ANTIDIABETIC AND HYPOLIPIDEMIC EFFECTS OF DIFFERENT FRACTIONS OF *COCCINIA CORDIFOLIA* L. ON NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The present study was carried out to observe the antidiabetic and hypolipidemic effects of petroleum-ether, ethyl acetate and chloroform fractions isolated from ethanolic extract of the leaves of *Coccinia cordifolia* Linn. (150 mg/kg body weight) on normal and streptozotocin (STZ)-induced diabetic rats for one day experiment. Single doses (150 mg/kg, i.p.) of *C. cordifolia* extracts were given to normal and diabetic rats. The fasting blood glucose (FBG), serum triglyceride (TG) and serum total cholesterol (TC) levels were investigated in normal and STZ-diabetic rats on 0, 1, 2, 3, 6, 10, 16, and 24th hours.

In normoglycemic rats the pet-ether and ethyl acetate fractions of *C. cordifolia* reduced blood glucose level significantly (39.66% and 40.68% at 16th and 24th hour respectively). In the STZ-diabetic rats pet-ether and ethyl acetate fractions also reduced blood glucose level significantly (50.39% and 50% at 10th and 24th hour respectively). Ethyl acetate fraction is most effective which reduced total cholesterol level by 31.04% and 36.69% in normal and STZ-diabetic rats respectively. Ethyl acetate fraction reduced triglyceride level by 43.82% and 42.01% in normal and STZ-diabetic rats respectively. Our results indicate that pet-ether and ethyl acetate fractions of *C. cordifolia* have potentiality against diabetes.

Keywords: *Coccinia cordifolia*, hypoglycemic, antihyperglycemic, hypolipidemic, streptozotocin induced diabetic rats.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system (David *et al*., 1997). Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Luzi, 1998). The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides (Larner, 1985). Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents has been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications (Larner, 1985, Momin, 1987 and Stenman *et al*., 1990). The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment (Spiller and Sawyer, 2006). The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. The World Health Organization also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate (WHO, 1980). There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Holman & Turner, 1991). The available literature showed that there are more than 400 plant species showing hypoglycemic activity (Mukherjee, 1981, Oliver-Bever, 1986, Rai, 1995). Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remains to be scientifically established.

Ivy gourd or *Coccinia cordifolia* (CC) is an aggressive vine in the Cucurbitaceae (cucumber) family. It is widely cultivated and has escaped to become a vigorous pest in Hawai'i, Australia, Saipan, Texas, and Florida. In Southeast Asia, ivy gourd is cultivated for its edible young shoots and edible fruits (Linney, 1986). It has been classified as one of the medicinal herbs in the traditional practice of the Bangladeshi as well as Indian medicine. The juice of the roots and leaves is used to treat diabetes while the leaves are also used as poultice to treat skin eruptions. In addition, the aqueous and ethanolic extracts of the plant have shown hypoglycemic action (Chopra *et al*., 1986). Another study has shown improvement in glucose tolerance of *C. cordifolia* in patients with maturity onset diabetes (Azad *et al*., 1979). The study suggested that *C. cordifolia* has a potential hypoglycemic action independent of energy/food intake or weight loss and thus could represent a possible dietary adjunct for the

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treatment of diabetes in patients with mild diabetes (Kuryian *et al*., 2008).

Some researchers have investigated the antidiabetic effect of ethanolic crude extract of the plant but the antidiabetic effects of petroleum-ether (Pet-ether), chloroform ($CHCl_3$) and ethyl acetate (EtAc) fractions isolated from the ethanolic extract of *C. cordifolia* (CC) have not been done yet. This fractionation effects will help us to determine which fraction is more potent. This finding will indicate which compounds are actually responsible for antidiabetic potentialities. In this study the effect (24 hours duration) of pet-ether, ethyl acetate and chloroform fractions from ethanolic extracts of *C. cordifolia* on fasting blood glucose (FBG) and lipid biochemical parameters such as serum total cholesterol (TC) and serum triglyceride (TG) were investigated in streptozotocin (STZ)-induced diabetic rats (SIDR) where metformin-HCl was used as standard drug. Thus the hypoglycemic, antihyperglycemic and hypolipidemic ettects of the plant fractions have been investigated.

MATERIALS AND METHODS

Plant material

Fresh leaves of *C. cordifolia* were collected from our University medicinal plant garden and were dried under shadow for several days. The dried leaves were then ground to a coarse powder. The authenticity of the *C. cordifolia* was identified by Mr. A.H.M. Mahbubur Rahman, Department of Botany, University of Rajshahi. Voucher specimens, collection # 33, dated 4/25/2002 for *C. cordifolia* kept in the Department of Botany, University of Rajshahi, Rajshahi.

Reagents

Metformin HCl was the generous gift sample from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Both the Streptozotocin-HCl (STZ) and dimethyl sulfoxide (DMSO) were purchased from Loba Chemie, Bombay, India.

Preparation of ethanol-extracts

Dried leaves of *C. cordifolia* were soaked for 5-7 days in 95% ethanol with occasional shaking and stirring. Then, they were passed through cotton and then filtered. The remaining parts were filtered again under the same procedure. Then the solvent i.e., ethanol was allowed to be evaporated using rotary evaporator at temperature 40-45°C. Thus the highly concentrated ethanolic extract was obtained.

Fractionation of ethanol extract

Crude rectified spirit extract was diluted by addition of DW to obtain aqueous solution. The aqueous solution was then treated with petroleum ether for three times. The upper fraction was collected in each time of fractionation by using separating funnel. The aqueous fraction was then treated with chloroform for three times. The lower fraction was collected for getting chloroform extract. The remaining aqueous fraction was again treated with ethyl acetate for three times. The upper fraction was collected for getting ethyl acetate fraction. The fractions of the different solvents were then evaporated by rotary evaporator. The remaining portions of the different fractions were then dried by mild sunlight. The dried extracts were then preserved in the freeze for the experimental use (Alam, 1999).

Phytochemical screening tests

The following phytochemical screening methods (Nayak & Pereira, 2006) were used for tests:

Test for saponins: Boiled 300 mg of extract with 5 ml water for two minutes. Mixtures was cooled and mixed vigorously and left it for three minutes. The formation of frothing indicates the presence of saponins.

Test for tannins: To an aliquot of the extract added sodium chloride to make to 2% strength. Filtered and mixed with 1% gelatin solution. Precipitation indicates the presence of tannins.

Test for triterpenes: 300 mg of extract mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulfuric acid and mixed properly. The appearance of red color indicates the presence of triterpenes.

Test for alkaloids: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature and examined the alcoholic layer for the pink colour which indicates the presence of alkaloids.

Test for flavonoids: The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnins and potassium hydroxide solution.

Animal experiments

A total number of 50 long-Evans female rats weighing about 150-180 gm, age 2 months were purchased from animal house of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature of 25°C and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water *ad libitum*. Animals were fasted for 16 h prior to drug administration allowing access only to water and were deprived of both food and water during the experiment.

Induction of diabetes

The rats were randomly divided into ten groups, each containing 5 rats. After fasting 16 h, rats of group (VI-X) were rendered diabetic by intraperitoneal injection of freshly prepared solution of STZ (45 mg/kg) in 0.1 mol/L citrate buffer, pH 4.5, in volume 1 ml/kg (Siddique *et al*., 1987), after a base line glucose estimation. After 48 hours blood glucose content was measured by BioLandG-423 Test Meter (BioLand, Germany) using blood sample from the tail vein of the rats. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol/L were selected for the study (Liang *et al*., 1993).

Effect on diabetic rats

Group I served as a normal control while group VI for diabetic control group. Group II served as normal metformin control while group VII served as diabetic metformin control group. Group II and VII were treated with metformin HCl (150 mg/kg, i.p.) for 24 hours experiment. Group III, IV, V and group VIII, IX and X were treated with the pet-ether, ethylacetate and chloroform fractions of ethanolic extract of *C. cordifolia* at 150 mg/kg for 24 hours experiment. The reference drug and the extracts were administered intraperitonially at single dose to the rats.

Collection of blood, serum and determination of blood glucose, serum total cholesterol (TC) and serum triglycerides (TG)

Blood samples were collected from tail vein of each rat of a group before and at 0, 1, 2, 3, 6, 10, 16 and 24th hours of one day experiment. The samples were analyzed for blood glucose content by using BioLand G-423 glucose test meter (BioLand Germany). Then the rats were sacrificed and about 1-2 ml of blood was collected directly from the heart by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was obtained for the determination of TC and TG. Serum TC and TG concentrations were analyzed by measuring absorbance by UV spectrophotometer (Shimidzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits (Boehringer Mannheim, GmbH) according to manufacturer's protocol.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (S.E.M). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test (DMCT) and the values were considered statistically significant when $p<0.01$. Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

RESULTS

The effects of different fractions of the ethanolic extract of *C. cordifolia* on the FBG, serum TC and serum TG levels were investigated in the control and STZ-diabetic rats using metformin HCl as standard antidiabetic agent. All treatment was done with a single dose of (150 mg/kg body weight).

Effect of different fractions of C. cordifolia on fasting blood glucose level in normoglycemic rats

The mean blood glucose concentration of control and fractions of *C. cordifolia* treated animals (after intraperitoneal administration of a single dose) on 0, 1, 2, 3, 6, 10, 16 and 24th hours are shown in table 1. Hypoglycemia was observed in animals treated with *C. cordifolia* extracts. The significant reduction ($p<0.01$) of 39.66% for Pet-CC occurs on 16th hour of the experiment. The $CHCl_3$-CC has no significant effect on the blood glucose level. The EtAc-CC reduced FBG by 40.68% at 24th hour only where a significant reduction ($p<0.01$) in blood glucose of 52.55% was observed for metformin-HCl at 6th hour after treatment in comparison to normoglycemic rats.

Effect of different fractions of C. Cordifolia on fasting blood glucose level in STZ-induced diabetic rats

The mean blood glucose concentration of control and fractions of *C. cordifolia* treated animals (after intraperitoneal administration of a single dose) on 0, 1, 2, 3, 6, 10, 16, and 24th hours are shown in table 2. Antihyperglycemia was observed in animals treated with *C. cordifolia* extracts. The significant reduction ($p<0.001$) of 50.39% for Pet-CC fraction occurs on 10th hour of the experiment. The Chloroform extract of coccinia reduced 23.31% of FBG on 16 hour of the experiment. . EtAc-CC fraction showed maximum reduction ($p<0.001$) of 50% at 24th hour. Metformin caused maximum reduction ($P<0.001$) of blood glucose level of 49.62% on 10th hour of experiment after treatment in comparison to controlled diabetic rats.

Effects of different fractions of C. Cordifolia on total cholesterol and triglyceride levels in normal rats

The mean serum total cholesterol and triglyceride levels of control and extracts of *C. cordifolia* treated animals (after intraperitoneal administration of a single dose) on the 24th hour are shown in figs. 1 and 2. Hypolipidemia was observed in animals treated with *C. cordifolia* extracts. The cholesterol level is reduced to 68.99%, 73.17%, 68.96% and 71.22% than normal control group by metformin, $CHCl_3$-CC, EtAc-CC and Pet-CC fractions respectively. The most significant reduction ($p<0.001$) was shown by the EtAc-CC fraction (31.04%), which is similar to metformin (31.01%).

In case of the effects of metformin and different fractions of *C. cordifolia* on serum triglyceride level of normal rats,

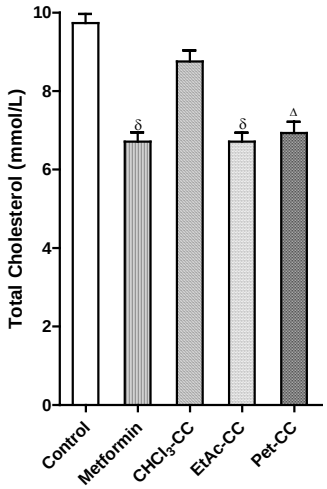

Fig. 1: Effect of different fractions of *C. cordifolia* on the total cholesterol (mmol/L) in normal rats.
The results are expressed as means ± S.E.M. Δ (**) indicates significant change in serum total cholesterol compared with normal control group (p< 0.01). δ (***) indicates significant change in serum total cholesterol compared with normal control group (p< 0.001).

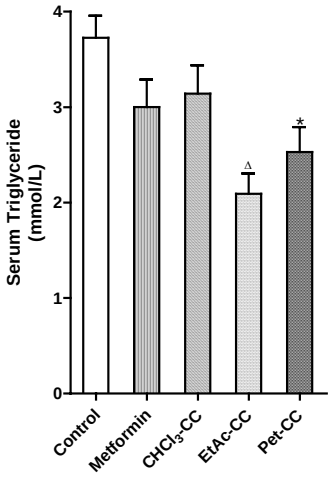

Fig. 2: Effect of different fractions of *C. cordifolia* on the triglyceride level (mmol/L) in normal rats.
The results are expressed as means ± S.E.M. Δ (**) indicates significant change in serum triglyceride compared with normal control group (p<0.01). * indicates significant change in serum triglyceride compared with normal control group (p< 0.05).

the metformin, $CHCl_3$-CC, EtAc-CC and Pet-CC reduced serum triglyceride level to 80.64% (not significant), 84.67%, 56.18% and 68.01% respectively. So the maximum reduction (p<0.01) of 43.82% was observed for EtAc-CC fraction.

Effects of different fractions of C. Cordifolia on total cholesterol and triglyceride levels in STZ-induced diabetic rats

The mean serum total cholesterol and triglyceride levels of control and extract of *C. cordifolia* treated animals (after intraperitoneal administration of a single dose) on the 24th hour of the experiment are shown in figs 3 and 4 respectively. In case of the effects of metformin and different fractions of *C. cordifolia* on total cholesterol level of STZ-diabetic rats, the metformin, $CHCl_3$-CC, EtAc-CC and Pet-CC reduced significantly (p<0.001) total cholesterol level to 64.61%, 72.48%, 63.31% and 78.32% respectively. Here the EtAc-CC showed maximum reduction (p<0.001) of 36.69%, which is almost similar to metformin (35.39%).

In case of the effects of metformin and different fractions of *C. cordifolia* on serum triglyceride level of STZ-diabetic rats, the metformin, $CHCl_3$-CC, EtAc-CC and Pet-CC reduced significantly (p<0.05) serum triglyceride level to 73.04%, 59.66%, 24.35% and 57.99% respectively than control diabetic group. The EtAc-CC fraction showed maximum reduction (p<0.05) of 42.01% serum triglyceride level.

However, the serum cholesterol and triglyceride lowering efficiency of ethyl acetate fraction of *C. cordifolia* was found higher than other fractions. All the plant fractions were found to have the antihyperlipidemic activity in SIDRs.

DISCUSSION

Plant medicines (phytotherapies) have a long history as treatment for diabetes. With a disturbing rise in the prevalence of this metabolic disease and associated healthcare costs, interest in alternative or complementary therapies has grown. Over the last 10-20 years data from controlled investigations in animal models and patients have validated the therapeutic values of numerous phytotherapies for diabetes. The aim of the present study was to evaluate the antidiabetic and hypolipidemic activity of intraperitonial ingestion of different organic solvent fractions of *C. cordifoia* on normal and STZ-induced diabetic rats. Our previous studies (Akhter *et al*., 2007) showed that the ethanolic extracts of the plant has potent hypoglycemic activity, when administered intraperitonially as a single dose/day in alloxan induced diabetic rats. We also found that the ethanolic extracts of this plant induces hypocholesterolemia and hypotriglyceridemia in both the normal and diabetic rats. For the present study, the experimental conditions (normoglycemic and STZ-induced diabetic) were selected with the aim to determine if the extracts have an insulin-like rapid onset of action to 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

Table 1: Fasting blood glucose (FBG) level (mmol/L) after treatment of metformin and different fractions of *C. cordifolia* on normal rats.

Treatment	0 hr	1 hr	2 hr	3 hr	6 hr	10 hr	16 hr	24 hr
Control	6.7±0.23	6.3±0.23	6.0±0.28	6.1±0.23	5.9±0.28	6.0±0.28	5.8±0.23	5.9±0.32
Metformin	7.0±0.23	6.5±0.23	4.6±0.28*	3.5±0.23$^{\Delta}$	2.8±0.28$^{\Delta}$	3.5±0.23$^{\Delta}$	4.6±0.28*	6.5±0.26
$CHCl_3$-CC	6.1±0.28	5.9±0.28	5.6±0.28	5.6±0.28	5.4±0.28	5.8±0.28	6.0±0.28	6.3±0.28
EtAc-CC	6.4±0.28	6.1±0.23	5.8±0.28	5.5±0.23	5.3±0.23	5.2±0.23	4.9±0.28	3.5±0.28$^{\Delta}$
Pet-CC	6.6±0.28	6.3±0.23	6.1±0.23	5.8±0.28	5.5±0.23	4.8±0.23*	3.5±0.23$^{\Delta}$	4.5±0.23*

* indicates significant changes in FBG level compared to normal rats after treatment (P< 0.05). Δ (**) indicates significant changes in FBG level compared to normal rats after treatment (P<0.01)

Table 2: Fasting blood glucose (FBG) level (mmol/L) after treatment of metformin and different fractions of *C. cordifolia* on normal control and STZ-induced diabetic rats

Treatment	0 hr	1 hr	2 hr	3 hr	6 hr	10 hr	16 hr	24 hr
Control	6.7±0.23	6.3±0.23	6.0±0.28	6.1±0.23	5.9±0.28	6.0±0.28	5.8±0.23	5.9±0.32
Diabetic	12.9±0.28$^{\Psi}$	13.2±0.28$^{\Psi}$	13.1±0.28$^{\Psi}$	13.5±0.23$^{\Psi}$	13.4±0.28$^{\Psi}$	12.9±0.17$^{\Psi}$	13.3±0.28$^{\Psi}$	12.8±0.23$^{\Psi}$
+Metformin	13.5±0.34	12.2±0.28	11.9±0.28	9.7±0.23 $^{\delta}$	8.5±0.23 $^{\delta}$	6.5±0.23 $^{\delta}$	6.9±0.28 $^{\delta}$	7.4±0.28 $^{\delta}$
+$CHCl_3$-CC	12.7±0.28	12.3±0.28	11.9±0.23*	11.6±0.28$^{\Delta}$	11.3±0.23$^{\Delta}$	10.5±0.23$^{\Delta}$	10.2±0.28$^{\Delta}$	10.1±0.28$^{\Delta}$
+EtAc-CC	12.4±0.28	12.0±0.28*	11.6±0.28*	11.3±0.28$^{\Delta}$	10.1±0.28$^{\Delta}$	8.9±0.28 $^{\delta}$	7.1±0.28 $^{\delta}$	6.4±0.28 $^{\delta}$
+Pet-CC	12.3±0.28	11.1±0.28$^{\Delta}$	10.30±0.23$^{\Delta}$	9.1±0.28 $^{\delta}$	6.9±0.28 $^{\delta}$	6.4±0.28 $^{\delta}$	7.1±0.28 $^{\delta}$	7.8±0.28 $^{\delta}$

Ψ (***) indicates significant changes in FBG level after diabetic induction (P<0.001).
* indicates significant changes in FBG level in SIDR after treatment (P< 0.05). Δ (**) indicates significant changes in FBG level in SIDR after treatment (P<0.01). δ (***) indicates significant changes in FBG level in SIDR after treatment(P<0.001).
Data are expressed as mean ± S.E.M., n = 5 rats in each group. All extracts were ingested intraperitonially at the rate of 150 mg/ kg body weight.

levels in the diabetic rats are about 22% of that in normal rats 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, as has been reported previously (Pari and Venkateswaran, 2004; Ruzaidi *et al*., 2005).

Metformin was used as a reference compound; Metformin is the most popular anti-diabetic drug in the world. It is a drug that is prescribed to treat type II diabetes. It works by decreasing the amount of sugar made by the liver and decreasing the amount of sugar absorbed into the body. As a result, metformin can help the body respond better to its own insulin and decrease blood sugar levels.

In the mid 1960s streptozotocin was found to be selectively toxic to the beta cells of the pancreatic islets, the cells that normally regulate blood glucose levels by producing the hormone insulin. This suggested the drug's use as an animal model of type I diabetes (Mansford *et al.*, 1968). It is well established that biguanides like metformin produce hypoglycemia by increasing the secretion of insulin from the pancreas (Yallow *et al*., 1960, Grodsky *et al.*, 1971) and these compounds are active in mild STZ-induced diabetes whereas they are inactive in intense STZ-induced diabetes (nearly all β-cells have been destroyed). However, since our results showed that metformin reduced the blood glucose levels in hyperglycemic rats, the state of diabetes is not severe. STZ-treated animals receiving the extracts of *C. cordifolia* showed rapid normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some β-cells are still surviving to exert their insulin releasing effect by different fractions. The composite extract has a protective therapeutic effect against diabetes through beta-cell regeneration capacity (Mallik *et al.*, 2009).

The present investigation showed that the different fractions of *C. cordifolia* when administered by intraperitoneal injection in normoglycemic and STZ-induced hyperglycemic rats for a day long experiment produced hypoglycemia in different extent like 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 (Akhtar *et al*., 2007), the ethanolic extract of *C. cordifolia* reduced blood sugar significantly in normoglycemic rats and produced more intense hypoglycemia in the diabetic rats. In the present study, among all the fractions of the ethanolic extract of *C.*

cordifolia, the ethyl acetate fraction is more active in controlling diabetes and then petroleum ether fraction is less active than the previous fraction, the chloroform fraction is relatively inactive. The mechanism of hypoglycemic action of the EtAc-CC and Pet-CC fractions are till unknown, but it is not via stimulation of insulin release from the pancreatic β-cells. However, since the STZ-diabetic rats do secrete some insulin (El-Hilaly and Lyoussi, 2002), the hypoglycemic action of the extracts of *C. cordifolia* may be insulin-mediated by mechanisms in common with metformin, 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 extracts of *C. Cordifolia* 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 extracts may also act via suppression of hyperglycemic hormones, such as epinephrine (Miura and Kato, 1997; Roman-Ramos *et al*, 1995).

During the experiments with the plant fractions in normoglycemic and STZ-induced diabetic rats, we found that EtAc-fraction of the plant is more active than other fractions, though it gives effect on 24th hour of the experiment. The Pet-CC fraction is also proved to be better both in normoglycemic and STZ-induced diabetic rats (tables 1 and 2). The relatively rapid onset of hypoglycemic action of continuous intraperitoneal injection of the *C. cordifolia*-extracts 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-

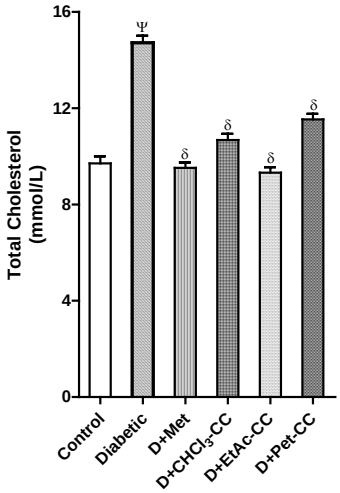

Fig. 3: Effect of different fractions of *C. cordifolia* on the total cholesterol (mmol/L) on diabetic rats compared to normal rats.

The results are expressed as means ± S.E.M. ψ (***) indicates significant change in serum total cholesterol compared with normal control group (p< 0.001). δ (***) indicates significant change in serum total cholesterol compared with diabetic control group (p< 0.001).

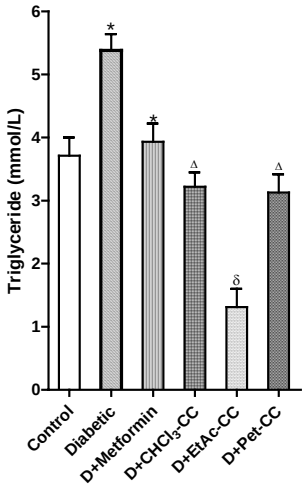

Fig. 4: Effect of different fractions of *C. cordifolia* on the serum triglyceride (mmol/L) on diabetic rats compared to normal rats.

The results are expressed as means ±S.E.M. * indicates significant change in serum triglyceride compared with normal control group (p< 0.05). * indicates significant change in serum triglyceride compared with diabetic control group (p< 0.05). Δ (**) indicates significant change in serum triglyceride compared with diabetic control group (p< 0.05). δ (***) indicates significant change in serum triglyceride compared with diabetic control group (p< 0.05).

Table 3: The phytochemical constituents of the experimental plant fractions obtained by phytochemical screening tests

	Saponin	Tanins	Triterpines	Alkaloids	Flavonoids
Petroleum ether-CC	(-)ve	(-)ve	(+)ve	(-)ve	(+)ve
Chloroform-CC	(+)ve	(-)ve	(-)ve	(-)ve	(+)ve
Ethyl acetate-CC	(-)ve	(-)ve	(+)ve	(+)ve	(+)ve

(-)ve = not detected (+)ve = detected

Suluybel, *et al*., 1995), or normalization of hepatic gluconeogenic enzymes (Pari and Venkateswaran, 2004).

During the short exposure of the animals to either extracts of *C. cordiflia* or metformin, hypolipidemia was pronounced in the diabetic rats and in the normal animals, and both substances had a greater effect on cholesterol than on triglyceride levels (figs. 1-4). Among the different fractions of the plant the serum total cholesterol and triglyceride lowering efficiency of ethyl acetate fraction of *C. cordifolia* was found higher than other fractions, which is very closer to the metformin preperation. The mechanism(s) of hypolipidemic effect of the extract may be similar to some of those proposed for metformin, including insulin-mediated lipolytic activity by inhibition of hormone-sensitive lipase 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 antihyperglycemic effects of the fractions of *C. cordifolia* were probably mediated by an enhanced secretion of insulin, like biguanides. The phytochemical screening test result showed that ethyl acetate fraction of coccinia contains triterpines, alkaloids and flavonoids compounds, which are known to be hypoglycemic. The pet ether fraction also has hypoglycemic potentialities may be due to the presence of triterpenes and flavonoids. Through chemical analysis, *C. cordifolia* is known to be rich in β-carotene, a major precursor of vitamin A from plant sources. Besides, β-carotene, *C. cordifolia* is a good source of protein, fiber and a moderate source of calcium (Wasantwisut *et al*., 2003). Further investigations are warranted to identify the hypolipidemic mechanism of the active principles in *C. cordifolia.*

In conclusion, this study is unique, in that this is the first study to show that the intraperitoneal administration of the plant fractions of *C. cordifolia* fractions caused rapid induction of hypoglycemia and hypolipidemia in normal and STZ-induced diabetic rats. Our studies have indicated that the EtAc fraction of *C. cordifolia* showed maximum reduction of blood glucose level and lipid level among the different fractions of the plant. On the other hand petroleum-ether fraction of the plant has also reduced significant blood glucose level. In the light of our pharmacological studies *C. cordifolia* appears to be a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes. The leaves of *C. cordifolia* seem to have a promising value for the development of potent phytomedicine for diabetes. We are carrying out additional chemical and pharmacological studies to determine the mechanism(s) of action of the EtAc-CC and Pet-CC fractions and to isolate the active principles responsible for the hypoglycemic action and hypolipidemic action.

REFERENCES

Ahmed I, Lakhani MS, Gillett M, John A and Raza H (2001). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabet. Res. Clin. Pract.*, **51**: 155-161.

Akhtar MA, Rashid M, Ibne Wahed MI, Islam MR, Shaheen SM, Islam MA, Amran MS and Ahmed M (2007). Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats. *Res. J. Med. Medical Sc.,* **2**(1): 29-34.

A.H.M. Khurshid Alam (1999). Antishigella activity of *Hemigraphis hirta* T. Anders and *Achyranthus ferruginea* Roxb. A dissertation submitted to the department of Pharmacy, University of Rajshahi, Bangladesh in partial fulfillment of the requirements for the degree of Master of Pharmacy.

Azad Khan AK, Akhtar S, and Mahtab H (1979). Coccinia indica in the treatment of patients with diabetes mellitus. *Bang.Med.Res. Coun. Bull.*, **5**: 60-66.

Chopra RN, Nayar SL and Chopra IC (1986). Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi, India, p.29.

Da Ros R, Assaloni R and Ceriello A (2004). The preventive anti-oxidant action of thiazolidinedione: a new therapeutic prospect in diabetes and insulin resistance. *Diabetic Med.,* **21**: 1249-1252.

David MN, James M and Daniel ES (1997). The epidemiology of cardiovascular disease in type 2 diabetes mellitus, how sweet it is . . . or is it? *Lancet*, **350**: S14-S19.

El-Hilaly J and Lyoussi B (2002). Hypoglycaemic effect of the lyophilised aqueous extract of *Ajuga iva* in normal and streptozotocin diabetic rats. *J. Ethnopharmacol*., **80**: 109-113.

Grodsky GM, Epstein GH, Fanska R and. Karam JH (1971). Pancreatic action of sulphonylureas. Fed. Proc., **36**: 2719-2728.

Holman RR and Turner RC (1991). Oral agents and insulin in the treatment of NIDDM. *In*: Pickup S and Williams G editors. Textbook of Diabetes. Blackwell Eds., Oxford, pp.467-469.

Kanigur-Suluybel G, Guven M, Onaran I, Tezcan V, Cenani A and Hatemi H (1995). The effects of metformin on insulin receptors and lipid peroxidation in alloxan and streptozotocin-induced diabetes. *J. Basic Clin. Physiol. Pharmacol.*, **6**: 271-281.

Kuriyan R, Bantwal G, Rajendran R and Kurpad AV (2008). Effect of supplementation of *Coccinia*

cordifolia extract on newly detected diabetic patient. *Diabet Care*., **31**(2): 216-220.

Larner J (1985). Insulin and oral hypoglycaemic drugs, glucagon. In: Gilman AG, Goodman LS, Rall TW Murad F editors. The Pharmacological Basis of Therapeutics, 7th ed., Macmillan Publishing Co., New York, pp.1490-1516.

Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J and Roufogalis BD (2005). *Punica granatum* flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J. Ethnopharmacol*., **99**: 239-244.

Liang XC, Xie MZ and Guo, SS(1993). Clinical and experimental study on effect of jin-qi-jiang-tang-pian or qi-yin deficiency and hyperactivity of diabetes mellitus. *Ch.J.Integ.Trad.West. Med.,* **13**(10): 587-590.

Linney G (1986). Coccinia grandis (L.) Voight: A new cucurbitaceous weed in Hawai'i. *Hawaii Bot. Soc. Newslet.,* **25**: 3-5.

Luo Q, Cai Y, Yan J, Sun M and Corke H (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sci.,* **76**: 137-149.

Luzi, L (1998). Pancreas transplantation and diabetic complications. *New Eng. J. Med.,* **339**: 115-117.

Mallick C, De D and Ghosh D (2009). Correction of protein metabolic disorders by composite extract of *Musa paradisiaca* and *Coccinia indica* in streptozotocin-induced diabetic albino rat: an approach through the pancreas. *Pancreas* **38 (3)**:322-329

Mansford KR and Opie L (1968). Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by Alloxan. *Lancet* **7544**:670-671.

Miura T and Kato A (1997). Hypoglycemic action of *Embelia madagascariensis* in normal and diabetic mice. *Am. J. Chinese Med.,* **25**:169-173.

Miura T, Itoh C, Iwamoto N, Kato M, Kawai M, Park SR and Suzuki I (2001). Hypoglycemic activity of the fruit of the *Momordica charantia* in type 2 diabetic mice. *J.Nutr. Sci. Vitaminol.,* **47**: 340-344.

Momin A (1987). Role of indigenous medicine in primary health care. In Proceedings of First International Seminar on Unani Medicine. New Delhi, India, pp: 54.

Mukherjee SK (1981). Indigenous drugs in diabetes mellitus. *J. Diabetes Assoc. Ind.,* **21**: 97-106.

Nagappa AN, Thakurdesai PA, Rao VN and Singh J (2003). Antidiabetic activity of *Terminalia catappa* Linn fruits. *J. Ethnopharmacol*., **88**: 45-50.

Nandhini ATA, Thirunavukkarasu V and Anuradha CV (2004). Stimulation of glucose utilization and inhibition of protein glycation and AGE products by taurine. *Acta Physiol. Scand.,* **181**: 297-303.

Nayak BS and Pinto Pereira LM (2006). *Catharanthus roseus* flower extract has wound-healing activity on Sprague Dawley rats. *BMC Complement Altern. Med.* **6:** 41.

Oliver-Bever B (1986). Oral hypoglycemic action of medicinal plants in tropical West Africa. Cambridge University Press, 245-267.

Pari L and Venkateswaran S (2004). Protective role of *Phaseolus vulgaris* on changes in the fatty acid composition in experimental diabetes. *J. Med. Food.,* **7**: 204-209.

Rai MK(1995). A review on some antidiabetic plants of India. *Ancient Sc. Life.,* **14**: 42-54

Rathi SS, Grover JK and Vats V (2002). The effect of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *Phytother. Res.,* **16**: 236-243.

Roman-Ramos R, Florez-Saenz JL and Alarcon-Aguilar FJ (1995). Antihyperglycemic effect of some edible plants. *J. Ethnopharmacol*., **48**: 25-32.

Ruzaidi A, Amin I, Nawalyah AG, Hamid M and Faizul HA (2005). The effect of malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. *J. Ethnopharmacol*., **98**: 55-60.

Sachdewa A and Khemani LD (2003). Effect of *Hibiscus rosa sinensis* Linn. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J. Ethnopharmacol*., **89**: 61-66.

Sharma SR, Dwivedi SK and Swarup D (1997). Hypoglycaemic, antihyperglycaemic and hypolipidemic effects of *Caesalpinia bonducella* seeds in rats. *J. Ethnopharmacol*., **58**: 39-44.

Siddique O, Sun Y, Lin JC and Chein YW (1987). Facilitated transdermal transport of insulin. *J Pharm Sci.* **76**: 341-345.

Spiller HA and. Sawyer TS (2006). Toxicology of oral antidiabetic medications. *Am. J. Health-Sys. Pharm.,* **63**: 929-938.

Stenman S, Groop PH, Laakkonen K, Wahlin-Boll E and Melander A (1990). Relationship between sulfonylurea dose and metabolic effect. *Diabetes*., **39**: 108A.

WHO Expert Committee on Diabetes mellitus (1980). Second report, Technical report series 646. World Health Organization, Geneva, pp.1-80.

Wasantwisut E and Viriyapanich T (2003). Ivy gourd (Coccinia grandis Voigt, Coccinia cordifolia, Coccinia indica) in human nutrition and traditional applications. *World Rev. Nutri. Diet*, **91**: 60-66.

Yallow RS, Black H, Villazan M and Berson SA (1960). Comparison of plasma insulin levels following administration of tolbutamide and glucose. *Diabetes*, **9**: 356-362.

Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Vilella D, Diez MT, Pelaez F, Ruby C, Kendall RL, Mao X, Griffin P, Calaycay J, Zierath JR, Heck JV, Smith RG and Moller DE (1999). Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Science* **284**: 974-977.