Prevention of High Fat Diet Induced Insulin Resistance in C57BL/6J Mice by Sida rhomboidea ROXB. Extract

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The present study investigates effect of Sida rhomboidea ROXB. (S. rhomboidea ROXB., SR) on high fat diet (HFD) induced insulin resistance in C57BL/6J mice and the results obtained have been compared with mice fed with rosiglitazone (ROS). Changes in bodyweight, food intake, fasting blood glucose, plasma insulin, plasma and hepatic triglyceride (TG), total cholesterol (TC) and free fatty acids (FFAs) have been investigated in various experimental groups. It was observed that feeding of SR extract to HFD fed mice (HFD+SR) reduced bodyweight (p < 0.05), food intake (p < 0.05) and feed efficiency ratio (p < 0.05). Plasma and hepatic TG, TC and FFA were also significantly lowered (p < 0.05) in HFD+SR groups. Efficient clearance of glucose in intraperitoneal glucose tolerance test (IPGTT), lowered area under curve (AUCglucose) values, low plasma insulin and fasting insulin resistance index (FIRI) coupled with higher KITT values were observed in HFD+SR groups. These findings were further justified by significant reduction of adipocyte diameter (p < 0.05) and surface area (p < 0.05) in HFD+SR groups. This study is a first scientific report on protective role of S. rhomboidea ROXB. extract against HFD induced insulin resistance in C57BL/6J mice and strengthens the folklore claim of use of SR leaves as alternative medicine against diabetes and obesity.

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INTRODUCTION

Prevalence of diabetes mellitus is now ubiquitous, affecting 150 million people worldwide. According to International Diabetes Federation (IDF) more than one fifth (33 million) of them are Indians and hence, India has been declared as “diabetic capital of the world.”1) Recent report have estimated that 80% of the total diabetic patients are suffering from type II diabetes2) and estimate of World Health Organisation (WHO) for year 2030, is of approximately 350 million of which 119.6 million are speculated of Asian origin.3) Since obesity play a central role in insulin resistance and induction of type II diabetes, consumption of high calorie diet is considered instrumental in induction of this pathological condition. Modern therapy involve expensive drugs for treating hyperlipidemia and hyperinsulinaemia in type II diabetes,4) with most of anti-diabetic drugs showing one or more side effects.5) WHO has recommended use of indigenous medicinal plants in lieu of expensive medicines for rural populace living in developing countries6) because of the easy availability and relatively less side effects.

Sida rhomboidea ROXB. (S. rhomboidea ROXB., Family Malvaceae) is a weed found in marshy places across India.7) In Ayurveda, it is known as Mahabala and is useful in fever, heart diseases, burning sensation, piles, urinary disorders and all kinds of inflammation.8) Studies have reported that its aerial parts have β-phenethylamine, N-methyl-β-phenethylamine, S-(-)-Nb-methyltrytophan methylester, vasicine, choline, betaine, ephedrine, ψ-ephrine,9) sigmasterol, sigmastenol, campesterol, 22-dehydrocampesterol, sitosterol, cholesterol, spinasterol, 24-methylencholesterol, 22-dihydrospinasterol and some n-alkanes and n-alcohols.10,11) It has been shown to have significant anti-inflammatory, and antimicrobial12,13) antinociceptive14) and hepatoprotective15) activity.

A decoction prepared from leaves of S. rhomboidea ROXB. (SR) is used as a folklore medicine against obesity and diabetes in parts of North Eastern India. Recent studies from our laboratory have reported its hypolipidemic16) and antihypertriglyceremic17) activities. However, there are no reports on therapeutic role of SR leaf extract against exper-
imentally induced type II diabetes and insulin resistance. In continuation with research from our laboratory this inventory is an effort to evaluate the protective effect of SR leaf extract against high fat diet induced insulin resistance in C57BL/6J mice.

**MATERIALS AND METHODS**

**Plant Material** —— SR leaves were collected from Imphal district India in month of June and shade dried. The plant was identified by Dr. Hemchand Singh, Taxonomist, Department of Botany, Dhana Manjuri College of Science, Imphal and a sample (voucher specimen No. 216) was deposited at the herbarium of the Department of Botany.

**Preparation of Extract** —— Leaves of SR were shade dried and powdered. A hundred gram of the leaf powder was boiled in distilled water for 3 hr at 100°C. Resulting filtrate was concentrated by heating till it formed a semisolid paste which was then freeze dried. The yield was 24% W/W. Different doses of freeze dried extract of SR were mixed with either low fat diet or high fat diet.

**Experimental Animals** —— Male C57BL/6J mice (6–8 weeks of age) were purchased from National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. They were housed and maintained in clean polypropylene cages and fed with either low fat diet or high fat diet and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the animal ethical committee of Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara (Approval No. 827/ac/04/CPCSEA).

**Experimental Procedure** —— A total of 36 animals were randomly allocated into 6 groups of 6 animals each group. Groups I and II were fed with low fat diet (LFD) while groups III, IV, V and VI were fed with high fat diet (HFD) for 16 weeks. SR was fed to the experimental animals by mixing it with their respective diets as follows: LFD: Mice fed with low fat diet, LFD + SR3: Mice fed with low fat diet containing SR extract (3% w/w), HFD: Mice fed with high fat diet, HFD + SR1: Mice fed with high fat diet containing SR extract (1% w/w), HFD + SR3: Mice fed with high fat diet containing SR extract (3% w/w), and HFD + ROS: Mice fed with high fat diet containing rosiglitazone (0.05% w/w).

At the end of the experimental period, animals were given mild ether anaesthesia and blood was collected by retro orbital sinus puncture in EDTA coated vials. Plasma was obtained by cold centrifugation (4°C) of the vials for 10 min at 3000 rpm. Later animals were sacrificed by decapitation and liver, pancreas, brain and epididymal fat pad were excised and stored at −80°C for further estimations.

**Body Weight, Food Intake and Feed Efficiency** —— Known quantity of food (LFD or HFD) was given to the respective experimental groups and food intake was measured daily. Feed efficiency ratio (FER) was expressed as the total weight gain of an experimental animal during 16 weeks / the energy intake.

**Plasma and Hepatic Lipids** —— Plasma free fatty acid (FFA) was estimated by the method of Itaya and Ui while triglyceride (TG), total cholesterol (TC) contents were estimated by enzymatic kits (Merck Diagnostics, Ltd., Mumbai, India) using semi autoanalyser (Micro lab 300 L, Mercck). Total lipids were extracted from liver of control and experimental animals with chloroform : methanol and hepatic FFAs were assayed in the same. Known quantity of lipid extract was then dissolved in 1% TritonX100 and TC and TG were assayed using above mentioned kits.

**Blood Glucose, Plasma Insulin and Fasting Insulin Resistance Index** —— Animals were fasted overnight (for 12 hr) and later blood glucose was measured in whole blood sample obtained from tail vein (by one touch glucometer, elegance CT-X 10, Convergent technologies, Frankenberg, Germany). Plasma insulin was assayed using Mouse ELISA kit (Mercodia Developing Diagnostics Ltd., Uppsala, Sweden). Fasting insulin resistance index (FIRI) was expressed as: fasting insulin (pmol/l) × fasting blood glucose (mg/dl) / 25.

**Intraperitoneal Glucose Tolerance Test (IPGTT)** —— Fasting (12 hr) blood glucose was measured in whole blood obtained from tail vein (by one touch glucometer, elegance CT-X 10) obtained from tail vein (0 min). Later, glucose solution was injected intraperitoneally (2 g/kg) and blood glucose was assayed at 30, 60, 90 and 120 min and a graph was plotted. Area under the curve (AUCglucose) was calculated based of trapezoid rule (Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, U.S.A.).

**Intraperitoneal Insulin Tolerance Test (ITT)** —— Overnight fasted mice received in-
insulin (Aventis Pharma Deutschland GmbH, Mumbai, India) 0.2 U/kg body by slow intraperitoneal injection. Blood samples were collected from tail vein at 0 min (before administration) and subsequently at 10, 20, 30 and 60 min after administration of insulin. Blood glucose was measured in whole blood (by one touch glucometer, elegance CT-X 10). $K_{ITT}$ was determined with the formula: $K_{ITT} = 0.693 \times \frac{T_1}{T_{1/2}}$. Where $T_{1/2}$ is half-life of plasma glucose decay was obtained with the formula: $T_{1/2} = \frac{\ln 2}{\omega}$. Where, $\omega$ constant of plasma glucose disintegration was obtained with the formula: $\omega = \frac{\ln C_1 - \ln C_2/T_2 - T_1}{T_1}$ with glucose concentration $C_1$ at time $T_1$ (10 min) and $C_2$ at $T_2$ (60 min).22)

**Histology of Epididymal Fat Pad** —— Epididymal fat pad was fixed in 4% buffered paraformaldehyde, dehydrated in graded alcohol series and embedded in paraffin wax. Five µm sections were cut (by Leica RM 2115 Microtome) and stained with hematoxyline and eosin and examined under Leica microscope. Photographs of adipocytes were taken with Canon power shot S72 digital Camera (400 X). Adipocyte diameter was measured using eye piece occulometer to calculate adipocyte surface area.23)

**Statistical Analysis** —— Statistical evaluation of the data was done by one way Analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test and results were expressed as mean ± Standard error of the mean (S.E.M.) (Using Graph Pad Prism version 3.0).

### RESULTS AND DISCUSSION

In the present study, C57BL/6J mice were chosen as the experimental model as it develops insulin resistance, hyperglycaemia and obesity when fed with high fat diet.24) The array of physiological events in C57BL/6J mouse closely parallels the progression of diabetes and obesity in humans.25,26) Hence, this experimental model has been used widely for conducting preclinical investigations of various anti-diabetic and anti-obesity agents belonging to herbal and synthetic origin.27,28)

Though, there was no significant difference in food intake of LFD and HFD groups, significantly higher weight gain ($p < 0.05$) was recorded in HFD fed mice (Table 1) due to intake of high calorie diet. This resulted in greater abdominal fat deposition ($p < 0.05$) than the LFD fed mice. However, HFD induced higher weight gain and feed efficiency were significantly ($p < 0.05$) reduced by SR supplementation (Table 1). Hence, observed decrease in body weight gain and feed efficiency ratio can be due to decreased food intake recorded in HFD+SR groups (Table 1).

Individuals suffering from type II diabetes are often prone to cardiovascular complications resulting from dyslipidemia.29) Storage of fats in non adipose tissue leads to cellular damage which is known as lipotoxicity; an integral part of pathogenesis of type II diabetes.30) Significant decrement ($p < 0.05$) in plasma TC, TG and hepatic lipids recorded in HFD+SR groups (Table 2) indicates at role of SR in preventing diabetic dyslipidemia and lipotoxicity. These results are also in accordance with its previously reported lipid lowering property in HFD fed hyperlipidemic rats.16) Synthetic antidiabetic drugs commercially available in the market are often not able to tackle dyslipidemia, thus raising the risk of cardiovascular complication. Hence, these results can be considered significant against diabetic dyslipidemia.

There exists a close correlation between visceral adiposity and insulin resistance in obese diabetic subjects.31) Higher accumulation of abdominal fat leads to elevated circulating levels of FFA. In-

### Table 1. Effect of SR Extract and Rosiglitazone on Bodyweight, Food Intake and Feed Efficiency

<table>
<thead>
<tr>
<th></th>
<th>Bodyweight</th>
<th>Weight gain</th>
<th>Food intake</th>
<th>Feed efficiency</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Initial $^a$</td>
<td>Final $^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LFD</td>
<td>22.07 ± 0.58</td>
<td>27.77 ± 0.88</td>
<td>5.70 ± 0.44</td>
<td>2.14 ± 0.12</td>
</tr>
<tr>
<td>LFD+SR3</td>
<td>22.09 ± 0.65</td>
<td>27.06 ± 0.71</td>
<td>4.97 ± 0.52</td>
<td>1.30 ± 0.16</td>
</tr>
<tr>
<td>HFD</td>
<td>19.63 ± 0.85</td>
<td>38.22 ± 1.61 $^c$</td>
<td>18.59 ± 0.78 $^c$</td>
<td>2.08 ± 0.13NS $^c$</td>
</tr>
<tr>
<td>HFD+SR1</td>
<td>19.62 ± 0.85</td>
<td>28.43 ± 1.33$^c$</td>
<td>8.81 ± 0.97$^c$</td>
<td>1.33 ± 0.13$^c$</td>
</tr>
<tr>
<td>HFD+SR3</td>
<td>20.65 ± 0.84</td>
<td>25.18 ± 0.59$^c$</td>
<td>4.53 ± 0.77$^c$</td>
<td>1.07 ± 0.08$^c$</td>
</tr>
<tr>
<td>HFD+ROS</td>
<td>19.50 ± 0.72</td>
<td>27.10 ± 0.73$^c$</td>
<td>7.6 ± 0.55$^c$</td>
<td>2.05 ± 0.16$^c$</td>
</tr>
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Where $^a$: g and $^c$: g/day per mice. a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$ and NS : non significant when, LFD vs. HFD. A) $p < 0.05$, B) $p < 0.01$, C) $p < 0.001$ and NS : non significant when, HFD vs. HFD+SR1, HFD+SR3 and HFD+ROS.
In the present study, HFD fed mice recorded significant increment \( (p < 0.05) \) in plasma FFA, insulin, blood glucose and FIRI (Table 3). Significant decrement \( (p < 0.05) \) in plasma FFA levels in HFD+SR groups (Table 2) can be contributing towards improved insulin signalling and preventing insulin resistance that can be correlated with observed decrease \( (p < 0.05) \) in blood glucose, plasma insulin and FIRI (Table 3).

Large adipocytes are associated with insulin resistance, whereas the smaller ones are associated with insulin sensitivity and hence adipocyte hypertrophy in obese subjects is reported to be closely associated with insulin resistance.\(^{33,34} \) HFD fed mice recorded significant increase \( (p < 0.05) \) in epididymal fat pad, adipocyte diameter and adipocyte surface area (Table 3 and Fig. 1). SR supplementation to HFD fed mice prevented fat deposition.
(p < 0.05) and decreased adipocyte diameter and surface area (Table 3). It can be speculated that SR may control insulin sensitivity possibly due to its ability to inhibit adipocyte hypertrophy in obese animals. However, this study falls short in providing information on the role of SR in adipogenesis and adipocyte differentiation. Further studies have been initiated on the similar line in our laboratory to decipher the role of SR in adipogenesis and adipocyte differentiation. The ability of SR extract to decrease fat depots in HFD-fed mice is of relevance in treating type II diabetes/insulin resistance.

ITT is useful to assess insulin sensitivity by exogenous administration of insulin using \( K_{ITT} \) index. HFD+SR group recorded significant (p < 0.05) increase in \( K_{ITT} \) values compared to that of HFD group (Fig. 2) suggesting improved insulin sensitivity possibly by improving one or more defects viz. insulin receptor, insulin receptor substrate, glucose transporters or enzymes involved in phosphorylation of glucose.\(^{35,36}\) Glucose tolerance test is a simple and widely accepted method for indirect assessment of in vivo peripheral insulin action and insulin resistance in animals\(^{37}\) and humans.\(^{38}\) IPGTT was evaluated in the present study and it was observed that HFD-fed mice recorded significantly (p < 0.05) higher AUC\(_{\text{glucose}}\) values compared to LFD mice (Fig. 3). However, HFD+SR group recorded lower AUC\(_{\text{glucose}}\) values compared to HFD-fed mice. Overall glycemic response recorded in HFD+SR group during both ITT and IPGTT indicate that SR supplementation to HFD-fed mice has the potential of improving insulin sensitivity.

Although SR extract did not induce any significant alterations in plasma and hepatic lipid profile and glycemic parameters of LFD-fed mice, it reduced food intake significantly. This observation is in accordance with our previous report\(^{16}\) and the same has been attributed to presence of ephedrine in SR extract.\(^{39}\) Oral acute and chronic toxicity studies performed in our laboratory with SR extract reveal that there were no adverse behavioural, biochemical and histopathological changes by oral administration of SR extract up to a dose of 3000 mg/kg bodyweight (unpublished observations). This inventory is a first report in confirming the role of SR extract in improving experimental diabetes and insulin resistance, but further studies are required to investigate its insulin sensitising action and to decipher its role in controlling visceral adipocity. However, results obtained herein validate the use of SR leaves by the general populace of North-East India against diabetes and obesity thus strengthening its folklore claim.

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