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ANTI-DIABETIC ACTIVITY OF *LAGENARIA SICERARIA* PULP AND SEED EXTRACT IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The aim of the present study was to evaluate the possible anti-diabetic potential of *Lagenaria siceraria pulp extract* (LSPE) *and Lagenaria siceraria seed extract* (LSSE) against the pancreatic damage from alloxan-induced diabetes in rats related to diabetes mellitus. *Lagenaria siceraria* induced significant reduction in blood glucose and increasing of serum insulin, our data indicate that the level of glucose in the animals that were subjected with alloxan was 210 mg/dl comparing with normal 70 mg/dl, the level of blood glucose in diabetic group when subjected with *Lagenaria siceraria* extract decreased to 89-106.5 mg/dl. These findings suggest that *LSPE* and *LSSE* treatment exerts therapeutic protective effect in diabetes by preserving pancreatic cell integrity and significant activity extract, which supports traditional usage of the plant to prevent diabetic complications.

INTRODUCTION: Diabetes is a growing epidemic around the world which is considered as a chronic incurable condition due to insulin deficiency that affects 10% of the population [1]. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025 [2].

Drug treatment is not completely successful in the people with diabetes and there is a compelling need for better prevention and treatment strategies [3]. Studies revealed that about 75 % of patients use some of form of complementary and alternative medicine even though physician knowledge, regulatory strands and evidence of safety and benefit are often lacking [4].

Nowadays herbal medicines are highly recommended for the treatment of diabetes in spite of the therapeutic options [5]. In the search of new opportunities for treatment of diabetes mellitus, researchers turn to the methods of popular medicine. Since antiquity people have used different medicinal herbs as ant diabetic remedy because it is considered to be less toxic and induce fewer side effects than synthetic ones [6]. One of the most popular herbal supplements which is used in ant diabetic phytotherapies is *Lagenaria siceraria.*

Lagenaria siceraria (Molina) Standley syn.*L. leucantha* Rusby; *L. Vulgaris* Ser. (Family: Cucurbitaceae) is commonly known as Bottle gourd, an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans [7]. *L. siceraria* fruits are also traditionally used for its cardio protective, cardio tonic, general tonic, aphrodisiac and acts as alternate purgative, diuretic [8, 9].

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It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders [8]. The fruits are edible and considered as a good source of vitamin C, carotene, vitamin B-complex, pectin and also contain high choline level- a lipotropic factor [7, 10, and 11]. Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B, D, G, H [10, 12, and 13] fucosterol, campesterol [14] and flavone C-glycosides [15]. *L. siceraria* seeds are used in migraine type headache and pain and are reported to contain saponins, essential fixed oils, vitamins [7, 11]. Lagenin- a novel ribosome inactivating protein has been isolated from the lyophilized water extract of seeds which is known to possess immunosuppressive, antitumour, antiviral, antiproliferative and anti-HIV activities [16].

Table 1: Plant profile of *Lagenaria siceraria*:

Calabash, *Lagenaria siceraria*	
Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	Lagenaria
Species:	***L. siceraria***
Binomial name ***Lagenaria siceraria* or** (Molina) Standl.	***Lagenaria vulgaris***

Synonyms: Calabash, bottle gourd, opo squash or long kalbas (Afr.); moraka (North Sotho); segwana (Tswana); iselwa (Xhosa, Zulu)

Biological Source: *Lagenaria siceraria*, the calabash, bottle gourd, opo squash or long melon is a vine grown for its fruit, which can either be harvested young and used as a vegetable, nor harvested mature, dried, and used as a bottle, utensil, or pipe. For this reason, the calabash is widely known as the bottle gourd. The fresh fruit has a light green smooth skin and a white flesh. Rounder varieties are called Calabash gourds. The bottle gourd may have been carried from Africa to Asia, Europe and the Americas in the course of human migration. It shares its common name with that of the calabash tree (*Crescentia cujete*). *L. siceraria* fruits are also traditionally used for its cardio protective, cardio tonic, general tonic, aphrodisiac and acts as an alternate purgative, diuretic. It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders [9].

Origin and dispersal: It is a commonly cultivated plant in tropical and subtropical areas of Eurasia and the Americas, now believed to have originated from wild populations in southern Africa. Stands of *Lagenaria siceraria* that may be source plants, and not merely domesticated stands run wild, were reported recently in Zimbabwe.This apparent domestication source plant produces thinner-walled fruits that, when dried, would not endure the rigors of use on long journeys as a water container [10].

Cultivation: Calabash had been cultivated in Asia, Europe and the Americas for thousands of years before Columbus's discovery of America. Historically, in Europe, Walahfrid Strabo (808–849), abbot and poet from Reichenau, advisor to the Carolingian kings, discussed it in his Latin Hortulus as one of the 23 plants of an ideal garden.

The rind of the domesticated calabash, unlike that of its wild counterpart, is thick and waterproof. It was therefore previously thought that calabash might have spread across oceans without human intervention, if the seeds were still able to germinate even after long periods at sea. This was the basis of the earlier, dominating theory, which proposed that the calabash had drifted across the Atlantic Ocean from Africa to North and South America.

The new research notes that domestication had led to changes in morphology (shape) of Asian and African specimens, potentially allowing the identification of the calabash from different areas. Now, both genetic and morphological considerations show that calabash found in American archaeological finds are closer to Asian calabash variants than to African ones. [9]

Toxicity: Like other members of the Cucurbitaceae family, calabashes contain cucurbitacins that are known to be cytotoxic. A toxin which is a tetracyclic

triterpenoid cucurbitacin compound, present in fruits and vegetables of the cucumber family, is responsible for the bitter taste and can cause ulcers in the stomach. In extreme cases, people have died from drinking calabash juice [12].

Chemical Constituents: The fruits are edible and considered as good source of vitamin C, carotene, vitamin B-complex, pectin and also contains high choline level- a lipotropic factor [13]. Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B, D, G, H [9, 10, and 11] fucosterol, campesterol [14] and flavone C-glycosides [13]. *L. siceraria* seeds are used in migraine type headache and pain and reported to contain saponins, essential fixed oils, vitamins [16]. Lagenin- a novel ribosome inactivating protein has been isolated from the lyophilized water extract of seeds which is known to possess immunosuppressive, antitumor, antiviral, anti- proliferative and anti-HIV activities [19].

Uses: The leaves are commonly eaten as a vegetable and are added fresh to maize porridge, or a relish is prepared from them, mixed with other plants. Dried leaves are stored for use in the lean season [20]. The young shoots seem to be an important vegetable, unlike the young fruits that are considered by some to be a famine food. The fruits of *L. siceraria* mature in bulk quantities, since they are needed for food and medicinal purposes, drying, milling and packing the product under hygienic environment can ensure a constant supply of antioxidant supplement.

The phytoconstituents consist the pharmacological actions as follows: Deshpande J.R. *et al* [23] (2008) has reported ant hyperglycemic activity of *Lagenaria siceraria*. The fruits of *Lagenaria siceraria* has antioxidant activity. It has also immunomodulatory effects, diuretic effects, cytotoxic activity, hepato-protective activity, Antihyperlipidemic effect, Cardio protective activity, Analgesic and Anti-Inflammatory activities, Hyperthyroidism, Hyperglycemia and Lipid Peroxidation. [21]

MATERIALS AND METHODS: Plant Collection: Aerial part of *Lagenaria siceraria* was collected from a local herb market in Shyambazar Kolkata, November 2011.

Preparation of LSPE: The LSPE extract was prepared by cutting the pulp into very thin and small pieces then boiling it in water for about 15 minutes, then allowing the decoction to stand for 30 minutes and then filtering it through Whatman filter paper. The filtrate was then heated in a Heating mantle so as to be concentrated for about 15 minutes. The extract was adjusted to 10 mg/ml of *Lagenaria Siceraria.*

Preparation of LSSE: The LSSE extract was prepared by taking the seeds separated from the pulp and then macerating it in Methanol until almost all the active principles had been separated from the seeds. Then the seeds were subjected to distillation and the methanolic extract was obtained as a thick concentrated mass in a beaker. The extract was adjusted to 10 mg/ml of *Lagenaria Siceraria.*

Amount of yield: LSPE: **328 ml** and LSSE: **235ml**

FIGURE 1

FIGURE 2

FIGURE 3

Phytochemical Screening: The LSPE was screened for presence of specific phytochemical constituents. The weight of the aqueous LSPE was observed as 110.0 gm. The following tests were conducted for the individual corresponding phytoconstituents as follows [22]:

1. **MOLISCH TEST**: To the test solution was added a few drops of alcoholic alpha-napthol, then a few drops of concentrated sulphuric acid was added through sides of test tube, purple to violet colour ring appeared at the junction.

2. **ALKALINE REAGENT TEST**: To the test solution was added a few drops of sodium hydroxide solution, an intense yellow colour was formed which turned colourless on addition of few drops of dilute acid.

3. **TEST FOR CATECHINS**: We dipped a matchstick in the test solution, dried it and lastly moistened it with concentrated hydrochloric acid. Then we warmed the stick near flame. The colour of the wood remained unchanged.

4. **FERRIC CHLORIDE TEST**: We treated the extract with Ferric Chloride solution, the colour remained unchanged.

5. **MAYER'S REAGENT TEST**: The test solution was treated with Mayer's Reagent (Potassium Mercuric Iodide solution), and a cream coloured precipitate was obtained.

6. **WAGNER'S REAGENT TEST**: The test solution was treated with Wagner's Reagent (Iodine-Potassium Iodide Solution), and a reddish brown precipitate was obtained.

7. **NINHYDRINE TEST**: To the test solution we added Ninhydrine solution, boiled, and a violet coloured appeared.

8. **FROTH FORMATION TEST**: We placed 2 ml solution of drug in water in a test tube, shaked well, and a stable froth (foam) was formed.

RESULTS AND DISCUSSION:

TABLE 2: **PHYTOCHEMICAL SCREENING**

PHYTOCONSTITUENT AND THE CORRESPONDING TEST CONDUCTED	OBSERVATION (LSPE)	CONCLUSION
CARBOHYDRATE		
Molisch Test	Deep violet colour ring appears	Presence is confirmed
FLAVONOID		
Alkaline reagent test	Intense Yellow colour formed on addition of NaOH which turns colourless on addition of dilute acid	Presence is confirmed
TANNIN		
Test for catechins	Woody piece colour does not change to blue	Presence is not confirmed
Ferric Chloride Test	Colour remains unchanged	Presence is not confirmed
ALKALOID		
Mayer's Reagent test	Cream coloured precipitate obtained	Presence is confirmed
Wagner's Reagent test	Reddish Brown precipitate obtained	Presence is confirmed
AMINO ACID		
Ninhydrin test (Test solution treated with Ninhydrin reagent and boiled)	Violet colour appears	Presence is confirmed
SAPONIN GLYCOSIDE		
2 ml solution of the drug was kept in test tube, shaken well and kept aside	Stable froth formation observed	Presence is confirmed

TABLE 3: VARIATION OF BODY WEIGHT

Group	Treatment	Body Weight (gm)	
		0 day	8th day
A	Normal Control	122.5 ± 12.5	122.5 ± 12.5
B	Alloxan Control	155 ± 25	125 ± 20.16
C	Alloxan+Treated (LSPE)	151.6 ± 16.6	155 ± 25
D	Alooxan+Treated (LSSE)	135 ± 14.43	145 ± 14.43

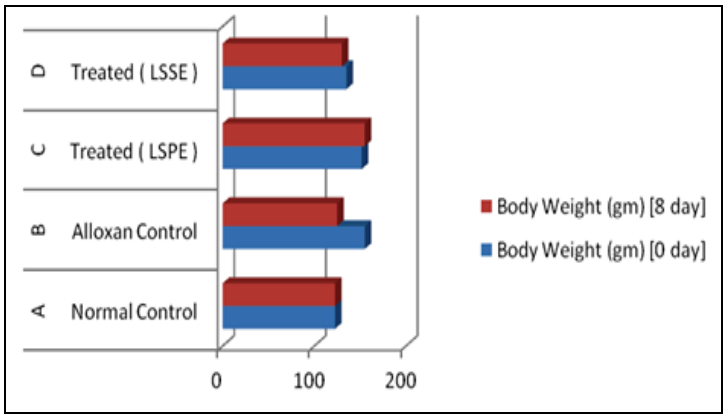

FIGURE 1: BAR DIAGRAM

Fig. 4 : Bar diagram of Table 3: It represents that the body weights of alloxan control rats were reduced from 155 ± 25 to 125 ± 20.16 and comparatively it is a higher reduction while the body weight in 8th day in treated LSPE & treated LSSE rats were 155 ± 25 & 145 ± 14.43 respectively.

TABLE 4: VARIATION OF FASTING BLOOD GLUCOSE LEVEL IN DIFFERENT GROUP

Group	Treatment	Fasting blood glucose level (mg/dl)	
		0 Day	8th Day
A	Normal Control	70 ± 2.26	70 ± 2.26
B	Alloxan Control	210	198 ± 4.08
C	Alloxan+Treated (LSPE)	70	106.5 ± 28.5
D	Alloxan+Treated (LSSE)	70	89.33 ± 15.37

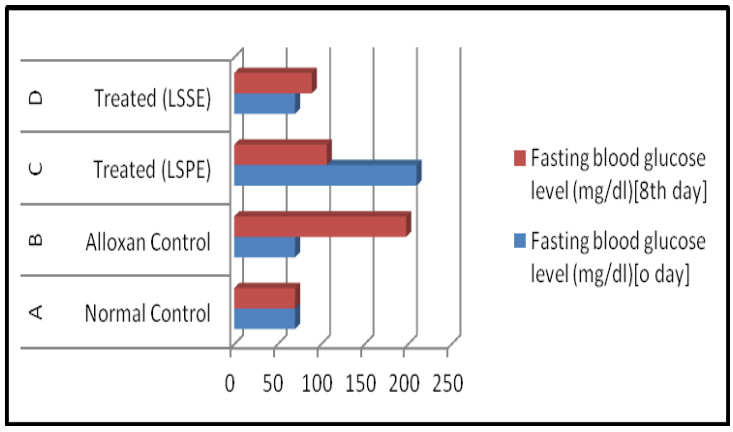

FIG. 5: BAR DIAGRAM

Fig. 5: Bar diagram of table 4: It represents that the percentage of reduction of FBG:

In Alloxan + treated group C: 46.21%

In Alloxan + treated group D: 54.88%

DISCUSSION: The results show that the baseline weights of the rats at the beginning of the study were similar in all groups but at the end of experiment significant weight loss observed in Alloxan induced diabetic rats than control normal rats. The finding of our study reports that treatment with extract of *Lagenaria siceraria* improved the weight gain compared to untreated diabetic rats (**Table 1**).

In this study, it is shown that LSPE and LSSE resulted in decreasing of blood glucose when subjected in diabetic animals 89-106.5 mg/dl, in comparison with Alloxan group 210 mg/dl. Numerous studies have found that the blood glucose level increased significantly in animals that were injected with Alloxan [24, 25, and 26].

Alloxan causes a massive destruction of β-cells of the Islets of Langherhans resulting in reduced synthesis and release of insulin [26]. The concentration of glucose in blood serves as the quantitative index in our study.

It is well known that during the evolution of diabetes mellitus in rats, after administration of Alloxan, the function of the insulin system is suppressed, which is expressed by high level of hyperglycemia [23].

Animals: Experiments were conducted on six adult albino female rats with the weights of 110-185g. All rats were fed normal laboratory diet containing protein, carbohydrates, fat and water was allowed *ad-libitum* under strict hygienic conditions. The rats were housed under standard environmental conditions as temperature; relative humidity and 12 hours dark/life cycle were maintained in the quarantine.

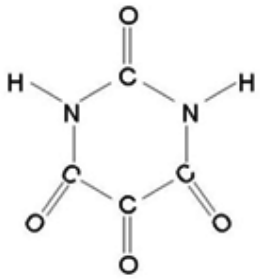

FIGURE 7

Induction of Diabetes: Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetitrone) is an oxygenated pyrimidine derivative [23] and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig [24]. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan monohydrate and all other chemicals used were of analytical grade and were acquired from commercial sources.

Experimental Design: Fasting blood glucose was determined after depriving food for 24 hrs with free access to drinking water. Hyperglycemia was induced by a single I.P. injection in dose of 100 mg/kg of body weight of Alloxan monohydrate in distilled water. After completion of Alloxan injection, the hyperglycemic rats (glucose level > 250 mg/dl) were separated and divided into 2 different groups comprising of 3 rats each for the anti-diabetic study. The treatment with LSPE and LSSE was started after a stabilization period of 48 hours from the day on which the animals were injected with Alloxan. During this period, animals in both groups had free access to standard diet and water. Body weight (by animal weighing balance) and blood glucose levels (from blood obtained by puncturing a minor portion of the tail and measured by Johnson and Johnson One-Touch Glucometer) were estimated on 7th day of the treatment. On the 12th day, blood sample was collected from overnight fasted rat by cardiac puncture under mild chloroform anesthesia for biochemical estimations.

Collection of Blood and Analytical Procedure: By the end of the 7 day period, the rats were reweighed and the blood glucose levels obtained. On the 12th day, after having starved the rat for 24 hours and sacrificed under chloroform anesthesia. 3 ml of blood was collected from one animal by cardiac puncture. The blood sample was put into test tube and allowed to clot for 30 minutes before centrifuging using a bench top centrifuge. This was stored at 4°C in refrigerator before the analysis of glucose by GOD-POD method using a visible spectrophotometer.

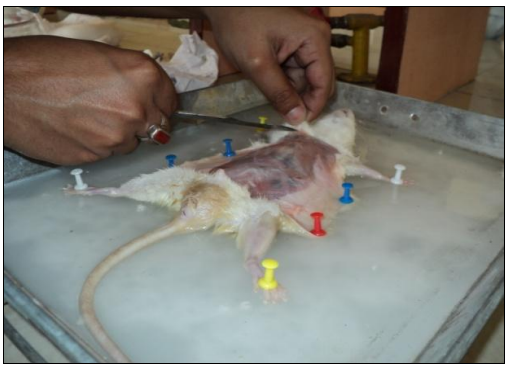

FIGURE 8

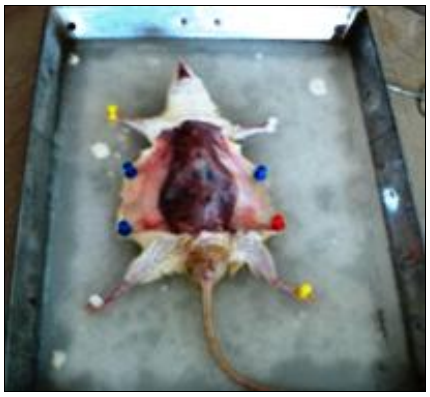

FIGURE 9

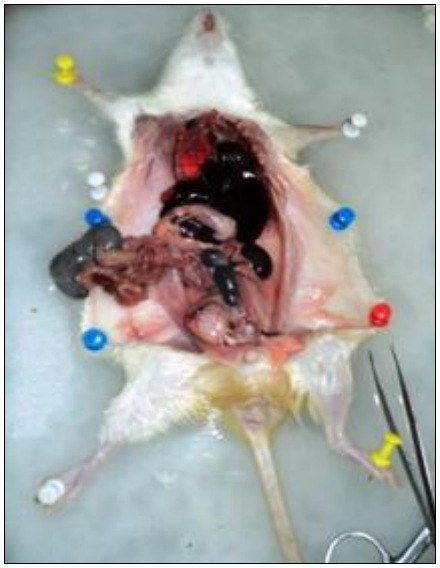

FIGURE 10

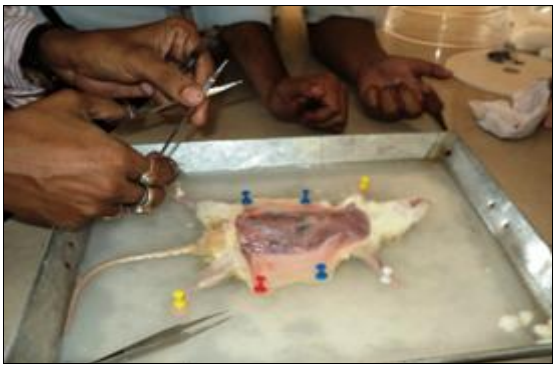

FIGURE 11

Groups of animals used in the Experiment:

Group I: Alloxan Monohydrate (100mg/kg of body weight) + LSPE (250 mg/kg body weight) {with some modifications to (19)}

Group II: Alloxan Monohydrate (100mg/kg of body weight) + LSSE (250 mg/kg body weight) {with some modifications [25]}

Treatment Schedule: Firstly 6 albino female rats of 100 gm - 185 gm are selected and divided into two groups which is indicated as group C and group D .At first Alloxan (100mg/kg) body weight was induced through IP to all the animals according to their body weight and stabilized for 24 hours and then from the very first day the LSPE and LSSE are fed orally every day as per their doses (250mg/kg) body weight. At the end of the 7 days the fasting blood glucose levels are checked.

Effect of *LSPE & LSSE* on biochemical parameters: Blood glucose levels were increased to 210 mg/dl significantly in a dose by inducing alloxan in the negatively treated rats of group 1 & group 2 while it is 70 mg/dl in average in normal rats and 106.5 mg/dl for the group 1 rats and 112 mg/dl for group 2 rats after 7 days treatment with LSPE and LSSE

Calculation:

Preparation of Stock Solution of Alloxan:

- For 150 gm of rat, we can inject through ip the amount of alloxan is 100mg/kg body weight.
- We prepare 90mg for 6 rats 0.4 ml alloxan IP dose is suitable for 150 gm of rats [26]
- 15 mg of alloxan is required for 150 gm of rats
- 15 mg alloxan is present in 0.4 ml. Then I dissolved 90 mg of alloxan in 2.4 ml of distilled water to prepare the stock solution which have to be injected through IP in 6 albino rats

Preparation of Stock Solution of LSPE & LSSE:

- 250mg/kg body weight of the test drug of LSPE & LSSE can be fed orally to rats
- 0.5 ml dose is required for 150gm of rats
- 37.5 ml of LSPE & LSSE were dissolved separately in 0.5 ml distiled water

So, for the oral feeding the stock solution has prepared where 787.5 mg drug is dissolved in 10.5 ml of distilled water

Effect of LSPE & LSSE on body weight in diabetic rat: Diabetic rats shown significant reduction of their body weight after 3 days when treated with alloxan mediated body weight reduction was significantly reversed by the methanolic extract.

Hence, we confirm the role of *Lagenaria siceraria* in the normalization of blood glucose: it is proved that the effect of *Lagenaria siceraria* on the secretion function of the pancreas is a result of their direction correlation with the cell membranes.

Comparative study of our experimental result with that of standard control rats: It is shown in the experimental practice that the FBG of diabetic rats treated with Standard drug (Alloxan + glibenclamide 10 mg/kg) is 183.18 ± 6.35 mg/dl after 7^{th} day and when treated with LSPE & LSSE the FBG are 106.5 ± 28.5 & 89.33 ± 15.37 respectively. So when treated with LSPE & LSSE the FBG value is quite close to normal FBG level of normal rats.

Effect of Standard drug (Alloxan + glibenclamide 10 mg/kg) on body weight in alloxan induced diabetic rats reduced 10±2.02 in the end of 7th day and while the body weight of LSPE & LSSE treated rats were 155±25 & 145±14.43 respectively.

Therefore it is experimentally proved from the above experiment that LSPE & LSSE are more effective medication than the Standard drug (Alloxan + glibenclamide) medication.

Methanolic LSSE is more efficacious than Aqueous LSPE because of FBG level of LSSE treated rats at the 8^{th} day is more close to the normal FBG of rats.

CONCLUSION: From above results, it was concluded that the *Lagenaria siceraria* pulp and seed extracts stimulate the changes of the functional state of pancreatic 13-cells. At the same time the capacity of the organism to produce and secrete insulin is increasing, the glucose level in blood is decreasing.

Our study clearly indicated a significant activity of pulp and seed extract of *Lagenaria siceraria* as an anti diabetic and supports traditional usage to prevent diabetic complications.

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