ORAL GLUCOSE TOLERANCE TEST (OGTT) IN NORMAL CONTROL AND GLUCOSE INDUCED HYPERGLYCEMIC RATS WITH COCCINIA CORDIFOLIA L. AND CATHARANTHUS ROSEUS L.

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ABSTRACT

The aim of this study is to investigate the hypoglycemic effects of petroleum ether, chloroform and ethyl acetate fractions isolated from ethanolic extracts of *Coccinia cordifolia* and *Catharanthus roseus* on normal control and orally glucose-induced hyperglycemic rats. Single doses (150 mg/kg) of different fractions of *C. cordifolia* and *C. roseus* extracts were intraperitonelly administered. The serum blood glucose level was obtained by pricking the tail vein using glucometer at time 0, 30, 60, 90, 150 and 270 minutes.

In the orally glucose induced hyperglycemic rats, chloroform-coccinia ($CHCl_3$-CC) fraction showed maximum reduction of blood glucose level by 21.94% on 60 minute of the experiment. On the other hand maximum reduction ($p<0.05$) of 17.92% was observed for petroleum ether-catharanthus (PET-CR) on 30 minute of the experiment. Metformin HCl was used as standard drug.

Our results indicate that the $CHCl_3$-CC fraction is relatively more potent than other fractions of *C. cordifolia*. Similarly the PET-CR is found to be better than other fractions of catharanthus. Phytochemical screening test results showed that chloroform fraction of *C. cordifolia* contain saponins and flavonoids compounds, which are known to be hypoglycemic. On the other hand petroleum ether fraction of *C. roseus* contains tannins, flavonoids and alkaloid compounds produced varying degree of blood sugar reduction. On the pharmacological point of view *C. cordifolia* and *C. roseus* appears to be a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes.

Keywords: *Coccinia cordifolia*, *Catharanthus roseus*, OGTT, metformin, hyperglycemic rats.

INTRODUCTION

The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose, that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Luzi, 1998). Ivy gourd or *Coccinia cordifolia* is an aggressive vine in the Cucurbitaceae (cucumber) family. The juice of the roots and leaves are used to treat diabetes in folk medicine. 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 (Khan *et al*., 1979). *Catharanthus* roseus Linn. (Apocyanaceae) is an herbaceous sub-shrub also known as Madagascar periwinkle, *Vinca rosea*, or *Lachnera rosea* worldwide. The leaves are used traditionally in various regions of the world to control diabetes (Cowley & Bennett, 1928). Significant antihyperglycemic and hypotensive activity of the leaf extracts (hydroalcoholic or dichloromethane-methanol) have been reported in laboratory animals (Pillay *et al*., 1959). Fresh leaf juice of *C. roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits (Nammi *et al*., 2003). Investigation on the fractionated portion of ethanolic extracts of *C. cordifolia* and *C. roseus* has not been performed. In this study the blood glucose lowering effects of the petroleum ether, ethyl acetate and chloroform fractions of the ethanolic extract of *C. cordifolia* and *C. roseus* leaves on oral glucose load has been investigated on normal rats.

MATERIALS AND METHODS

Fresh leaves of *C. cordifolia* and *C. roseus* were collected and dried under shadow for several days. After grinding into coarse powder, dried leaves of *C. roseus* and *C. cordifolia* were soaked for 5-7 days in 2 liter of 95% ethanol with occasional shaking and stirring. The ethanolic extract was obtained by the same method as previously reported (Akhtar *et al*., 2007). DMSO (dimethyl sulfoxide) was used to dissolve metformin and

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the extracts, since these substances are insoluble in water and other available inert solvents (Akhtar *et al*., 2007). Crude ethanolic extract was dissolved in 100 ml distilled water. To the solution almost equal volume of pet-ether, ethyl acetate and chloroform were added. After proper shaking the separated layers were collected. The process was repeated for three times and collected, respective layers 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 at 4ºC until use. Phytochemical screening tests for saponins, tannins, triterpenes and alkaloids were performed according to the standard methods (Nayak *et al*., 2006).

A total number of 40 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). The animals were prepared for study as before (Akhtar *et al*., 2007). Group I served as a normal control group while group II for metformin control group. Group III, IV and V were treated with pet-ether, ethyl acetate and chloroform fractions of *C. cordifolia*. Group VI, VII and VIII were treated with pet-ether, ethyl acetate and chloroform fractions of *C. roseus.* The reference drug and the extracts were administered intraperitonially to the rats.

OGTT for nondiabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925). In short, Group I to Group VIII was selected for OGT test after starving at water for 16 hours. The baseline glucose level was measured by glucometer (BioLand G-423 glucose test meter). Group I stands for normal control group. Group II is treated with metformin (150 mg/kg body weight). The extract of different fractions was then administered intraperitonially to the glucose fed (2 gram/kg body weight) rats at the dose of 150 mg/kg body weight. Serum glucose of blood sample from tail vein was estimated by using glucometer at 0, 30, 60, 90, 150 and 270 minutes. Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test and the values were considered statistically significant when $P<0.05$. 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 AND DISCUSSION

Experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a 1.5 to 2-fold increase in plasma glucose levels (comparing bar groups of 0 minute with the bar groups of 30 minute, fig. 1).

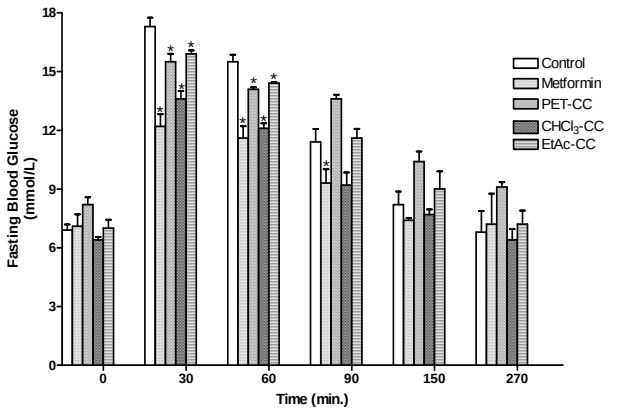

Fig. 1: Effect of different fractions of *C. cordifolia* on the glucose-induced hyperglycemia in normal rats. The results are expressed as means ± SEM. * indicates significant change in blood glucose level (mmol/L) compared with normal control group ($p< 0.05$).

Most significant reduction was observed for $CHCl_3$-CC and then PET-CC and EtAc-CC on 60 minute. In OGTT (fig. 1) metformin showed reduction of blood glucose level of 70.52%, 74.83%, and 81.57% at 30 min, 60 min and 90 minutes respectively. So, the maximum reduction ($p<0.05$) of 29.48% for metformin was observed on 30 min. PET-CC fraction showed blood glucose level of 89.59% and 88.38% at 30 and 60 min respectively. $CHCl_3$-CC fraction showed blood glucose level of 78.61% and 78.06% at 30 min and 60 min respectively. EtAc-CC showed blood glucose level of 91.90% and 92.90% at 30 min and 60 min respectively. So among the different fractions isolated from *C. cordifolia*, $CHCl_3$-CC fraction is more potent for hypoglycemic activity. Experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a 1.5 to 2-fold increase in plasma glucose levels (fig. 2).

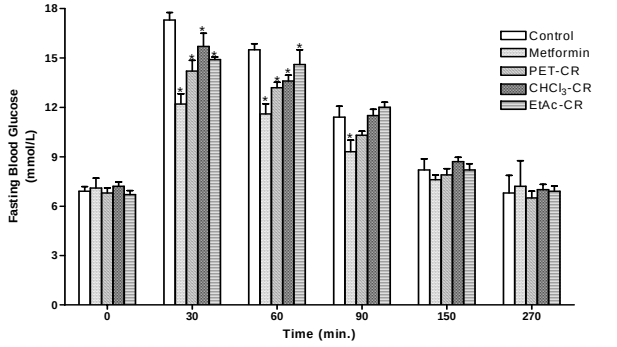

Fig. 2: Effect of different fractions of *C. roseus* on the glucose-induced hyperglycemia in normal rats. The results are expressed as means ± SEM. * indicates significant change in blood glucose level (mmol/L) compared with normal control group ($p< 0.05$).

PET-CR fraction showed blood glucose level of 82.08% and 85.16% at 30 min and 60 min respectively. $CHCl_3$-

CR fraction showed blood glucose level of 90.75% and 87.74% at 30 min and 60 min respectively. EtAc-CR fraction showed blood glucose level of 86.12% and 94.19% at 30 min and 60 min respectively. Among the different fractions of *C. roseus*, PET-CR is more potent for hypoglycemic activity. Maximum reduction ($p<0.05$) of 17.92% was observed for PET-CR on 30 min of the experiment. This may be due to the presence of hypoglycemic alkaloids (leurosine, vindolinine etc) and flavonoids. Maximum reduction ($p<0.05$) of blood glucose level of 21.94% occurs for $CHCl_3$-CC fraction was observed at 60 min. This may be due to the presence of saponin glycoside and flavonoids.

A study in our laboratory showed that the crude ethanolic extract of *C. roseus* and *C. cordifolia* reduced blood sugar significantly in normoglycemic rats and produced more intense hypoglycemia in the diabetic rats (Akhtar *et al*., 2007). In the present study, among the all fractions of the ethanolic extract of *C. roseus* and *C. cordifolia*, the $CHCl_3$ fraction of *C. cordifolia* and petroleum ether fraction of *C. roseus* are relatively more active; ethyl acetate fractions are relatively inactive. No histological studies were carried out to prove the antihyperglycemic effects and it is not possible to explain the detailed mechanism of antidiabetic action of the fractions of *C. cordifolia* and *C. roseus.* But it can be suggested that the mode of action of the active constituent(s) of *C. cordifolia* and *C. roseus* is probably mediated by an enhanced secretion of insulin, like biguanides.

The phytochemical screening test result showed that chloroform fraction of *C. cordifolia* contains saponins and flavonoids compounds, which are known to be hypoglycemic. On the other hand, petroleum ether fraction of *C. roseus* contains tannins, flavonoids and alkaloid compounds (catharanthin, leurosine, lochnerine, tetrahydroalstonin, vindoline and vindolinine) produced varying degree of blood sugar reduction (Svoboda *et al.,* 1964).

Through chemical analysis, *C. cordifolia* is known to be rich in β-carotene, a major precursor of vitamin A from plant sources. The hypoglycemic effects have resulted from the antioxidant effect of the different fractions of ethanol extract of *C. roseus*, whose phytochemical components include flavonoid, which is known for antioxidant effect (Afanas'ev *et al*., 1995). Further investigations are warranted to identify the hypoglycemic mechanism of the active principles in C. *cordifolia* and *C. roseus.*

This is the first study to show that the intraperitoneal administration of the plant fractions of *C. cordifolia* and *C. roseus* ethanolic extract cause rapid induction of hypoglycemia in orally glucose induced hyperglycemic rats. Our studies have shown that the $CHCl_3$ fraction of *C. cordifolia* showed maximum reduction of blood glucose level among the different fractions of the plant. On the other hand petroleum ether fraction of *C. roseus* have reduced maximum blood glucose level in comparison with other fractions. In the light of our pharmacological studies we can assume that further experiment should be carried out for isolating the possible hypoglycemic compounds and then explain the actual mechanism of hypoglycemic actions of the plant fractions. The present study has given some preliminary idea of the hypoglycemic compounds present in the reported plant fractions.

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