Effect of *Salacia reticulata* Wight extracts on drug induced diabetes mellitus in rats

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Summary

**Background.** Experimentally, the root extract of *Salacia reticulata* has been found to have potent hypoglycemic activity both in normal and in streptozotocin-induced diabetic rats. Furthermore, the decoction of *S. reticulata* roots is used in the treatment of rheumatism,
gonorrhea, itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea.

The aim of the study. The efficacy of the plant extract of *Salacia reticulata* Wight (hippocrataceae) was tested on albino rats (Wistar strain) in which diabetes were induced by administration of alloxan.

Methods. The rats were grouped into diabetic control, insulin treated and various extracts of *Salacia reticulata* Wight treated. The effect of extract on blood glucose level after 15 and 30 days of treatment, serum cholesterol and triglyceride level was assessed after 30 days of treatment.

Results. The fall in the blood glucose level in rats treated with ethyl acetate fraction was more followed by ethanol extract, insulin treatment, water extract and diethyl ether extracts. The insulin treated rats had a significant fall in serum cholesterol level followed by ethanol, diethyl ether and petroleum ether extracts. Serum triglyceride level was not influenced by any of the extract treatment.

Conclusions. Our study confirms the percentage of hypoglycemic effect exerted by all the different fractions almost correspond with the level of action exerted by insulin. Hence we strongly recommend the use of *Salacia reticulata* Wight extract in diabetic condition.

Key words: Salacia reticulate, diabetes mellitus

INTRODUCTION

Diabetes mellitus is an increasingly common, potentially devastating, expensive, treatable but incurable life long disease. It affects an estimated 5% of the population in USA [1]. The incidence is almost similar in Orientals as well [2].

Insulin and other antidiabetic drugs have been used in allopathic practice of medicine for about last 75 years. Of late there is an increased interest to understand the utility of plant and plant products as therapeutic agents. The utility of the plant and plant products as therapeutic agents, which can be taken orally for diabetes, is in vogue in different parts of the world.

Experimentally, the root extract of *Salacia reticulata* has been found to have potent hypoglycemic activity both in normal [3] and in streptozotocin-induced diabetic rats [4]. Furthermore, the decoction of *S. reticulata* roots is used in the treatment of rheumatism, gonorrhea, itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea [3].

However, the hypoglycemic effect of *Salacia reticulata* Wight was not studied in an animal model with strong supporting control groups and also mechanism involved in its action. Hence, the efficacy of the plant extract was tested on albino rats (Wistar strain) in which diabetes were induced by administration of alloxan. The effect of the extract after 15 and 30 days of treatment was assessed. We further measured levels of serum cholesterol and triglyceride after 30 days of treatment.

The results of this study will have an impact in delivery of better health care which is less cumbersome, more functional and would be within the reach of common man from the economy aspect as well.
METHODS

**Animals.** In-house bred albino Wistar strain male rats aged two- to three-month-old (120–180 g) were used in the study. All the rats were maintained under 12 h day light environment. For housing the animals, polypropylene cages (29 x 22 x 14 cm) were used (3 animals/cage) with paddy husk bedding at 26°C±1°C. All the rats were maintained on the standard rat food and water *ad libitum*. All the experimental procedures were approved by the Institutional ethics committee. Maintenance of the animals was done as per the guidelines of Government of India for use of Laboratory animals (Government of India notifies the rules for breeding and conducting animal experiments, proposed in the Gazette of India Dec 15, 1998: which was reproduced in Ind J Pharmacol 1999; 31:92-5).

**Induction of diabetes.** Alloxan solution was prepared at a very low temperature by placing ice cubes around the beaker, as alloxan is known to get oxidized at room temperature. Alloxan was dissolved in saline (0.9%). Injection of either saline or alloxan was carried out on rats that had been deprived of food for about 24 hours. After about 30 minutes of injection, food was provided to animals *ad libitum*.

**Confirmation of diabetes.** After seven days of stabilization blood samples were obtained from rats fasted overnight. Blood was drawn from intra orbital plexus by inserting a “mucap” capillary. About 1 ml of blood was drawn into a test tube having sodium fluoride. The sample of blood was mixed with the sodium fluoride and allowed to clot. After about an hour the sample of blood was centrifuged (R & C centrifuge) at 2000 rpm for about 15 min. After centrifugation the supernatant serum was collected and the blood glucose level was estimated. The alloxan-injected group, which had more than 200 mg% of blood glucose, was included in the study. The diabetes-induced animals were randomly assigned into different groups with eight animals in each group. The day of confirmation of diabetes was taken as a day 1 for further course of treatment as per the group. The blood glucose estimation was done again on 15th and 30th day from rats fasted overnight.

**Preparation of extract using root + bark.** *Salacia reticulata* roots were obtained locally and were authenticated by Dr. Gopalakrishna Bhat, Professor of Botany, Poorna Prajna College, Udupi. A voucher specimen has been deposited in the College of Pharmaceutical Sciences, Manipal. Shade dried finely powdered root+bark (2 kg) was soaked in 95% ethanol for four days. The cold extract was decanted off and the soaked material was refluxed in 5 batches of about 400 g with 95% ethanol (3 l X 3 hr). Both the cold and warm extracts were boiled and concentrated under reduced pressure till a syrupy consistency was obtained. It was then spontaneously evaporated to dryness (yield 50.8 g). This was fractionated using petroleum ether (PE), diethyl ether (DE) and ethyl acetate (EA) in succession (yield was 4.58 g, 7.01 g and 4.9 g, respectively for different fractions).

**Treatment schedule.** The treatment schedule of the various groups is as detailed. On the 15th and 30th day of treatment, blood from animals fasted overnight was drawn and the biochemical parameters were assessed.
NC – normal control – vehicle (propylene glycol)
DC – diabetic control – vehicle (propylene glycol)
IN – insulin treated – 6 units/kg body weight
EE – ethanol extract treated – 50 mg/kg bodyweight
PE – petroleum ether extract – 50 mg/kg body weight
DE – diethyl ether extract – 50 mg/kg body weight
EA – ethyl acetate extract – 50 mg/kg body weight
WE – water extract – 50 mg/kg body weight

The various extracts were dissolved in propylene glycol and the drug was administered intragastrically using Ryle’s tube. While preparing the drug the concentration of drug obtained was 10 mg/ml of propylene glycol. The weight of the animal was recorded everyday and drug was administered as per dosage required, in relation to body weight. Normal control and diabetic control groups were also given the vehicle as per their body weight range. The insulin groups were injected with insulin subcutaneously with a dose of 6 units/kg body weight [5]. Dilution of insulin to obtain the required concentration was done by diluting insulin with 0.9% sodium chloride solution.

All the solutions (various extracts/insulin) once prepared usually lasted for about 8–10 days. The various drugs/vehicle/insulin were stored in refrigerator at 8 to 10°C temperature. They were taken out of refrigerator and allowed to come to room temperature before administration.

While preparing the solution of various extract in propylene glycol (where the drug did not dissolve at room temperature), the solution was warmed in water-bath at about 60–70°C for about 20–30 min. to ensure total dissolution.

The treatment used to be done once everyday between 8 and 10 a.m. The groups would receive the treatment for 30 days from the day of confirmation of diabetes.

**Recording of body weight.** On the day the diabetic state was confirmed the animals were weighed using a scale with a resolution of 10 g and the weight was noted down. This was recorded as weight of animal on day 1. After 15 and 30 days of treatment the weight of the animal was recorded again.

**Estimation of blood glucose.** was done by glucose oxidase-peroxidase method (kit marketed by Pointe Scientific Inc., USA).

Test principle:

\[
\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{guconic acid} + H_2O_2
\]

\[
H_2O_2 + 4 \text{ amino antipyrine} \xrightarrow{\text{peroxide}} \text{coloured complex} + H_2O
\]
The red colored complex formed is measured at 505 nm and the intensity of the color formed is directly proportional to the concentration of glucose in the sample [6].

**Estimation of cholesterol (kit marketed by Pointe Scientific Inc., USA).** The cholesterol estimation was based on the hydrolysis of cholesterol esters by cholesterol esterase to free cholesterol and fatty acids. Free and liberated cholesterols are oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The liberated hydrogen peroxide couples with 4-amino antipyrine and p-hydroxy benzene sulfate in the presence of peroxidase to form red quinone. The intensity of this red quinone was measured photometrically at 520 nm [7].

**Estimation of triglyceride (kit marketed by Pointe Scientific Inc., USA).** The method is based on the hydrolysis of serum triglycerides to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerokinase, the glycerol is converted to glycerol–3–phosphate, which is then oxidized to glycerol–phosphate oxidase to yield hydrogen peroxide. Hydrogen peroxide formed, then reacts with ESPAS (N-ethyl-N-sulfopropyl-M-anicidine) and 4-amino antipyrine in the presence of peroxidase to form coloured complex. The intensity of the quinoneimine was measured photometrically at 546 nm [8].

**Statistical analysis**

The data were expressed as mean±SE. The significance of differences among the groups were assessed using one-way analysis of variance (ANOVA) test followed by Bonferroni multiple comparison test. The values of $p<0.05$ were considered as significant. Comparison of data within the group at different intervals was assessed by paired T-test. The difference of freedom has been mentioned at appropriate places.

**RESULTS**

**Body weight changes in the different groups**

The animals included in the study belonged to the age group of 2–3 months. Hence, a further increase in the body weight was expected during the course of study of about 37 days (7 days of stabilization period after injection of alloxan and 30 days of treatment period) (fig. 1).

The normal control group in which diabetes was not induced did gain an average weight of about 29 g during the period of study. The diabetic control group, which was not given any treatment other than vehicle, lost an average of about 25 g during the same period.
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ANNOVA significance: day of confirmation $F=6.31$; after 15 days $F=8.23$; after 30 days $F=25.87$. Error bar indicates ±SE.

Normal control (NC) v/s others $^a$ – p<0.05, $^{**}$ – p<0.01, $^{***}$ – p<0.001
Diabetic control (DC) v/s others $^a$ – p<0.05, $^b$ – p<0.01, $^c$ – p<0.001
Insulin treated (IN) v/s others $^a$ =p<0.05, $^{**}$ = p<0.01, $^{***}$ =p<0.001

**Figure 1.** Comparison of the body weight of rats at different intervals treated with *Salacia reticulata* extract.

In the other groups treated with either insulin or any of the different extracts, the body weight change observed is given below:

Ethyl acetate extract treated group gained about 22 g weight. In diethyl ether extract treated group the gain of weight was about 14 g. In water extract or petroleum extract treated groups or in insulin treated group, the average weight gain was about 5 g. The ethanol extract treated group did not show weight gain.

Diabetic control group was compared with the groups treated with insulin or any of the extracts. The gain in body weight was highly significant (p<0.001) with ethyl acetate extract treated group, moderately significant (p<0.001) with insulin treated group and least significant (p<0.05) in diethyl ether extract treated group. $F = 25.87$

**Comparison of percentage gain in the body weight at the end of 30 days**

While the percentage weight gain in normal control was 18%, the same could be obtained only with ethyl acetate extract treated group (tab. 1). It was slightly less (11%) in diethyl ether extract treated group and least (5%) with water extract or petroleum ether extract treated groups.
Table 1. Comparison (T values) of body weight of the rats at different intervals (paired’ test)

<table>
<thead>
<tr>
<th>animal groups</th>
<th>day 1 vs. day 15</th>
<th>day 1 vs. day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control (nc)</td>
<td>7.48***</td>
<td>8.21***</td>
</tr>
<tr>
<td>diabetic control (dc)</td>
<td>5**</td>
<td>4.93***</td>
</tr>
<tr>
<td>insulin treated (in)</td>
<td>2.65*</td>
<td>1.87</td>
</tr>
<tr>
<td>ethanol extract treated (ee)</td>
<td>4.97**</td>
<td>4.97***</td>
</tr>
<tr>
<td>petroleum ether treated (pe)</td>
<td>4.77**</td>
<td>1.66</td>
</tr>
<tr>
<td>diethyl ether treated (de)</td>
<td>2.65*</td>
<td>3.28*</td>
</tr>
<tr>
<td>ethyl acetate treated (ea)</td>
<td>4.25**</td>
<td>7.18***</td>
</tr>
<tr>
<td>water extract treated (we)</td>
<td>0.68</td>
<td>1.18</td>
</tr>
</tbody>
</table>

\[ t > 2.36, p < 0.05 = *, t > 3.49, p < 0.01 = **, t > 5.40, p < 0.001 = *** \]

Blood glucose level in the different groups

In normal control group, which was not alloxan-administered, the blood glucose level was about 80 mg/dl throughout the 30-day study period. The diabetic group that was alloxan-administered but not the extracts or insulin treated had sustained blood glucose from 322 to 332 mg/dl during the same period. In other groups in which either insulin or any of the extracts was given, the blood glucose level was as follows (fig. 2):

a) insulin treatment: decreased from 275 to 85 mg/dl;
b) ethanol extract treatment: decreased from 345 to 141 mg/dl;
c) diethyl ether extract treatment: decreased from 265 to 116 mg/dl;
d) ethyl acetate extract treatment: decreased from 274 to 57 mg/dl;
e) water extract treatment: decreased from 323 to 140 mg/dl.

ANOVA significance: day of confirmation F = 6.81; after 15 days F = 12.19; after 30 days F = 19.97. Error bar indicates ±SE.

normal control (NC) v/s others * – p<0.05, ** – p<0.01, *** – p<0.001
diabetic control (DC) v/s others a – p<0.05, b – p<0.01, c – p<0.001
insulin treated (IN) v/s others $ – p<0.05, $$ – p<0.01, $$$ – p<0.001

Figure 2. Comparison of the blood glucose level of the rats at different intervals treated with Salacia reticulata extract
However, in petroleum ether extract treated group the hyperglycemic state increased further from around 276 to 300 mg/dl.

The diabetic group was compared with groups treated with insulin or any of the extracts. The fall in blood glucose level was highly significant (p<0.001) in ethyl acetate and diethyl ether extract treated groups. It was moderately significant (p<0.01) in water and ethanol extract treated groups; F=19.97.

Comparison of percentage change in blood glucose level at the end of 30 days

When compared to about 3% increase in blood glucose level in diabetic group between day 1 and day 30, the falls in the various groups were as follows:

a) 69% with insulin treatment;
b) 59% with ethanol extract treatment;
c) 56% with diethyl ether and water extracts treatment;
d) 79% with ethyl acetate treatment.

In petroleum ether extract treated group there was about 8% increase of blood glucose level during the same period. The fall in blood glucose level was highly significant (p<0.001) with insulin, diethyl ether, ethyl acetate and water extract treated groups. It was moderately significant (p<0.01) in ethanol extract treated group (tab. 2).

<table>
<thead>
<tr>
<th>animal groups</th>
<th>day 1 vs. day 15</th>
<th>day 1 vs. day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control (NC)</td>
<td>0.41</td>
<td>0</td>
</tr>
<tr>
<td>diabetic control (DC)</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>insulin treated (IN)</td>
<td>7.4</td>
<td>6.45**</td>
</tr>
<tr>
<td>ethanol extract treated (EE)</td>
<td>2.12</td>
<td>5.04**</td>
</tr>
<tr>
<td>petroleum ether treated (PE)</td>
<td>4.37**</td>
<td>0.54</td>
</tr>
<tr>
<td>diethyl ether treated (DE)</td>
<td>7.62**</td>
<td>9.41**</td>
</tr>
<tr>
<td>ethyl acetate treated (EA)</td>
<td>8.54**</td>
<td>25.17**</td>
</tr>
<tr>
<td>water extract treated (WE)</td>
<td>2.73*</td>
<td>12.81**</td>
</tr>
</tbody>
</table>

Serum cholesterol level in the different groups

Serum cholesterol level was estimated in the different groups after the study period of 30 days from the induction of diabetes or in normal controls. The serum cholesterol level of the diabetic group has been compared with other groups treated with either insulin or any of the extracts. The fall in the cholesterol level was highly significant (p<0.001) in insulin treated group, moderately significant (p<0.01) in ethanol extract, diethyl ether and petroleum ether extracts treated groups. It was the least significant (p<0.05) in water extract treated group (fig. 3). There was no significant difference in ethyl acetate extract treated group; F=7.73.
Serum triglyceride level in the different groups

There was no significant difference in triglyceride level when compared with diabetic group at the end of 30 days in all groups (fig. 3); F=3.44.

Figure 3. Comparison of cholesterol & triglyceride levels treated with Salacia reticulata extract

DISCUSSION

One of the parameters to consider the amelioration of the diabetic state is to ascertain the effect of treatment on the body weight. An increase in body weight implies that anabolic effects have overridden the catabolic ones. No variation means protection against weight loss. Decrease in body weight would mean that catabolism has persisted. Effects of certain plant/plant products in body weight gain in diabetic state have been reported by Stanley et al. [9]. In present study groups treated with water extract and petroleum ether extract were effective in exerting protection against body weight loss. Groups treated with ethyl acetate and diethyl ether extracts gained body weight.

Insulin is a major anabolic hormone in the body. Its deficiency not only affects glucose metabolism but also protein and fat metabolism. Unopposed actions of
the counter-regulatory hormones also play an important role in metabolic derangements. With deficiency of insulin the scale swings from insulin promoted anabolism to catabolism of proteins and fats. Proteolysis follows and gluconeogenic amino acids are removed by liver and used as building blocks for glucose. The catabolism of proteins and fat tend to induce negative nitrogen balance. This results in increased appetite (polyphagia). The combination of polyphagia coupled with weight loss is paradoxical and always raise the suspicion of diabetes [10]. Disturbance in the metabolisms results in wasting of muscles and early fatiguability [11]. Either a protection against weight loss alone or an increase of body weight has their own distinctive role to play. The normalization of carbohydrate, protein and fat metabolisms would alleviate the diabetic symptoms like loss of weight and fatiguability. This would certainly improve the quality of life of the individual. From the present study it can be concluded that ethyl acetate and diethyl ether extracts are more effective not only in preventing body weight loss but also in helping to gain weight.

Current treatments for diabetes are far from ideal. Diet control and exercise are difficult for many people. Oral hypoglycemic agents often become ineffective and sometime have side effects. Furthermore, all insulin replacement regimens are grossly imperfect [1].

In present study it has been observed that hypoglycemic effect exerted by diethyl ether, ethyl acetate and water extracts and petroleum ether are quite effective. The percentage hypoglycemic effect exerted by all the different fractions almost corresponds with the level of action exerted by insulin.

Petroleum ether extract of the root bark of Salacia oblonga Wall [12], water extract of the root of Tinospora cordifolia [13], effect of Opuntia megacantha [14], bio tea [15], leaf of Hibiscus rosa-sinensis [16], root of Withania somnifera [17], herbal formulation [18], aqueous extract of Eichostemma littorale Blume [19] are a few studies where hypoglycemic effects have been demonstrated in the recent past.

Diabetes in general is associated with accelerated atherosclerosis and predisposes to certain microvascular abnormalities. It doubles the risk for stroke, increases the risk of heart attack two to three fold and peripheral vascular problems, particularly in feet, by about fifty fold. There is a reason to believe that elevated total cholesterol level is having equal atherogenic potential in diabetic as in non diabetic subjects. These factors led the American Diabetes Association to convene a special consensus conference to discuss the role of modification of cardiovascular risk factors in prevention and treatment of macrovascular changes in patients with diabetes mellitus. The group came to the conclusion that it was reasonable to assume that lowering of cholesterol in patients with diabetes would have beneficial effect on cardiovascular changes [20].

In our study a highly significant (p<0.001) effect has been observed with petroleum ether extract followed by ethyl acetate and ethanol extracts. Ethanol extract of the leaf of Gymura procumbens [21], ethanol extract of the leaves of Averrhoa bilimbi [22], seeds of Eruca sativa [23], root of Withania somnifera [17] have demonstrated hypocholesteremic effects of the plant extracts in diabetes in the recent past.
Administration of ethanol extract of the leaf of *Gynura procumbens* [21], seeds of *Eruca sativa* [23], ethanol extract of the leaves of *Averrhoa bilimbi* [22] etc. have also demonstrated the hypotriglyceridemic effects in diabetes in the recent past.

Salacinol, a naturally occurring sulfonium ion, is one of the active principals in the aqueous extracts of *Salacia reticulata*. This compound inhibits recombinant human maltase: glucoamylase, one of the key intestinal enzymes involved in the breakdown of glucose oligosaccharides in the small intestine [24].

In our study it has been found that treatment with petroleum ether extract was able to register hypotriglyceridemia even though there could not be a significant increase in triglyceride level in diabetic group or in any other group. The hypotriglyceridemia observed in petroleum ether extract treated group was least significant when compared with normal control.

To conclude, the ethyl acetate and diethyl ether extracts not only prevent loss of body weight but also help to gain weight. None of the extracts produced a decrease in the triglyceride levels of blood. Hence we strongly recommend the use of *Salacia reticulata* Wight extract in diabetic condition.

REFERENCES

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Wpływ ekstraktu z korzeni *Salacia reticulata* Wight na cukrzycę polekową u szczurów

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Streszczenie

Wstęp. Stwierdzono doświadczalnie, że wyciąg z korzenia *Salacia reticulata* może mieć działanie hipoglikemiczne zarówno u zdrowych szczurów, jak i tych z cukrzycą indukowaną streptozotocyną. Wywaru z korzenia *Salacia reticulata* używa się w leczeniu reumatyzmu, rzeżączki, świądu, obrzęków, astmy, odwodnienia, braku menstruacji i bole- snego miesiączkowania.

Cel badania. Badano skuteczność wyciągu z rośliny *Salacia reticulata* Wright na białych szczurach (szczep Wistar), u których wywołano cukrzycę alloksanem.

Metody. Szczury podzielono na grupy: cukrzycową (kontrolną), leczoną insuliną i leczoną różnymi wyciągami z *Salacia reticulata* Wright. Po 15 i 30 dniach leczenia oznaczano wpływ ekstraktu na poziom cukru we krwi, cholesterolu w surowicy i poziom triglicerydów.

Wyniki. Obniżenie poziomu glukozy we krwi szczurów leczonych frakcją etyloacetylową było większe niż u tych leczonych wyciągiem etanolowym, insuliną, wyciągiem wodnym i dietyloeterowym (kwas acetylooctowy). U szczurów leczonych insuliną dodatkowo zanotowano istotny spadek poziomu cholesterolu w surowicy, większy niż po zastosowaniu wyciągu etanolowego, dietyloeterowego i na eterze ropopochodnym. Poziom triglicerydów w surowicy nie zmieniał się pod wpływem leczenia żadnym z tych ekstraktów.


Słowa kluczowe: *Salacia reticulata*, diabetes mellitus