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## Effect of okra seed in treatment of hypoglycemia: A research framework using STZ induced rat

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**Abstract**

Diabetes mellitus is a metabolic disorder characterized by abnormal elevated levels of blood glucose due to complete or relative insufficiency of insulin secretion or insulin resistance as well as disturbances in carbohydrate, fat and protein metabolism. *Abelmoschus esculentus*, AE (okra or lady's finger) is a flowering plant and cultivated throughout the tropical and temperate region in the world. Okra is an important tropical vegetable and source of dietary medicine. This plant is popular with various health benefits which include anti-diabetic properties. This paper will provide the overview of the research framework and give an insight of the experimental procedure to be implemented to investigate the differential parameter in the blood of streptozotocin-induced diabetic rat in response to *Abelmoschus esculentus* (AE) treatment.

**Keywords:** Diabetes mellitus, *Abelmoschus esculentus*, streptozotocin, insulin resistance

**Introduction**

Diabetes can be described as a disease in which glucose in the blood increased. Diabetes affected considerable percentage of population throughout the world. Epidemiologic data indicated that 2.8% of the world's population was diabetic in the year 2000 and it may progress to 4.4% of the world's population by 2030. It affects all age groups of people and ethnic groups [1]. It's become a major health challenge worldwide [2]. Diabetes is fundamentally a condition of disordered glucose metabolism, it is reasonable to ask whether the type of dietary carbohydrate can influence the risk and course of this disease [3]. The management of diabetes without any side effects is a challenge to the present medical system as the treatment for diabetes is relatively limited with significant side effects. There is a lot of interest growing in the use of natural products as an alternative approach to current medications. Plant sources have become a major target to explore new drugs [4]. The streptozotocin-induced diabetic rat is still considered as an important means for the pathophysiology and pharmacology studies of diabetes mellitus [5].

**Review of Literature**

**Okra**

Okra (*Abelmoschus esculentus* L.) belongs to family Malvaceae and is grown as a summer vegetable throughout the tropical and sub-tropical region of the world [6]. It contains vitamins A, B, C as well as fat, carbohydrate, fiber, iron, iodine and is a major protein source in nearly all developing countries. Its fruits contain glycosides, a small amount of Ca, P, Mg and K. A mucilaginous preparation from the fruit has set up an application as a plasma substitute or blood level expander [7]. The high contents of linoleic acid and amino acid in the seed render it an adequate supplement to legume based diets [8]. Young capsules are emollient, demulcent and diuretic. Seeds are stimulants, cordial and antispasmodic. Okra leaves are considered good cattle feed and are also used as an emollient, antiscorbutic, chronic ulcers, spermatorrhoea and continual dysentery [7, 9].

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## Nutritive and phytochemical profiling of different parts of okra

Parts	Form	Name of the Medicinal system where it is used	Used for	References
Fruit	Infusion of the fruit mucilage	Indian ethno medicine	For treating dysentery and diarrhoea in acute inflammation and irritation of the stomach, bowels, and kidneys catarrhal infections, ardour urinae, dysuria, diuret ic, plasma replacement and gonorrhoeaa.	Odedra, & Nathabhai, 2009; Lim, 2012; Maramag, 2013; Smit, Neeraj, & Preeti, 2013; Sayana <i>et al.</i> , 2014 [10, 11, 12, 13, 14]
	Infusion of the fruit mucilage	Indian ethno medicine	Antipyretic and plasma replacement.	
	A decoction of the Immature fruit	Indian ethno medicine	Demulcent and emollient poultice.	
Leaves	Extract of leaves and roots	Indian ethno medicine	Demulcent, though less so than that of okra fruit.	Babu & Srinivasan, 1995; Odedra, & Nathabhai, 2009 [15, 10]
	Extract of leaves	Indian ethno medicine	Extract of leaves mixed with egg albumin and applied on hair which makes black and silky hair.	
	Leaves	Latin Amrica	Remedies for tumour	
Root	Extract of roots	Indian ethno medicine	Demulcent and emollient poultice.	Barrett, 1994; Yesilada <i>et al.</i> , 1951; Babu, & Srinivasan, 1995; Odedra, & Nathabhai, 2009; Lim, 2012 [ 15, 18, 10, 11, 16]
	The juice of the roots	Nepal	To treat cuts, wounds and boils.	
	An infusion of the Roots	Indian ethno medicine, Malaya	Treatment of syphilis.	
	Infusion of the roots	Traditional medicine of Nicoragua's Atlantic Coast and Turkey	Used as stomachic, to treat diabetes, ulcer, used as laxative and treatment of jaundice.	
Seed	Seeds	Indian ethno medicine	Antispasmodic, cordial and stimulant.	Crossley & Hilditch, 1952; Martin, 1982; Vaidya & Nanoti, 1989; Calisir <i>et al.</i> , 2005; Jarret <i>et al.</i> , 2011; Lim, 2012; Smit <i>et al.</i> , 2013 [20, 21, 22, 23, 11, 13]
	Infusion of the roasted seeds	Indian ethno medicine	Has sudorific properties	
	Okra seed	Indian ethno medicine	Treatment of spermatorrhoea	
	Okra seed	Turkish folk medicine	In managing increased blood glucose concentration	
	Seeds	Latin America	Remedies for tumour	
	Infusion of roasted okra seeds	Turkey	Diabetes mellitus therapy.	
Flower	The decoction of the leaves and flowers	Indian ethno medicine	Used for the treatment of bronchitis and pneumonia.	Lim, 2012; Marwat <i>et al.</i> , 2011. [11, 24]

**Diabetics and okra seed relation**

Author Bishambar Dayal *et al* (2012) [25] have propounded that an advanced Glycation End Products (AGEs) were associated with the micro-vascular complications in diabetes and other age-related neurodegenerative diseases. Authors have investigated the effect of glycosylation of bovine serum albumin (BSA) in the presence of okra seed extracts. The degree of protein glycation with glucose was assessed by tryptophan AGE, AGE-induced cross-linking by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Nano Drop spectrophotometry. Fluorescence spectra (excitation at 360 nm and read at 460nm) of BSA solution incubated for 90 days with okra seed extracts showed significant inhibitory potential (45-50%) at 0.1mg/ml concentration in a dose dependent manner. Intensity of fluorescence spectra combined with densitometry measurements exhibited 50% inhibition of glycation of BSA. Authors propose that the fluorescence emission spectra were altered by glycation when incubated with okra seed extracts and thus inhibited the advanced glycation end products. Further studies were however, needed to understand the bioactive compounds present in okra seed extracts in *in vivo* models.

Priya Singha *et al* (2014) [26] had recrded an overview on okra (*Abelmoschus esculentus*) and it's importance as a nutritive vegetable in the world. Okra a commercial vegetable crop belongs to family Malvaceae. It originates from Ethiopia and is widely spread all over tropical, subtropical and warm

temperate regions of the world. It plays an important role in the human diet and is a good source of protein, carbohydrates, vitamins, calcium, potassium, enzymes, and total minerals which are often lacking in the diet of developing country. Its medicinal value has also been reported in curing ulcers and relief from hemorrhoids. Okra has found medical application as a plasma replacement or blood volume expander and also useful in genito-urinary disorders, spermatorrhoea and chronic dysentery. The fruits of okra have reawakened beneficial interest in bringing this crop into commercial production. Okra (*Abelmoschus esculentus* (L.) Moench) is a medicinal plant of immense importance with large pharmacological applications. Besides having the above mentioned nutritional and medical, industrial properties, it has been used as an ingredient of many herbal formulations, which are used for the cure of various ailments, in particular the regulation of blood pressure, fat, diabetes, chronic dysentery genitourinary disorders, simple goiter and ulcer.

**Research frame work****Primary treatment of raw materials**

The cleaning of okra seed doen manually remove damaged seeds, dust particles, seeds of other grains/crops and other impurities such as metals and weeds washed in tab water.

**Preparation of okra seed flour**

The okra seeds were placed into the dry grinder. It was grinded until seeds became flaky.

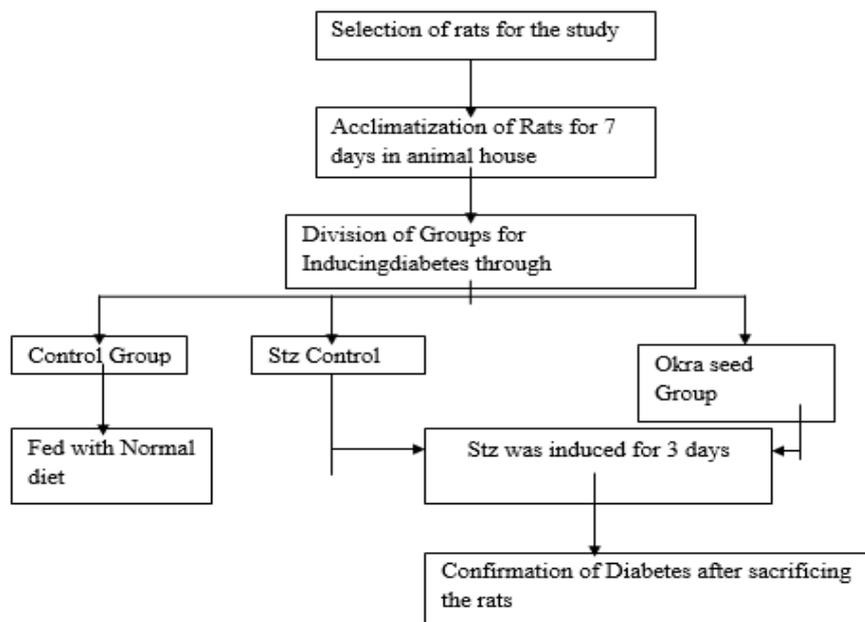
### Experimental design for checking efficacy of okra seed

**Animals and Maintenance:** This study 46 male albino rats has been taken, with the weight of 180 gm-200 gm. They have maintained in a well ventilated room exposed to ambient condition. Environmental conditions such as humidity, heat, light, and ventilation were kept constantly for 24 hours daily during the period of the study. Soaked *Cicer arietinum* have provided to the animal as food and clean water ad libitum. The animals has access to pellet diet for a week after this washout period. The rats were allowed to acclimatize for a period of 07 days before the commencement of treatments. Handling of animals done accordance with relevant institutional and ethical guidelines as approved for scientific study.

### Experimental diabetes induction

Animals fasted overnight and diabetes had induced diabetic by single intraperitoneal injection of streptozotocin (60 mg/kg body weight) prepared in 0.1 M Citrate buffer at pH 4.57. To overcome drug induced hypoglycemia, animals were allowed to drink 5% glucose solution overnight. Citrate buffer alone injected to control rats. In this group total 46 rats were taken and 36 rats were induced with STZ and after induction period of 3 days 6 rats were sacrificed for confirmation of diabetes. Animals with fasting blood glucose levels > 200 mg/dl were considered as diabetic and taken for the study. Further the rats were divided into following groups:

### Experimental Design for Inducing Diabetes in Rats



**Control Group (Negative Control):** In control group 10 rats taken. They had only fed with the pellet diet and water throughout the study period. The weights of the rats checked biweekly.

**Stz Control (Positive Control):** In this group 10 rats taken and, acclimatized for 7 days and reweighed. They were induced with Stz. In between feeding period the weight of the rats checked biweekly.

**Okra seed Group:** There were 20 rats taken in this group. They were also induced with Stz. The rats were acclimatized for 7 days and reweighed. In between feeding period the weight of the rats were checked biweekly. The group will further divided into two subgroups (n=10) in each group as low okra seed group (given 250 mg of okra seed) and high okra seed group (given 500 mg of okra seed).

### Collection of blood specimen

After completion of the treatment period, the animals were kept on overnight fast before sacrificed from each animal in the control and test group via cardiac puncture after each of the animal had been anaesthetized with ether at the end of the exposure. The blood will be collected from heart. After that blood centrifuged at 2500 rpm for 25 minutes to separate serum. After centrifugation by using clean pipette, serum collected and placed into 1.5ml of eppendorf tube. The serum was stored at -20°C until the analysis.

### Biochemical Analysis

From the collected blood serum, the biochemical markers such as Blood Glucose Level, Total Cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Serum Creatinine (Cr), SGOT and SGPT were determined by using enzopak kit for different estimation.

### Conclusion

This paper provides a research framework for streptozotocin-induced diabetic rat after the treatment of okra seed investigate by the analysis of blood serum in. The expected findings may reveal the anti-diabetic properties of okra seed. The limitation of the stude is the study include the animals experimental ranging from ethical consideration, The physiological status of every rat may not be the same and will give different results.

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