ANTIHYPERGLYCEMIC ACTIVITIES OF LEAVES OF THREE EDIBLE FRUIT PLANTS (*AVERRHOA CARAMBOLA*, *FICUS HISPIDA* AND *SYZYGIUM SAMARANGENSE*) OF BANGLADESH

Shejuty Shahreen, Joyanta Banik, Abdul Hafiz, Shahnaz Rahman, Anahita Tanzia Zaman, Md. Abu Shoyeb, Majeedul H. Chowdhury*, Mohammed Rahmatullah**

Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka-1205, Bangladesh *Present address: New York City College of Technology The City University of New York 300 Jay Street, Brooklyn, NY 11201, USA.
***Email:** rahamatm@hotmail.com

Abstract

Averrhoa carambola L. (Oxalidaceae), *Ficus hispida* L.f. (Moraceae), and *Syzygium samarangense* (Blume) Merr. & L.M. Perry (Myrtaceae) are three common plants in Bangladesh, the fruits of which are edible. The leaves and fruits of *A. carambola* and *F. hispida* are used by folk medicinal practitioners for treatment of diabetes, while the leaves of *S. samarangense* are used for treatment of cold, itches, and waist pain. Since scientific studies are absent on the antihyperglycemic effects of the leaves of the three plants, it was the objective of the present study to evaluate the antihyperglycemic potential of methanolic extract of leaves of the plants in oral glucose tolerance tests carried out with glucose-loaded mice. The extracts at different doses were administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (p.o.) using glucose oxidase method. Significant oral hypoglycemic activity was found with the extracts of leaves of all three plants tested. The fall in serum glucose levels were dose-dependent for every individual plant, being highest at the highest dose tested of 400 mg extract per kg body weight. At this dose, the extracts of *A. carambola*, *F. hispida*, and *S. samarangense* caused, respectively, 34.1, 22.7, and 59.3% reductions in serum glucose levels when compared to control animals. The standard antihyperglycemic drug, glibenclamide, caused a 57.3% reduction in serum glucose levels versus control. Among the three plants evaluated, the methanolic extract of leaves of *S. samarangense* proved to be the most potent in demonstrating antihyperglycemic effects. The result validates the folk medicinal uses of *A. carambola* and *F. hispida* in the treatment of diabetes, and indicates that the leaves of *S. samarangense* can also possibly be used for amelioration of diabetes-induced hyperglycemia.

Key words: *Averrhoa carambola*, *Ficus hispida*, *Syzygium samarangense*, antihyperglycemic

Introduction

The fruits of *Averrhoa carambola* L. (Oxalidaceae, local name: dumur, English name: hairy fig), *Ficus hispida* L.f. (Moraceae, local name; koromcha, English name; star fruit), and *Syzygium samarangense* [(Blume) Merr. & L.M. Perry (Myrtaceae, local name: jamrul, English name: Java apple] are edible and enjoy high consumer demand in Bangladesh. All three plants are also used in the folk medicinal system of Bangladesh; the leaves and fruits of *A. carambola* are used for treatment of diabetes, colic and fever, while the leaves and fruits of *F. hispida* are used for the treatment of diabetes. The leaves of *S. samarangense* are used to treat colds, itches, and waist pain. The roots of *F. hispida* are also used by folk medicinal practitioners (Kavirajes) for treatment of diseases of the gall bladder (Rahmatullah et al., 2009a). The leaves of the same plant are further used by the Kavirajes for treatment of jaundice and dermatitis, while fruits are used as carminative (Rahmatullah et al., 2009b; Rahmatullah et al., 2010).

The antioxidant properties of fruits of *A. carambola* and *S. samarangense* have been reported (Soubir, 2007). Hypotensive effects have been reported for aqueous extract of leaves of *A. carambola* in rats (Soncini et al., 2010). Methanol extract of *F. hispida* reportedly demonstrated significant inhibitory activity against castor oil-induced diarrhea in rats (Mandal and Kumar, 2002). The phenanthroindolizidine alkaloid, *O*-methyltylophorinidine, isolated from leaves and twigs of the plant reportedly showed potent cytotoxic activity against human cancer cell lines (Peraza-Sánchez et al., 2002). The protective effect of methanol extract of leaves has also been shown against paracetamol-induced hepatotoxicity in rats (Mandal et al., 2000). From the leaves of *S. samarangense*, two antihyperglycemic flavonoids have been reported, namely, 2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone, and its isomeric flavone, 5-*O*-methyl-4′-desmethoxymatteucinol (Resurreccion-Magno et al., 2005). Immunomodulatory effects have also been described for a number of flavonoids isolated from the acetone extract of leaves of the plant, as demonstrated through their inhibitory potency on human peripheral blood mononuclear cells proliferation activated by

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phytohemagglutinin (Kuo et al., 2004). Four flavonoids isolated from hexane extract of the plant showed spasmolytic and calcium antagonist activities, validating the anti-diarrheal use of the plant in folk medicine (Ghayur et al., 2006).

The objective of the present study was to investigate the antihyperglycemic activity of methanol extracts of leaves of the three plants, *A. carambola*, *F. hispida*, and *S. samarangense*. The leaves of the first two plants are in use in the folk medicinal system of Bangladesh for treatment of diabetes, but scientific studies are yet to be carried out. The third plant reportedly contains antihyperglycemic flavonoids in its leaves, and we decided to include the leaves of this plant also in the present antihyperglycemic study. The earlier study on antihyperglycemic activity was carried out with plants growing in the Philippines. It was of interest to see whether such antihyperglycemic activity also exists in plants growing in Bangladesh.

Materials and Methods

Collection of plant material

The leaves of *A. carambola*, *F. hispida*, and *S. samarangense* were collected, respectively, during May 2010 from Dhaka district, Bangladesh, December 2009 from Brahmanbaria district, Bangladesh and July 2010 from Dhaka district, Bangladesh. The leaves were identified by the Bangladesh National Herbarium, Mirpur, Dhaka (Accession Nos. 34,972, 34,490 and 35,070) and sample specimens have been kept over there.

Preparation of the test samples

The leaves of the plants were separately air-dried in the shade and pulverized into a fine powder and were mixed with methanol at a ratio of 1:3 (w/v). After 24 hours, the mixtures were filtered; the filtrate was collected and the residue was again mixed with methanol at a ratio of 1:2 (w/v) for 24 hours. After filtration, filtrates were combined and evaporated to dryness (approximate yields 10.4, 2.2 and 5.8% for *A. carambola*, *F. hispida*, and *S. samarangense*, respectively) using rotary evaporator. Extracts were suspended in 1% Tween 80 in water prior to administration.

Animals

Swiss albino mice (male), weighing 15-20 g bred in the animal house of ICDDR,B (International Centre for Diarrheal Disease and Research, Bangladesh) were used for the present experiments. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2^0C, and 12 hrs light-dark cycles). The animals were fed with standard diet from ICDDR,B and had free access to water. The study was approved by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.

Anti-hyperglycemic activity test

Antihyperglycemic activities of the extracts were studied through the glucose tolerance test method. Glucose tolerance test was performed following the procedure as described by Joy and Kuttan (1999) with slight modifications (Rahman et al., 2011; Ahmed et al., 2011). In brief, fasted mice were divided into fourteen groups. Each group received a particular treatment: group-I served as control and received vehicle (1% Tween 80 in water, 10 $ml.kg^{-1}$ body weight), while group-II received standard drug (glibenclamide, 10 $mg.kg^{-1}$ body weight). Groups III-VI received leaf extract of *A. carambola* at four different doses of 50, 100, 200 and 400 mg $extract.kg^{-1}$ body weight, respectively. Groups VII-X was administered the leaf extract of *F. hispida* at doses of 50, 100, 200 and 400 mg $extract.kg^{-1}$ body weight, respectively. Groups XI-XIV was administered the leaf extract of *S. samarangense* at doses of 50, 100, 200 and 400 mg $extract.kg^{-1}$ body weight, respectively. Each mouse was weighed properly and the doses of the test samples, standard drug, and control materials were adjusted accordingly. Test samples, control, and glibenclamide were given orally. After one hour, all mice were orally treated with 2 $g.kg^{-1}$ of glucose. Blood samples were collected two hours after glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method (Venkatesh et al., 2004).

Statistical analysis for anti-hyperglycemic activity

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Acute toxicity study

The study was carried out as previously described (Ganapaty et al., 2002) with minor modifications. For each plant leaf extract, selected animals were divided into nine groups of six animals each. The control group received 1% Tween 80 in normal saline (2 $ml.kg^{-1}$ body weight). The other groups received respectively, 100, 200, 300, 600, 800, 1500, and 3000 mg leaf methanolic $extract.kg^{-1}$ body weight. Animals were monitored closely after dosing for the next 8 hrs for any behavioral changes and were kept under observation up to 14 days to find out if there is any mortality.

Table 1: Effects of methanol extract of *A. carambola*, *F. hispida*, and *S. samarangense* (leaf) on serum glucose level in hyperglycemic mice.

Treatment	Dose	Serum glucose level ($mg.dl^{-1}$)	% inhibition
Group I (Control, vehicle)	1% Tween 80 in water ($10\ ml.kg^{-1}$ body weight)	162.3 ± 6.3	-
Group II (glibenclamide)	$10\ mg.kg^{-1}$ body weight	69.3 ± 4.2*	57.3
Group III (*A. carambola*)	$50\ mg.kg^{-1}$ body weight	118.8 ± 12.4*	26.8
Group IV (*A. carambola*)	$100\ mg.kg^{-1}$ body weight	113.6 ± 9.8*	30.0
Group V (*A. carambola*)	$200\ mg.kg^{-1}$ body weight	110.5 ± 10.4*	31.9
Group VI (*A. carambola*)	$400\ mg.kg^{-1}$ body weight	107.0 ± 7.2*	34.1
Group VII (*F. hispida*)	$50\ mg.kg^{-1}$ body weight	146.5 ± 8.1	9.7
Group VIII (*F. hispida*)	$100\ mg.kg^{-1}$ body weight	140.8 ± 7.8*	13.2
Group IX (*F. hispida*)	$200\ mg.kg^{-1}$ body weight	125.9 ± 4.2*	22.4
Group X (*F. hispida*)	$400\ mg.kg^{-1}$ body weight	125.4 ± 5.2*	22.7
Group XI (*S. samarangense*)	$50\ mg.kg^{-1}$ body weight	106.5 ± 3.8*	34.4
Group XII (*S. samarangense*)	$100\ mg.kg^{-1}$ body weight	85.8 ± 6.5*	47.1
Group XIII (*S. samarangense*)	$200\ mg.kg^{-1}$ body weight	85.4 ± 4.7*	47.4
Group XIV (*S. samarangense*)	$400\ mg.kg^{-1}$ body weight	66.0 ± 2.2*	59.3

Extracts and drug were given orally one hour before glucose administration and serum glucose level was measured two hours after glucose administration. Values are given as Mean ± S.E.M. from six mice in each group (Groups I-X) and seven mice in Groups XI-XIV. * $P < 0.05$ is significant compared to hyperglycemic control animals.

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Results and Discussion

Any mortality of mice was not observed in any of the extracts at tested doses till the end of 14 days of observation.

The results obtained from this study indicate that the methanol extract of the leaves of *A. carambola*, *F. hispida*, and *S. samarangense* lowered serum glucose levels significantly when compared to control (group-I) at nearly all doses examined in a dose-dependent manner. The only exception, where there was not a significant reduction of serum glucose levels compared to control animals, was in the case of *F. hispida* at a dose of 50 $mg.kg^{-1}$ body weight. The anti-hyperglycemic activity was more pronounced with methanolic leaf extract of *S. samarangense* than the other two plants. Maximum hypoglycemic activity of methanol extract of *S. samarangense* leaves in glucose-induced hyperglycemic mice was observed with a 400 $mg.kg^{-1}$ dose (59.3% inhibition), while the standard drug, glibenclamide produced 57.3 % inhibitory activity at 10 $mg.kg^{-1}$ dose (Table 1) under the experimental conditions of the present study. A comparative analysis of the results obtained with the three plants indicated that methanol extract of leaves of *S. samarangense* produced the highest antihyperglycemic effect, followed respectively, by *A. carambola* and *F. hispida*. It is to be noted that leaves of *S. samarangense* contain two antihyperglycemic flavonoids (Resurreccion-Magno et al., 2005), which could account for the antihyperglycemic effects obtained with the leaves of this plant in the present study. While anthyperglycemic constituents are yet to be reported in the leaves of the other two plants studied, nevertheless, the results indicate the presence of such constituents.

Reduction of serum glucose levels by a plant extract can stem from several factors. The extract may influence in a positive manner the pancreatic secretion of insulin, or the extract may increase the glucose uptake (Nyunai et al., 2009; Farjou et al., 1987). It is also possible that the extract may inhibit glucose absorption in gut, thus reducing the presence of glucose in serum (Bhowmik et al., 2009). The exact mechanisms through which the extracts lowered serum glucose levels in hyperglycemic mice in the present study, as well as the identification of phytochemical constituent(s) responsible for the antihyperhyperglycemic effects, is currently under investigation in our laboratory.

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