MATERIAL AND METHODS
Healthy female albino rats of Wistar strain and spontaneously hypertensive (SH) rats of Okomoto Okai strain weighing 200-250g were used in the present investigation. All the animals were housed in a group of 3 rats per cage at 27-30 °C with 12 hour alternating light and dark cycles. The animals had free access to food and water. All animals were kept under uniform diet of the following composition:

- Wheat (60 %)
- Maize (20 %)
- Gram (10 %)
- Milk powder (2.5 %)
- Vegetable oil (5 %)
- Salt (5 %)

We have used four models of diabetes-mellitus and hypertension. For diabetes-mellitus we used rat models of both STZ-induced insulin dependent diabetes-mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). For hypertension we used the DOCA-salt hypertensive rat and the genetically hypertensive SH rat.

**Induction of insulin dependent diabetes-mellitus (IDDM) in adult rats:** Type I diabetes (IDDM) was induced by chemical destruction of pancreatic beta-cells using streptozotocin (STZ). STZ (45 mg/Kg) dissolved in citrate buffer (pH 4.5) was injected into the tail vein. Control rats received citrate buffer (pH 4.5) alone. Three days after the injection of STZ, rats were checked for extent of glycosuria using enzymatic test strips. Rats showing glucosuria greater than 2% were considered as diabetic and used for experiments.

**Induction of hypertension in STZ-diabetic or Wistar rats:** Although STZ-induced diabetic rats exhibited hypertension, deoxycorticosterone acetate (DOCA) was used to induce hypertension in STZ-diabetic or Wistar rats. DOCA (5 mg/Kg) was administered subcutaneously every day to rats. These animals were also given 2.5 % NaCl in drinking water. Treatment with DOCA was initiated 7 days after STZ treatment. DOCA was suspended in a vehicle containing 1.8 g NaCl, 1.8 g benzyl alcohol, 1.0 g carboxymethylcellulose (CMC), 0.8 g polysorbate 80, made upto 200 ml with distilled...
water. Control animals received equivalent amount of the vehicle. Animals showed development of hypertension ten days after initiation of DOCA treatment.

**Induction of non-insulin dependent diabetes mellitus (NIDDM):** Neonatal administration of STZ to rat pups produces a condition of NIDDM in later life (Wier et al 1981). Most of the beta-cells are destroyed by STZ treatment to pups, however, there is subsequent regeneration of beta-cells resulting in 25-50% beta-cell mass when compared to control rats (Weir et al 1981). To induce NIDDM, healthy albino Wistar rats were allowed to breed. Rat pups to be made diabetic were administered 90 mg/Kg (i.p.) STZ dissolved in normal saline on day 5 after birth. Control rat pups were administered saline alone. Pups were weaned a month later and reared under standard conditions. At 16 weeks of age, the female STZ treated Wistar rats were checked for serum glucose levels. Rats showing serum glucose levels greater than 150 mg/dl were considered as neonatal STZ-diabetic and used for the experiments. Rats that had received only saline on day 5 of birth were considered as control rats.

**Grouping of animals and treatment schedule:** Rats belonging to each experimental model group (namely IDDM, NIDDM, DOCA hypertensive and SH) were divided into subgroups: control and treated. For each experimental set, a group of control Wistar rats was maintained and subjected to similar experimental procedures as the rat models of diabetes and/or hypertension. Thus in all sets of experiments there were three groups as follows:

<table>
<thead>
<tr>
<th>STZ-diabetic</th>
<th>Neonatal STZ-diabetic</th>
<th>SH</th>
<th>DOCA-hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar control</td>
<td>Wistar control</td>
<td>Wistar control</td>
<td>Wistar control</td>
</tr>
<tr>
<td>STZ-diabetic control</td>
<td>Neonatal STZ-diabetic control</td>
<td>SH control</td>
<td>DOCA-hypertensive control</td>
</tr>
<tr>
<td>STZ-diabetic treated</td>
<td>Neonatal STZ-diabetic treated</td>
<td>SH treated</td>
<td>DOCA-hypertensive treated</td>
</tr>
</tbody>
</table>

Treatment with antihypertensive drug was given daily for six weeks. The antihypertensive agents used were nifedipine (5 mg/Kg), amlodipine (5 mg/Kg), ramipril (1 mg/Kg) and spirapril (2 mg/Kg). Rats were treated with these agents for six weeks. Nifedipine was suspended in 1% CMC for per oral administration. Amlodipine, ramipril or
spirapril were dissolved in distilled water and administered by the per oral route. Experimental control rats received an equivalent volume of distilled water or 1% CMC in case of nifedipine.

To observe any change in body weight, food and water intake of rats these were measured initially and then every week. Before initiation of treatment and at the end of six weeks, systolic blood pressure and heart rate were measured by the indirect tail-cuff method. At the end of treatment blood samples were collected from the retro-orbital plexus and serum was analyzed for glucose, insulin, T₃, TSH, total cholesterol, triglyceride, GPT and creatinine. On termination of treatment 18-hour fasted rats were given an oral glucose load (1.5 g/Kg) and serum levels of insulin and glucose were estimated at 0, 30, 60 and 120 minutes. Animals were also subjected insulin tolerance test at the end of six weeks. Finally, animals were sacrificed, their liver and skeletal muscle (gastrocnemius/diaphragm) were dissected and liver phosphorylase and glycogen content as well as skeletal muscle glycogen content were estimated spectrophotometrically.

**Blood pressure and heart rate measurements:** Indirect blood pressure measurement (systolic) was done using the tail-cuff method. Rats were transferred from animal house and taken to a quiet room in the morning hours. They were allowed free access to food and water. Rats were then placed in a comfortable restrainer. Rats were preconditioned to the blood pressure measurement procedure. The tail cuff used was selected by taking into consideration the thickness of tail. The cuff was selected such that it was neither too tight nor too loose when placed 1 cm below the base of the rat tail. The Harvard blood pressure monitor cum students’ oscillograph (Harvard Apparatus Ltd, UK) was used for measuring systolic blood pressure and heart rate. Rat tail was introduced into the cuff. Baseline of the pressure pen in the oscillograph was adjusted. Pressure was so calibrated (using the 'calibrate' knob in the monitor and gain knob in the oscillograph) that 10 divisions on the chart paper were equivalent to 100 mmHg pressure. After calibration of the device, the pulse pen was adjusted to give minimum pulsations by adjusting the gain knobs in the monitor and oscillograph simultaneously. The chart drive was set on speed 0.5 mm/sec and the cuff was inflated till the monitor showed 200-250 mmHg pressure. The pressure in the cuff started decaying gradually since the valve in the pressure bulb had previously been adjusted. At this stage the pulse pen did not show any pulsations.
The reappearance of pulsations (on gradual deflation) signified the systolic blood pressure. At this point the chart speed was increased to 20 mm/sec for approximately 80 mm of the chart paper. Number of peaks in 75 mm of the chart paper were multiplied by 20 to get the heart rate in beats/minute.

**Collection of blood samples:** At the end of treatment, blood samples were collected from the retino-orbital plexus of rats fasted for 18 hours. Serum was obtained and analyzed for glucose, insulin, total cholesterol, triglycerides, T₃, TSH, glutamate pyruvate translocase (GPT), and creatinine. Rats were initially sedated by light ether anesthesia. Immediately, heparinized glass capillaries were introduced into the plexus after exposing the eye ball. Blood was collected in storage tubes. After clotting, samples were centrifuged at 300 rpm for 15 minutes to separate the serum which was transferred carefully into clean storage tubes. A small sample of serum was analyzed for glucose on the same day. 100 μl of the serum sample was stored in another set of clean storage tubes and stored at -20 °C until analyzed for insulin. The rest of the collected serum was stored at -20 °C until other analyses.

**Oral glucose tolerance test (OGTT):** OGTT was conducted to study the glucose-stimulated insulin release and the insulin-mediated glucose disposal in rats since this would give some idea about insulin sensitivity in rats. At the end of treatment animals were fasted for 18 hours. Animals were then given a glucose challenge by administering 1.5 g/Kg glucose (dissolved in water) by per oral route. Blood samples were collected at 0, 30, 60 and 120 minutes of oral glucose load. Serum was collected and analyzed for glucose immediately. Serum samples were stored at -20 °C until analyzed for insulin. Insulin levels were measured within 10 days of collection of serum by radioimmunoassay. Glucose concentrations at 0, 30, 60 and 120 minutes were calculated and expressed in mg/dl of blood. Insulin levels were calculated by linear regression obtained by plotting the standard B/B₀ values on a logit-log scale. Insulin concentrations were expressed in uU/ml. Plotting the glucose or insulin concentrations versus time would give only a vague idea about alterations in insulin release in response to oral glucose load and insulin-mediated glucose disposal. Therefore, the integrated area under OGTT curves were calculated by the trapezoid rule:

\[
\text{AUC} = C_1 + C_2 \int_{t_1}^{t_2} \frac{1}{2} (t_2 - t_1)
\]
and changes in glucose and insulin concentrations during OGTT were expressed as AUC glucose (mg/dl . min) and AUC insulin (uU/ml . min) respectively.

**Determination of insulin sensitivity index (K_{ITT}):** Insulin sensitivity was measured by insulin tolerance test (ITT) described by Alford et al (1971) using K_{ITT} as an index of insulin mediated glucose metabolism. Rats were fasted for 18 hours. Initial, or 0 minute blood samples were collected to determine the basal glucose level. Thereafter, 0.2 U/100 g body weight of purified porcine insulin (Actrapid Novo, Ahmedabad, India) was injected intravenously. Blood samples were collected at 5, 10, 20, and 30 minutes for estimation of glucose. The K_{ITT} was derived from the slope of the linear portion of the regression line of % reduction in glucose versus time. The formula used was as follows:

\[
K_{ITT} = \frac{0.693 \times 100}{T^{1/2}},
\]

where \(T^{1/2}\) represents the half life of plasma glucose decay. The half life of plasma glucose was obtained by plotting % reduction in plasma glucose concentrations and time.

**Glycogen content and liver phosphorylase activity (LPA):** Insulin affects the peripheral glucose uptake and any alteration in skeletal muscle glycogen content would give information about changes in peripheral glucoregulatory action of insulin. Moreover, insulin resistance is associated with defective peripheral glucose disposal. Further, since insulin has significant effects on hepatic output of glucose, changes in insulin sensitivity could alter liver glycogen content and liver phosphorylase enzyme activity which is responsible for glycogenolysis and is normally inactivated by insulin. In the light of these, the liver and skeletal muscle glycogen content and the liver phosphorylase activity were measured.

**Statistical analysis:** All data are presented as means ± SEM. Whenever comparison between two values was carried out the first independent variable was the untreated Wistar control whereas, in the second comparison the independent variable was the antihypertensive treated group. One-way analysis of variance (ANOVA) was used to test...
statistical significance of the several dependent variables (body weight, blood pressure, heart rate, glucose, insulin, lipids and other biochemical parameters. Once the F ratio was computed from the ANOVA, the within mean square for the ANOVA was used for the Tukey's multiple range test. Q value was computed and compared with standard table at degrees of freedom (d.f.) for error for the within mean square in the one-way ANOVA. If computed Q was greater than the tabulated Q value then the independent variable was considered significantly different. For the present work statistical significance was tested at 5% level.