Antihyperglycemic and hypolipidemic effects of *Hibiscus schizopetalus* (Mast) Hook in alloxan-induced diabetic rats

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**Abstract**: The antihyperglycemic and hypolipidemic activities of *Hibiscus schizopetalus* (Mast) Hook (Malvaceae) flower and leaves extracts were investigated in alloxan-induced diabetic rats. The hypoglycemic activity of both the extracts (100mg/kg, body weight) was tested in fasting normal rat, glucose loaded rats. Observation on body weight was also recorded. The extracts showed a significant (P<0.001) reduction in blood glucose level in normal fasting rats. In glucose tolerance test, significant (P<0.01) decreased observed in all glucose loaded animals. While in alloxan induced diabetic rats, the percent blood glucose reduction was 59.94% and 45.14% in extracts treated groups. The results obtained were compared with the reference standard drug Tolbutamide (100mg/kg, body weight). The diabetic rats showed sign of decreased in their body weight during the treatment period. Cholesterol and triglycerides levels were significantly decreased (P<0.001) by HFE. The results obtained demonstrated the potential hypoglycemic activity of methanolic extracts of *H. schizopetalus*. There is need of bioassay-directed assay of the active principles responsible for the anti-diabetic activity. The methanolic extracts showed the presence of carbohydrates, alkaloids, steroids, terpenes, saponins and glycosides.

**Keywords**: *Hibiscus schizopetalus* (Mast) Hook, alloxan-induced diabetic rats, hypoglycemic activity, hypolipidemic, phytochemicals.

**INTRODUCTION**

Diabetes mellitus is a major disorder affecting nearly 10% of the population all over the World (Bruke *et al.*, 2003). Diabetic complications arise due to the glycosylation damage to structural and functional proteins and causes chronic failure to maintain blood glucose homeostasis. Other complications includes diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and diabetic cardiomyopathy prevail as a result of hyperglycemia (Annupurna *et al.*, 2001).

The conventional drugs used for diabetes mellitus such as sulfonylureas, biguanides, α-glucosidase inhibitors, glinides, are associated with several side effects and fail to significantly alter the course of diabetic complications. Management of diabetes without any side effect is still a challenge (Sy *et al.*, 2005; Kameswara *et al.*, 1999). The search for new pharmacologically active agents obtained by natural sources such as medicinal plants has led to the discovery of many useful drugs that play a major role in the treatment of human diseases. A number of medicinal plants and their formulations are used for the treatment of diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices (Grover *et al.*, 2002). In accordance with the recommendation of the WHO expert committee on diabetes mellitus the hypoglycemic agent of plant origin used in traditional medicine have become more significant. There are many herbs and plant products possess hypoglycemic action like *Allium cepa*, *Allium sativum*, *Syzygium cumini*, *Eugenia jambolan*, *Momordica charantia*, *Gymnema sylvestre*, *Pterocarpus marsupium* etc (Ajgonkar, 1979; Mukherjee, 1981; Bailey and Day, 1989).

The genus Hibiscus comprises about 275 species in the tropics and subtropics. With attractive and colorful flowers, plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine (Dasuki, 2001). *Hibiscus schizopetalus* (Mast) Hook belongs to the family Malvaceae (Yasin, 1979). It is one of the least examined specie of this genus. It is a shrub with spreading or usually drooping branches found in east of tropical Africa. It is also a common ornamental shrub cultivated in Pakistan. Coral Hibiscus, Chinese Hibiscus, Japanese lantern, Fringed Hibiscus (English), Tanglong (Malay), Arana (Spanish) are its common names. From April to September it bears red or orange-red flowers, drooping with deeply fringed petals. It is used as male parent in the crosses with Hibiscus rosa-sinensis Linn. and its varieties. Colombians use the infusion of flower to treat cold and cough (Jalan, 2002).

There is no antidiabetic screening reported in literature for *H. Schizopetalus*. Analgesic and antipyretic activities have been evaluated (Zahid *et al.*, 2012). The basic aim of this study was to investigate the hypoglycemic and hypolipidemic potential of this plant.
MATERIAL AND METHODS

Collection, identification and extraction
Hibiscus schizopetalus (Mast) Hook leaves and flower was collected from the premises of University of Karachi, Pakistan, in the month of July, 2009. The plant materials were identified and authenticated by Prof. Dr. Suriya Khatoon, Chairperson Department of Botany, University of Karachi, Pakistan. A voucher specimen No. 082 was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

After the collection and weighing, leaves and flower were washed with distilled water to remove dirt and dried under shade separately. Leaves (1000g) and flower (500g) were chopped and soaked in absolute methanol at room temperature for 7-10 days. The resulting mixtures were filtered and filtrate was removed under controlled temperature (40±2°C) and reduced pressure on a rotary evaporator (Buchi, Switzerland) to get 40.1 g of HFE and 73.8 g of HLE respectively. The resultant extracts were used in the experiment.

Experimental animals
Adult albino rats weighing between 130-200 g of either gender were used in the study. Animals were maintained under standard environmental conditions at a temperature of 27 ± 2°C. They were exposed to 12 h of dark and light cycle and fed with standard laboratory diet (PCSIR Laboratories, Karachi, Pakistan) and water. Diet was removed from the animal cages at least 12 h before each experiment but allowed access to water. The study was conducted after the approval from the institutional ethical committee.

Chemicals
Absolute Methanol (Merck, Germany), Tolbutamide (Merck, Germany), Glucose (Merck, Germany), Alloxan monohydrate (Sigma Chemical, USA).

IDENTIFICATION OF PHYTOCHEMICAL

The extracts were tested for the presence of bioactive compounds by using standard methods (Harborne, 1973; Trease and Evan, 1989).

Test for carbohydrates
The extracts (100 mg) were dissolved in 5ml of water and filtered. The filtrate was then subjected to the following tests.

Molish’s test
In 2 ml of filtrate, two drops of alcoholic solution of α-naphthol are added, the mixture was shaken well and then 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. Formation of violet ring indicates the presence of carbohydrates.

Fehling’s Test
One ml of filtrate was boiled with 1 ml each of Fehling solutions A and B on water bath. A red precipitate appears that indicates the presence of sugar.

Barfoed’s Test
To 1 ml of filtrate, 1 ml of Barfoed’s reagent was added and heated on a boiling water bath for 2 min. red precipitate indicates presence of sugar.

Benedict’s Test
In 0.5 ml of filtrate, 0.5 ml of Benedict’s reagent was added. This mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of carbohydrates.

Test for Fixed Oils
Spot Test
A small quantity of each extract was pressed between two filter papers. Oil stain on the filter paper indicates the test is positive.

Test for Alkaloids
0.5 g of the extract was mixed with 5 ml of 1% aqueous hydrochloric acid on a water bath. The filtrate is carefully tested with different alkaloidal reagents for the presence of alkaloids.

Dragendroff’s Test
1 or 2 drops of freshly prepared Dragendroff’s reagent was added to few ml of filtrate and then observed for formation of yellow or orange precipitate.

Mayer’s Test
One drop or two drops of Mayer reagent was added to few ml of filtrate by the side of the test tube. Formation of white or yellowish color precipitate indicates the presence of alkaloids.

Test for Flavonoids
Shinoda Test
In an alcoholic solution of the respective extracts two to three pieces of Magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

Ferric chloride Test
Extracts was boiled with water and filtered to 2 ml of the filtrate, two drops of freshly prepared ferric chloride solution was added. The presence of phenolic hydroxyl group is confirmed by green, blue or violet colorations.

Alkaline reagent Test
Extracts (2 ml) was dissolve in 10% aqueous sodium hydroxide solution it gives yellow color, a change of color from yellow to colourless on addition of dilute HCL indicate the presence of flavonoids.
**Test for Terpenes**

*Liebermann-Burchard Test*

Anhydrous acetic acid (1 ml) was added to 1/ml chloroform and cooled to 0°C then 1-2 drops of concentrated sulphuric acid was added followed by the extract. The solution was observed for blue, green, red or orange colour that changes with time.

**Test for Steroids**

*Salkowski Test*

A little quantity of the each extracts was dissolved in 1 ml chloroform and to it 1 ml of concentrated sulphuric acid was added down to form two phases. Formation of red colouration was taken as an indication for the presence of sterols.

**Test for tannins**

*Lead acetate Test*

Few drops of 1% lead acetate were added in five ml of the extracts. A yellow precipitate was showed the presence of tannins.

**Test for Saponins**

*Frothing Test*

The extracts were shaken with distilled water in separate test tubes. Formation of froth which persisted for 15 min indicates the presence of saponins.

**Test for Glycosides**

The extracts were hydrolyzed with concentrated HCL for few hours on a water bath, filtered and hydrolysate was subjected to following tests.

*Legal’s Test*

To the hydrolysate, one ml of pyridine was added with 1-2 ml of sodium nitroprusside. It was then made alkaline with 10% sodium hydroxide solution. Presence of glycoside is indicated by pink colour.

*Borntrager’s Test*

Filtered hydrolysate (2 ml) was added in 3/ml of chloroform and then shaken. 10% of ammonia solution was added to it. Presence of pink colour in the lower phase indicates the presence of glycosides.

**Test for gum and mucilages**

*Alcohol 95% Test*

The extract 100 mg is dissolved in 10ml of distilled water, 25 ml of absolute alcohol was added to this with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

**INDUCTION OF DIABETES**

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg, body weight) in normal saline. To avoid drug induced hypoglycemia the animals were allowed to drink 5% glucose solution overnight. After 72 h these animals were tested for diabetes and the animals with fasting blood glucose level between 170-300 mg/dl were selected for further study.

**Fasting blood glucose (FBG) in normal rats**

The hypoglycemic activity was performed in overnight fasted normal rats (Jarald et al., 2008). All animals were randomly divided into three groups of six rats each. Group I kept as control and given normal saline through oral intubation. Group II and III were orally administered with 100 mg/kg body weight of HFE and HLE respectively. The blood glucose concentration was measured just prior to 1, 2 and 3 h of administration. Blood samples were collected from tail tip of rats and measured the glucose concentration using glucometer.

**Oral glucose tolerance test (OGTT)**

All rats were randomly divided into three groups, each group consist of six animals. Group I orally administered with normal saline. Group II and III orally received 100 mg/kg body weight of HFE and HLE respectively. After 30 min glucose 2 g/kg body weight was given orally to all rats. Blood sample were taken from tip of the tail prior to glucose administration and at 30 min and 90 min after glucose loading (Vats et al., 2002).

**Effect of extracts on alloxan-induced diabetic rats**

Diabetic rats were divided into four groups. Group I served as diabetic control and orally administered with normal saline. Group II received Tolbutamide (100 mg/kg body weight) orally. Group III and IV received 100 mg/kg body weight of HFE and HLE respectively. The treatment was continued once daily for seven days. After seven days treatment blood were collected from tip of tail for the estimation of blood glucose level by glucometer (Vats et al., 2002; Dhanabal et al., 2008).

**Effect of extracts on body weight in alloxan-induced diabetic rats**

Body weight of all animals in each group was taken before alloxan induction. Then body weights of diabetic rats were taken on day 4 and day 7 of post-treatment by electronic balance (Electronic Scale, TH-1002).

**Estimation of biochemical parameters**

On day 7, the blood was collected from heart puncture and centrifuged at 3000 rpm for 10 minutes and clear serum was aspirated, stored frozen and then used for desired analysis. Serum total cholesterol and triglyceride were measured using kit available from Erba diagnostics (Adhire and Laddha, 2005).

**Collection of blood sample**

Blood samples were collected via tip of the tail at the defined time intervals and blood glucose levels were determined by blood glucose test strips with Elite-Glucometer (Bayer, USA).
STATISTICAL ANALYSIS

The results were expressed as mean ± S.E.M. The statistical evaluations were made using ANOVA followed by LSD post hoc multiple comparison tests, in order to compare control and treated groups. P≤0.05 was considered as significant. All data were processed with SPSS software version No.19.

RESULTS

Identification of phytochemical
The methanolic extracts of flower and leaves showed the presence of carbohydrates, alkaloids, steroids, terpenes, saponins and glycosides tabulated in table 1. Tannins and fixed oil are absent in flower extract while flavonoids are absent in leaves extract.

Fasting blood glucose (FBG)
Effect of leaves and flower extracts of Hibiscus schizopetalus (Mast) Hook was showed in fig. 1. Administration of each extracts (100 mg/kg, body weight) in normal fasting rats was found to reduce blood glucose level. The significant (P<0.001) reduction in blood glucose level was noted within 2 hours after oral administration of extracts.

Oral glucose tolerance test (OGTT)
The results of the effect of extracts of H. schizopetalus on oral glucose tolerance were shown in fig. 2. Administration of 100 mg/kg body weight of HFE and HLE extracts respectively produced significantly decrease (P<0.01) in elevated glucose level in all glucose loaded rats. In control group, highly impaired glucose tolerance was observed. However, in the extracts treated group, significant blood glucose attenuation was observed from 30 min onwards.

Effect of extracts on blood glucose level in alloxan-induced diabetic rats
The effect of HFE and HLE on blood glucose level in alloxan-induced diabetic rats were shown in fig. 3. The blood glucose level in diabetic control group significantly increased from 85.83 to 205.66 mg/dl after alloxan injection. The percentage blood glucose reduction with 100mg/kg, body weight of H. schizopetalus extracts and tolbutamide (100 mg/kg, body weight) was 59.94%, 45.14%, 35.50% respectively. Both the extracts treatment groups showed effective lowering of blood glucose level.

Effect of extracts on body weight in alloxan-induced diabetic rats
The changes of body weight in alloxan-induced diabetic rats were showed in fig. 4. Untreated diabetic rats showed a progressive fall in body weight throughout the experiment. A normal body weight gain (5.24%) was
observed in Tolbutamide (100 mg/kg, body weight) group compared to that of diabetic control. The treated diabetic animals showed sign of decreased in their body weight during the treatment period.

**Fig. 4**: Effect of extracts on body weight in alloxan-induced diabetic rats. Initial weights of all rats were taken before treatment and then at day 4 and 7. Values were expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by LSD post hoc test * \( p<0.05 \); **\( p<0.01 \); ***\( p<0.001 \).

**Effect of extracts on Lipid profile**
The effects on cholesterol, triglycerides concentration compared to the diabetic control were illustrated in fig. 5. Blood cholesterol was significantly decreased by tolbutamide and HFE (\( P<0.001 \)). While no significant reduction was observed in HLE treated group. Triglycerides level significantly (\( P<0.001 \)) decreased in diabetic rats, treatment with HFE and HLE at a dose of 100mg/kg, body weight for 7 days compared to the untreated group.

**Fig. 5**: Effect of extracts on blood cholesterol and Triglycerides levels.

**DISCUSSION**
Diabetes mellitus is a serious chronic disorder (Zhou, 2009). It is characterized by high blood glucose level due to absolute and relative lack of insulin (Villasenor et al., 2006). Alloxan is a cytotoxic agent to the insulin secreting \( \beta \)-cells of the pancreas and effectively induced diabetes in wide variety of animal models (Etuk, 2010; Lazarow, 1964). Thus, it allows elucidation of antihyperglycemic agent in the treatment of diabetes (Tanquilut et al., 2009). Traditional medicinal plants are used throughout the world for the treatment of wide range of diabetic complications. The plants extracts that are used for anti diabetic activity may contain one or more compound to decrease blood sugar level suggesting that the natural constituents could act separately or synergistically to produce hypoglycemic effect (Marles and Farnsworth, 1995).

The result obtained in normal fasting rats demonstrated that both the extracts produced significant reduction in blood glucose level within 2 h of oral administration of extract. In oral glucose tolerance test, all treated rats with extracts showed significant improvement in glucose tolerance. The onset of action for HFE was found at 30 min and at 90 min for HLE after glucose loading. Sulfonylureas are the compound produced hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes (Yallow et al., 1960). Since results showed that tolbutamide reduced blood glucose levels in alloxan induced diabetic animals, the state of diabetes is not severe. After 7 days treatment period with extract and tolbutamide blood glucose were decrease significantly. HLE was more effective in decreasing blood glucose as compare to HFE.

There may be more than one mechanism for the antihyperglycemic effect of the extracts. The possible mechanism by which the extracts causes hypoglycemic condition may probably due to increasing the insulin effect of plasma by stimulating insulin release from the pancreatic \( \beta \)-cells. (Mahmod and Ojewola, 2003). Beside this, other mechanism might include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Andrade-Cetto and Wiedenfeld, 2004).

Weight gain is indicator of efficient glucose homeostasis in normal condition; but in diabetics, glucose is not available for the cell therefore the cells utilizes alternatively proteins to produce energy. Consequently due to excessive breakdown of tissue protein results in loss of body weight (Gupta, 1994). Treatment with tolbutamide showed an increase in the body weight in diabetic rats, which can be attributed to the improvement in insulin secretion. Extracts treated groups did not showed increase in body weight.

Diabetes mellitus is also associated with hyperlipidaemia.
with profound alteration in the concentration and composition of lipid (Odetola et al., 2006). Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran et al., 2006). Alloxan significantly increased TG, phospholipids and cholesterol levels. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyper lipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony et al., 1994).

The decreased in cholesterol and triglycerides by HLE and HFE might be directly or indirectly related with the decreased of blood glucose levels in alloxan-induced diabetic rats.

It could be speculated that *H. schizopetalus* possess hypoglycemic activity in alloxan-induced diabetic rats. It may offer a valuable therapeutic agent for the treatment of diabetes mellitus. Further studies are necessary to determine the exact mechanism of action and active constituents of plant that are involved in hypoglycemic activity.

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