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# EVALUATION OF HYPOGLYCEMIC ACTIVITY OF *ANNONA RETICULATA* L. STEM BARK EXTRACTS AGAINST STREPTOZOTOCIN INDUCED DIABETIC RATS

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**ABSTRACT**

This study is carried out in a view to evaluate hypoglycaemic activity of stem bark extracts of Annona reticulata L. in streptozotocin induced diabetic rats. Oral administrations of petroleum ether, chloroform, ethyl acetate and ethanol extract of the plant stem bark up to 2000mg/kg body weight did not show any signs of toxicity and mortality in this study. In Oral Glucose Tolerance Test, administration of glucose (2.5g/kg,) produced significant change in blood glucose level of normal mice. Treatment with A. reticulata ethanolic extract (200 and 400mg/kg) and standard drug glibenclamide (10mg/kg.) significantly reduced serum glucose level in normal fasting, at 0min, 30min, 60min and 120min compared to normal control group. Ethanolic extract of the plant showed more significant blood glucose lowering activity at 400mg/kg dose level. But Petroleum ether, ethyl acetate and chloroform extracts of the plant did not show significant decrease in the blood glucose levels. So, in this study 400mg/kg of ethanolic extract of A. reticulata was selected for the evaluation of anti-diabetic activity in streptozotocin induced diabetic rats. Oral administration of ethanolic plant extract of A. reticulata stem bark at 400mg/kg/day and standard drug Glibencamide for 15 consecutive days to streptozotocin induced diabetic rats significantly reversed decreased body weight, increased water consumption and increased fasting plasma glucose level in comparision to diabetic control animals. Phytochemical analysis of the plant sample showed the presence of different chemical compounds like alkaloids, flavanoides, Phenolic glycosides and lignans.

**Keywords:** *Annona reticulata* L., diabetic, stem bark, rats, streptozotocin.

## INTRODUCTION

*Annona reticulata* L. commonly known as Custard Apple, bullock's heart is a small deciduous or semi-evergreen tree in the plant under the family Annonaceae and is native to the West Indies and tropical America. The plant is naturalized and commonly cultivated in Bengal, Assam, Khasi Hills of Meghalaya and some area of southern India. Leaves, fruits, root and stem barks of *A. reticulata* are used as source of medicine and also for industrial products. It possesses several medicinal properties such as anthelmintic, analgesic, anti-inflammatory, antipyretic, wound healing and cytotoxic effects. Leaves and seeds are used in boils, bark as astringent, antidysnteric and vermifuge; fruits are in dysentery, biliousness and in various diseases of blood. Leaves are made into paste and applied to ulcers. Leaf juice is useful to kill lice. Leaves and seeds are insecticidal. It is widely distributed with phytochemicals like alkaloids, glycosides, phenols, flavonoids and steroids[1].

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. It is a chronic metabolic disorder mainly affects the carbohydrate metabolism, but has marked secondary effects on protein, fat, water and electrolytes. Diabetes occurs when the body cannot produce enough insulin or cannot use insulin effectively. Insulin is a hormone produced in the pancreas that allows glucose from food to enter the body's cells where it is converted into energy needed by muscles and tissues to function. Till to- day in the field of modern medicines, a good number of research works carried out by different scientists to find out a satisfactory management of diabetes mellitus and try to prevent its complications. In Ayurvedic classics there is also abundant literature found about the disease comprising of the measures for its effective management. In the present time, there is much research going on all aspects of this disease in the field of diabetes. Despite insulin therapy and oral drugs, beta cells transplants, allopathy in the develop countries is on look out for preventive intervention in diabetes and its complications. But still no perfect remedy is available while the incidence is increasing alarmingly. Diabetes mellitus is a fast-growing global problem with huge social, health, and economic consequences[2,3]. WHO estimates an increase of 3.9 billion diabetes cases by 2030[4]. Keeping all these aspect the present study was undertaken to screen out the anti-diabetic efficacy of *Annona reticulate* stem bark in established case of diabetes mellitus through experimental study.

## MATERIALS AND METHODS

### Drug and chemicals

The standard drug i.e. Glibenclamide obtained from Ranbaxy laboratories. Streptozotocin is obtained from sigma Aldrich, Germany. The all other chemicals were of analytical grade and obtained commercially. In the experimental study the Glucometer (Infopia Co., Ltd., India) was used to determine the blood glucose level.

**Collection and extraction of plant materials**
**Plant Materials**

The specific indicated part of Plant materials stem bark of *Annona reticulata* which are taken for research study were collected from the different parts of Kamrup district of Assam before study were initiated. The Plants are authenticated by a Taxonomist from Gauhati University and Survey of Medicinal Plant Unit (SMPU) of North Eastern India Ayurveda Research Institute (CCRAS), Ministry of AYUSH, Govt. of India, Guwahati, Assam. The herbariums were prepared and deposited as voucher specimen (No. IASST/MEP/HNo. 27 & 19) in the Medicinal Plant and Biochemistry Laboratory of IASST for future reference.

**Preparation of plant extracts**

Freshly collected plant materials were dried under shade and 500gm of the fine dried powdered materials were then extracted with Petroleum ether, Chloroform, Ethyl acetate and ethanol (Merck) by means of cold maceration procedure at room temperature. The extracts were concentrated in a rotary evaporator (Buchi R124, German) at $<40^0$ C was kept in refrigerator at $4^0$ C for future use.

**Phytochemical screening**

In this study, the phytochemical screening of the plant extract *A. reticulata* was done by different chemicals as follows[5]

**Test for Alkaloids**

**1) Mayer's Test**
To a few ml. of filtrate, two drops of Mayer's reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

**2) Wagner's Test**
To a few ml. of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate indicates test as positive.

**3) Hager's Test**
To a few ml. of filtrate 1 or 2 ml. of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

**4) Dragendorff's Test**

To a few ml. of filtrate, 1 or 2 ml. of Dragendorff's reagent was added. A prominent reddish brown precipitate indicates positive test.

**Test for Flavonoids**

**NaOH test:** A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

**$H_2SO_4$ test:** A fraction of extract was treated with concentrated $H_2SO_4$ and observed for the formation of orange colour.

**Lead acetate test:** A small amount of extract was treated with lead acetate and observed for the formation of white precipitate.

**Test for lignans**
0.5ml. of aqueous solution of extract was added to 2ml. of 2% (V/V) furfuraldehyde in a test tube– Red color indicates the presence of flavonoids.

**Test for Glycosides**

**Keller Killiani Test –** Test solution was treated with fewdrops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluishgreen would indicate a positive test for glycosides.

**Bromine water test:** Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

**Acute Oral Toxicity Study**

Acute oral toxicity studies were performed according to Organisation for Economic Co-operation and Development (OECD) guidelines to test chemicals. Randomly selected Swiss albino mice of either sex (6 animals) were used for this study. Animal was kept overnight fasting with free access to water but not food, next day morning *A. reticulata* extracts at the dose ranging from 200mg, 400mg, 600mg, 1000mg,1500mg 2000mg/kg body weight were administered orally to groups of three animals each. Animals were observed for 14 days, if mortality was observed in 2 out of 3 animals, then the dose was identified as toxic dose. If mortality was observed in 1 animal, experiment was repeated again with same dose to confirm the toxic dose. If mortality observed again experiment was continued with low doses (300, 50 and 5mg/kg body weight).

**Oral Glucose Tolerance Test (OGTT)**

Mice divided into four groups (n = 6) were administered with distilled water 10 ml/kg, glibenclamide (standard drug) 10 mg/kg, and with Petroleum ether, Chloroform, Ethyl acetate and ethanol extracts of *Annona reticulata* at dose of 200 and 400mg/kg p.o. by help of oral gavages, respectively. OGTT was carried out after 14 days of treatment, during which the animals were fed with normal diets. Glucose (2.5 g/kg) was fed 30 min after the administration of extracts. On completion of 14 days of treatment, the mice were fasted over night and blood was withdrawn from tail-vein just prior to the drug administration (normal fasting) and at 0, 30, 60 and 120 min of glucose loading. Blood glucose level was measured immediately by using glucose oxidase-peroxidase reactive strips and a glucometer[6].

**Grouping**
Group I : Untreated control (D.w)
Group II : glibenclamide (10 mg/kg)
Group III : *A. reticulata* petroleum ether extract (200 mg/kg)
Group IV : *A. reticulata* petroleum ether extract (400 mg/kg).
Group V : *A. reticulata* chloroform extract (200 mg/kg).
Group VI : *A. reticulata* chloroform extract (400 mg/kg).
Group VII: *A. reticulata* ethyl acetate extract (200 mg/kg).
Group VIII: *A. reticulata* ethyl acetate extract (400 mg/kg).
Group IX : *A. reticulate* ethanol extracts (200 mg/kg).
Group X : *A. reticulate* ethanol extracts (400 mg/kg).

**Experimental Animals**

Male rats (Wistar strain) weighing 150–220g were used in this study and were group-housed (n = 6 per cage) in a room with controlled temperature (21–22°C), and a reversed light–dark cycle (12 h/12 h), and they had free access to food and water adlibitum. The experimental protocols were approved by the Institutional Animal Ethical Committee of IASST and experiments were performed according to the Committee for the

purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines.

**Evaluation of hypoglycemic activity of plant extracts against streptozotocin induced diabetic rats**

Diabetes was induced by a single intra peritoneal injection of streptozotocin (STZ, 45 mg/kg; i.p.) freshly dissolved in citrate buffer pH 4.4 to overnight fasted male Wistar rats. Three days later, fasting blood glucose level was measured, the rats which displayed fasting blood glucose >250 mg/dl was considered as diabetic and grouped. The blood samples were taken by cutting the tail tip of rat and letting a drop of blood on the test strip. Fasting blood glucose (FBG) was determined by Glucometer. After that 400mg/kg body weight dose of the plants extracts were orally administered to the Streptozotocin induced diabetic rat (n = 6) for 15 days with using a feeding tube[7]. Blood glucose was measured at 0, 5, 10, 15 and 20 days during the drug treatment. Diabetic control groups were administered with 0.3% CMC [Carboxy methyl cellulose, (oral)], Glibenclamide (10mg/kg bwt, oral) group treated as standard.

**Grouping:**
Group I : Untreated control (D.W)
Group II : Diabetic control STZ + (0.3% CMC)
Group III : STZ induced diabetic rats + glibenclamide (10mg/kg)
Group IV : STZ induced diabetic rats + *A. reticulata* ethanol extract (200mg/kg).
Group V : STZ induced diabetic rats + *A. reticulata* ethanol extract (400 mg/kg).

**RESULTS**

Phytochemicals present in different plant extracts of *A. reticulata* are given in Table 1.

**Acute Toxicity Studies**

Oral administration of *A. reticulata* petroleum ether, chloroform, ethyl acetate and ethanol up to 2000mg/kg body weight did not show any signs of toxicity, like salivation, lighting reflex, corneal reflex (Table 2). After 14 days of observation period no mortality was found. So, 1/5$^{th}$ and 1/10$^{th}$ of the non toxic dose was selected for the experimental study i.e 200 and 400mg/kg.

**Oral Glucose Tolerance (OGT) Test**

Administration of glucose (2.5g/kg,) produced significant change in blood glucose level of normal mice. Treatment with *A. reticulata* ethanolic extract (200 and 400mg/kg) and glibenclamide (10 mg/kg.) significantly reduced serum glucose level in normal fasting, at 0min, 30min, 60min and 120min compared to normal control group. Ethanolic extract of the plant showed more significant blood glucose lowering activity at 400 mg/kg dose level. But Petroleum ether, ethyl acetate and chloroform extracts of the plant did not show significant decrease in the blood glucose levels. So, in this present study 400mg/kg of ethanolic extract of *A. reticulata* was selected for the evaluation of anti-diabetic activity in Streptozotocin induced diabetic rats. Results of OGTT of the different extracts of the plant were given in Table 3.

**Effect of Plant extract on Diabetic rats**
**Body weight changes**

Treatment with Streptozotocin causes decrease in body weight of the animals. Treatment with standard drug and ethanolic plant extract of *A. reticulata* at 400mg/kg showed significant increase in body weight in comparision to diabetic animals (STZ + 0.3% CMC) group (Table 4).

**Effect on water intake**

Treatment with Streptozotocin causes increased in water intake in diabetic animals. Treatment with standard drug and ethanolic plant extracts at both 200 &400 mg/kg showed significant decrease in water intake in comparison to diabetic animals (STZ + 0.3% CMC) group. The plant extract at 200 mg/kg showed less significant results than high dose (Table 5).

**Effect of plant extracts on blood glucose levels in diabetic rats**

After STZ injection, rats exhibited significant increase in fasting plasma glucose levels as compared to non-diabetic control rats ($p<0.001$) indicates induction of hyperglycemia in rats. Standard drug and plant extract treatment administration from 5$^{th}$ day exhibited significant reduction in fasting plasma glucose level ($p<0.001$) as compared to diabetic rats (Table 6).

**Table 1: Phytochemical investigation of different fraction of extracts of *A. reticulate***

| Plant | Extract | Alkaloids | Flavonoids | Phenolic glycosides | Lignans |
|---|---|---|---|---|---|
| *A. reticulata* | Petroleum ether | + | - | + | - |
| | Chloroform | + | + | - | - |
| | Ethyl acetate | - | - | + | + |
| | Ethanol | + | + | + | + |
| | Ethanol | + | + | + | + |

**Table 2: Dose and Toxicity of the different plant extracts of *A.reticulata***

| Sl. No. | Dose (mg/kg body wt.) | Toxicity signs | | | Remarks |
|---|---|---|---|---|---|
| | | Salivation | Lighting reflex | Corneal reflux | |
| 1 | 400 | _ | _ | _ | No any sign of toxicity found |
| 2 | 600 | _ | _ | _ | |
| 3 | 1000 | _ | _ | _ | |
| 4 | 1500 | _ | _ | _ | |
| 5 | 2000 | _ | _ | _ | |

**Table 3**: **Oral glucose tolerance test (OGTT) of different extracts of *A. reticulate***

| Sl. No. | Group | F BG level | 0 min | 30 min | 60 min | 120 min |
|---|---|---|---|---|---|---|
| 1 | Control (D.W) | 114± 1.61 | 265 ± 2.24 | 348 ± 3.67 | 297 ± 2.13 | 236 ± 2.22 |
| 2 | Standard Drug treated (10 mg/kg | 96 ± 2.61 | 176 ± 1.84*** | 242 ± 2.86 *** | 171± 3.11*** | 147 ± 3.42*** |
| 3 | *A. reticulata* pet ether extract (200mg/kg) | 106± 1.26 | 249 ± 2.18 | 351 ± 4.39 | 289 ± 2.95 | 239 ± 3.39 |
| 4 | *A. reticulata* pet ether extract (400mg/kg) | 119± 2.81 | 255 ± 3.19 | 331± 4.15 | 278 ± 3.91 | 229 ± 2.99 |
| 5 | *A. reticulata* chloroform extract (200mg/kg) | 122 ± 2.22 | 272 ± 3.14 | 359 ± 4.24 | 281 ± 3.81 | 248 ± 2.81 |
| 6 | *A. reticulata* chloroform extract (400mg/kg) | 119± 1.89 | 281 ± 5.36 | 359 ± 4.81 | 301 ± 3.99 | 242 ± 2.91 |
| 7 | *A. reticulata* ethyl acetate extract (200mg/kg) | 120± 2.11 | 241 ± 2.82 | 366 ± 4.77 | 274 ± 3.81 | 229 ± 3.18 |
| 8 | *A. reticulata* ethyl acetate extract (400mg/kg) | 118± 2.66 | 255 ± 3.31 | 329 ± 4.11 | 276 ± 3.97 | 241 ± 3.38 |
| 9 | *A. reticulata* ethanolic extract (200mg/kg) | 108 ± 2.11 | 237 ± 1.34 | 316 ± 2.94 | 263 ± 2.43 | 207 ± 3.26*** |
| 10 | *A. reticulata* ethanolic extract (400mg/kg) | 102 ± 3.07 | 196 ± 2.72 | 277 ± 4.42 | 207 ± 3.28** | 169 ± 1.98*** |

All the results are expressed in mean ± S.D (n=6). *** p<0.001 in comparison between extract treated group to control group.

**Table 4: Effect of ethanolic extract of *A. reticulata* on body weight changes in streptozotocin induced diabetic rats**

| Sl. No. | Group | 0 Day (gm) | 20 day (gm) |
|---|---|---|---|
| 1 | Control (D.W) | 175 ± 3.11 | 182 ± 2.67 |
| 2 | Diabetic (STZ in0.3 % CMC) | 177 ± 2.47 | 132 ± 2.68$$$ |
| 3 | Standard Drug treated (10 mg/kg) STZ (45 mg/kg body weight. (B.W) | 174 ± 1.74 | 166 ± 2.17*** |
| 4 | *A. reticulata* (200mg/kg) STZ (45 mg/kg body weight. (B.W) | 168 ± 3.11 | 147 ± 2.65 |
| 5 | *A. reticulata* (400mg/kg) STZ (45 mg/kg body weight. (B.W) | 175 ± 2.66 | 156 ± 3.15*** |

All the results are expressed in mean ± S.D (n=6). $$$ p<0.001 comparison between diabetic rats and control rats, * p< 0.05, *** p<0.001 in comparison between drug treated group to diabetic animals.

**Table 5: Water intake by the rat's administered with ethanolic extract of *A. reticulata* streptozotocin induced diabetic rats**

| S. No | Group | in ml. |
|---|---|---|
| 1 | Control (D.W) | 11 ± 2.7 |
| 2 | Diabetic (STZ in 0.3 % CMC) | 42 ± 2.3$$$ |
| 3 | Standard Drug treated (10 mg/kg) STZ (45 mg/kg body weight. (B.W) | 18 ± 2.4*** |
| 4 | *A. reticulata* (200mg/kg) STZ (45 mg/kg body weight. (B.W) | 36 ±2.26* |
| 5 | *A. reticulata* (400mg/kg) STZ (45 mg/kg body weight. (B.W) | 25 ± 3.12*** |

All the results are expressed in mean ± S.D (n=6). $$$ p<0.001 comparison between diabetic rats and control rats, * p< 0.05, **p<0.01, *** p<0.001 in comparison between drug treated group to diabetic animals.

**Table 6**: **Effect of ethanolic extract of *A. reticulata* at different doses on blood glucose levels of streptozotocin induced diabetic rats**

| Sl. No. | Group | 0 Day | 5 Day | 10 Day | 15 Day | 20 day |
|---|---|---|---|---|---|---|
| 1 | Control (D.W) | 102 ± 2.41 | 99 ±3.44 | 100 ± 2.78 | 103 ± 1.98 | 101 ± 2.22 |
| 2 | Diabetic (STZ (45 mg/kg in 0.3 % CMC) | 96 ± 3.18 | 321 ± 4.21$$$ | 349 ± 3.98$$$ | 372 ± 2.99$$$ | 396 ± 4.78$$$ |
| 3 | Standard Drug treated (10 mg/kg+ STZ (45 mg/kg bw)) | 92 ± 2.61 | 342 ± 3.76 | 211 ± 2.91*** | 178 ± 4.49*** | 137 ± 3.11*** |
| 4 | *A. reticulate* (200mg/kg+ STZ (45 mg/kg bw) | 104 ± 2.44 | 349 ± 5.28 | 292 ± 4.19* | 276 ± 3.87** | 259 ± 3.85*** |
| 5 | *A. reticulate* (400mg/kg+ STZ (45 mg/kg bw) | 98 ± 3.11 | 352 ± 4.68 | 248 ± 5.12*** | 215 ± 2.89*** | 171 ± 5.45*** |
| 6 | *A. reticulate* (600mg/kg+ STZ (45 mg/kg bw) | 107 ± 2.47 | 362 ± 5.28 | 238 ± 4.81*** | 202 ± 3.17*** | 166 ± 4.21*** |

All the results are expressed in mean ± S.D (n=6). $$$ p<0.001 comparison between diabetic rats and control rats,* p<0.05, **p<0.01, *** p<0.001 in comparison between drug treated group to diabetic animals.

## DISCUSSION

In this present study oral administration of *A. reticulata* in all forms petroleum ether, chloroform, ethyl acetate and ethanol did not show any toxicity sign in model mice ranging from 200-2000mg/kg body weight, which is observed up to the 14 days. It is suggested that the plant extract studied is safe even at higher doses. In Oral glucose tolerance test, the ethanolic extract of *A. reticulata* in both200mg/kg, body weight and 400mg/kg b.wt significantly reduced fasting blood glucose level. It is suggested that the extract have some inhibiting action over hepatic glycogenosis and glucogenosis.

Streptozotocin (STZ) is commonly used for experimental induction of type-I diabetes mellitus, which causes elective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide. This results in rapid reduction in pancreatic islet pyridinenucleotide concentration and subsequent β-cell necrosis. There by reduction in insulin secretion by pancreases and cause elevated levels of blood glucose[8]. In the present study assessment of the anti-diabetic activity of the ethanolic extracts

of *Annona reticulata* showed significant ($p<0.001$) fall in Fasting Blood Glucose level in streptozotocin induced diabetic rats compared to diabetic control. The anti-diabetic property of *Annona reticulata* may be due to decreasing the damage of pancreatic β-cell and improving the insulin production from the β-cell of pancreas.

Diabetic animals used to have symptoms like polyphagia, polydipsia and polyuria. After induction of the diabetes water intake of animals was significantly increased, this is the main symptom of diabetes. Treatment of diabetic animals with standard drug Glibenclamide and the plant extract showed significant reduction in intake of the water compared to diabetic animals was observed, which clearly showed that both the plant extracts at higher dose have the ability to reduce the symptoms of diabetic condition.

In diabetic condition muscle cells do not have the ability to uptake the glucose because of low levels of the insulin production. Due to this condition muscle wasting and drastic reduction in the body weight of the animals was observed. Streptozotocin induction leads to destruction of the pancreatic beta cells and low levels of insulin is main reason for this condition. Treatment with *A.reticulata* ethanolic extract showed siginifican gain in the body weight compare with diabetic animals with no drug treatment, this might be due to the ability of the plant extract to stimulate the pancreatic beta cells and stimulate the production of insulin.

After the complete evaluation of the plant extracts the ethanol extract of *A.reticulata* stem bark showed significant anti diabetic properties. Phytochemical analysis of the plant sample showed the presence of different chemical compounds. The anti-diabetic properties of the plant may be due to the presence of the polar compounds like alkaloids, flavanoids and phenolic glycosides in the extracts of the plant.

## CONCLUSION

Oral administration of ethanolic plant extract of *A. reticulata* stem bark at 400mg/kg/day and standard drug Glibencamide (10mg/kg body weight) for 15 consecutive days to streptozotocin induced diabetic rats significantly reversed decreased body weight, increased water consumption and increased fasting plasma glucose level in comparision to diabetic control animals. Phytochemical analysis of the plant sample showed the presence of different chemical compounds like alkaloids, flavanoides, Phenolic glycosides and lignans. Anti diabetic activity of the plant extracts may be due to the presence of these chemical molecules or synergestic activity of the all the chemical compounds present in the sample.

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## REFERENCES

1. Satyanaraya T, Gangarao, B, Surendra G, Rajesh K, 2013. Pharmacognostical and Phytochemical Studies of *Annona reticulata* Linn. International Journal of Research in Pharmacy and Chemistry 2013; 3(2): 477-482.
2. Ahuja MMS. Learning to live with DIABETES 2nd edition 1994 Published by National books trust India, New Delhi.
3. Ada P.Kahn-' Diabetes causes, prevention and treatment, 1994 published by Orient Papers backs, New Delhi.
4. Kanagavalli U, Bhuvaneshwari B, Sadiq, A M. Anti Diabetic Activity 0f Boerhaavia Diffusa Against Alloxan Induced Diabetic Rats. International Journal of Pharma and Bio Sciences 2015; 6(4): (B) 1215 – 1219.
5. Akhtar N, Haq IU, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian Journal of Chemistry, 2015; http://dx.doi.org/10.1016/j.arabjc.2015.01.013.
6. Badole SL, Bodhankar SL, Thakurdesai PA. Study of interaction of aqueous extract of Pleurotus pulmonarius (Fr.) Quel-Champ with rosiglitazone in alloxan induced diabetic mice. Pharmacologyonline 2006; 3: 64-72.
7. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D. Development of a New Model in Adult Rats Administered Streptozotocin and Nicotinamide. Diabetes 1998; 47: 224-229.
8. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Verdi AA, Mofidian SMA, Rad BL. Induction of Streptozotocin in rats. Indian Journal of Clinical Biochemistry 2007; 22(2): 60-64.

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