# Antihyperglycemic Activity on stems and Tendril parts of Methanolic extract of Plant *Lagenaria siceraria* standley on Alloxan induced Diabetic rats

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***Abstract*** **:** The above mentioned studies is done to know about all the facts and explanations of stems and tendril parts of the plant *Lagenaria siceraria* and with the help of this, to evaluate the Antihyperglycemic Activity of its Methanolic Extract. First of all, the diabetes is induced in the male Wister Albino Rats by Alloxan administration of dose about 150mg/kg i.p. The standard reference drug used is Glibenclamide of dose about 500µg/kg. The plasma fasting blood glucose level is monitored. The antihyperglycemic potential of the test drug was determined by its moderate doses that are lower dose of 200mg/kg and higher dose of 400mg/kg in various intervals of days that are on day 2$^{nd}$, day 4$^{th}$, day 8$^{th}$, and day 15$^{th}$ after the induction of diabetes in the rats. The sufficient and notable changes have been monitored due to the attributing flavonoidal content in the Methanolic Extract of *Lagenaria siceraria* (MELS).

***Index Terms*** **:** Hyperglycemic, Phytochemicals, *Lagenaria siceraria* Standl., MELS.

***Abbreviations*** **:** MELS- Methanolic Extract of *Lagenaria siceraria*

## 1. INTRODUCTION

Diabetes mellitus is known to be that metabolic chronic disorder that leads to the symptom of elevation of plasma blood glucose level in the body. It may cause various types of body complications and can cause damage of renal cells as well as Cardiovascular, retinal and neurological systems (Marles RJ, 1995; (Bracken P etal, 2003). The problem of diabetes has been raised from Asia to Africa and emerging into the rest of the world. Due to the worldwide distribution of this disorder, many indigenous plants has been use in order to cure the diabetes mellitus because of the unaffordable prices of the synthetic antihyperglycemic agents and they have worked well also (King H, etal, 1998).

## 2. PLANT REVIEW

Our Vedic literature Charak Samhita has reported a number of plant species and their effective parts in order to cure the diabetes and it's been effectively used by almost the two third of the world's population (Kirtikar KR, 2003). And the studies done over them has shown the effectiveness of more than seven hundred species over this chronic metabolic disorder. Some of these species are-

- *Aegle marmelos*
- *Allium cepa*
- *Allium sativum*
- *Andrographis paniculata*

- *Gymnema sylvestre* etc.

Similarly Almost the whole plant of *Lageneria siceraria* is very effective in diabetes disorder. Hence the Methanolic Extract of Stem and tendrils of *Lageneria siceraria* have been selected for study the of Antihyperglycemic Activity in experimental animals, the male Wister Albino rats.

Lagenaria plant that is *Lagenaria siceraria* Molina (standley) is commonly known as bottle gourd belonging to the family Cucurbitaceae. Geographically it is cultivated worldwide and found almost all the parts of India by their different vernacular names. This property of Lagenaria makes it cheap and easily available. It has great diuretic and antiobesitic properties for which it is indigenous to the world. However, the Lagenaria plant shows good antioxidant property. It chiefly contains Polyphenols and Flavone C glycoside (Sonja S etal, 2000; Baranawska MK, 1994) for its antioxidant property (Shirwaiker A. etal,1996; Ghule BV, etal,2006; Deshpandey JR, 2007; Deshpandey JR, Choudhary AA, etal , 2008; Shah BN, 2010; Duke JA, 1999). It also contains Lagenin, Cucurbitacin, some proteins and Ascorbic Acid. Lagenin is responsible for the antitumor and immunoprotectant activity. Thus, there is a number of effects has been shown over the fruit extract of Lagenaria plant including its pulps and seeds also. Therefore this actually shows how effective and novel the Lagenaria plant is. Now the present work wants to emerge the other important and crucial Antihyperglycemic activity of the Methanolic Extract of *Lagenaria siceraria* plant.

## 3. MATERIAL AND METHODS

### 3.1 Collection of plant material:

The stems and tendrils of *Lagenaria siceraria* standley, is collected from the local area in Civil Lines Allahabad, Uttar Pradesh, India. The plant has been authenticated by Botanical Survey of India, Ministry of Environment, forest and climate change Chaitham Lines, Allahabad, under the reference number 100228.

### 3.2 Preparation of plant extract:

The plant extract is prepared by shade drying the plant and powdering it to the mechanical grinder and passing it to 40 mesh sieve. The powdered material (25g) is extract by subjecting it to Cold Maceration and successively powdered material was defatted with petroleum ether (60-80ºC) and successively extracted for 24 hr using chloroform and methanol and distilled water solvents in the increasing order of polarity.

The solvent was distilled under reduced pressure, controlled temperature (40-50°c) and the resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue put in airtight container and stored in refrigerator.

The extraction of stems and tendril parts were done by Successive solvent extraction technique. The cold maceration technique was done for the successive solvent extraction. The liquid is evaporated in Rotatory evaporator at 75º±5ºC for about 4-5 hours and dried in vacuum drier to get the semisolid extract.

### 3.3 Animals:

Healthy Wistar Albino Rats 160-180g is to be purchased from NIN, Hyderabad used for study. Housed individually in propylene cages, maintained under standard conditions (12h light; 12 h dark Cycle; 23±2ºc , 50±5% RH), they will be fed with Standard Rat Pellet diet. The institutional Animal Ethics Committee (TIT/IAEC/P'Col/2013-17) approved the study.

### 3.4 Preliminary phytochemical screening:

Table: 1 Presence of various phytochemicals like alkaloids glycosides, saponins, tannins etc. has been identified by many phytochemical screening methods(Kokate CK, 1994).

| Sr. No. | Name of the test | Pet. Ether extract | Chloroform extract | Methanolic extract | Aqueous extract |
|---|---|---|---|---|---|
| 1. | **Alkaloid test**<br>Mayer's test<br>Dragendorff's test | <br>Negative<br>Negative | <br>Negative<br>Negative | <br>Negative<br>Negative | <br>Negative<br>Negative |
| 2. | **Carbohydrate test**<br>Barfoed test<br>Molisch Test | <br>Positive<br>positive | <br>Positive<br>Positive | <br>Positive<br>Positive | <br>Positive<br>Positive |
| 3. | **Phytosterols Test**<br>Burchard's reaction | Positive | Positive | Positive | Positive |
| 4. | **Flavonoids Test**<br>Lead Acetate Test<br>Ferric Chloride Test<br>Alkaline Reagent Test | <br>Positive<br>Positive<br>Positive | <br>Positive<br>Positive<br>Positive | <br>Positive<br>Positive<br>Positive | <br>Positive<br>Positive<br>Negative |
| 5. | **Tannin Test**<br>Ferric chloride test | Positive | Positive | Positive | Negative |
| 6. | **Amino acid Test**<br>Ninhydrin Test | Positive | Positive | Positive | Negative |
| 7. | **Phenol Test**<br>Lead acetate test | Positive | Positive | Positive | Positive |

### 3.5 Acute toxicity studies:

Acute oral toxicity study (Ghosh MN, etal, 1984) was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-423 [9]. The animals were given the successive dose of Methanolic extract of test drug as 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg. All the doses of the extract given to the animals were found to be safe hence according to the basis of the review of the literature, the dose of 200mg/kg body weight and 400mg/kg body weight of the animal has been taken as the Low Dose and High Dose respectively.

### 3.6 Test dose selection and preparation

The experimental design consisted of 24 rats grouped as follows:

1. Group 1– Diabetic untreated (negative control);
2. Group 2 – Diabetic treated with standard drug (500µg/kg body weight of glibenclamide);
3. Group 3 – Diabetic treated with 200 mg/kg body weight of crude ethanol extract of *L. siceraria*
4. Group 4 – Diabetic treated with 400 mg/kg body weight of crude methanol extract of *L. siceraria.*

1%w/v aqueous Suspension of Tween 80 was made. The doses were freshly prepared prior to administration.

### 3.7 Induction of Diabetes

The diabetes was induced (Brosky J, etal, 196by Alloxan Induced Diabetic Model in which All the groups were given 150mg/kg of Alloxan freshly made in 0.5M citrate buffer at pH 4.5. After 2nd day of Alloxan administration the blood was withdrawn by Tail Vein Puncturing and blood glucose level was observed. The blood glucose level > 150mg/dL then the animals were considered to be diabetic.

### 3.8 Testing of fasting blood glucose level

The fasting blood glucose level is to be monitored on day 2, 4, 8, 15. The drop of blood was collected from the tip of the tail vein of each rat and fasting blood glucose level was measured by One Touch Glucometer. Initial and final body weights were also recorded.

### 3.9 Preparation of Dosage form of the extract

For the further studies two dosage forms has been prepared in order to make the lower dose and higher dose of Methanolic Extract of *Lagenaria siceraria*. The shade dried powder of the crude plant has gone through the success solvent extract technique. After that the liquid of the extracted material is evaporated through Rotatory Evaporator. The condition of Rotatory evaporator was set to be 75±5ºC for about 4-5 hours and dried in vacuum drier to get the desired mass.

The lower dose of MELS was set about 200mg/kg and the higher dose was 500mg/kg.

## 4. STATISTICAL ANALYSIS

The statistical analysis was done by expressing the values as Mean ± SEM. The method of evaluating the data is One Way Analysis of Variance (ANOVA) by GraphPad InStat Software. P values less than 0.01 were considered to be the significant data.

| Sr. no. | Treatment/ dose | Blood glucose level mg/kg day 2 | Blood glucose level mg/kg day4 | Blood glucose level mg/kg day8 | Blood glucose level mg/kg day15 |
|---|---|---|---|---|---|
| 1 | Diabetic control | 220.88±0.1219 | 235.33±0.1904 | 270.71±0.1011 | 260.01±0.0722 |
| 2 | Glibenclamide 500µg/Kg | 217.42±0.1584 | 204.98±0.0315 | 130.57±0.0823 | 89.99±0.0601 |
| 3 | Lower dose 200mg/Kg | 212.38±0.1879 | 190.99±0.060 | 161.04±0.0645 | 110.95±0.0455 |
| 4 | Higher dose 400mg/Kg | 207.45±0.1860 | 170.40±0.1582 | 152.96±0.0606 | 93.06±0.0532 |

All the values of statistical analysis are expressed as ±SEM for n=6 rats for each group when compared to standard**p<0.01

Figure 1: Diagrammatic representation of the action of various treatment doses over Wister Albino Rats on day 2

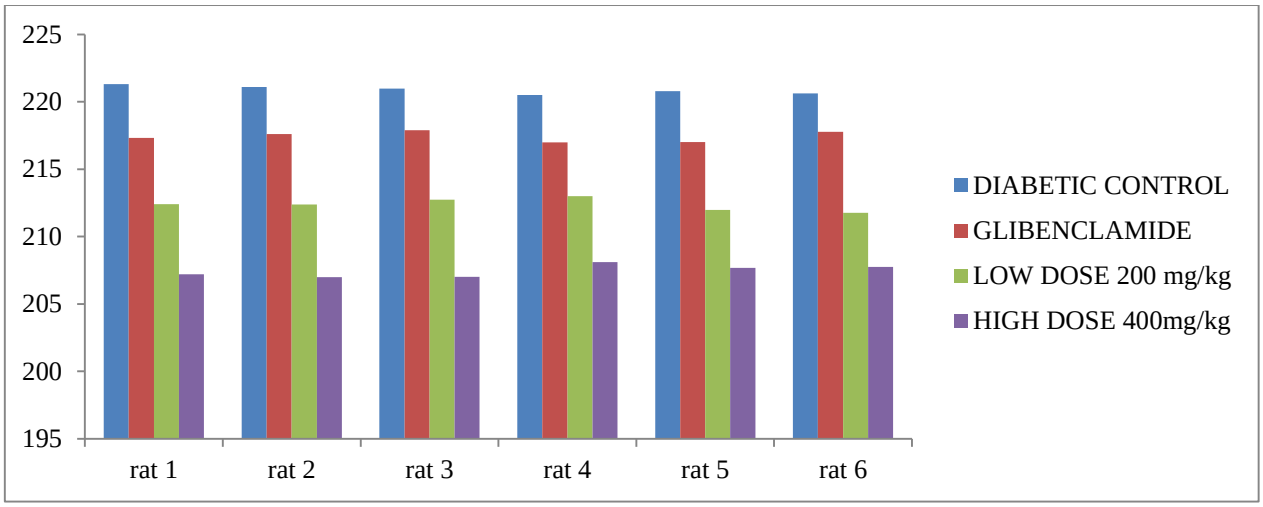



Figure 2: diagrammatic representation of the action of various treatment doses over Wister Albino Rats on day 4

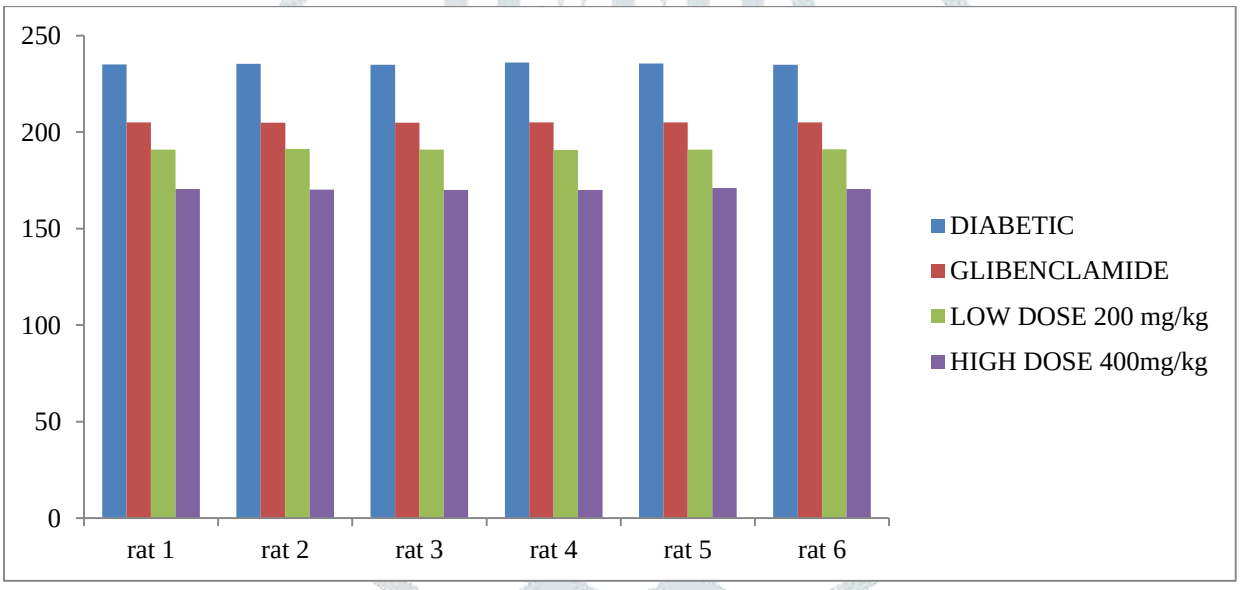



Figure 3: diagrammatic representation of the action of various treatment doses over Wister Albino Rats on day 8

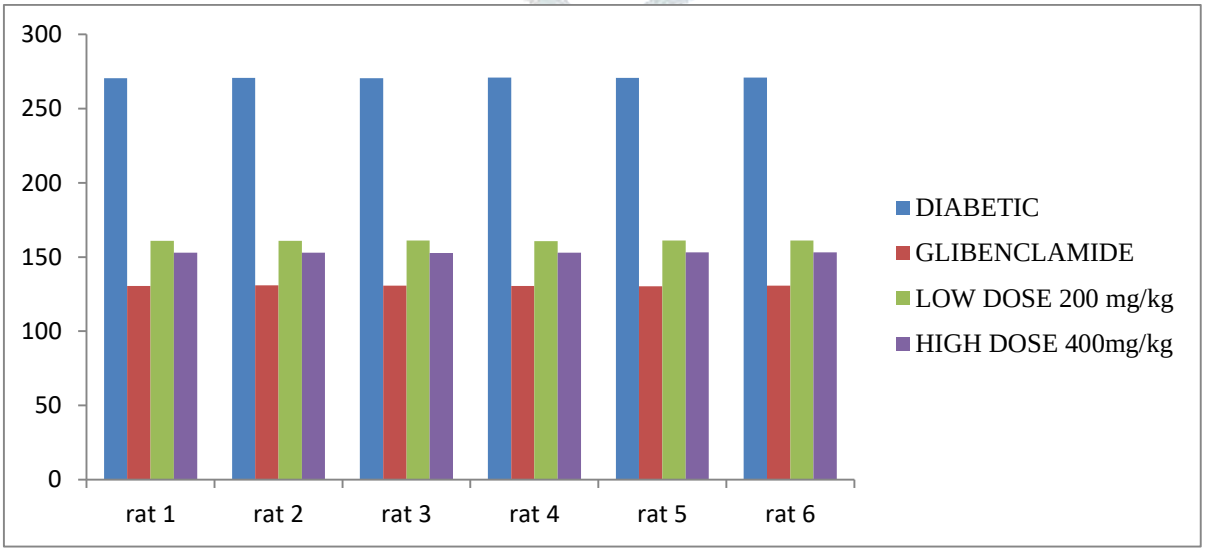
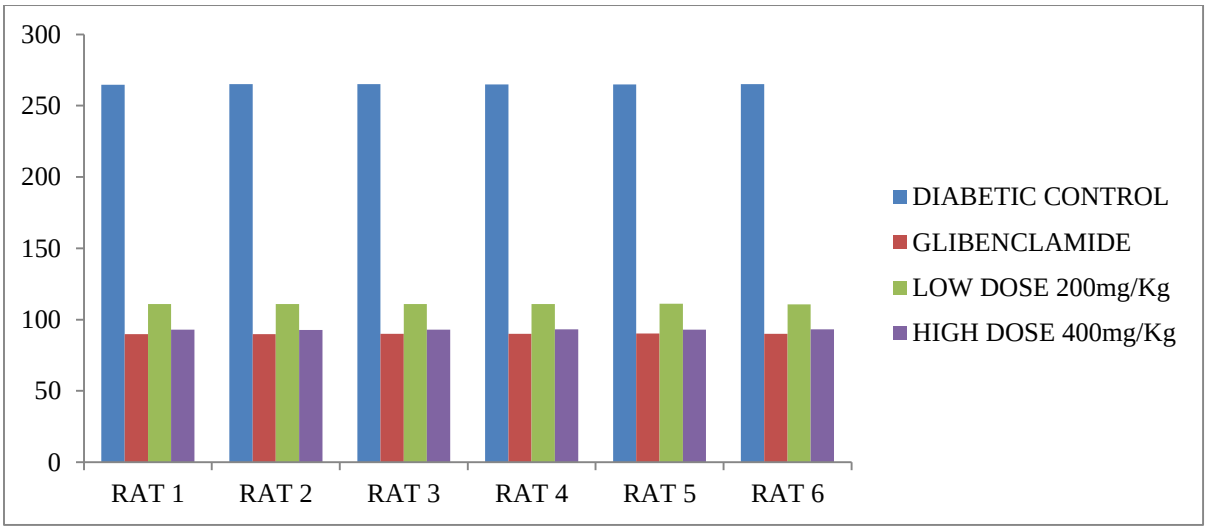



Figure: Diagrammatic representation of the action of various treatment doses over Wister Albino Rats on day 15




## 5. RESULTS AND DISCUSSION:

The study evaluated the Anti hyperglycemic activity of Methanolic extract of Lagenaria siceraria on Alloxan induced diabetic rats.

Alloxan induced hyperglycemia ( Saha P, 2008; Gillman L, 1985; Aslan M, 2007; Haldar PK 2010; Kamalakkannan N, 2006) is a useful experimental modal for studying antihyperglycemic activities

From the present investigation it can be included that Methanolic extract of *Lageneria siceraria* supplementation is quite beneficial in controlling blood glucose level without producing hypoglycemia.

Pancreas is therefore a very crucial organ to the body. It produces insulin and chiefly which are principally concerned with control of blood glucose level of the body that is done by the specialized organelle present in it known as β cell islets of Langerhans. Thus insulin is the hormone that helps to regulate blood sugar levels by assisting the transport of glucose from the blood into neighboring cells.

Failing to the proper regulation of insulin inside the body leads to diabetes mellitus. Therefore it is crucial to regulate the level of blood glucose but due to the unaffordable prices of synthetic hypoglycemic agents for the underdeveloped and developing countries, the natural crude indigenous substances become the only way to stabilize this hyperglycemic condition.

During the above studies, this has been emerged out that the Methanolic extract of *Lagenaria siceraria* standley is completely able to regulate the plasma blood glucose level. It is clear that the flavonoidal content present in the Lagenaria plant shows the notable effect over the glucose metabolism on rats. They have the specialized ability to regulate the glucose uptake. It reduces the intestinal absorption of blood glucose inside the body. And the reduction of intestinal absorption of glucose constitutes a possible means of controlling diabetic hyperglycemia. These findings demonstrate that flavonoidal content directly acts on the pancreatic β islets leading to the activation of cAMP/ PKA signaling cascade to exert an insulinotropic effect. Thus finally helps to regulate the blood glucose.

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