10.84	260.20 ± 14.55	255.80 ± 10.83	258.50 ± 9.71
30 min	84.00± 5.29^{NS}	257.00 ± 11.58	187.00 ± 12.62***	249.0 ± 9.90^{NS}	238.5 ± 6.41**	234.30 ± 5.20**
120 min	83.00 ± 5.62^{NS}	257.70 ± 13.02	178.80 ± 13.14***	248.50 ± 10.45^{NS}	237.20 ± 6.61***	226.30 ± 8.38***
240 min	85.50 ± 4.76^{NS}	257.20 ± 12.25	174.30 ± 9.61***	242.50 ± 5.68*	233.80 ± 7.83***	208.00 ± 13.91***

Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV−VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. * P<0.05, **P<0.01, *** P<0.001 comparing with Group II. NS: Non significant. Unit : mg/dL.

Table 2
Effect of *S. asper* petroleum ether extract (SAPE) on glycolytic and gluconeogenic enzyme activities of the liver and its glycogen content in diabetic rats(Duration of treatment −30 days).

Content	Group I	Group II	Group III	Group IV	Group V	GROUP VI
Hexokinase	581.20±17.88	275.50 ± 63.95$^{\#}$	409.3 ± 24.74***	338.7 ± 22.11*	352.0 ± 19.45**	356.0 ± 22.91**
Glucose 6 phosphatase	35.00± 2.83	89.50 ± 9.52$^{\#}$	53.83 ± 10.63***	73.83 ± 5.71*	66.50 ± 9.18**	62.83 ± 12.16***
Glycogen	45.33 ± 2.73	19.00 ± 2.19$^{\#}$	31.00 ± 2.83***	23.00 ± 3.03^{NS}	26.00 ± 4.90*	26.50 ± 5.82*

Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV−VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. $^{\#}P$<0.001 comparing with group I.* P<0.05, ** P<0.01, *** P<0.001 comparing with group II. NS: Non significant. Units Hexokinase: nmoles of Glucose−6−phosphate formed/min/g protein, Fructose 1,6 diphosphate and Glucose 6 phosphatase : nmoles of P liberated/gm protein at 37 ℃. Glycogen: mg per 100 g tissue.

Table 3
Effect of *S. asper* petroleum ether extract (SAPE) on the glycolytic and gluconeogenic enzyme activities of the kidney and its glycogen content in diabetic rats (Duration of treatment −30 days).

Content	Group I	Group II	Group III	Group IV	Group V	Group VI
Hexokinase	58.33 ± 3.33	119.7 ± 21.75$^{\#}$	74.67 ± 8.62***	97.00 ± 5.18*	91.17 ± 10.30**	89.33 ± 13.95**
Glucose 6 phosphatase	33.67 ± 5.92	85.33 ± 7.99$^{\#}$	49.17 ± 6.73***	67.33 ± 4.13*	61.33 ± 12.86**	60.83 ± 14.43**
Glycogen	1.23 ± 0.22	2.90 ± 0.53$^{\#}$	1.53 ± 0.26***	2.55 ± 0.29^{NS}	2.20 ± 0.42^{NS}	2.13 ± 0.58*

Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV−VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. $^{\#}P$<0.001 comparing with group I. * P<0.05, **P<0.01, ***P<0.001 comaprign with group II. NS: Non significant. Units Hexokinase: nmoles of Glucose−6−phosphate formed/min/gm protein, Fructose 1,6 diphosphate and Glucose 6 phosphatase: nmoles of P liberated/gm protein at 37 ℃. Glycogen: mg per 100 g tissue.

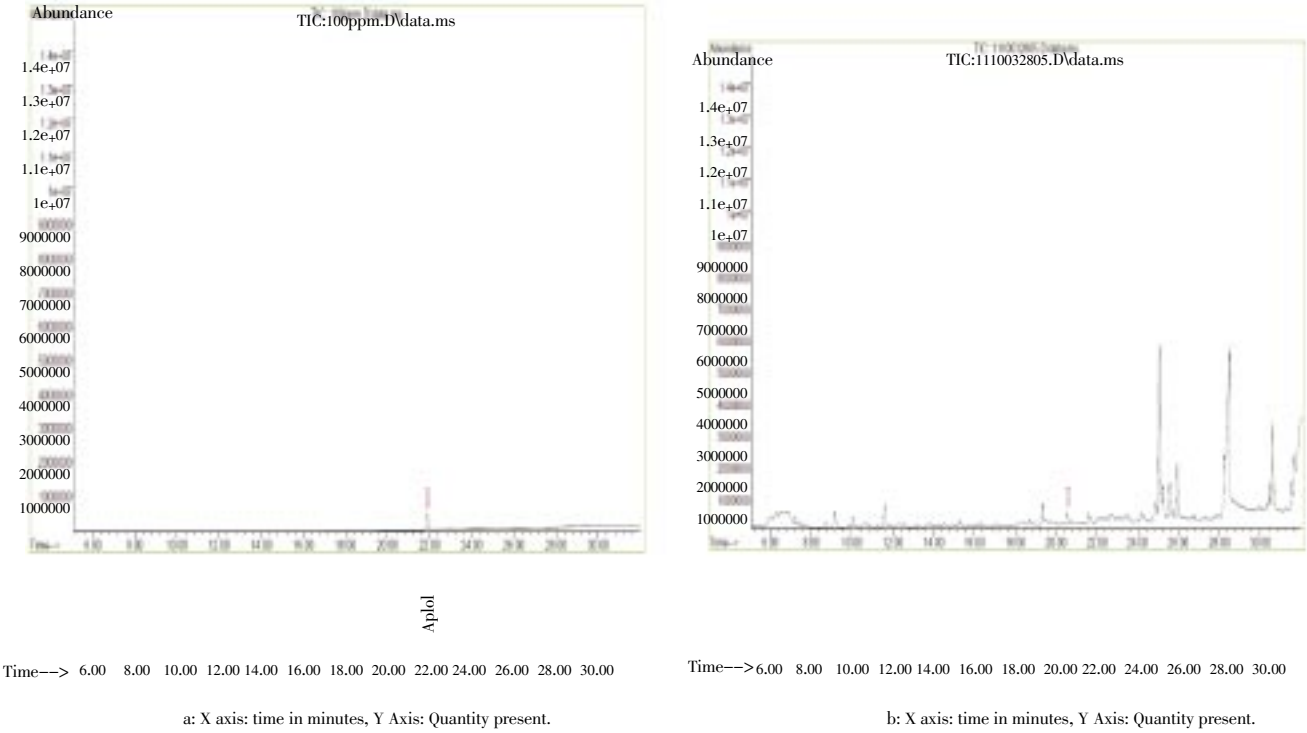

Figure 1. a. GCMS Spectrum for the standard curve of Apiol ; b. GCMS Spectrum for the Quantification of Apiol in *Streblus asper* petroleum ether extract (SAPE) by GCMS analysis

GCMS analysis quantified apiol concentration in SAPE at 81.74 ppm. (Figure 1).

3.3. Anti-diabetic effect of Apiol

Pure Apiol at the dose of 50 mg/kg bw, 75 mg/kg bw and 100 mg/kg bw exhibited remarkable hypoglycemic activity and it could able to reduce the fasting blood sugar levels of the diabetic animals by 22%, 25% and 27% respectively after four hours of treatment (Figure 2).

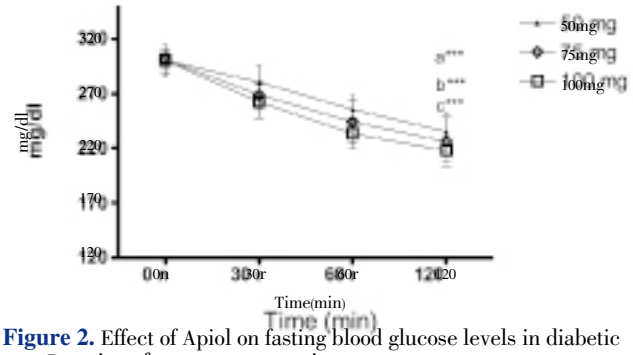

Figure 2. Effect of Apiol on fasting blood glucose levels in diabetic rats (Duration of treatment -120 minutes).
a: 0 min v/s 120 min for 50 mg/kg bw, b: 0 min v/s 120 min for 75 mg/kg bw and c: 0 min v/s 120 min for 100 mg/kg bw respectively. n=6 .
* P<0.05, ** P<0.01, *** P<0.001

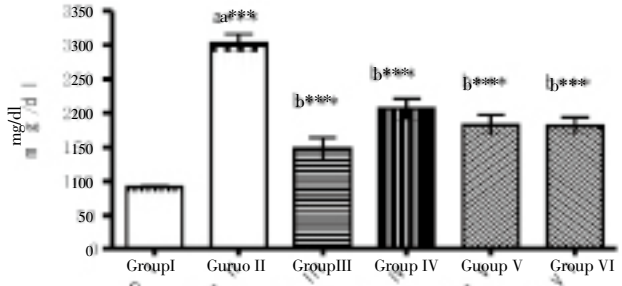

Figure 3. Effect of *Streblus asper* petroleum ether extract (SAPE) on fasting blood glucose levels in diabetic rats(Duration of treatment -30 days).
Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV-VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. a: Group II v/s Group I. b: Group III,IV,V,VI v/s Group II. *** P<0.001.

3.4. Metabolic enzyme activities and glycogen content in liver

The reduction in the blood glucose levels was reflected in the glycolytic and gluconeogenic enzyme activities of the liver and kidney of the experimental groups. SAPE treatment at dosages of 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg bw increased the liver hexokinase activity by 23%, 28% and 29% while reducing the glucose 6- phosphatase activity by 18%, 26% and 30% respectively. The glycogen level in these tissues was also increased by 21%, 37% and 37% respectively. On the other hand, glibenclamide treatment increased the hexokinase activity and glycogen content of the liver by 48% and 63% respectively while reducing the glucose 6 phosphatase activities by 40% (Table 2).

3.5. Metabolic enzyme activities and glycogen content in kidney

The elevated hexokinase activity in the kidney of the diabetic rats was reduced by 18%, 24% and 25% with 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg SAPE treatment respectively, while their elevated glucose 6 phosphatase activity was reduced by 21%, 28% and 29%. SAPE also reduced the elevated kidney glycogen content by 12%, 24% and 27%. The better blood glucose regulation was seen in Glibenclamide treated animals as compared with SAPE and therefore, the hexokinase, glucose 6 phosphatase and glycogen content of the diabetic kidney were reduced by 38%, 42% and 47% respectively (Table 3).

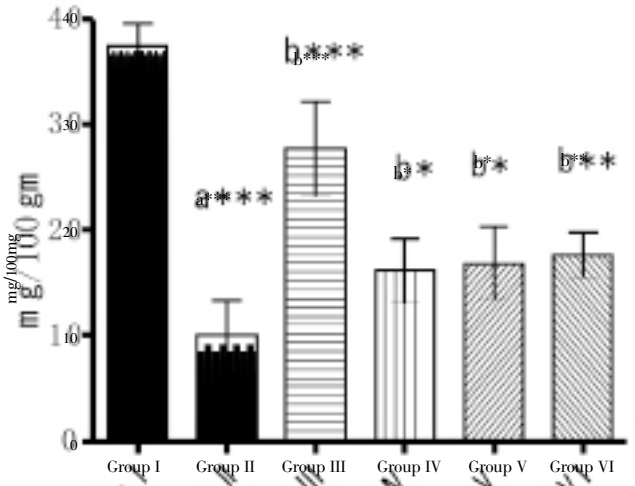

Figure 4. Effect of *Streblus asper petroleum* ether extract (SAPE) on the glycogen content of muscle (diaphragm) in diabetic animals (Duration of treatment -30 days)
Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV-VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. a: Group II v/s Group I. b: Group III,IV,V,VI v/s Group II. * P<0.05, **P<0.01, ***P<0.001.

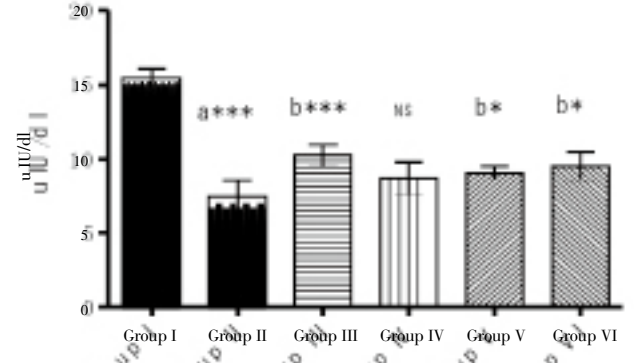

Figure 5. Effect of *Streblus asper* petroleum ether extract (SAPE) on plasma insulin levels in diabetic rats (Duration of treatment -30 days)
Group I: Normal animals, Group II: Diabetic animals, Group III: Glibenclamide treated diabetic animals, Group IV-VI: Diabetic animals treated with 100, 250 and 500 mg/kg bw SAPE. n=6. a: Group II v/s Group I. b: Group III,IV,V,VI v/s Group II. * P<0.05, *** P<0.001. NS: Non significant.

3.5. Muscle glycogen content and plasma insulin level

Muscle (diaphragm) glycogen content was decreased in

the diabetic animals whereas SAPE treatment at the doses of 100 mg/kg bw, 250 mg/kg bw and 500 mg/kg increased its level by 61%, 68% and 76% respectively (Figure 4) along with increased insulin levels by 17%, 21% and 27% respectively (Figure 5). The plasma insulin levels of the glibenclamide treated diabetic animals were restored by 37% and the muscle glycogen content by 80%.

4. Discussion

Streblus asper is well known for its therapeutic efficacy and has already been used for the treatment of different ailment in the Indian System of Medicine(ISM) since long back.

The leaves of *Streblus asper* is being used by the native people of Assam for improving the weakness and wound management. In the present study, qualitative GCMS analysis using GCMS library reviled that one of the major fractions in the SAPE was Apiol and later it was quantified using SAPE.

Petroselinum crispum is frequently being used in the Turkey as anti- diabetic agent and it has been reported that Apiol is the major phytoconstituent [19]. Earlier reports suggested the lowest LD 50 of Apiol to be 1 gm/kg bw [20], therefore 100 mg/kg bw Apiol was taken as the highest dose for the study while two other dosages below 100 mg/kg bw were also selected for comparison. Apiol exhibited a significant anti hyperglycemic activity and could be accounting for the anti diabetic activity of SAPE.

After 30 days of treatment the blood sugar of the glibenclamide treated fasting diabetic animals were found significantly decreased as compared with SAPE treated animals and it was also reflected by the glycolytic and gluconeogenic enzyme activities of the treated animals. Carbohydrate metabolizing enzymes viz. hexokinase and glucose 6 phosphatase play a major role in the controlling the blood glucose levels and insulin can influence their activities [21-23].

The level of glycogen in the tissues depends upon the hexokinase and glucose 6 phosphatase activities [24] and all these parameter plays a pivotal role in peripheral glucose utilizing capability of the anti diabetic drugs. During diabetes, liver and kidney controls the endogenous glucose production (EGP) [25] and thus, the regulations of these enzymes are responsible for the blood glucose levels. Therefore, the increased concentration of plasma insulin in SAPE treated diabetic rats might have increased the liver hexokinase activity and glycogen content together with reduced in glucose 6 phosphatase activity.

An increased level of plasma insulin was observed in the glibenclamide treated diabetic rats and the values were higher than the SAPE treated diabetic rats which may due to the insulin secretory property of glibenclamide[26]. Hence, the liver glucokinase activity was higher along with elevating liver glycogen levels in glibenclamide treated group and it could able to controlled blood glucose level better than SAPE. Muscle (diaphragm) is considered to be an insulin dependent tissue [27] and the increased level of glycogen in the muscle by SAPE clearly indicates its peripheral glucose utilizing property.Kidney is an insulin independent tissue and its level of hexokinase and glucose 6 phosphatase enzyme activities are dependent on the blood glucose level [28]. Due to the better management of fasting blood glucose by glibenclamide, hexokinase and glucose 6 phosphatase activities were significantly reduced and may have resulted significantly decreased glycogen content in the diabetic kidney. Even though SAPE significantly reduced the hexokinase and glucose 6 phosphatase activities of the diabetic kidney along with, their glycogen level reduced significantly at the dose of 500 mg/kg bw thereby indicating that SAPE at higher dose can be as effective as glibenclamide.

The major causes for insulin resistance diabetes are hyperglycemia, hyperlipidemia, loss of antioxidants and increased anti- inflammatory activities [29]. In the present study, anti-hyperglycemic as well as peripheral glucose utilizing properties of SAPE was observed and it's anti-oxidant activity and anti- inflammatory activity [30] has already been reported [14]. Thus, it appears that *Streblus asper* can probably reduce insulin resistance by its multifarious therapeutic efficacy. Overall SAPE exhibited promising results against insulin resistance than glibenclamide and therefore, this plant medicine needs full consideration and can be included in the group of anti-diabetic plants against insulin resistance diabetes [31].

Since, Apiol exhibited anti- hyperglycemic activity and is also present in the SAPE, it appears to be one of the major phytoconstituents responsible for anti-diabetic activity of SAPE. Apiol is a polyvalent active natural product which probably can target different anti diabetic receptors. Therefore, it is difficult to predict the exact mode of action of apiol. Further investigation in this direction is required to determine the anti- diabetic mechanism of Apiol against insulin resistance at the molecular level.

Conflict of interest statement

We declare that we have no conflict of interest.

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