ACUTE HYPOGLYCEMIC, HYPOCHOLESTEROLEMIC AND HYPOTRIGLYCERIDEMIC EFFECTS OF CONTINUOUS INTRAVENOUS INFUSION OF A LYOPHILISED AQUEOUS EXTRACT OF AJUGA IVA L. SCHREBER WHOLE PLANT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

JAOUAD EL-HILALY, ADIL TAHRAOUI, ZAFAR H. ISRAILI* AND BADIÂA NYOUSSI

UFR Physiology–Pharmacology, Laboratory of Animal Physiology, Department of Biology, Faculty of Sciences Dhar El Mehraz, Fez, Morocco
*Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA

ABSTRACT
The hypoglycemic and hypolipidemic effect of continuous intravenous infusion of a lyophilised aqueous extract of the whole plant Ajuga iva (L.) Schreber (Labiatae) (AI-extract) was investigated in anesthetized normal and streptozotocin (STZ)-induced diabetic rats. The AI-extract was administered to a group of rats by continuous intravenous infusion for 4 h at a dose of 4.2 µg/min/100g body weight; another group was infused with taurine, the reference compound, at the same dose. In normal rats, AI-extract infusion had no effect on plasma glucose or triglycerides, but plasma cholesterol levels were significantly decreased (22%; P<0.05). However, taurine infusion produced significant hypoglycemic, hypocholesterolemic and hypotriglyceridemic effects (all changes, P<0.05).

In STZ-diabetic rats, AI-extract infusion reduced plasma levels of glucose by 24 % (P<0.05), cholesterol by 35% (P<0.01) and triglycerides by 13% (P<0.05). Infusion with taurine produced a greater fall in plasma glucose (72%, P<0.001) and cholesterol (54%; P < 0.001) and triglyceride (24%; P<0.001) levels.

Our results indicate that intravenously administered AI-extract exerts hypoglycemic and hypolipidemic effects in diabetic rats by mechanism(s) which appear to be similar to that of taurine, which involve insulin sensitization or an insulin-like effect. The identity and the exact mechanism(s) of action of the active component(s) of the AI-extract are not known.

Ajuga iva appears to be a useful plant in the therapy of diabetes, a condition in which hyperglycemia and dyslipidemia coexist quite often.

Keywords: Ajuga iva; intravenous infusion; streptozotocin-induced diabetic rats; hypoglycemic effect; hypolipidemic effect.

INTRODUCTION
The prevalence of diabetes mellitus (DM) is increasing at a much faster rate in the developing countries than in the developed nations. DM is a leading cause of end-stage kidney disease, cardiomyopathy and heart attacks, strokes, retinal degeneration leading to blindness, and non-traumatic amputations (Engelgau et al., 2003; American Diabetes Association, 2004). Dyslipidemia, quite common in diabetic patients, is the main risk factor for cardiovascular and cerebrovascular diseases. About 10% of DM patients have type 1 diabetes (insulin-dependent diabetes mellitus, IDDM). An animal model of human IDDM is produced by the injection of streptozotocin (STZ) in rats (Tomlinson et al., 1992).

Ethnopharmacological and ethnobotanical surveys indicate that more than 1200 plants are used worldwide in traditional medicine to treat diabetes (see for example, Rahman and Zaman, 1989; Roman-Ramos et al., 1995; Ziyyat et al., 1997; Bnouham et al., 2002; El-Hilaly et al., 2003; Tahraoui et al., 2007). The hypoglycemic activity of many of these plants has been confirmed in hundreds of studies in experimental animals (see for example, Al-Shamaony et al., 1994; Jouad et al., 2000; Benwahoud et al., 2001; El Hilaly and Lyoussi, 2002; Sachdewa and Khemani, 2003; Nagappa et al., 2003; Afifi et al., 2005), and in numerous studies in diabetic patients (for instance, Herrera-Arellano et al., 2004; Jayawardena et al., 2005).

According to our inquiry and other surveys realized in Morocco, Ajuga iva (L.) Schreber is widely used in Moroccan traditional pharmacopoea to treat diabetes and other disorders (Ziyyat et al., 1997; Bennagh-mouch et al., 2001; Bnouham et al., 2002; El-Hilaly et al., 2003; Tahraoui et al., 2007). We have convincingly demonstrated the hypoglycemic activity of a lyophilized aqueous extract of Ajuga iva whole plant (AI-extract), given as a single oral dose or repeated daily doses for 3 weeks, in normal and STZ-diabetic rats (El-Hilaly and

Corresponding author: E-mail: lyoussi@rocketmail.com
Preparation of the aqueous extract of *Ajuga iva* whole plant

The whole plant was washed well with water, dried at room temperature in the dark and then ground in an electric grinder to obtain a coarse powder. Then 50 g of the plant powder was suspended in 500 mL distilled water and heated under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at –20°C and then lyophilised (FreeZone® Dry 4.5, USA). The yield of the dry product was about 25% w/w, which was stored at –20°C until used.

For intravenous infusion, the lyophilized material prepared as above was dissolved at a concentration of 16.8 mg/100 mL (AI-extract) in isotonic physiological saline (0.9% NaCl) and sterilised by filtration using a bacterial filter (Millipore HAWPO4700, 0.45µm).

**Experimental animals and induction of diabetes**

Adult male Wistar rats, approximately of the same age weighing between 200g and 260g were housed in an air-conditioned animal room at 23 ± 2°C with 12 hr/12 hr light/dark photoperiod. They were allowed free access to tap water and laboratory rat chow. Some of the rats were artificially made diabetic by intravenous injection of STZ (Sigma, St. Louis, MO) through the tail vein (60 mg/kg BW dissolved in citrate buffer, 0.1M, pH 4.5). After 3 days, the rats with fasting blood glucose levels > 250 mg/dL were considered as diabetic and selected for the study. Rats were randomly divided into groups of 6 each: three groups of normal rats and three groups of diabetic rats.

The care and handling of the animals were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our institutional committee on animal care following the French Technical Specifications for the Production, Care and Use of the Laboratory Animals.

**Surgical procedure**

The rats were fasted overnight with free access to water. On the following day, they were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg BW). Then, the animals were placed on a thermostated table to keep their body temperature at about 37°C. A tracheotomy was performed in order to facilitate respiration. Two catheters (PE50; Intramedic, Clay-Adams), one filled with physiological saline (0.9% NaCl) and the other with heparinized physiological saline solution, were introduced respectively in the right femoral vein and the left femoral artery. The former was used for subsequent infusion of test solutions while blood samples were collected from the latter.

**Experimental protocol and infusions**

After the surgical procedure, while the rats were under anesthesia, physiological saline was infused continuously at a flow rate of 25 µL/min/100g BW during the control phase (1 hr). Three control measurement periods (C–60, C–30, C0) of 30 min each were started (Chart 1). At the end of these periods, saline infusion was stopped and the experimental phase of the study was started, during which solutions of the test materials (AI-extract or taurine) were infused at the flow rate of 25 µL/min/100g BW. After an

**MATERIALS AND METHODS**

**Plant material**

The plant, *Ajuga iva* L. Schreb. was collected during our ethnobotanical survey undertaken in north Morocco (El-Hilaly et al., 2003). Professor M. Fennan from the Scientific National Institute, Rabat, confirmed its identity and a voucher specimen (Reference No. H63) was deposited in the herbarium of the Institute.

**Preparation of the aqueous extract of *Ajuga iva* whole plant**

Lyoussi, 2002; El-Hilaly et al., 2006), and thus, confirmed the ethnomedical use of this plant in diabetes. We have also demonstrated the relatively non-toxic nature of the AI-extract in mice and rats (El-Hilaly et al., 2004). In our recent study (El-Hilaly et al., 2006), we discovered that the AI-extract also decreases plasma cholesterol and triglycerides when given as a single or repeated oral doses in normal and STZ-diabetic rats. Thus, *Ajuga iva* L. Schreb. appears to be ideally suited to be investigated further to develop an antidiabetic agent in humans, since this relatively non-toxic plant, in addition to its hypoglycemic activity, also possesses hypolipidemic activity, and thus, would potentially improve lipid profile along with normalization of plasma glucose with the use of a single agent.

Insulin is usually given parenterally to rapidly control highly elevated blood glucose, such as found in critically ill (Nasraway, 2005) and stroke (Vinychuk et al., 2005) patients; no other antidiabetic drug is given parenterally. Although insulin is quite safe, severe adverse effects of insulin have been reported (Physicians Desk Reference, 2007). There are no studies which show that a plant extract can reduce plasma glucose when given parenterally. A new relatively non-toxic substance, which can be given intravenously, could be useful for rapid control of elevated glucose in critically ill patients. Therefore, the main aim of the present study was to determine if a short-term continuous intravenous infusion of the AI-extract would reduce plasma glucose in STZ-diabetic rats. Since, our previous studies show that the AI-extract also has hypolipidemic effect in diabetic rats (El-Hilaly et al., 2006), the effect of continuous intravenous infusion of the AI-extract in lipid levels was also investigated. Taurine, which has both hypoglycemic (Tenner et al., 2003) as well as hypolipidemic (Yokogoshi and Oda, 2002) effect in diabetic animals, was used as the positive control.

Although insulin is quite safe, severe adverse effects of insulin have been reported (Physicians Desk Reference, 2007). There are no studies which show that a plant extract can reduce plasma glucose when given parenterally. A new relatively non-toxic substance, which can be given intravenously, could be useful for rapid control of elevated glucose in critically ill patients. Therefore, the main aim of the present study was to determine if a short-term continuous intravenous infusion of the AI-extract would reduce plasma glucose in STZ-diabetic rats. Since, our previous studies show that the AI-extract also has hypolipidemic effect in diabetic rats (El-Hilaly et al., 2006), the effect of continuous intravenous infusion of the AI-extract in lipid levels was also investigated. Taurine, which has both hypoglycemic (Tenner et al., 2003) as well as hypolipidemic (Yokogoshi and Oda, 2002) effect in diabetic animals, was used as the positive control.

**MATERIALS AND METHODS**

**Plant material**

The plant, *Ajuga iva* L. Schreb. was collected during our ethnobotanical survey undertaken in north Morocco (El-Hilaly et al., 2003). Professor M. Fennan from the Scientific National Institute, Rabat, confirmed its identity and a voucher specimen (Reference No. H63) was deposited in the herbarium of the Institute.

**Preparation of the aqueous extract of *Ajuga iva* whole plant**

The whole plant was washed well with water, dried at room temperature in the dark and then ground in an electric grinder to obtain a coarse powder. Then 50 g of the plant powder was suspended in 500 mL distilled water and heated under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at –20°C and then lyophilised (FreeZone® Dry 4.5, USA). The yield of the dry product was about 25% w/w, which was stored at –20°C until used.

For intravenous infusion, the lyophilized material prepared as above was dissolved at a concentration of 16.8 mg/100 mL (AI-extract) in isotonic physiological saline (0.9% NaCl) and sterilised by filtration using a bacterial filter (Millipore HAWPO4700, 0.45µm).

**Experimental animals and induction of diabetes**

Adult male Wistar rats, approximately of the same age weighing between 200g and 260g were housed in an air-conditioned animal room at 23 ± 2°C with 12 hr/12 hr light/dark photoperiod. They were allowed free access to tap water and laboratory rat chow. Some of the rats were artificially made diabetic by intravenous injection of STZ (Sigma, St. Louis, MO) through the tail vein (60 mg/kg BW dissolved in citrate buffer, 0.1M, pH 4.5). After 3 days, the rats with fasting blood glucose levels > 250 mg/dL were considered as diabetic and selected for the study. Rats were randomly divided into groups of 6 each: three groups of normal rats and three groups of diabetic rats.

The care and handling of the animals were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our institutional committee on animal care following the French Technical Specifications for the Production, Care and Use of the Laboratory Animals.

**Surgical procedure**

The rats were fasted overnight with free access to water. On the following day, they were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg BW). Then, the animals were placed on a thermostated table to keep their body temperature at about 37°C. A tracheotomy was performed in order to facilitate respiration. Two catheters (PE50; Intramedic, Clay-Adams), one filled with physiological saline (0.9% NaCl) and the other with heparinized physiological saline solution, were introduced respectively in the right femoral vein and the left femoral artery. The former was used for subsequent infusion of test solutions while blood samples were collected from the latter.

**Experimental protocol and infusions**

After the surgical procedure, while the rats were under anesthesia, physiological saline was infused continuously at a flow rate of 25 µL/min/100g BW during the control phase (1 hr). Three control measurement periods (C–60, C–30, C0) of 30 min each were started (Chart 1). At the end of these periods, saline infusion was stopped and the experimental phase of the study was started, during which solutions of the test materials (AI-extract or taurine) were infused at the flow rate of 25 µL/min/100g BW. After an
equilibration period of 1 hr, four experimental measurement periods (E50s, E120s, E180s, E240s) of 1 hr each were realised.

One group of normal rats and a group of diabetic rats received only normal saline, while a second set of normal and diabetic rats received continuous infusion (4 hr) of the AI-extract at a dose of 4.2 µg/min/100g BW. The third set of normal and diabetic rats was infused (4 hr) with the reference compound, taurine, at a dose of 4.2 µg/min/100g BW.

**Analytical methods**

Blood glucose levels were measured by a reflective glucometer using the glucose oxidase method (GlucoMen Glyco, A. Menari Diagnostic MTB, Germany). Plasma insulin concentrations were determined by radioimmunoassay (RIA kit, BetaMatic, Kontron, Montigny le Bretonneaux, France). Plasma triglycerides and cholesterol were determined enzymatically by colorimetric specific kits (Randox, UK).

**STATISTICAL ANALYSIS**

All data are expressed as mean ± SEM. Within group comparisons were performed by analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by the student’s t-test; P-values of less than 0.05 were considered to be significant.

**RESULTS**

**Effect of STZ treatment on plasma glucose, insulin, cholesterol and triglyceride levels in anesthetised rats**

Experimental induction of diabetes by injection of STZ resulted in a 3- to 4-fold increase in plasma glucose levels (comparing Panel A with Panel B, fig. 1). Plasma insulin levels decreased to about 22% of the levels in control animals (table 1). There was no significant change in plasma cholesterol concentration, but there was a 2-fold increase in triglyceride levels (table 2).

**Effect of continuous intravenous infusion of the AI-extract or taurine on plasma glucose and insulin levels in anesthetised normal and STZ-diabetic rats**

The effect of continuous intravenous administration of AI-extract or taurine on blood glucose levels in normal rats is shown in Figure 1, Panel A; also shown is the effect of saline. Infusion with either saline or AI-extract did not affect glycemia in normal rats. However, infusion with taurine produced a small (18%) but significant hypoglycemic effect in these rats (4.0 ± 0.3 mmol/L (at 180 or 240 min) versus 4.9 ± 0.3 mmol/L at baseline (0 min); P<0.05).

In the STZ-diabetic rats, intravenous infusion of the AI-extract progressively decreased (fig. 1, Panel B) blood glucose levels (9% at 1 hr, P = NS; 14% at 2 hr, P = NS; 18% at 3 hr, P < 0.05) reaching the maximum decline (24%) at the end of infusion (12.4 ± 0.8 mmol/L at 4 hr versus 16.3 ± 1.2 at 0 min; P<0.05). However, taurine perfusion at the same dose, as the AI-extract, caused a far greater decline in blood sugar (14% at 1 hr, P = NS; 19% at 2 hr, P = NS; 57% at 3 hr, P < 0.01; 72% at 4 hr, P < 0.01), with normalisation of blood glucose levels at the end of the experimental period [5.1 ± 2.1 mmol/L at 4 hr versus 18.2 ± 1.3 mmol/L (baseline, 0 min); P<0.01] (fig. 1, Panel B); saline had no effect on glucose levels.

None of the treatments (saline, AI-extract or taurine) had any effect on insulin levels in either normal or diabetic rats (table 1).

**Effect of continuous intravenous infusion of the AI-extract or taurine on plasma cholesterol levels in anesthetised normal and STZ-diabetic rats**

The effect of continuous intravenous infusion (4 hr) of the AI-extract, taurine and normal saline on plasma total cholesterol levels in normal rats and the STZ-diabetic rats is presented in table 2. In normal rats, infusion with either the AI-extract or taurine caused a small but significant decrease in total cholesterol levels after 4-hr of infusion as compared to the baseline value [AI-extract: 1.8 ± 0.1 mmol/L versus 2.3 ± 0.4 mmol/L (0 min); P < 0.05; taurine: 1.7 ± 0.1 mmol/L versus 2.1 ± 0.3 mmol/L (0 min); P< 0.05]. But, the values were not significantly different from that in control rats given saline (table 2); saline had no effect.

In the STZ-diabetic rats, the AI-extract caused a significant reduction (35%) in total cholesterol levels in plasma (table 2) after 4-hr of infusion as compared to the baseline values [1.5 ± 0.2 mmol/L versus 2.3 ± 0.1 mmol/L (0 min); P<0.01]; the levels were significantly lower than in rats treated with saline. Taurine infusion produced a greater reduction in cholesterol levels (54%) than with the AI-extract [(1.1 ± 0.1 mmol/L (at 4 hr) versus 2.4 ± 0.2 mmol/L at baseline (0 min); P < 0001]; saline infusion had no effect (table 2).

**Effect of continuous intravenous infusion of the AI-extract or taurine on plasma triglyceride levels in anesthetised normal and STZ-diabetic rats**

The effect of infusion of various substances on plasma triglycerides of normal and diabetic rats is presented in table 2. Infusion of the AI-extract for 4 hr caused a small (17%) and insignificant decline in plasma triglyceride levels [1.5 ± 0.1 mmol/L versus 1.8 ± 0.3 mmol/L (0 min); P = NS] in normal rats, while taurine infusion produced a small (25%) but significant decrease [1.2 ± 0.2 mmol/L versus 1.6 ± 0.1 mmol/L (0 min); P<0.05]. In the diabetic rats, infusion of the AI-extract for 4 hr...
Acute hypoglycemic, hypocholesterolemic and hypotriglyceridemic effects

Table 1: Effect of intravenous infusion of the aqueous extract of Ajuga iva or taurine on plasma insulin levels in normal and STZ-diabetic rats

<table>
<thead>
<tr>
<th>Treatment#</th>
<th>Dose (µg/min/100g)</th>
<th>Plasma levels of insulin (µU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Normal rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (0.9%)</td>
<td>-</td>
<td>35.2 ± 0.7</td>
</tr>
<tr>
<td>Ajuga iva extract§</td>
<td>4.2</td>
<td>37.5 ± 0.9</td>
</tr>
<tr>
<td>Taurine§</td>
<td>4.2</td>
<td>34.6 ± 0.6</td>
</tr>
<tr>
<td>STZ-diabetic rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (0.9%)</td>
<td>-</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>Ajuga iva extract§</td>
<td>4.2</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>Taurine§</td>
<td>4.2</td>
<td>7.7 ± 0.9</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M., n = 6 rats in each group.
# All substances were infused intravenously at the rate of 25 µl/min/100 g BW.
§ Concentration of the infusion solutions of both the aqueous extract of Ajuga iva L. Schreb., and taurine = 16.8 mg/100 mL.
The differences in the values after treatment with Ajuga iva or taurine compared to the baseline (0 min) values or in saline-treated rats are non significant in all groups of rats.

Table 2: Effect of intravenous infusion of an aqueous extract of Ajuga iva or taurine on plasma cholesterol and triglycerides levels in normal and STZ-diabetic rats

<table>
<thead>
<tr>
<th>Treatment#</th>
<th>Dose (µg/min/100g)</th>
<th>Plasma levels (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Normal rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (0.9%)</td>
<td>-</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Ajuga iva extract§</td>
<td>4.2</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Taurine§</td>
<td>4.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>STZ-diabetic rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (0.9%)</td>
<td>-</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Ajuga iva extract§</td>
<td>4.2</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Taurine§</td>
<td>4.2</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M., n = 6 rats in each group.
# All substances were infused intravenously at the rate of 25 µl/min/100 g BW.
§ Concentration of the infusion solutions of both the aqueous extract of Ajuga iva L. Schreb. and taurine = 16.8 mg/100 mL.
Significantly different from pre-treatment (0 min) levels: * P < 0.05; ** P < 0.1; *** P < 0.001

resulted in a significant reduction in plasma triglycerides (13%, P<0.05). Infusion of taurine produced higher hypotriglyceridemia (24%, P<0.01). Saline had no effect on triglyceride levels in normal or diabetic rats (table 1).

DISCUSSION

The aim of the present study was to evaluate the hypoglycemic and hypolipidemic activity of short-term continuous intravenous infusion of an aqueous extract of Ajuga iva L. Schreb. whole plant in normal and STZ-diabetic rats. Our previous studies (El-Hilaly and Lyoussi, 2002; El-Hilaly et al., 2006) show that the AI-extract has potent hypoglycemic activity, when administered orally as a single dose or sub-chronically in normal and STZ-diabetic rats. We also found that acute and sub-chronic oral administration of the AI-extract induces hypocholesterolemia and hypotriglyceridemia in both normal and diabetic rats (El-Hilaly et al., 2006). For the present study, the experimental conditions (short term treatment and the intravenous route of administration) were selected with the aim to determine if the AI-extract has an insulin-like rapid onset of action in the moderate to severe diabetic state.

In the STZ-induced diabetic model used [in which there is selected destruction of pancreatic islet β-cells (Ruzaidi et al., 2005)], some β-cells do survive since plasma insulin levels in the diabetic rats are about 22% of that in normal rats (this study, table 1 and El-Hilaly and Lyoussi, 2002),
and that insulin secretion can be stimulated in the residual β-cells of these diabetic animals by glibenclamide (Sachdewa and Khemani, 2003). In addition to marked hyperglycemia, the STZ-diabetic rats also developed notable hypertriglyceridemia (table 2), as has been reported previously (Pari and Venkateswaran, 2004; Ruzaidi et al., 2005). Anesthesia did not have any effect on plasma glucose, insulin, cholesterol or triglyceride levels in control or diabetic rats (values compared with historical data in unanesthetised rats).

Taurine was used as a reference compound, since oral administration of this amino acid decreases serum glucose (Tenner et al., 2003) and lipids (Yokogoshi and Oda, 2002; Zhang et al., 2004) in humans as well as in diabetic animals. Although, taurine is relatively safe (http://www.nutros.com/nsr-02015.html; accessed June 2007), there is some toxicity associated with taurine accumulation in patients with renal failure (Suliman et al., 2002) or overdose (http://www.nutros.com/nsr-02015.html; accessed June 2007). Also, taurine supplementation with a high cholesterol diet has been reported to result in adverse lipid profile in normal human volunteers (Tanno et al., 1989).

The present investigation shows that the AI-extract when administered by continuous intravenous infusion in anesthetized animals for a relatively short time (4 hr) produces both hypoglycemia and hypolipidemia, especially in the diabetic rats. Several other plant preparations such as, Momordica charantia (Ahmed et al., 2001), Hibiscus rosa sinensis (Sachdewa and Khemani, 2003), Lycium barbarum (Luo et al., 2004), Phaseolus vulgaris (Pari and Venkateswaran, 2004), have been reported to produce both hypoglycemia and hypolipidemia in STZ-diabetic rats, but only after chronic/sub-chronic oral administration.

In our previous studies, an oral dose of the AI-extract reduced blood sugar significantly from 2-hr to at least 6-hr post dose in normoglycemic rats and produced more intense hypoglycemia in the diabetic rats (El-Hilay and Lyoussi, 2002; El-Hilaly et al., 2006). In the present study, continuous intravenous infusion did not have any effect on glucose levels in the normal rats, but caused significant hypoglycemia in the diabetic rats. The reason for the difference in these observations could be that the normal (glucose/carbohydrate) homeostasis mechanisms, such as by the release of counter-regulatory hormones...
Acute hypoglycemic, hypocholesterolemic and hypotriglyceridemic effects

(Roman-Ramos et al., 1995; Miura and Kato, 1997) may be operating in the normoglycemic rats.

The mechanism of hypoglycemic action of the AI-extract is not known, but it is not via stimulation of insulin release from the pancreatic β-cells, since acute (table 1) or chronic administration (El-Hilaly and Lyoussi, 2002) of the AI-extract has no effect on plasma insulin levels in either normal or diabetic rats. However, since the STZ-diabetic rats do secrete some insulin (table 1 and El-Hilaly and Lyoussi, 2002), the hypoglycemic action of the AI-extract may be insulin-mediated by mechanisms in common with taurine, such as by increasing glucose utilization via insulin sensitization in the peripheral tissues (Nandhini et al., 2004), as has also been proposed for Momordica charantia fruit (Miura et al., 2001), and thiazolidinediones (Da Ros, et al., 2004). Alternatively, the AI-extract may have a direct insulin-mimetic effect, such as suggested for the hypoglycemic activity of a fungal quinone (Zhang et al., 1999), and an aqueous extract of Momordica charantia (Rathi et al., 2002). The AI-extract may also act via suppression of hyperglycemic hormones, such as epinephrine (Miura and Kato, 1997; Roman-Ramos et al., 1995).

The relatively rapid onset of hypoglycemic action of continuous intravenous infusion of the AI-extract would exclude mechanisms, such as by decrease in glucose absorption from the small intestines by various mechanisms (Li et al., 2005), or regeneration of pancreatic β-cells (Nagappa et al., 2003), increase in the expression of insulin receptors in the liver plasma membrane (Kanigur-Sultuybek, et al., 1995), or normalization of hepatic gluconeogenic enzymes (Pari and Venkateswaran, 2004).

During the short exposure of the animals to either AI-extract or taurine, hyperlipidemia was more pronounced in the diabetic rats than in the normal animals, and both substances had a greater effect on cholesterol than on triglyceride levels (table 2). At the doses used, the AI-extract was less potent than taurine in decreasing plasma cholesterol and triglycerides. However, it may be recognized that the AI-extract is a crude mixture of some active substances of unknown concentrations, and it is possible that some of the active moieties in the pure form would be more potent than taurine.

The mechanism(s) of hypolipidemic effect of the AI-extract may be similar to some of those proposed for taurine, including insulin-mediated lipolytic activity by inhibition of hormone-sensitive lipase (Al-Shamaony et al., 1994) or lipogenic enzymes (Pari and Venkateswaran, 2004), and/or activation of lipoprotein lipase (Ahmed et al., 2001), as has been proposed for some anti-diabetic plants exhibiting hypolipidemic activity, such as Ceasalpinea bondecella (Sharma et al., 1997) and Momordica charantia (Ahmed et al, 2001).

The active principles in the AI-extract, which may be responsible for the hypolipidemic and hypoglycemic actions, are unknown. Our preliminary phytochemical analysis has indicated that flavonoids are the major constituents of the AI-extract (unpublished data); the flavonoids have been reported to exert potent hypoglycemic and hypolipidemic effects (Khushbaktova et al, 2001). In addition, the observed pharmacological activity may be due to any one or more of the compounds found in Ajuga iva L. Schreb., such as 8-O-acetyl harpagide, ajugarine, apigenin-7-O-neohesperidoside, barpagide, caffeine, clorogenes, cyasterone, diglycerides, 14,15-dihydroajugapitin, ecdysones, ecdysterones, flavonoids, iridoides, makisterone A, naringin, neohesperidoside, phenylcarboxylic acids, tannin-polyphenols (Khafagy et al., 1979; Iannet et al., 1997; www.hipernatural.com/en/plitiva.html, accessed June 2007). It needs to be determined as to which components of Ajuga iva L. Schreber are responsible for the hypoglycemic and hypolipidemic activity exhibited by the AI-extract.

Although, we have not carried out toxicological studies of the intravenously administered AI-extract, no apparent toxicity was observed under the experimental conditions. Also, our previous acute and chronic toxicological investigations lead us to suggest that Ajuga iva L. Schreb. is “non-toxic,” since the calculated median lethal dose (LD₅₀) of the acute intraperitoneally administered AI-extract is quite high (3.6 g/kg), and it did not cause lethality or organ toxicity after single oral doses of as high as 14 g/kg in mice or up to 600 mg/kg given daily for 3 months in rats (El-Hilaly et al., 2004).

In conclusion, this study is unique, in that this is the first study to show that intravenously administered plant extract causes rapid induction of hypoglycemia and hypolipidemia in diabetic rats. This study is also the first to demonstrate the hypoglycemic and hypolipidemic action of intravenously administered taurine in diabetic rats. The mechanism(s) of action of the hypoglycemic and hypolipidemic activity appear to be similar for the AI-extract and taurine, which involve insulin sensitization or an insulin-like effect. In the light of our pharmacological and toxicological studies, Ajuga iva L. Schreb. appears to be a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes, a condition in which hyperglycemia and dyslipidemia coexist quite often. We are carrying out additional chemical and pharmacological studies to determine the mechanism(s) of action of the AI-extract and to isolate the active principles responsible for the hypoglycemic and hypolipidemic action of the AI-extract.
REFERENCES


Pari L and Venkateswaran S (2004). Protective role of *Phaseolus vulgaris* on changes in the fatty acid.
Acute hypoglycemic, hypocholesterolemic and hypotriglyceridemic effects


