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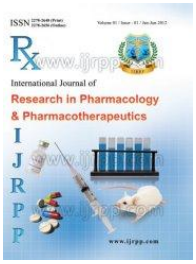

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Evaluation of phytochemicals and anti-diabetic activity of *abelmoschus esculentus*

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ABSTRACT

Okra *(Abelmochus esculentus*) is an economically important vegetable crop grown in tropical and subtropical parts of the world. In the present we investigated the phytochemical analysis of aqueous and ethanol extract of okra and the *invivo* antidiabetic effect of aqueous extract of *Abelmoschus esculentus* against Alloxan induced diabetic rat. The standard drug glibenglimide (5 mg/kg) and *Abelmoschus esculentus* (150 mg/kg) were dissolved in water in given orally. The blood glucose levels of rats were noted at regular intervals of time. A gradual decrease in the blood glucose levels was observed by regular feeding of "okra" *Abelmoschus esculentus* (ladies finger) fruit extract for about twelve days.

Keywords: *Abelmoschus esculents,* Diabetes, study on rats, Blood sugar level.

INTRODUCTION

Diabetes mellitus, a non-communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world. It is a metabolic disorder affecting carbohydrate, fat, and protein metabolism. A worldwide survey reported that diabetes mellitus is affecting nearly 10% of the population every year [1].

Type 1 DM results from the pancreas' failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown. Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "noninsulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise (Jump 2013). Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood sugar level [2].

Type 2 makes up about 90% of the cases. This is equal to 8.3% of the adult population with equal rates in both women and men. In 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths [3].

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged

period Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma [4].

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes [5].

Low blood sugar is common in persons with type 1 and type 2 diabetes. Most cases are mild and are not considered medical emergencies. Effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior, seizures, unconsciousness, and (rarely) permanent brain damage or death in severe cases [6].

Mechanism of insulin release in normal pancreatic beta cells —insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose. Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus [7].

The body obtains glucose from three main places: the intestinal absorption of food, the breakdown of glycogen, the storage form of glucose found in the liver, and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen [8].

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in the decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin [9].

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis [10].

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) [11].

This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia) [10].

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease. The primary complications of diabetes due to damage to small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness [12].

Prevention and treatment involve a healthy diet, physical exercise, not using tobacco and being a

normal body weight. Blood pressure control and proper foot care are also important for people with the disease. Type 1 diabetes must be managed with insulin injections. Type 2 diabetes may be treated with medications with or without insulin. Insulin and some oral medications can cause low blood sugar (Rippe *et al*., 2010). Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM. Gestational diabetes usually resolves after the birth of the baby [13].

Diabetes is a common ailment in people all over the world. These days we get to hear that small children are also diabetic and they need to maintain their health with lots of restrictions in diet. For a few people, the sugar levels are high and they need to take insulin to maintain sugar levels. People with diabetes need to go thru' lot of pain and expensive treatment with regular medicines. Here is some good news; Ayurveda recommends a natural method to bring sugar levels down. Ladies finger is the vegetable that has proven great results in reducing diabetes. Ladies finger is known as okra, bhendi, vendakaya or bendakaaya [14].

MATERIALS AND METHODS

The material and methods pertaining to the study on "Evaluation of Phytochemicals And Anti-Diabetic Activity of *Abelmoschus Esculentus*"is presented under the following headings,

Collection of plant materials

The okra was obtained from a local supermarket of Mayiladuthurai, Nagappattinam District, Tamil Nadu, South India. The mature (5 to 10 days from fruit set and dark green color) and medium size classification (10 to 20 g) of the okra was used.

Preparation of Okra extracts

*Abelmochus esculentus vegetable*s were collected from in and around the area of Mayiladuthurai. Take two raw ladies finger, wash them. Cut the ends of the ladies finger and slit the ladies finger lengthwise without separating the 4 slits at the broad base. Immerse the ladies finger in a glass of water. Cover the glass such that the ladies finger gets immersed. Leave it overnight. In the morning remove the ladies finger from the water and drink the water on an empty stomach. The water will be slimy with the addition of the mucilage sticky content of ladies finger. Repeat this method for 2 weeks and then analysis of antidiabetic activity.

25 ml of slimy water of okra was dissolved in 100 ml of 10 % aqueous and ethanol the resultant extract was boiled in a water both until a syrupy consistency was obtained this syrupy consistency was used screen the phytochemical analysis.

Preliminary phytochemical screening

Qualitative phytochemicals examinations were carried out for all the extract as per the standard methods [15].

ANTIDIABETIC ACTIVITY

Animals

Male *Albino Wistar* strain rats (50-60 days old) were obtained from "Sri Venkateswara Enterprises", Bangalore, India. They were housed in plastic cages under controlled conditions fed with normal rat chow and were provided with clean drinking water *ad libitum*.

Diabetes induction in rats

Alloxan monohydrate was used to induce diabetes. Animals were allowed to fast for 16hr and were injected intraperitoneally (i.p.) with freshly prepared Alloxan monohydrate in normal saline in a dose of 150mg/kg. Rats with a blood glucose level above 200mg/dl were considered to be diabetic and were used in this experiment.

Experimental design

The rats were divided into four groups with 4 rats in each as follows:

Group I : Normal rats
Group II : Diabetic Control rats
Group III : Diabetic + plant extract (150mg/Kg body weight) for 12days (Given orally).
Group IV : Diabetic + glibenclamide (5mg/kg). for 12 days (Given orally).

At the end of the experimental period all rats were sacrificed by cervical decapitation and the serum was collected from the blood. Immediately liver was removed and washed with ice-cold physiological saline. They were homogenized in 0.1M tris-Hcl buffer pH 7.4 to give a 10% homogenate. This homogenate was used for the appropriate parameter estimation.

Biochemical analysis

Estimation of glucose

Glucose was estimated by the method of GOD-POD method [16]. To the 0.02 ml of blood, 2ml of the dilution reaction mixture was added and mixed well. The mixture was incubated at 20-25$^0_{c.}$ After 30 minutes the absorbance was read at 510 nm against the reagent blank. The blood glucose level was expressed as mg/dl.

Estimation of glycated haemoglobin

Carbohydrate content in globln solutions was estimated by phenol: sulphuric acid method [17]. To one ml aqueous globin solution, 3ml concentrated sulphuric acid was added and vortexed. The solution was allowed to cool for 30 minutes at room temperature before the addition of 0.025 ml of 80% phenol. The optical density values were measured at 485 nm, 30 minutes the addition of phenol to the system.

Assay of superoxide dismutase

Superoxide dismutase was assayed according to the method of Misra and Fridovich. [18]. To 0.05 ml tissue supernatant, 1.5 ml of the buffer was added. The reaction was initiated by the addition of 0.4 ml of epinephrine and change in optical density per minute was measured at 480 nm in a Shimadzu spectrophotometer. One unit of superoxide dismutase activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

Assay of catalase

Catalase was assayed according to the method [19]. To 1.2ml of phosphate buffer, 0.05ml of the tissue homogenate was added. The enzyme reaction was started by the addition of 1.0ml of the hydrogen peroxide solution. The decrease in absorbance was measured at 240nm at 30 seconds

Assay of lipid peroxidation

The extent of lipid peroxidation was estimated according to the method [20]. A 20% liver homogenate was prepared in phosphate buffer (pH 7.2). With 0.5ml of the homogenate, 1.0ml of TCA and 1.0ml of TBA was added and mixed thoroughly. The mixture was heated in a boiling water bath for 20 minutes. The tubes were centrifuged at 1000gfor 10 minutes and the absorbance was read at 535nm on a spectrophotometer against a blank containing all the reagents except the homogenate. The lipid peroxide concentration was expressed as n moles of MDA/mg protein intervals for 3 minutes. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as n moles of hydrogen peroxide decomposed/minute/mg protein.

RESULTS

Qualitative phytochemical studies

Different phytochemical components and anti-oxidant are present in the herbal extract that can be significantly therapeutic uses. Much of the protective effect of fruit has been attributed by phytochemical which are the non- nutrient plant compound [21].

In the present study ethanolic and aqueous extract of *abelmoschus esculentus vegetable* used for screening phytochemical compound. This plant was chosen or the basis of their medicinal value. Table. 1 represents the qualitative phytochemical analysis of ethanol and aqueous extract of *abelmoschus esculentus vegetable.*

Table: 1 Phytochemical screening of *Abelmoschus esculentus:*

S.No	Name of the test	Aqueous	Ethanol
1	Steroids	+	+
2	Phenols	+	+
3	Resins	+	+
4	Tannins	+	+
5	Flovonoids	+	+
6	Alkaloids	-	+
7	Glycosides	+	+
8	Saponins	+	+
9	Coumarins	+	+
10	Quinine	+	+
11	Xanthoprotein	+	+
12	Phlobatannins	-	-
13	Carbohydrate	+	+
14	Terpenoids	+	+
15	Anthocyanidine	-	-

Presence(+)
absence (-)

Medicinal plants are important sources of biologically active antioxidants. Natural antioxidants, which are ubiquitous in fruits and vegetables, have also received great attention and have been studied extensively, since they are effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants. Green leafy vegetables provide a high amount of carotene, ascorbic acid, and microelements which play important roles in nutrient metabolism and slowing down of degenerative diseases. *Abelmoschus esculentus* L. (Family: Malvaceae), also known as *Hibiscus esculentus*, is an important vegetable, widely distributed from Africa to Asia, Southern Europe, and America that is more commonly known as ladies finger, okra, or gumbo. The fibres in ladies finger help to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. Previous studies reported that ladies finger polysaccharide possesses hepatoprotective, antidiabetic, antiulcer, anticancer, anti-inflammatory, laxative, antihyperlipidemic, antifungal, and analgesic activities. Recently, some quercetin derivatives, well-known antioxidants, were identified and isolated from ladies finger. Nutritionally, the richest part of the ladies finger plant is the dried seeds. The oil of ladies finger seeds is edible and the residual meal after oil extraction is rich in protein. With this background, the present investigation aims at exploring the natural antioxidant, antistress, and nootropic activities of the aqueous and methanolic seed extracts of *Abelmoschus esculentus* (AE, ME) [22].

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12 000 items used by traditional healers, about 500 are in common use [21]. The screening of aqueous,

ethanolic extract indicates the presence of Steroids, Phenols, Resins, tannins, Flovonoids, Glycosides, Saponins, coumarins, Quinine, Xanthoprotein, carbohydrate, Terpenoids and absence of phlobatannins, Anthocyanidine, Alkaloids are absent in Aqueous extract of *Abelmochus esculentus* is given in table1.

Examine the antidiabetic activity.

Group I : Indicates Normal rats

Group II : Indicates Diabetic control rats

Group III : Indicates Diabetic+ plant extract

Group IV : Indicates Diabetic+ globenclamide

The hypoglycemic activity of ***Abelmoschus esculentus*** extract was shown in Table 2 and figure 1. The concentration of glucose was significantly higher in alloxan treated rats (Group II), as compared to normal control animals (Group I). These constituents were found to attain a near normal level in plasma in glibenclamide treated rats (Group IV) and ***Abelmoschus esculentus***treated rats (Group III).

Table: 2 Effect of Aqueous Extract of *Abelmoschus Esculentus*on Blood Glucose

S.No	Groups	Blood glucose (mg/dl)
1.	Group I	74.3±0.5
2.	Group II	212.5±1.
3.	Group III	110.8±0.1
4.	Group IV	85.4±3.9

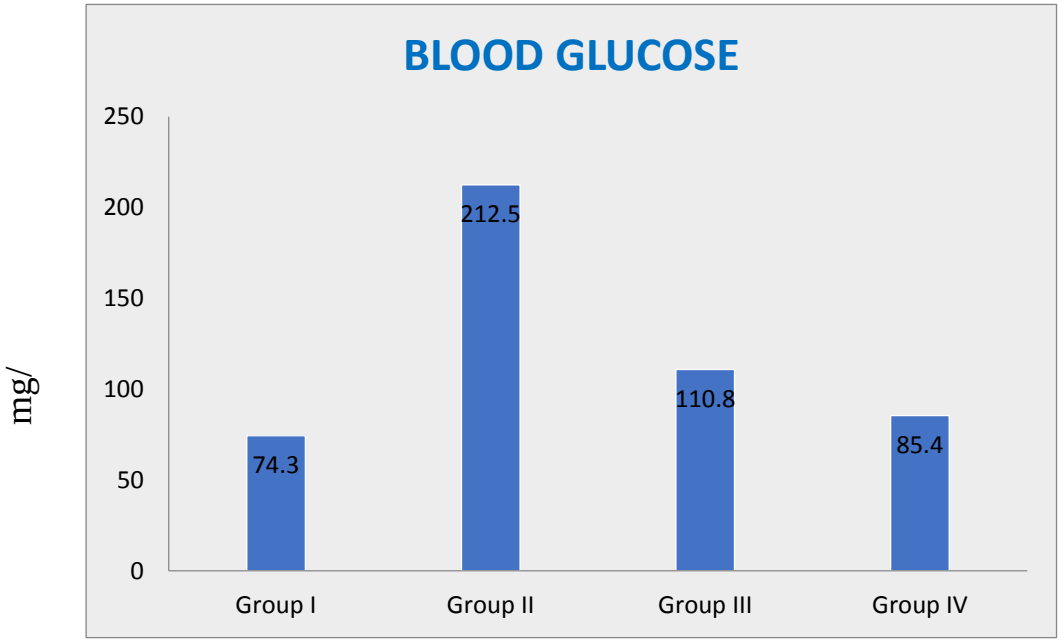

Figure 1

Table: 3 Effect of Aqueous extract of *Abelmoschus esculentus* *on* Glycated Hemoglobin

S.No	Groups	Glycated Hemoglobin(mg/dl)
1.	Group I	6.2±0.1
2.	Group II	10.4±0.18
3.	Group III	7.4±0.1
4.	Group IV	6.6±0.2

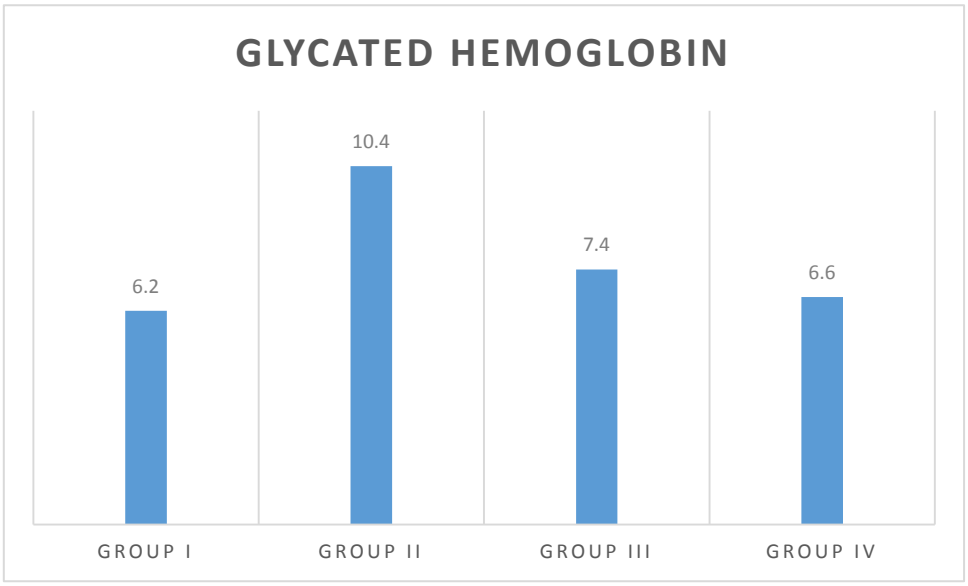

Figure: 2

Glycated hemoglobin is increased in alloxan treated diabetic rats. Administration of by aqueous extract of ***Abelmoschus esculentus*** (150mg/kg b.w.) for 12 days showed significant control in glycosylated hemoglobin, thereby increasing the levels of total hemoglobin in diabetic rats (Table 3 and figure 2). This could be due to the result of improved glycemic control produced by the plant extract.

Alloxan produces diabetes by liberating oxygen free radicals, which cause lipid peroxide mediated pancreatic injury. The aqueous extract of ***Ablemoschus esculentus*** may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces an antidiabetic effect. The diabetic rats showed a significant decrease in the levels of total haemoglobin and a significant increase in the level of glycated haemoglobin (HbA1c).

The concentration of tissues SOD and CAT were significantly decreased in diabetic rats and LPO was increased when compared to the control group. Administration of aqueous extract of ***Abelmoschus esculentus*** to diabetic rats tends to bring the activities of these enzymes to near normal level, (Tables 4, 5 and 6, and figure 3, 4, 5).

Table: 4 Effect of Aqueous extract of *Abelmoschus esculentus*on SOD

S.No	Groups	SOD(units/mg protein)
1.	Group I	5.6±0.4
2.	Group II	4.1±0.3
3.	Group III	5.4±0.1
4.	Group IV	5.4±0.1

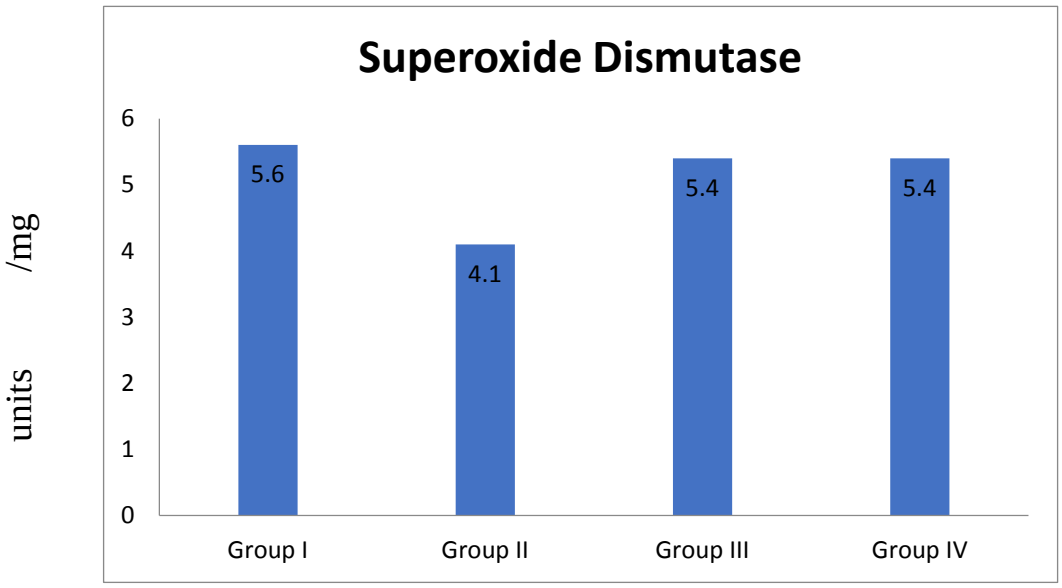

Figure: 3

Table: 5 Effect of Aqueous Extract of *Abelmoschus Esculentus*on Catalase

S.No	Groups	Catalase(µ mole of H_2O_2consumed/min/mg protein)
1.	Group I	67.2±1.3
2.	Group II	40.6±0.3
3.	Group III	58.4±1.2
4.	Group IV	67.6±1.3

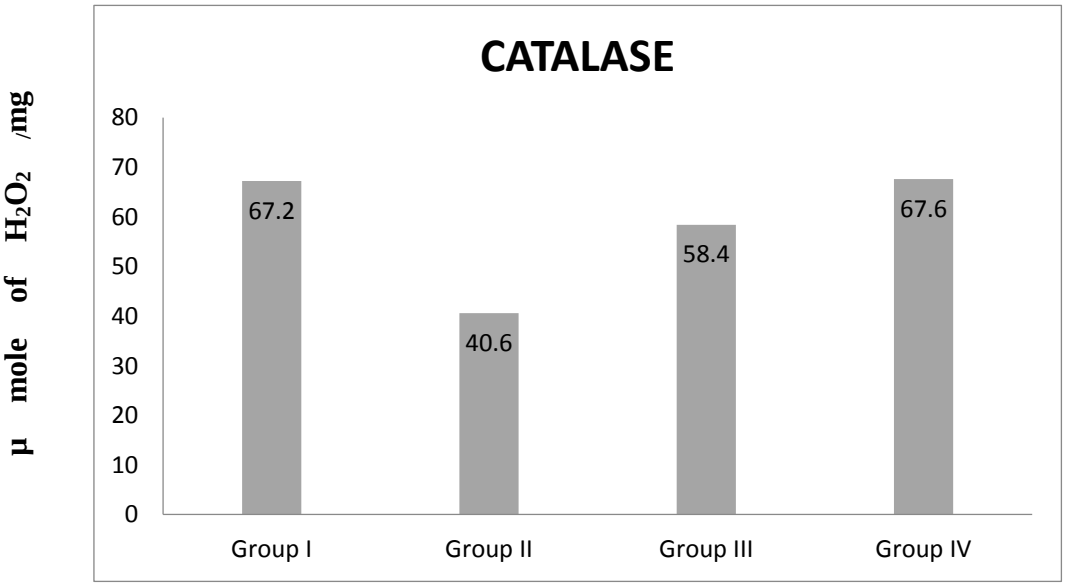

Figure: 4

Table 6 Effect of aqueous extract of *Abelmoschus esculentus*on LPO

S.No	Groups	LPO(n moles of MDA/mg protein)
1.	Group I	2.3±0.4
2.	Group II	5.4±0.18
3.	Group III	3.8±0.4
4.	Group IV	3.2±0.1

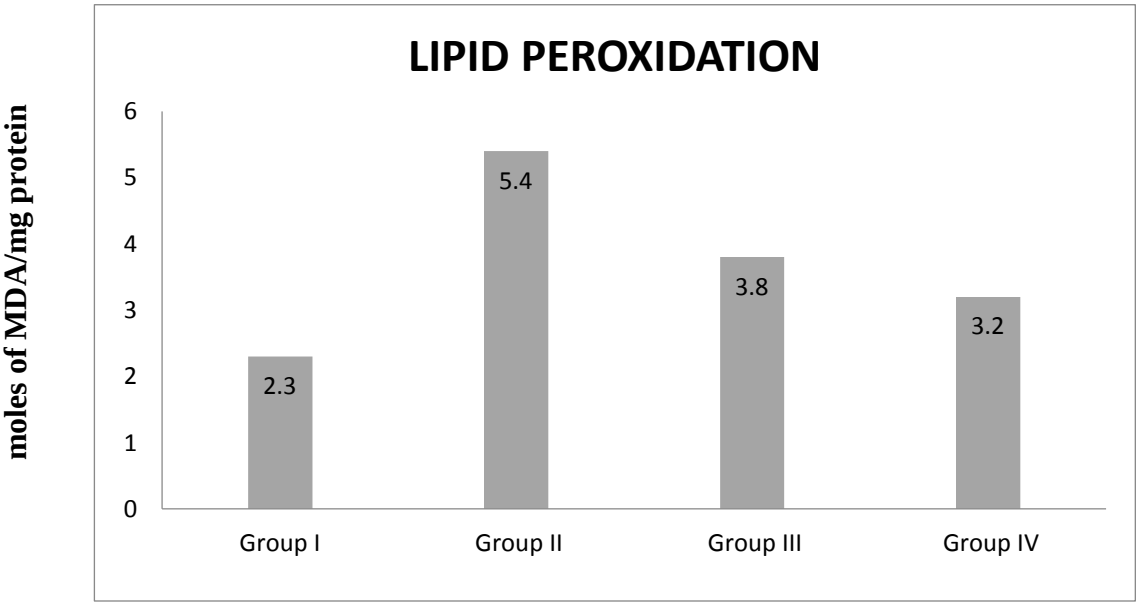

Figure: 5

DISCUSSION

The pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted. However, Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes mellitus in a wide variety of animal species through the damage of pancreatic β-cells. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results. In the present study, the results of the experiment indicated the significant antidiabetic activity of *Abelmoschus esculentus*Since, the experiment focused on exploring the competence of aqueous extract of *Abelmoschus esculentus*for the treatment of diabetes and relative complications like oxidative stress to substantiate folklore claim.

The elevated glucose level was successfully controlled by the *Abelmoschus esculentus* extract and several investigators have recommended that glycosylated hemoglobin to be used as an indicator for glycohemoglobic control of diabetes since glycohemoglobin levels approach normal values in diabetics in metabolic control. So in our case also the *Abelmoschus esculentus* controlled the glycated hemoglobin.

The increase in the levels of lipid peroxidation might be indicative of a decrease in the enzymatic antioxidant defense mechanism. Several studies have indicated that oxygen free radicals are generated in diabetic β-cells, and that the overexpression of antioxidant enzymes, such SOD and CAT plays an important role in protecting cells from oxidative damage. In the present study, it was observed that the TGM extract could increase the SOD and CAT activities in the liver tissues of diabetic rats. This indicates that *Abelmoschus esculentus*extract could inhibit or reduce the oxidative stress in diabetes. The activities of both SOD and CAT were augmented in diabetic rats, which could be attributed to the strong antioxidative properties

Researchers are interested in search of new drugs from medicinal plants for their biological activities like antidiabetic. In this study, we have evaluated the antioxidant and antidiabetic effect of *Abelmoschus esculentus* extract in alloxan induced rats. The enriched secondary metabolites may be responsible for the anti-diabetic and activity of *Abelmoschus esculentus.* The aqueous extract of *Abelmoschus esculentus* exhibited significant antidiabetic activity in alloxan induced diabetic rats.

SUMMARY AND CONCLUSION

From the present study it can be summarized as follows,

Screening of extracts for different type of phytoconstituents indicates the presence of Steroids, Phenols, Resins, tannins, Flovonoids, Glycosides, Saponins, coumarins, Quinine, Xanthoprotein, carbohydrate, Terpenoids and absence of phlobatannins, Anthocyanidine, Alkaloids are absent in Aqueous extract of *Abelmochus esculentus.*

The concentration of glucose was significantly higher in alloxan treated rats, as compared to control rats. These constituents were found to attain near normal levels in plasma in *Abelmochus esculentus* treated rats.

Glycated hemoglobin is increased in alloxan treated diabetic rats. Administration of aqueous extract of *Abelmochus esculentus* significantly control in Glycated hemoglobin.

The concentration of tissues SOD and CAT, were significantly decreased in diabetic rats and LPO was increased when compared to the control group. Administration of aqueous extract of *Abelmoschus esculentus* to diabetic rats tends to bring the activities of these enzymes to near normal level,

Hence it can be concluded that, okra is a natural product and it has antidiabetic activity. So the usage of the soaked okra water is not harmful to human health. Our in vivo condition experiments in rats shown a good result on Antidiabetic activity.

Furthermore, this vegetable can also be used as an indispensable tool when it comes to reducing then prevalence of malnutrition, especially among resource constrained urban households in addition to rural household. Consumption of Okra by both low-income and high-income groups can also use as a means of dietary diversification approach.

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