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Okra (*Abelmoschus esculentus*), a possible intervention for diabetes

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Abstract
Okra or Lady's fingers (*Abelmoschus esculentus*, synonym; *Hibiscus esculentus*) is a popular vegetable of the Malvaceae family. Several pharmaceutical benefits of okra fruit have been documented. Nevertheless, only a little attention has paid on the therapeutic use of this vegetable. Okra is prepared in various recipes throughout the world. This study investigated the blood glucose regulating effect of mucilage of okra (crude water extract) with a view to improve the therapeutic use while cooking.
Crude water extract (500 and 1000 mg/kg body weight), hot water extract (500 mg/kg body weight) and the water fraction of okra (50 mg/kg body weight) was provided to normoglycaemic rats for one week. The effect of treatment of okra fruit (500 mg/kg body weight) on serum glucose levels of rats subjected to a glucose challenge was also determined.
Crude water extract (500 and1000 mg) and the water fraction of okra (50 mg) had the potential to reduce ($p< 0.01$) serum glucose levels of rats in the test group. The maximum hypoglycaemic effect was shown by 500mg/ kg dose which is equivalent to the human therapeutic dose. The hot water extract was not effective in reducing blood glucose levels of normoglycaemic, rats.
The present study confirms that the fruit of okra possess hypoglycaemic and antihyperglycaemic properties. Application of mild heat, while cooking is needed to preserve the therapeutic efficacy.

Keywords: *Hibiscus esculentus*, *Abelmoschus esculentus*, diabetes, antihyperglycaemia, hypoglycaemia

Introduction
Diabetes mellitus (DM) is a chronic metabolic disease, debilitating populations both in affluent and less affluent societies alike. The hall mark of DM is high levels of glucose in blood due to relative or absolute deficiency of insulin or reduction of insulin sensitivity [1]. Currently there are over 370 million diabetic patients worldwide and this is likely to increase to 590 million or more by 2035 [2]. Therefore, it is an urgent need for investigation and development of novel therapeutically active anti-diabetic agents from natural/ herbal sources and this has become a major research area at present.
Although many studies have been conducted to evaluate the hypoglycaemic potential of okra (*Abelmoschus esculentus* L.), no single scientific literature was available on methods of preparation of this popular green vegetable preserving its hypoglycaemic potential despite its wide use across the world [3, 4, 5, 6]. Therefore, the present study was focused on the hypoglycaemic and anti-hyperglycaemic effect of mucilage of okra fruit (crude extract and its water fraction) in Wistar rats with an emphasis to analyze the effect on modern culinary methods.
Abelmoschus esculentus L. (Synonym; *Hibiscus esculentus*) or okra (Family: Malvaceae, English: Lady's fingers, Tamil: Shabdkosh) is a flowering plant and is cultivated throughout the tropical, sub-tropical and warm temperate regions around the world [7, 8, 9]. It is valued for its edible green seed pods which serve as a delicious vegetable worldwide.
Okra is widely used in ethno medicine in diverse cultures [10]. Different parts of the plant are employed in the treatment of human diseases throughout the world. It has been documented that the infusion of the fruit mucilage has relieving effect on dysentery in acute inflammation and irritation of the stomach [10]. Anti hyperglycaemic effect of okra has been widely studied by several workers during the recent past. A significant reduction in blood glucose levels of diabetic rats on administration of peel and seed powder of the fruit of okra has been reported by Sabitha *et al*., (2011) [3].
On administering the water-soluble fraction of fruit of okra, a significant reduction in glucose absorption was noted by Khatun *et al*., (2011) [11]. Further it was revealed that the presence of two major flavonioid glucosides [isoquercetin and quercetin-3-O-beta-glucopyranosyl-(1-6) glucoside] in okra seeds exhibit the α- glucosidase inhibitory activity and this may responsible for the reduction of blood glucose in experimental rats [12].

Materials and Methods
Collection of plant materials
The fresh pods of okra (*Abelmoschus esculentus*) were purchased from the local market of Kelaniya, Western Providence of Sri Lanka. The plant and the fruit were authenticated by a botanist from the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka. A voucher specimen was deposited therein for future reference purposes (voucher No. 6/01/H/03).

Preparation of extracts

Aqueous crude extraction procedure

Finely washed, fresh pods were immersed in water and heated for 10-15 minutes at about 60˚C in a clay pot. Pods were gently smashed and filtered through a piece of cotton cloth to remove insoluble material. The filtrate (water extract) had a mucilaginous nature and it was freeze-dried to obtain a dry powder. This filtrate (water extract) was freeze-dried to obtain a dry sample. The dry residue was weighed and stored in air and water proof container and kept in a refrigerator at 4˚C for future experimental procedure.

Heat-treated water extract

The heat-treated water extract was prepared daily by boiling raw fruit of okra in water. The mixture was boiled up to 100ºC for more than 1 hour. The heated mixture was filtered and concentrated by heating again at 100ºC for more than 1 hour to reduce the volume. The concentrated fresh crude extract (hot water extract) was used in the experiments.

Experimental Protocol

The male Wistar rats weighing 150-200 g were purchased from the Medical Research Institute, Colombo, Sri Lanka. The animal studies were initiated after obtaining permission from the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (protocol approval No. 538/11). Proir to initiation of experiments the rats were allowed one week of acclimatization under standard laboratory conditions.

The doses of okra to be administered to rats were derived by referring the method described by Dhawan and Srimal (2010) [13]. Daily intake of okra by an adult human was considered as 100 g/75 Kg body weight when extrapolating the doses. Freeze - dried powder of the crude water extract or water fraction was dissolved in distilled water to make the extractives for oral feeding. Hot water extract was daily prepared for the feeding trials. The oral administration was done by using Sondi needles.

Effect of different doses of crude water extract of okra on fasting serum glucose levels of rats

Male rats were randomly assigned into three groups (Test I, II and control) with six rats each. The respective doses of crude water extract (500 and 1000 mg/Kg) were orally administered to the test groups for one week to establish the dose curve. The effective time duration for the administration of the extract was considered as one week according to the available literature [3]. To each of the rats in the control group distilled water was given.

Effect of crude heat-treated water extract of okra on fasting blood glucose levels of rats

Heat- treated water extract of okra was orally fed at a dose of 500mg/Kg body weight to the rats of the test group. The control group received the distilled water.

Effect of crude water extract of okra on serum glucose levels following an oral glucose load

Twelve male rats were used in this experiment which was divided randomly into two groups. The rats were made to fast for eight hours. One milliliter of crude water extract (dose: 500mg/ Kg) and distilled water were administered orally to the test and control groups, respectively. Serum glucose concentration after one hour and 30 minutes post glucose load was determined.

Blood collection and determination of serum glucose

Prior to termination of the above experiments on day eight, the rats were fasted overnight. Blood samples (0.5 ml) were collected by tail vein puncture under light ether anesthesia and serum was separated. The glucose concentration in the serum samples was analysed immediately by the glucose oxidase method using DiaSys Diagnostic Kits, Germany by using a Shimadzu- UV 200 Japan, spectrophotometer.

Statistical analysis

All the results are presented as mean±S.E.M. Statistical analysis was carried out in Microsoft Excel. The significance was tested by Student's t-Test. A probability level of $p<0.05$ was chosen as the criterion of statistical significance.

Results

Effect of different doses of crude water extract of okra on fasting serum glucose concentration in normal rats

As shown by the Table 1, both tested doses were effective in reducing serum glucose concentration in normoglycaemic rats. The observed reduction was 40.4% and 37.9% respectively by the doses of 500 and 1000 mg/ kg. However, the maximum reduction was observed with 500mg/kg and this dose was selected as the most effective dose and provided the basis for the dose in other experiments.

Table 1: Effect of oral administration of crude water extract of okra (doses; 500 and 1000 mg) on fasting serum glucose concentration of rats (mean ± SEM)

Treatment/ Dose (mg/kg)	Serum glucose concentration (mg/dl)	% reduction compared with control
Crude water extract/ 500	48.8 ± 2.4**	40.4
Crude water extract/ 1000	50.8 ± 7.3*	37.9
Control/distilled water	87± 6.6	-

Values are significant at $**p<0.00001$ and $**p<0.0002$ compared with the control

Effect of crude hot water extract of okra on fasting serum glucose concentration in normoglycaemic rats

Administration of the crude hot water extract (dose: 500mg/kg) to the test group of rats did not produce any significant reduction ($p>0.05$) in the serum glucose concentration when compared with those of the control group (98.6± 19.7 vs. 107.7± 12.1, test vs. control).

Effect of crude water extract of okra on serum glucose levels after an oral glucose load in normoglycaemic rats

As depicted in Table 2, the fasting serum glucose concentration of the test and control were 92.5±11.2 and 90.4±8.9 while the values after glucose challenge were 180.4±7.4 and 222.6±24.4 mg/dl in the two groups respectively. The reduction in the test compared with the control was 19% with the $p<0.002$.

Table 2: Effect of crude water extract of okra (dose; 500mg) on serum glucose concentration of rats; at 0 hour and 2 hour post glucose load

Treatment/ Dose (mg/kg)	Serum glucose levels (mg/dl)		% reduction compared with control
	Pre-treatment fasting	Post treatment 2nd hour	
Crude water extract/ 500	92.5± 11.2	180.4±7.4*	20
Control/distilled water	90.4±8.9	225.6±24.4	-

Significantly different from control at *p<0.002

4. Discussion

This study examined the blood glucose regulating properties of crude water extract and water fraction of fruit of okra using oral administration to rats with a scientific view to extrapolate the data obtained to humans preserving the biological activity.

The results confirmed that the crude water extract of okra possess both hypoglycaemic and antihyperglycaemic activities. These results are comparable with the previous studies. The hypoglycaemic activity of the dried fruit had reported by Saha *et al*., (2011) [4]. Antidiabetic and antihyperlipidemic potential of okra peel and seed powder was observed by Sabitha *et al.*, (2011) by using diabetic rats as the experimental model [3].

It is interesting to report that the effect which was shown by the crude water extract could not observed when it was heated and for the first time, we report that the heat labile nature of the active principle of okra.

Okra is prepared into different recipes among various cultural backgrounds throughout the world. In the present study, the mucilage prepared (slightly boiled) by edible portion of okra was used to simulate the cooking method of this vegetable. It is worth mentioning, that the method of preparation during cooking is the most important piece of evidence gathered from the present study. Due to the heat **l**abile nature of the hypoglycaemic principle, the modern culinary techniques for okra (drinking the boiled water of okra, deep frying and other recipes prepared using high heat) may not be of little or no benefit. Therefore, the cooking processes which apply low heat may preserve the therapeutic potential of okra. Okra prepared preserving the green colour could be recommended, to improve the glucose tolerance in diabetic patients and the present study provides a dietary intervention for the management of diabetes without untoward drug interactions.

Since the green pod is used by many parts of the world as a day today dish, the therapeutic use of the vegetable can be applied widely even on a daily basis.

5. Conclusion

The present study provides evidence to support that fruit of okra improve the glucose tolerance normoglycaemic rats. Recipes made by applying low heat could be appreciated as they preserve the therapeutic potential and may have a value in improving glycaemic control in diabetics.

Conflicts of interest: The authors declared that there is no conflict of interest regarding publication of this paper

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