

EFFECT OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* (BURM. F.) NEES ON A COMBINATION OF FAT-FED DIET AND LOW DOSE STREPTOZOTOCIN INDUCED CHRONIC INSULIN RESISTANCE IN RATS

Rammohan Subramanian¹, Mohd. Zaini Asmawi¹, Amirin Sadikun²

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oral administration of Andrographis paniculata ethanolic extract may have the ability to improve insulin sensitivity and delay the development of insulin resistance, and may thus have a role in amelioration of insulin resistance in patients. However, its potential use in humans can only be validated by thorough investigation.

SUMMARY

Andrographis paniculata ethanolic extract was evaluated to screen the effect on insulin resistance using a combination of fat-fed diet and low dose streptozotocin. The glucose-insulin index as a measure of insulin action on glucose disposal rate was calculated during the intraperitoneal glucose tolerance test. Oral administration of 1000 mg/kg extract to rats was able to cause a significant ($p < 0.05$) reduction of elevated glucose-insulin index, signifying a potential insulin sensitizing effect. Oral administration of the extract at a dose of 1000 mg/kg once daily for 30 days to streptozotocin-diabetic rats increased the hypoglycemic responses to incremental dosing of exogenous insulin, thus causing an increase in insulin sensitivity. The results seem to suggest that

INTRODUCTION

Insulin resistance is defined as a state where normal or elevated insulin level produces a reduced biological response (1). It classically refers to impaired sensitivity to insulin mediated glucose disposal (2). Insulin resistance syndrome implies a series of abnormalities that occur frequently in insulin resistant individuals. These include glucose intolerance, dyslipidemia, endothelial dysfunction, elevated procoagulant factors, hemodynamic changes, elevated inflammatory markers, abnormal uric acid metabolism, increased ovarian testosterone secretion and sleep-disordered breathing (2). Clinical syndromes associated with insulin resistance include type 2 diabetes, cardiovascular disease, essential hypertension, polycystic ovary syndrome, non-alcoholic fatty liver disease, certain forms of cancer and sleep apnea. Type 2 diabetes and metabolic syndrome are the most common clinical syndromes associated with insulin resistance and frequently indicated in the etiology of hypertension,

Corresponding author: Rammohan Subramanian, Department of Pharmacology, School of Pharmacy, University Sains Malaysia, Penang, Malaysia
E-mail: rmohans02@yahoo.co.in

polycystic ovarian syndrome, non-alcoholic fatty liver disease, certain forms of cancer and obstructive sleep apnea (2).

It is of utmost importance to establish an insulin resistance animal model, in order to have better understanding of the pathological process of insulin resistance and to develop therapeutic drugs. Several insulin resistance animal models are available, including hereditary ob/ob mice and Sprague Dawley or Wistar rat models developed by either injecting low-dose dexamethasone (2 µg/day) into abdominal cavity or feeding food rich in fructose and sucrose (3-8). The major disadvantages of all these animal models are the long experimental cycles (4-30 wk) and the less relevant pathologic status of insulin resistance induced by a sole factor as opposed to the natural multi-factorial process.

Rat models of only high-fat fed diet develop obesity, hyperinsulinemia, and insulin resistance and not frank hyperglycemia or diabetes, thus limiting the screening of agents that control only the blood glucose level (9). High dose streptozotocin model of diabetes predominantly causes an insulin deficient condition resembling type 1 diabetes. Thus, we embarked upon the objective of developing a suitable insulin resistant rat model that would closely mimic the natural human disease progression on the one hand, and on the other show metabolic features of type 2 diabetes, at the same time being simple, inexpensive, easily inducible and useful for both the investigation and preclinical testing of various compounds for the treatment of type 2 diabetes. The rationale for combining a high fat-fed diet and low dose streptozotocin (10-12) was to produce insulin resistance and also cause the initial beta cell dysfunction with subsequent frank hyperglycemia in adult Sprague-Dawley rats. The present study was carried out to evaluate the effects of ethanolic extract of *Andrographis paniculata* on chronic insulin resistance in adult rats with similar pathophysiological characteristics as humans.

MATERIALS AND METHODS

Plant material and preparation of extracts

Dried leaves and aerial parts of *Andrographis paniculata* were obtained from cultivated nurseries of the Malaysian Agriculture Development Institute (MARDI), Kelantan. The dried leaves were powdered using a milling machine and extracted with 95% v/v ethanol (R&M Chemicals, Essex, UK) using the technique of cold maceration. The solvent was replenished every 24 h for 7 days. The extracts from previous days were pooled, filtered, and separated from the marc through a porous plug of absorbable cotton and concentrated at 60°C by a rotatory evaporator (Buchi Labortechnik, Flawil, Switzerland). To obtain dry powder, the extract was placed in a freeze drier (Labconco Corporation, Kansas City, Missouri, USA) under controlled conditions of low pressure and temperature for 24 hours after been kept in a freezer at -40 to -50°C for 8 hours (overnight).

Chemicals

Stripped lard (Acros Organics, Geel, Belgium), 6n-propyl- 2-thiouracil (Sigma Aldrich Chemical Co., US) cholesterol (95% purity, Acros Organics, Geel, Belgium), sodium glutamate (99-100% purity, Sigma Aldrich Chemical Co., US), sucrose (BDH Chemicals Ltd., Poole, England), Tween 80 (R&M Chemicals, Essex, UK), oleic acid (99% purity, Sigma Aldrich Chemical Co., US), streptozotocin (98% purity, Sigma Aldrich Chemical Co., US), and sodium citrate (Sigma Aldrich Chemical Co., US).

Preparation of fat emulsion (13)

Fat emulsion was prepared according to Jing *et al.*, with some slight modification: 500 mL of fat emulsion containing 100 g lard, 5g 6n-propyl-2-thiouracil, 25 g cholesterol, 5 g sodium glutamate, 50 g sucrose, 100 mL Tween 80, 150 mL oleic acid and remaining volume made up by adding distilled water. The fat components and water components were added along with Tween 80, oleic acid, triturated slowly and steadily in a mortar and pestle with minimum quantity of water to effect a milky white primary emulsion. The process was carried on until there was a distinct crackling noise resulting in primary

emulsion with a soft, milky white appearance. Volume was made up with addition of water to 500 mL with further mixing. The emulsion was stored in an amber color bottle at 4°C.

Experimental animals

Female Sprague Dawley rats weighing around 200-275 g were randomly divided into groups receiving oil vehicle (normal control group), and high fat emulsion daily for 10 days. After 10 days of oral administration of fat emulsion, all rats were administered a single low dose intraperitoneal injection of 35 mg/kg streptozotocin (14) in ice cold citrate buffer pH 4.5 (Cyberscan® 500 pH meter, Eutech Cybernetics, Singapore); the above normal control group injected with ice cold citrate buffer pH 4.5 and rats showing blood glucose around 8-12 mmol/L were included in the study.

Measurement of the glucose-insulin index (15)

Fat-fed and low dose streptozotocin-injected rats were used as insulin-resistant animals (14). The insulin resistant rats were orally treated with various doses of 95% v/v 250 mg, 500 mg, 1000 mg/kg designated as D1, D2, and D3, pioglitazone (PG) 30 mg/kg, andrographolide (AG) 10 mg/kg, normal control rats (NC). Insulin resistant control rats (IRC) were administered distilled water daily for 30 days. The intraperitoneal glucose tolerance test (IPGTT) was performed in insulin-resistant rats after 30 days of respective treatments.

Intraperitoneal glucose tolerance test (IPGTT) (16)

After 30 days, body weights were measured for each rat. Overnight fasted animals were used for the i.p. injection of glucose 1.0 g/kg. Blood was collected without anticoagulant, allowed to clot, and then centrifuged at 5000 rpm for 5 min. The supernatant serum was collected and separately stored in individual disposable Eppendorf® microcentrifuge tubes. Serum glucose was determined immediately using blood samples from the tail vein taken at time 0 (before glucose injection), 15, 30, 45, 60, 90, and 120 min after glucose administration. Serum was stored at -70 °C until insulin analysis. The serum glucose concentration was

determined using the glucose oxidase method with a portable Accu-Chek® Advantage II Clinical Glucose meter (Roche Diagnostics, Mannheim, Germany). An ELISA test was performed to measure serum insulin using commercially available kit (Crystal Chem Inc., IL, US). The glucose-insulin index was calculated as the product of the serum glucose and serum insulin areas under the curve (AUC), described by Peth *et al.* (15).

Tolbutamide-induced hypoglycemic challenge test (17)

Fat-fed and streptozotocin injected rats were designated into respective treatment groups of D3 (1000 mg/kg) of the extract, pioglitazone 30 mg/kg (PG) (Oglo 30, Panacea Biotec, India), norm cntrl (NC), insulin resistant cntrl (IRC) (distilled water), and AG (10 mg/kg) and treated orally. The 1000 mg/kg (D3) dose of the extract was selected based on the above positive IPGTT results. Oral treatment was also continued with pioglitazone (30 mg/kg) (Oglo 30, Panacea Biotec, India), normal cntrl rats (NC), insulin resistant cntrl (distilled water), and AG (10 mg/kg) for 21 days. The development of insulin resistance was identified using the loss of tolbutamide-induced hypoglycemic activity. Briefly, rats received an i.p. injection of 10 mg/kg tolbutamide (Orinase® Pharmacia & Upjohn Company, US) at 5 h of the treatment with 1000 mg/kg of the extract. Effects on fasting serum glucose and serum insulin were determined using blood samples collected from tail vein of rats at 1 h after tolbutamide injection. The hypoglycemic activity of tolbutamide was calculated as percentage decrease of the initial value according to the following formula:

$$\left(\frac{C_i - C_t}{C_i} \right) \times 100 \%$$

where C_i is the initial serum glucose concentration (the blood sample was taken before treatment during the fasting state) and C_t is the serum glucose concentration after treatment with tolbutamide.

Insulin sensitivity test (16)

Streptozotocin-diabetic rats were used to investigate the response to exogenous insulin. Streptozotocin at a dose of 45 mg/kg in ice cold citrate buffer pH 4.5 (Cyberscan®

500 pH meter, Eutech Cybernetics, Singapore) was injected intraperitoneally and resulting diabetic rats received an i.p. injection of long-acting human insulin 1 IU/kg (Lantus[®], Insulin glargine, Aventis Pharma Deutschland GmbH, Frankfurt am Main, Germany) once daily. Rats were also orally administered their respective treatments with 1000 mg/kg of extract, metformin 500 mg/kg (Glucophage[®], Lipha Pharm Ltd., United Kingdom), normal ctrl rats (NC), diabetic control (DC; 4 mL/kg ice cold citrate buffer pH 4.5), and AG (10 mg/kg) daily for 30 days with i.p. injection of long-acting human insulin continued for 30 days. After 30 days, a subcutaneous insulin challenge test was performed by injecting short-acting human insulin (Actrapid HM[®], Novo Nordisk, Bagsvaerd, Denmark) at doses of 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 IU/kg in these rats. Each of the rats was given all the doses of insulin one after another at 2-hour intervals. Blood samples were drawn at 30 min following insulin injection for measurement of blood glucose and serum glucose lowering action was found by applying the formula:

$$\left(\frac{C_0 - C_i}{C_0} \right) \times 100 \%$$

where C_0 is the initial serum glucose concentration (the blood sample was taken before treatment during the fasting state) and C_i is the serum glucose concentration after treatment with insulin.

Statistical analysis

All data were expressed as mean \pm SEM. Differences in the means between the groups were statistically analyzed by one way ANOVA followed by Tukey's test for post hoc analysis; $p < 0.05$ was considered significant.

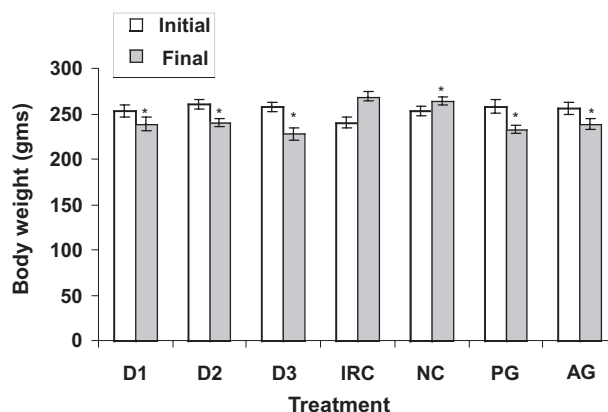
RESULTS

Body weight

IRC group showed a significant ($p < 0.05$) increase in body weights after 10-day administration of fat emulsion followed by a single intraperitoneal injection of streptozotocin. NC group showed a moderate but significant ($p < 0.05$) increase in body weight. Extract treated groups D1, D2, and D3 of 95% v/v ethanolic

extract showed a significant ($p < 0.05$) decrease in body weights in comparison with IRC group. Figure 1 illustrates the effect of treatment with various doses of 95% v/v extract. PG also showed a significant ($p < 0.05$) reduction in body weight upon daily treatment for 30 days. AG also exhibited a significant ($p < 0.05$) decrease in body weight.

Figure 1. **Body weight changes of rats administered the extract after 30 days.** D1 = 250 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata* (AP); D2 = 500 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D3 = 1000 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg.



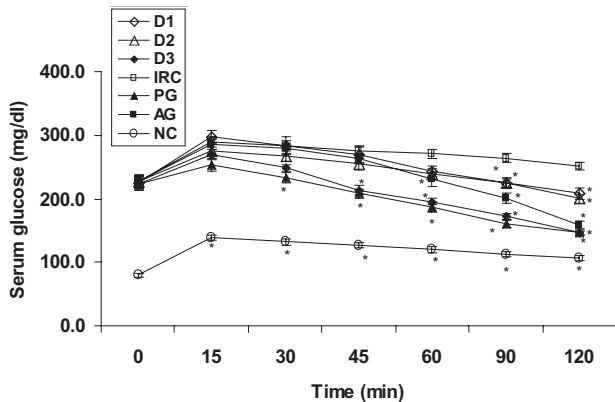
Values are mean \pm SEM (n=6) rats per group; * $p < 0.05$ compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

Measurement of the glucose-insulin index and IPGTT

Insulin resistant control rats demonstrated significant ($p < 0.05$) increments in serum glucose and insulin levels when compared to normal rats at all time points (Figs. 2 and 3). There were also significant ($p < 0.05$) increases in AUC level of serum glucose (Fig. 4) (increased to more than double compared to NC rats) and serum insulin (Fig. 4) when compared with AUC levels of serum glucose and insulin concentration in normal rats. There was also a significant ($p < 0.05$) increase in the measure of glucose-insulin index (Fig. 5) in IRC rats compared to NC group.

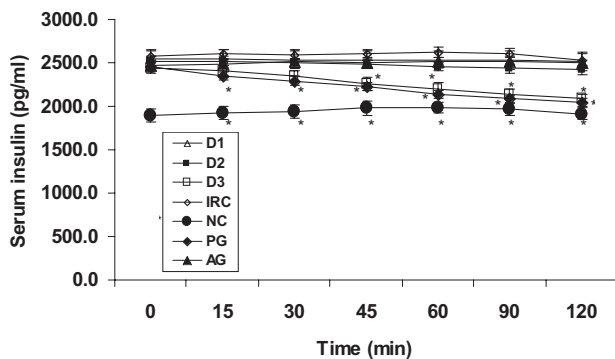
D1 (only at 90 and 120 min), D2 (only at 90 and 120 min), and D3 (at 45, 60, 90 and 120 min) dose levels of 95% v/v ethanolic extract treated groups showed

Figure 2. Serum glucose responses during the intraperitoneal glucose tolerance test (IPGTT) in insulin resistance rats administered the extract. D1 = 250 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D2 = 500 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D3 = 1000mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg. Glucose at 1.0 g/kg was used for the IPGTT.



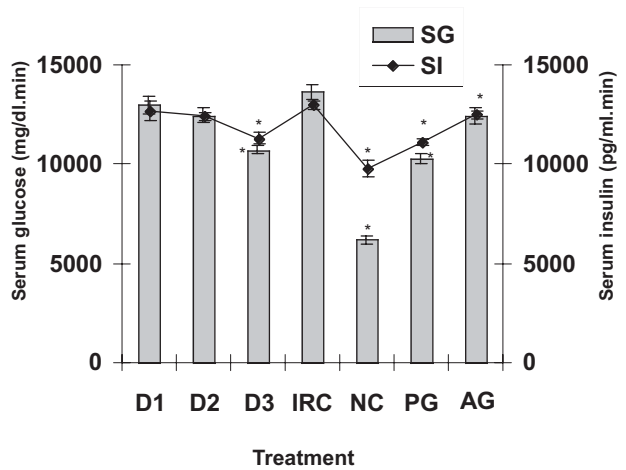
Values are mean±SEM (n=6) rats per group; *p<0.05 compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

Figure 3. Serum insulin responses during the intraperitoneal glucose tolerance test (IPGTT) in insulin resistance rats administered the extract. D1 = 250 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D2 = 500 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D3 = 1000 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg; glucose at 1.0 g/kg was used for the IPGTT.



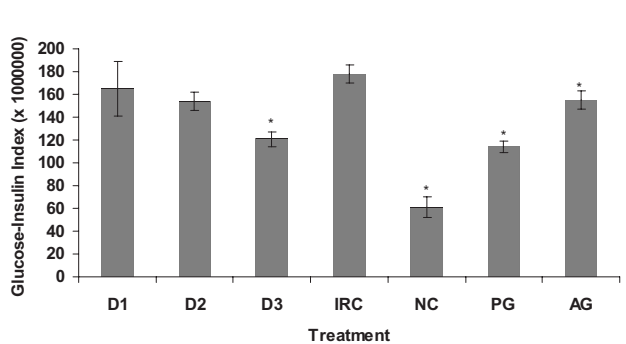
Values are mean±SEM (n=6) rats per group; *p<0.05 compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

Figure 4. Incremental areas under the curves (AUC) for serum levels of glucose and insulin in rats administered the extract during the IPGTT. D1 = 250 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D2 = 500 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D3 = 1000 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg; glucose at 1.0 g/kg was used.



Values are mean±SEM (n=6) rats per group; *p<0.05 compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

Figure 5. Glucose-insulin index calculated as the product of serum glucose AUC and serum insulin AUC in rats administered extract. D1 = 250 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D2 = 500 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; D3 = 1000 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg; glucose at 1.0 g/kg was used.



Values are mean±SEM (n=6) rats per group; *p<0.05 compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

Table 1. Effect of tolbutamide on serum glucose, insulin levels, and serum glucose lowering activity pretreated with extract during tolbutamide-induced hypoglycemic challenge test

| Treatment | Serum glucose (mmol/L) | | Serum insulin (ng/mL) | | Hypoglycemic activity (%) |
|-----------|------------------------|-----------|-----------------------|-------------|---------------------------|
| | Day 0 | Day 21 | Day 0 | Day 21 | |
| D3 | 10.9±0.27 | 8.4±0.17 | 2.465±0.045 | 2.375±0.046 | 22.2* |
| IRC | 10.6±0.30 | 10.3±0.26 | 2.631±0.072 | 2.634±0.071 | 2.5 |
| NC | 4.4±0.04 | 3.3±0.04 | 1.785±0.040 | 1.791±0.041 | 24.0* |
| PG | 11.0±0.45 | 8.7±0.21 | 2.521±0.044 | 2.387±0.051 | 20.2* |
| AG | 11.0±0.43 | 10.5±0.35 | 2.469±0.060 | 2.480±0.063 | 4.4 |

D3 = 1000 mg/kg 95%v/v ethanolic extract of *Andrographis paniculata*; IRC = insulin resistant control; NC = normal control; PG = pioglitazone 30 mg/kg; AG = andrographolide 10 mg/kg; values are mean±SEM (n=6) rats per group; *p<0.05 compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

significant (p<0.05) reduction in serum glucose levels. In case of D1 and D2 groups there was a significant (p<0.05) reduction in serum glucose levels only at 90 and 120 min, whereas in case of D3 group significant (p<0.05) reductions in serum glucose were observed at 45, 60, 90 and 120 min during IPGTT. AUC for the serum glucose response showed a significant (p<0.05) decrease in comparison to IRC rats only for D3 group. Neither D1 nor D2 group showed any significant reduction in serum glucose AUC levels. Serum insulin AUC levels after treatment with D1 and D2 revealed no significant increase after treatment compared to IRC rats. However, only D3 group showed a significant (p<0.05) decrease in serum insulin level when compared to vehicle treated IRC rats. Only D3 showed a significant (p<0.05) decrease in the glucose-index levels compared to IRC group. D1 and D2 showed no significant reductions in the glucose-insulin index values.

Treatment with PG produced significant (p<0.05) reductions in serum glucose levels at all time points during IPGTT compared to IRC rats. There were also significant (p<0.05) reductions in serum insulin concentration in comparison to IRC group. The incremental AUC for serum glucose concentration showed significant (p<0.05) reduction. Incremental AUC for serum insulin level also exhibited significant (p<0.05) reductions during IPGTT. There was also a significant (p<0.05) improvement in the glucose-insulin index.

Treatment with AG led to significant (p<0.05) reductions in serum glucose levels at 60, 90 and 120 min compared to IRC rats. However, AG treatment induced no significant reductions in serum insulin levels. Incremental AUC levels for serum glucose showed a

slight but significant (p<0.05) reduction in comparison to IRC rats, but no significant reduction was observed in case of incremental AUC level of serum insulin.

Tolbutamide-induced hypoglycemic challenge test

IRC rats showed a significantly (p<0.05) higher serum glucose concentration of 10.6±0.30 mmol/L on day 0 compared to NC rats. The serum glucose lowering activity was also significantly (p<0.05) less compared to NC rats (Table 1). Serum insulin concentration was also found to be significantly (p<0.05) increased compared to NC rats.

D3 group rats showed a significant (p<0.05) lowering of serum glucose concentration in response to tolbutamide compared to IRC rats (Table 1). The serum glucose lowering activity was also observed to be significantly (p<0.5) higher compared to IRC rats. Furthermore, a slight but significant (p<0.05) lowering of serum insulin concentration was observed in comparison with IRC rats.

In normal rats, serum glucose decreased significantly (p<0.05) from day 0 value of 4.4± 0.04 mmol/L to 3.3±0.04 mmol/L, along with a significant increase (p<0.05) in serum glucose lowering activity (around 21.4±3.5%) compared to IRC rats. The serum insulin concentration did not change significantly from day 0 value of 1.785±0.040 ng/mL to 1.791±0.041 ng/mL on day 21 compared to IRC rats (Table 1).

In case of PG group, serum glucose decreased significantly (p<0.05) from day 0 value of 11.0±0.45 mmol/L to 8.7±0.21 mmol/L. At the same time, there was no significant difference in serum glucose level of IRC

rats. The serum glucose lowering activity in response to tolbutamide challenge was also observed to be significantly ($p < 0.05$) higher than in IRC rats. Furthermore, a significant ($p < 0.05$) decrease in serum insulin level was found as compared to IRC group rats. PG also showed a tendency to delay the occurrence of insulin resistance as shown by a higher serum glucose lowering activity than in IRC rats that exhibited complete loss of serum glucose lowering activity in response to tolbutamide (Table 1).

AG did not show any significant decrease in serum glucose concentration compared to IRC rats. Furthermore, no significant decrease of serum glucose lowering activity was found in response to tolbutamide (Table 1). Serum insulin levels were not significantly affected compared to IRC rats either.

Insulin sensitivity test

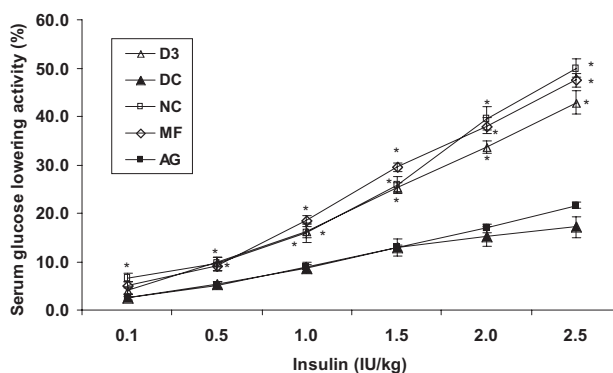
The basal serum glucose concentration in DC rats before initiation of insulin treatment was found to be 11.6 ± 0.34 mmol/L (Fig. 6). Upon administration of 0.1 IU/kg insulin, the serum glucose lowering activity was $2.6 \pm 0.8\%$. The serum glucose lowering activity rose to double value of $5.2 \pm 0.7\%$ upon increasing insulin dose five times to 0.5 IU/kg. In DC rats, administration of 1 IU/kg dose of insulin led to further increase of serum glucose lowering activity to $8.5 \pm 0.9\%$. A $13 \pm 1.8\%$ serum glucose lowering activity was found upon injecting 1.5 IU/kg to DC rats (Fig. 6). The serum glucose lowering activity increased marginally to $15.2 \pm 2.0\%$ upon dosing with 2 IU/kg insulin s.c. Administration of 2.5 IU/kg dose of insulin increased fractionally the serum glucose lowering activity to $17.1 \pm 2.2\%$.

NC rats before streptozotocin administration were found to have serum blood glucose of 4.5 ± 0.22 mmol/L. After 0.1 IU/kg dose of insulin in NC rats, the serum glucose lowering activity was at $6.7 \pm 1.5\%$, which was also significant ($p < 0.05$). Upon increasing the dose of insulin five times to 0.5 IU/kg, the serum glucose lowering activity also rose sharply to $9.5 \pm 1.3\%$, which was significant ($p < 0.05$). Administration of 1 IU/kg dose of insulin increased significantly ($p < 0.05$) the serum glucose lowering activity to $15.9 \pm 2.0\%$ (Fig. 6). At 1.5 IU/kg dose of insulin, the serum glucose lowering

activity of NC rats showed a significant increase to $25.8 \pm 1.7\%$. Furthermore, the serum glucose lowering activity showed an upward trend rising significantly ($p < 0.05$) to $39.6 \pm 2.3\%$ upon s.c. injection of insulin at 2 IU/kg. And finally, at a dose of 2.5 IU/kg insulin, the serum glucose lowering activity rose significantly ($p < 0.05$) by around 11% to $50 \pm 1.9\%$ (Fig. 6).

In D3 group, serum glucose concentration was found to be 11.6 ± 0.34 mmol/L before treatment with insulin. After injection with 0.1 IU/kg dose of insulin, the serum glucose lowering activity was $4.0 \pm 0.7\%$ (Fig. 6), which was also significant ($p < 0.05$). Upon treatment with 0.5 IU/kg dose of insulin, the serum glucose lowering activity increased significantly ($p < 0.05$) to more than double at $9.9 \pm 1.0\%$. The serum glucose lowering activity further increased significantly ($p < 0.05$) to $16.3 \pm 1.3\%$ upon treatment with 1 IU/kg. A significant ($p < 0.05$) increase to $25.4 \pm 1.0\%$ was found upon treatment with 1.5 IU/kg. Further significant ($p < 0.05$) increase to $33.7 \pm 1.3\%$ in serum glucose lowering activity was observed upon s.c. injection with 2 IU/kg dose of insulin. Finally, the highest dose of s.c. insulin 2.5 IU/kg produced a significant ($p < 0.05$) increase to $42.9 \pm 2.3\%$ in serum glucose lowering activity.

Figure 6. Effect of 95% v/v ethanolic extract of *Andrographis paniculata* on insulin sensitivity test in streptozotocin-induced diabetic rats. D3 = 1000 mg/kg 95% v/v ethanolic extract of *Andrographis paniculata*; DC = diabetic control; NC = normal control; MF = metformin 500 mg/kg; AG = andrographolide 10 mg/kg.



Values are mean \pm SEM (n=6) rats per group; * $p < 0.05$ compared with insulin resistant control rats; one way ANOVA followed by Tukey's test for post hoc analysis.

The initial serum glucose level in MF group before initiating insulin treatment was 11.7 ± 0.39 mmol/L (Fig. 6). In metformin treated rats, 0.1 IU/kg dose of insulin produced serum glucose lowering activity of $5.1 \pm 0.6\%$, which was significant ($p < 0.05$). The 0.5 IU/kg dose of insulin in metformin treated rats showed lowering of serum glucose to $9.0 \pm 0.8\%$ and was also significant ($p < 0.05$) (Fig. 6). On administering a dose of 1 IU/kg insulin, serum glucose lowering activity of $18.4 \pm 1.2\%$ was observed and was also significant ($p < 0.05$). The serum glucose lowering activity increased to $29.5 \pm 0.9\%$ and was also significant ($p < 0.05$) after administering a dose of 1.5 IU/kg insulin. A further significant ($p < 0.05$) increase in serum glucose lowering activity to $38.1 \pm 0.16\%$ was observed in metformin treated rats administered 2 IU/kg insulin dose. Finally, 2.5 IU/kg dose of exogenous insulin produced $47.5 \pm 1.4\%$ in serum glucose lowering activity and was also significant ($p < 0.05$).

Basal serum glucose in AG treatment group before insulin administration was around 11.2 ± 0.50 mmol/L. Upon administration of 0.1 IU/kg dose of insulin, the serum glucose lowering activity was $2.5 \pm 0.4\%$. The serum glucose lowering activity slightly increased to $5.1 \pm 0.8\%$ upon increasing insulin dose to 0.5 IU/kg. Upon treatment with 1 IU/kg dose of insulin, AG treated rats showed serum glucose lowering activity of $8.8 \pm 1.0\%$. A moderate increase in serum glucose lowering activity of $12.8 \pm 0.7\%$ (Fig. 6) was observed upon increasing the dose of insulin by half to 1.5 IU/kg. AG treated rats administered 2 IU/kg dose of insulin produced a serum glucose lowering activity of $16.9 \pm 0.9\%$. Finally, upon injecting with a high dose of 2.5 IU/kg, the serum glucose lowering activity increased to $21.6 \pm 0.7\%$ (Fig. 6).

DISCUSSION

In the present study, we developed successfully an insulin resistance model in adult rats based on feeding fat emulsion for 10 days (13), followed by a single low dose of streptozotocin (14) at a dose of 35 mg/kg. The fat emulsion was prepared by admixing saturated fats with sucrose along with oleic acid as a vehicle. To control the daily fat intake, the fat emulsion was administered to rats

by oral gavage and not by mixing with food. Hence the possibility of imbalanced food intake due to decreased appetite as a result of high fat diets was totally avoided.

The rats fed with fat emulsion developed weight gain, hyperinsulinemia and insulin resistance, and a single low dose of streptozotocin contributed to controlled hyperglycemic status. Hence the induced insulin resistance developed in rats in this study would mimic the natural history of disease initiation and development and demonstrate the characteristic metabolic features of type 2 diabetes and insulin resistance. Moreover, the model was cheaper to develop, easy to handle and available, useful for regular screening as well as preclinical testing of various compounds like insulin sensitizers and insulinotropics for the treatment of type 2 diabetes and insulin resistance (14). Hence, many investigators try to establish an ideal animal model for type 2 diabetes and insulin resistance either by way of manipulating existing methods or by developing new methodologies or using a combination of both.

To assess insulin sensitivity in fat-fed and streptozotocin injected animals, IPGTT was carried out to find out the AUC of glucose and insulin during the test. There was a significant increase in circulating insulin and glucose values during IPGTT in IRC rats. The disposal of ingested glucose, a primary function of insulin hormone, into peripheral tissues was markedly reduced in rats receiving fat emulsion along with a single i.p. injection of streptozotocin, as shown by the high glucose-insulin index, the product of the AUC of glucose and insulin, and an indirect index of *in vivo* insulin action. IPGTT measures the ability of insulin to stimulate glucose disposal, and was found to be markedly impaired in this study in rats receiving fat emulsion and a single i.p. injection of streptozotocin, indicating the presence of insulin resistance. Thus, these rats could serve as a suitable model for insulin resistance.

The sluggish glucose disposal observed in insulin resistant rats was attenuated by the D3 dose of extract as indicated by a reduction in the glucose-insulin index. AG also showed a mild insulin sensitizing effect in the IPGTT test. This shows that the ethanolic extract of *Andrographis paniculata* may have an insulin sensitizing effect, by attenuating the impairment of insulin stimulated glucose disposal in insulin resistant rats. This

probably indicates that the extract may increase the activity of endogenous insulin to improve insulin resistance condition. However, low doses of the extract, D1 and D2 group, did not cause any significant reduction in the glucose-insulin index.

The loss of the serum glucose-lowering response to tolbutamide has been interpreted as the development of insulin resistance (16). Thus, the serum glucose-lowering activity of tolbutamide was used in the present study as an indicator to evaluate the induction of insulin resistance in rats. Since the D1 and D2 doses of extract did not demonstrate any beneficial reduction in glucose-insulin index, the tolbutamide-challenge test was not performed in these groups. Only D3 dose of the extract was included in the tolbutamide challenge test. The serum glucose-lowering activity of tolbutamide is believed to depend on the secretion of endogenous insulin. It was observed that the serum glucose-lowering activity of tolbutamide (10.0 mg/kg) disappeared rapidly in vehicle-treated rats receiving fat emulsion and injected with streptozotocin, whereas the loss of response to tolbutamide was delayed in rats treated orally with D3 dose of the extract during the same time. The principal marker AG did not cause any appreciable increase in serum glucose lowering activity, meaning that it does not show any signs of delay in insulin resistance either. The serum insulin increase before and after tolbutamide treatment in the extract treated groups showed no significant difference, except for D3 dose of extract, which showed a slight but significant decrease, suggesting that the stimulation of endogenous insulin release in rats with tolbutamide treatment was not influenced significantly by the ethanolic extract treatment. So, the high hypoglycemic activity of tolbutamide in the extract treated group compared to IRC vehicle treated rats was attributed not to the increase of serum insulin concentration, but to the increase of insulin sensitivity.

It seems that the extract has the ability to delay the induction of insulin resistance in rats; however, on the other hand, AG did not cause any delay in the induction of insulin resistance. The possible mechanism of action is related to the change in insulin sensitivity. Thus, we investigated the effect of ethanolic extract of *Andrographis paniculata* on insulin sensitivity.

To demonstrate the insulin sensitizing effect, streptozotocin-induced diabetic rats were used to assess the serum glucose lowering activity upon treatment with incremental doses of short-acting insulin and with D3 dose of extract (16). Since streptozotocin is a selective β -cell toxin, there would be no endogenous insulin and the serum glucose lowering activity would be a direct consequence of exogenous insulin. It was observed that D3 dose of the extract caused a significant increase in the serum glucose lowering activity in the presence of incremental doses of insulin. It was also observed that AG did not cause any significant increase in serum glucose lowering activity in the presence of insulin. The ability of ethanolic extract of *Andrographis paniculata* to improve insulin sensitivity seems to be similar to that of metformin used as positive control, because the serum glucose lowering activity in response to metformin was similar to the response generated by D3 dose of 95% v/v extract treated group in streptozotocin-diabetic rats. The results of the increased action of exogenous insulin produced by the extract were similar to those obtained by the insulin sensitizing effect of metformin.

Defects in the insulin signal cascade leading to impaired glucose utilization have been proposed to have a key role in the pathogenesis of insulin resistance (18). It has been indicated that metformin improves insulin sensitivity in insulin-resistant subjects by activating post-receptor insulin signaling pathways. Insulin sensitizers may improve insulin sensitivity *via* many mechanisms such as direct effect on muscle insulin sensitivity, stimulation of insulin-sensitive fat cells, or regulation of leptin expression. Hence, further studies are required to determine how exactly the extract causes improvement in insulin resistance.

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