

TO CHECK THE ANTI DIABETIC STRENGTH OF SEEDS OF OKRA SEEDS (*Abelmoschus esculentus*) AND GARLIC BULB (*Allium sativum*) on male wistar albino rats .

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ABSTRACT

Diabetes mellitus is gradually globally spreading metabolic disorder with high incidence rate. In diabetes patient elevation of blood glucose level occurs due to deformation of insulin receptor action. There are many treatment regimens available. To overcome these types of problems it becomes important to find different types of treatments and therapeutic targets . Okra *Abelmoschus esculentus* genus is a plant species which is square in measures used for various medicated functions. This plant is widespread in Republic of all over the world and in India. Okra is celebrated in several communication and all over the world which is known as the ladies' fingers. Garlic *Allium sativum* genus is a species as onion genus. Its also known as shallot, leek, Chinese onion etc.

INTRODUCTION

Diabetes is a broad term used for a group of diseases. Its lead to prolonged hyperglycaemia. Diabetes mellitus is divided into two main types-Type 1 diabetes and, Type 2 diabetes

Other type of diabetes mellitus can be like following:

1. Gestational diabetes
2. Diabetes Latent Autoimmune Diabetes in Adult,
3. Diabetes Maturity Onset Diabetes of the Young,
4. Diabetes Maternally Inherited Diabetes and Deafness,
5. Double diabetes mellitus,
6. Steroid induced diabetes mellitus,
7. Brittle diabetes **mellitus**,
8. Diabetes Insipidus etc.

1. **Type 1 diabetes mellitus.**

–Type 1 diabetes is a resistant framework ailment in which the β -cells of the pancreas don't make satisfactory insulin, a hormone which empowers use to glucose for imperativeness.

There are two types of Type 1 DM. one is a safe intervened sicknesses with immune system marker, for example, islet cell. Upwards of 85-90% of patients with fasting hyperglycemia are certain for no less than one of these markers. Solid human leukocyte antigen (HLA) connection moreover exists. A minute kind of sort 1 DM, now called idiopathic diabetes, has no known reason Just a minority of patients fall into this gathering, which happens predominantly in people of African and Asian root. Idiopathic diabetes is emphatically heritable, yet it needs immune system markers and isn't HLA related. Despite the fact that it can happen at any age, type 1 DM is more typical in people under 30 years old. The rate of pancreatic obliteration is variable and is for the most part more fast in babies and kids and slower in grown-ups.

2. Type 2 diabetes –

It is caused by a mix of hereditary factor identified with hindered insulin emission and insulin assurance and common variables, for instance, bulkiness, reveling, nonappearance of action and stress, and developing. It is routinely a multifactorial ailment including different characteristics and natural components to changing degrees. **(Brophy S, 2011)**

Other types of Diabetes:

1. **Gestational diabetes**-Pregnant ladies regularly create diabetes. Amid pregnancy vast amounts of hormone are delivered, these hormones diminish insulin activity in body of a mother .Hence , causing insulin protection. Ladies which create diabetes mellitus amid pregnancy and ladies with undiscovered asymptomatic write 2 diabetes mellitus that is found amid pregnancy are grouped with gestational diabetes mellitus.**(Guglielmi C et al., 2012)**Clinical significance of gestational diabetes mellitus is lies in the way that it is related with huge maternal and fetal grimness.**(Banerjee et al., 2016)**
2. **Diabetes latent Autoimmune Diabetes in Adult-**
Latent immune system diabetes in grown-up (LADA) wins as a diabetes ask about in spite of the wayit was arranged as the 2nd silliest name two or 3years back..**(O. Rolandsson and J. P. Palmer, 2010)**

Patients with LADA have a more treacherous beginning, and the turmoil is described by an immune system assault of insulin-creating beta cells of the pancreas, which are viewed as outside. In 2005, LADA was characterized as grown-up period of beginning, pancreas of autoantibodies and insulin after no less than a half year of finding.**(SK Aggarwal et al., 2002)**The world health organization (WHO) grouped LADA under sort 1 diabetes since

patients progress toward becoming insulin subordinate following a half year(**Tattersall RB , 1974 & Banerjee et al, 2016**)

3. **Diabetes Maturity onset diabetes of the Young-**

NIDDM is a heterogeneous issue made out of subtypes with scope of variable inheritance going from polygenic to monogenic legacy. MODY, first portrayed since 1974 by Tatter should is an uncommon, familial type of β -cell brokenness. (**B Mory et al., 2012**) The established qualities of MODY incorporate autosomal predominant legacy, youthful period of beginning (more often than not analyzed before the age of 45 years) (**Yung-Nien Chen et al., 2003**) absence of β -cell autoimmunity and highlights of insulin protection and managed endogenous insulin discharge(**Kavvoura et al., 2012 & U. Nwosu , 2013**)

4. **Diabetes MIDD (Maternally inherited diabetes and deafness) -** This disorder was in this way recognized in diabetic patients from different racial roots and is alluded to as an alternate phenotype of A3243G transformation related mitochondrial encephalomyopathy, lactic acidosis and stroke-like scene (MELAS) disorder. It was later meant as maternally acquired diabetes and deafness (MIDD) disorder with a specific end goal to imply its distinction from the conventional diabetic disorder.

A current multicenter imminent investigation of 54 patients with type 2 DM and the mtDNA 3243 transformation closed the MIDD patients were youthful at diabetes beginning and gave a typical or low weight list(**Yung-Nien Chen et al., 2003**).

5. **Double diabetes** –Another subset of diabetes, called double diabetes is ending up progressively pervasive because of the scourge of youth stoutness. In twofold diabetes, components of both T1D and T2D exist together. In this condition, people with T1D have cold-heartedness to insulin that is regularly connected with stoutness and people with T2D have antibodies against the pancreatic beta cells.(**Benjamin U. Nwosu 2013 & G. Pagno et al., 1983**)

Double diabetes (DD) was a term authored to depict people with type 1 diabetes indicating clinical highlights good with type 2 diabetes. It has been fluidly utilized as a part of writing, to portray the two people with stoutness and other insulin protection (IR) qualities since finding and the individuals who have put on weight amid development, getting to be hefty after some time (**Gluffrida et al., 2016**)

6. **Steroid induced diabetes** – Glucocorticoids are regularly used to treat a wide assortment of both intense and endless ailments. Their utilization can be joined by a large number of reactions; including hyperglycaemia and can decline previous diabetes or accelerate new steroid-instigated diabetes. (**Lisa R. Simmons et al., 2012, Sieno S ,2012 & S. Kalra et al.,2015**)

The expression "steroid diabetes" was authored by Ingle in the 1940s to depict the hyperglycaemia noted in rats getting glucocorticoids.

Another Classification of Diabetes mellitus – (Oral Hypoglycaemic drugs)

A. Enhance Insulin Secretion

1. Sulfonylureas

1st generation:

Tolbutamide

2nd generation:

Glyburide,

Glipizide,

Glimepiride

2. Meglitinide/phenylalanine analogues is also type of diabetes mellitus for example

Repaglinide,

Nateglinide

3. Glucagon- like peptide-1 receptor agonists or can say Injectable drugs:

Exenatide,

Liraglutide

4. Dipeptidyl peptidase-4 or DPP-4 inhibitors:

Sitagliptin,

Vildagliptin,

Saxagliptin,

Linagliptin

B. Overcome Insulin resistance is classified as below:

1. Biguanides or AMPK activator for example- Metformin

2. Thiazolidinediones activator for example- Pioglitazone

C. Miscellaneous antidiabetic drugs are as below-

1. α - Glucosidase inhibitors-for example; Acarbose Miglitol, Volibo

2. Amylin analogue for example- Pramlintide

3. Dopamine-D2 receptor agonist- Bromocriptine 2.5 mg

PLANT PROFILE-



Fig 2: OKRA

Okra illustrious in several communicatory countries as ladies' fingers , could be a phanerogam within the dilleniid dicot family. Okra is valuable for its edible in experienced seed pods. The geographical origin is controversial, with supporters of Ethiopian, and South Asian origins. *Abelmoschus esculentus* is cultivated in tropical, subtropics and heat temperate regions round the world.

Botanical Name	<i>Abelmoschus esculentus</i>
Common Name	Bhindi
Classification	<p>Kingdom: Plantae</p> <p>Unranked: Angiosperms</p> <p>Clade: Eudicots</p> <p>Order: Malvales</p> <p>Family: Malvaceae</p> <p>Genus: <i>Abelmoschus</i></p>

Synonyms: gumbo, okra plant, lady's-finger

Benefits of Okra

- 1)Alleviates Asthma,**
- 2)Lowers cholesterol,**
- 3)Manages Diabetes,**
- 4)Boosts Immune System,**
- 5)Prevents renal disorder.**

1.3 GARLIC-

Garlic (*Allium sativum*) is a species within the onion genus, *Allium*. Its shut relatives embody the onion, shallot, leek, chive etc.



Botanical Name	<i>Allium sativum</i>
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Common Name	Lehsun
Classification	<p>Kingdom: Plantae</p>
	<p>Unranked: Angiosperms</p>
	<p>Clade: Monocots</p>
	<p>Order: Asparagale</p>
	<p>Family: Amaryllidaceae</p>
	<p>Genus: <i>Allium</i></p>

Synonyms: garlic, ail(*noun*)

Uses of garlic:

1. Calorie for calorie, garlic is improbably nutritive. One clove (3 grams) of raw garlic contains (5):
 - Contains Manganese: 2%
 - Contains Vitamin B6: 2%
 - Contains Vitamin C: 1% o
 - Contains Selenium: 1%
 - Contains Fiber: 0.6 grams
2. Garlic supplements are far-famed to spice up the operate of the system.
3. Vessel diseases like heart attacks and strokes are the world's biggest killers. High pressure level, or high blood pressure, is one in all the foremost vital drivers of those diseases.
4. Garlic allium sativum lowers the total and cholesterol. For those with high sterol, garlic supplements seem to cut back total and/or cholesterol by regarding 10–15%.
5. The combined effects on reducing sterol and pressure level, likewise because the inhibitor properties, might scale back the danger of common brain diseases like Alzheimer's disease} and dementia.

MATERIALS AND METHODS

All the drugs which are going to use in this experiment as well as chemicals were of analytical grade and were obtained commercially.

4.1 DRUGS

- Streptozotocin
- Glibenclamide

4.2 CHEMICALS

- Solvents for extraction
 - Petroleum ether
 - Ethyl acetate
 - Methanol

4.3 APPARATUS

- Beaker
- Conical flask
- Measuring cylinder
- Round bottom flask
- Petri dish
- China dish
- Funnel
- Volumetric flask
- Pipette
- Glass rod

4.4 EQUIPMENTS

S. N0.	Equipments Name	Brand Owner
1.	Glucometer	Johnson & Johnson, one touch ultra
2.	U.V. Spectrophotometer	Shimadzu, USA
3.	Water bath	Remi, Mumbai
4.	Electronic balance	J. Mitras & Bros, New Delhi

4.5 COLLECTION, IDENTIFICATION AND AN AUTHENTICATION OF PLANT MATERIAL

The okra *Abelmoschus esculentus* and garlic *allium sativum* were collected from New Delhi in 2018. The formal authentication and identification was done by Dr.Manish k kandwal scientist D and Dr.Ansari scientist E , botanical garden of Indian public.

The seeds and buds were allowed to air dry, away from sunlight. The dried material was grounded coarsely to a powder and transferred to labeled brown bottles until required.

4.5 EXTRACTION PROCEDURE

Petroleum ether extraction of okra seeds and garlic bulb

Powdering: The fresh and dried seeds of okra and dried bulb of garlic of were crushed by using the grinder.

Sieving: The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair.

Soxlation: The dried, powdered the plant material (90g) is extracted with petroleum ether at 60°C for 24 hr using a soxhlet apparatus. The collected mass was subjected to drying to evaporate the excess of solvent. The collected dark brownish colour material was termed as petroleum ether extract of okra seeds and garlic bulb

2. Ethyl acetate extraction of okra and garlic

Powdering: The fresh and dried seeds of okra and dried bulb of garlic were crushed by using the grinder.

Sieving: The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair.

Soxlation: The dried, powdered of the plant material (90g) is extracted with an ethyl acetate at 60°C for 24hr using a soxhlet apparatus. The collected mass subjected to drying to evaporate the excess of solvent. The collected dark brownish colour material was termed as ethyl acetate extract of okra seeds and garlic bulb.

3. Methanolic extraction of okra seeds and garlic bulb

Powdering: The fresh and dried fruits of okra and garlic were crushed by using the grinder.

Sieving: The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair.

Soxlation: The dried, powdered plant material (90g) was extracted with methanol at 60°C for 24 hr using a soxhlet apparatus. The collected mass was subjected to drying to evaporate the excess of solvent. The collected black colour material was termed as methanol extract of okra seeds and garlic bulb.

4.7 IDENTIFICATION OF PHYTOCONSTITUENTS BY CHEMICAL TESTS

An individual concentrates are subjected to subjective synthetic examinations for recognizable proof of phytoconstituents. The preparatory phytochemical tests were performed for each concentrate(Kokate C.K. et al., 2008).

Preliminary phytochemical screening of extracts Okra

Test for Phytoconstituents	Methanolic extract	Aqueous extract
<u>Carbohydrate</u>		
Molisch's Test	+ve	+ve
Fehling's Test	+ve	+ve
Benedicts' Test	+ve	+ve
<u>Gums & Mucilages</u>		

Ruthenium red test	+ve	+ve
Lead subacetate	+ve	+ve
Gelatine solution	+ve	+ve
<u>Protein & Amino acid</u>		
Ninhydrin,s test	+ve	+ve
Millon's test	+ve	+ve
<u>Alkaloid</u>		
Mayer's test	-ve	-ve
Dragendroff's test	-ve	-ve

Hager's test	-ve	-ve
Wagner's test	-ve	-ve
<u>Glycoside</u>		
Legal's test	-ve	-ve
Brontager's test	-ve	-ve
<u>Phytosterol</u>		
Salkowaski test	+ve	+ve
Libermann's test	+ve	+ve
<u>Flavonoid</u>		
Alkali & Acid test	+ve	+ve
Ferric chloride test	+ve	+ve
<u>Tannin & Plant phenol</u>		
5% ferric chloride test	+ve	+ve
Lead acetate test	+ve	+ve
Copper sulphate test	-ve	-ve
<u>Fixed oil</u>		
Spot test	-ve	-ve
Saponification test	-ve	-ve
Volatile oil	+ve	+ve



Preliminary phytochemical screening of extracts Garlic

Inference	Test for Garlic(+,-)
<p style="text-align: center;"><u>Saponin</u> Foam will remain for 10 mints, presence of Saponin</p>	+++
<p style="text-align: center;"><u>Tannin</u> Blue or green colour indicates presence of tannin</p>	-ve
<p style="text-align: center;"><u>Phenol</u> Dark blue colour indicates presence of phenol</p>	-ve
<p style="text-align: center;"><u>Alkaloids</u> Orange indicates presence of alkaloids</p>	-ve
<p style="text-align: center;"><u>Terpenoid</u> Brown colour ring formation at the junction of two liquid indicates the presences of Terpenoid</p>	++
<p style="text-align: center;"><u>Flavonoids</u> Intense yellow colour appears. Then yellow colour disappears</p>	+ve
<p style="text-align: center;"><u>Amino acid and protein</u> Violet colour indicates presence of protein</p>	+++
<p style="text-align: center;"><u>Carbohydrate</u> Red precipitate indicates the presence of carbohydrate</p>	-ve
<p style="text-align: center;"><u>Phlobatannins</u> Red precipitate indicates the presence of phlobatannin</p>	-ve
<p style="text-align: center;"><u>Volatile oil</u> White precipitate indicates presence of volatile oil</p>	+++

<u>Hydrolysable tannin</u> Emulsion formation precipitate indicates the presence of hydrolysable tannin	-ve
<u>Glycosides</u> Brick red precipitate indicates presence of glycosides	-ve
<u>Cardiac glycosides</u> Formation of three layer of different color indicates the presence. Upper layer – Green color Middle layer – Brown color Lower layer – Violet.	+++
<u>Vitamin C</u> Violet colour presence of Vit C	-ve

(+++ High concentration of the compound ++ Moderate concentration of the compound Low concentration of the compound _ ve absence of the compound,)

RESULTS, DISCUSSION AND CONCLUSION

5.1 RESULT

Effect of different extracts of *okra (abelmoschus esculentus)* and *garlic (allium sativum)* level of blood glucose against streptozotocin induced diabetic male wistar albino rats.

S.NO.	Group	Treatment (mg/kg b.w.)	Blood glucose (mg/dl)			
			0 day	7 th day	14 th day	21 st day
1.	I	Normal saline (0.5ml/kg)	88.16±5.7	85.96±3.9	81.88±4.5	80.78±4.8
2.	II	Normal saline +Streptozotocin (0.5ml/kg+60mg/kg)	275.7±9.5	265.8±8.3**	268.7±9.6**	256.0±14.6*
3.	III	Petroleum ether extract + Streptozotocin (400mg/kg)	213.8±10.1	202.2±9.3***	194.6±12.4***	184.7±9.8***
4.	IV	Ethyl acetate extract + Streptozotocin (400mg/kg)	210.5±8.1	151.7±5.4**	148.2±8.2**	143.7±7.5**
5.	V	Methanol extract + Streptozotocin (400mg/kg)	202.1±9.7	106.7±6.8**	120.6±8.4**	115.9±4.7**
6.	VI	Glibenclamide + Streptozotocin (60mg/kg)	200.9±8.4	111.0±9.8***	109.7±10.8***	106.5±8.9***

All the upper table values were expressed as mean ± SD (n=5). *p<0.001 when we compared to control group **p<0.002. And when compared to standard (one way ANOVA followed by Dunnett’s Test)

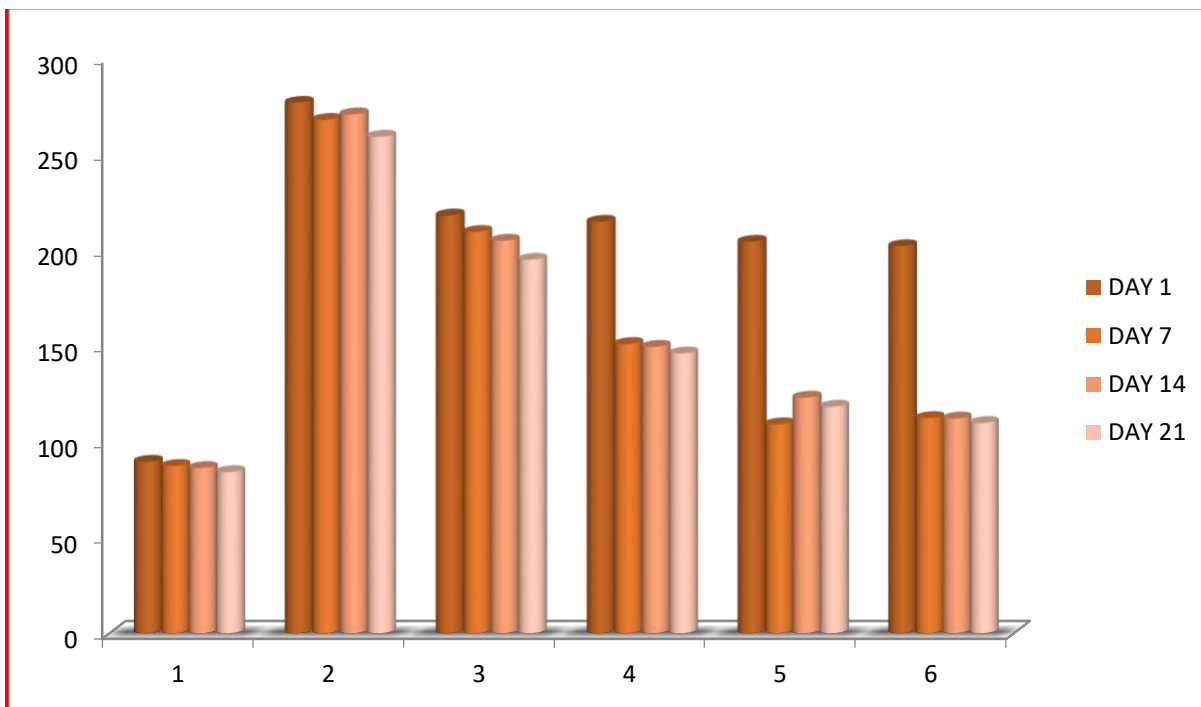


Figure 5.1: Histogram represents the effect of different extracts of *okra and garlic* on blood glucose level against streptozotocin induced diabetes.

1. Normal saline + streptozotocin (Group 2)
2. Petroleum ether extract + streptozotocin (Group 3)
3. Ethyl acetate extract + Streptozotocin (Group 4)
4. Methanol extract + streptozotocin (Group 5)
5. Glibenclamide + streptozotocin (Group 6)

5.2 DISCUSSION

When we Administerd STZ taken in quantity $60 \text{ mg kg}^{-1} \text{ b/w}$.The blood glucose level $\geq 200 \text{ mg dL}^{-1}$ in all treated male wistar albino rats. The blood glucose level monitored in all male wistar albino rats for 48 hours. The Fasting blood glucose of non diabetic male wistar albino rats was higher then normal control male wistar albino rats. Usually the pancreas cells maintains the blood glucose level concentrations within a narrow range by modulating insulin secretion rate.

5.3 CONCLUSION

Its the evidence by an experimental studies and data collected that okra seed *abelmoschus esculentus* genus and garlic bulb *allium sativum* genus both are together has potential of hyporglycemic activity in Streptozotocin which is induced in diabetic male wistar albino rats. Our results confirm that okra seeds (*Abelmoschus esculentus*) and garlic bulb (*allium sativum*) were significantly sufficient for body weight loss.

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