*Available online at www.JGTPS.com*

*Research Article*

*ISSN:2230-7346*
*Journal of Global Trends in Pharmaceutical Sciences*

*Vol.4, Issue 1, pp -973-982, January-March 2013*

# CLINICAL EVALUATION OF ANTI-HYPERGLYCEMIC ACTIVITY OF *BOERHAAVIA DIFFUSA* IN COMPARISON WITH GLIBENCLAMIDE IN THE RAT MODEL OF T2DM

**Dwividendra Kumar Nim[1], Pratap Shankar*[2], Rakesh Chaurasia[3], Bhaumic Goel1, Rakesh Kumar Dixit[4]**

1. Junior Resident, Pharmacology, Moti Lal Nehru Medical College Allahabad
2. Ph.D Scholar, Pharmacology, King George's Medical University Lucknow
3. Assistant Professor, Pharmacology, Moti Lal Nehru Medical College Allahabad, U.P, INDIA.
4. Professor, Pharmacology, King George's Medical University Lucknow

***Corresponding Author E-mail: pratap.mbi@gmail.com**

## ABSTRACT

Diabetes Mellitus is one of the most common metabolic disorders. It is one of the leading causes of morbidity worldwide. International Diabetic Federation (IDF) 2006 report stating that around 246 million people worldwide are suffering from the disease and the prevalence is expected to be 380 million by the year 2026. Diabetes Mellitus, along with its associated complications cause enormous medical and socioeconomic burden on the healthcare system. As most of the antidiabetic drugs possess some serious side effects, this group of drug is also not free of such side effects. On administration acute and chronic complication may develop if the therapy is not monitored regularly, this can lead to noncompliance and worsening of disease condition. Here we used *Boerhaavia diffusa* an herbaceous plant of the family *Nyctaginaceae as* anti-hyperglycemic and compare its effect with a standard drug Glibenclamide. The purpose of this study is to evaluate the anti-hyperglycemic activity of above plant in the rat model of Diabetes Mellitus Type-2.

**Keywords:** anti-hyperglycemic, *Boerhaavia diffusa,* Glibenclamide, T2DM

## INTRODUCTION

In last century, advancement of science successfully developed treatment for several diseases. But developments of human beings also led to adoption of a life style that flourished some uncommon diseases of the past to the most important causes of morbidity and mortality in present. Diabetes Mellitus is one such important disease to be cured. It is one of the most common metabolic disorders. It is one of the leading causes of morbidity worldwide. International Diabetic Federation (IDF) 2006 report stating that around 246 million people worldwide are suffering from the disease and the prevalence is expected to be 380 million by the year 2026[1].

Diabetes Mellitus, along with its associated complications cause enormous medical and socioeconomic burden on the healthcare system. Considering the enormous morbidity and mortality associated with diabetes, search for effective drugs for its treatment are undergoing in all parts of the world. In fact, in India also, the search for the treatment of this disease is undergoing since ancient times when this disease was referred to as "Madhumeha". Though pathophysiology of this disease has been studied extensively and many drugs are available which act at different levels, still the disease continues to progress imposing enormous strains on healthcare systems.

*Boerhaavia diffusa* is an herbaceous plant of the family *Nyctaginaceae*. The whole plant or its specific parts (leaves, stem, and roots) are known to have Medicinal properties and have a long history of use by indigenous and tribal people in India. The Medicinal value of this plant in the treatment of a large number of human ailments is mentioned in "The Ayurveda", "Charaka Samhita" and "Sushruta Samhita". It has many ethno botanical uses (the leaves are used as vegetable; the root juice is used to cure asthma, urinary disorders, leucorrhea, rheumatism and encephalitis) and is medicinally used in the traditional Ayurvedic system of medicine.

Previous studies suggest that *Boerhaavia diffusa* possess anti-hyperglycemic activity. But only a very few studies have been done on this plant to establish their anti-hyperglycemic property. The purpose of this study is to evaluate the anti-hyperglycemic activity of this plant (if they do have any) in the rat model of Diabetes Mellitus Type-2.

## MATERIALS AND METHODS

This study was conducted at Department of Pharmacology and the Department of Biochemistry, Moti Lal Nehru Medical College, Allahabad and albino rats of both sexes (male and female) weighing between 100 - 150 gm. were used and obtained from registered seller (Reg. No.- B-37/0605003769) and kept in animal house under the supervision of veterinary doctor. All rats were housed at an ambient temperature of 25$^{o}$C± 2°C with a 12 hour light/dark cycle, and provided with standard pellet diet/high fat diet and water *ad libitum*. The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of Institutional Animal Ethics committee and the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH

Publication. No. 85-23 revised 1996, Latest revision in 2011).

**Test Drugs and Chemicals:**

All the drugs were administered orally with the help of feeding cannula after preparing suspension in distilled water (vehicle).

***Boerhaavia diffusa* extract** –It was procured as commercially available crude extract in dry powder form, from The Himalaya Drug Co., Banglore, India. It was given in doses of 100 mg/kg and 200 mg/kg[2].

**Glibenclamide** – It was given in a dose of 0.6 mg/kg[3].It was procured from USV Pharma Ltd, India.

**Streptozocin** (minimum assay 97%) was procured from Spectrochem Pvt. Ltd., Mumbai. Glucose estimation kit used for estimation of plasma glucose was purchased from Span Diagnostic limited, Surat, India. All the chemicals and reagents used were of analytical grade.

**Study Design**

The study was started with 24 rats. Baseline fasting plasma glucose (FPG) levels of all the rats were determined. All the animals were fed on high fat diet (58% energy as fat) for two weeks. After two weeks fasting plasma glucose levels were taken and all the rats (24) were injected intraperitonially with 35 mg/kg of streptozocin in citrate buffer (single dose)[4]. The FPG levels were estimated in all the rats after 1 week. The rats with plasma glucose level > 200 mg/dl were considered to be diabetic[5] and were included in the study. They were randomly (by using random number table) divided into 4 groups of 6 rats each, so that a total of 4 groups were formed. The drugs were administered orally once daily after preparing suspension in distilled water for further 10 weeks. Fasting plasma glucose of all the rats was taken every two weeks. Blood samples were drawn from the tail vein and plasma glucose estimation was done by the Glucose-Oxidase method. The observations of the test groups (2 & 3) were compared with that of the standard (glibenclamide) and the diabetic control (vehicle) as shown in Table1

***Table 1-: Grouping of study animals***

| Group No. | Group name (n=6) | Drug administered | Dose |
|---|---|---|---|
| 1 | Diabetic Control (DC) | Vehicle only (distilled water) | 0.5 ml/day |
| 2 | Low dose *Boerhaavia diffusa* crude extract (BDL) | *Boerhaavia diffusa* crude extract | 100 mg/kg/day |
| 3 | High dose *Boerhaavia diffusa* crude extract (BDH) | *Boerhaavia diffusa* crude extract | 200 mg/kg/day |
| 4 | Standard drug (SD) | Glibenclamide | 0.6 mg/kg/day |

**Statistical Analysis:**

The observations were analyzed using one way "ANOVA" and "student t test" where ever needed.

## OBSERVATIONS AND RESULT

All the groups were observed and fasting plasma glucose (FPG) levels were measured at the start of study and same after 2 weeks they were injected with streptozocin in citrate buffer or plain citrate buffer depending upon the group. After this, rats were continued on their respective diet and drugs. FPG levels were determined every 2 weeks till the end of tenth week. The values of the test groups were compared with that of the control and standard groups. FPG levels were again measured before administration of streptozocin. At this time, FPG in Diabetic Control, group 2 & 3 and standard group (SD) ranged from 80-91, 83-88 and 80-88 respectively with mean±S.D. being 86.17±3.97, 85.5±1.87 and 84.5±3.45. On comparing the mean FPG together, ANOVA again revealed similar mean FPG among the groups ($F=0.378$, $p>0.05$) i.e. mean FPG did not differ significantly between the groups. Mean fasting plasma glucose levels of diabetic control group did not varied much over the period of 10 weeks. Mean values at any point of the time did not varied more than 11.33 mg/dl from mean baseline value of 360.17 mg/dl. Group given Glibenclamide (Standard Drug), showed consistent improvement in FPG level over 10 weeks with the maximum improvement of 57% from baseline values at the end of 10 weeks. Maximum reduction in mean FPG level (34.11%) was seen in first two weeks. Our test drug *Boerhaavia diffusa*, at low dose of 100 mg/kg was able to decrease the fasting plasma glucose levels consistently from 2 weeks (11.17%) to 10 weeks (27.83%). On comparing FPG level of low dose *Boerhaavia diffusa* (100 mg/kg) with diabetic control group, it was observed that from 4 week onwards *Boerhaavia diffusa* was able to decrease the FPG levels significantly ($p<0.001$ at 4, 6, 8 and 10 weeks). On comparing low dose *Boerhaavia diffusa* with Glibenclamide, we found that anti-hyperglycemic *Boerhaavia diffusa* is less than glibenclamide at all the time and the difference was significant ($p<0.001$) in whole study duration. At high dose (200 mg/kg) of *Boerhaavia diffusa* the fasting plasma glucose levels decreased consistently till the end of the study with a maximum reduction of 38.06% at the end of 10 weeks. The reduction in glucose level was significant from 4 weeks onwards ($p<0.001$ at weeks 4, 6, 8 and 10). On comparing effect of high dose *Boerhaavia diffusa* with glibenclamide it was found that *Boerhaavia diffusa* decreased plasma glucose level to a lesser extent and the difference was significant ($P<0.001$ at all points of obervation) in whole study duration Fig. 2.

*Fig-2: Bar diagram showing effect of low and high doses of Boerhaavia diffusa extract on Fasting Plasma Glucose with standard and diabetic control group*

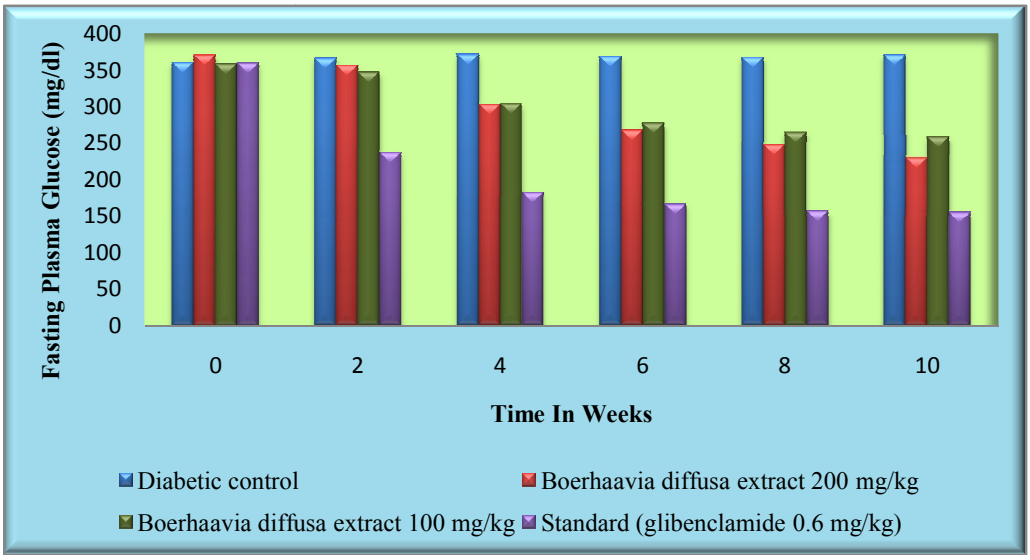
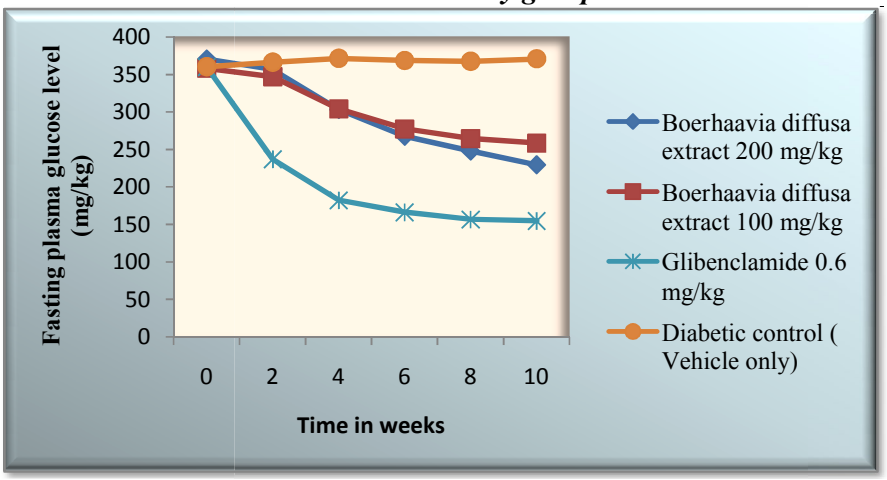



*Table-2: Comparison of effect of all the drugs in decreasing fasting plasma glucose levels*

| Groups | Time in Weeks | | | | |
|---|---|---|---|---|---|
| | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 |
| Diabetic control (vehicle only) | -1.67% | -3.15% | -2.31% | -2.04% | -2.96% |
| *Boerhaavia diffusa* extract 100 mg/kg | 3.12% | 15.12% | 22.57% | 26.15% | 27.83% |
| *Ocimum sanctum* extract 400 mg/kg | 4.08% | 15.55% | 23.06% | 27.47% | 30.53% |
| Standard (glibenclamide 0.6 mg/kg) | 34.11% | 49.31% | 53.75% | 56.44% | 57% |

*Fig-3: Line diagram showing fasting plasma glucose levels over the period of 10 weeks in all 6 study groups*



On simultaneous comparison of all the groups the maximum mean % reduction in fasting plasma glucose of all the groups, the order of their effects is-Glibenclamide >*Boerhaavia diffusa* (200 mg/kg) >*Boerhaavia diffusa* (100 mg/kg). This trend in reducing plasma glucose levels can be better visualized in the line diagram of fig. 3 and Table 2.

## DISCUSSION

The present study was conducted to evaluate the anti-hyperglycemic activity of extracts of *Boerhaavia diffusa* in rats as well as to provide an introductory approach for the evaluation of their traditional preparations in order to scientifically validate the therapeutic preparation of these plants in the control of hyperglycemia in Diabetes mellitus type-2.

Glucose is the primary insulin secretagogue, and insulin secretion is tightly coupled to the extracellular glucose concentration. Islets are richly innervated by both adrenergic and cholinergic nerves. Stimulation of $\beta_2$adrenergic receptor agonists and vagal nerve stimulation enhance insulin release.

The pancreatic cell is a highly specialized cell that has considerable structural and functional similarities to a sensory neuron: Both cell types quickly sense and respond to external stimuli. The molecular events controlling glucose-stimulated insulin secretion begin with the transport of glucose into the cell via a facilitative glucose transporter. In rodents, this is the GLUT2, which has a distinctive, low affinity for glucose and is also the primary glucose transporter in hepatocytes. Human cells express primarily GLUT1 but little GLUT2. Upon entry into the cell, glucose is quickly phosphorylated by glucokinase (GK; hexokinase IV). GK's distinctive affinity for glucose leads to a marked increase in glucose metabolism over the range of 5-10 mM glucose, where glucose-stimulated insulin secretion is most pronounced. The glucose-6-phosphate produced by GK activity enters the glycolytic pathway, producing changes in NADPH and the ratio of ADP/ATP. Elevated ATP inhibits an ATP-sensitive $K^+$ channel ($K_{ATP}$ channel), leading to cell membrane depolarization. This $K_{ATP}$ channel is a heteromeric protein that consists of an inward rectifying $K^+$ channel (Kir6.2) and a closely associated protein known as the sulfonylurea receptor (SUR). Membrane depolarization then leads to opening of a voltage-dependent $Ca^{2+}$channel and increased intracellular $Ca^{2+}$, resulting in exocytotic release of insulin from storage vesicles. These intracellular events are modulated by a number of processes, such as changes in cAMP production, amino acid metabolism, and the level of transcription factors. GIP and GLP-1 couple to $G_s$ to stimulate adenylyl cyclase and insulin secretion[6].

There are some drug groups that can cause increase in insulin secretion and thus called insulin secretogogues. These groups are – sulfonylureas, meglitinides, GLP-1 agonists and Dipeptidyl Peptidase-4 (DPP-4) inhibitors. Sulfonylureas are further divided in two groups according to generation of agents. The first generation sulfonylureas (Tolbutamide, Tolazamide and Chlorpropamide) are very rare now a days for treatment of Diabetes Mellitus type-2. The second generation sulfonylureas

(Glibenclamide, Glipizide and Glimepiride) are more potent anti-hyperglycemic and hypoglycemic agents than first generation sulfonylureas[6]. As most of the antidiabetic drugs possess some serious side effects, this group of drug is also not free of such side effects. On administration acute and chronic complication may develop if the therapy is not monitored regularly, this can lead to noncompliance and worsening of disease condition.

There are many known plants that possess anti-hyperglycemic activity. *Boerhaavia diffusa* is a plant which is shown to possess anti-hyperglycemic activities by few studies. The present study was conducted to evaluate their anti-hyperglycemic activity in high fat diet and streptozocin induced diabetic rats. This effect was compared with the anti-hyperglycemic activity of glibenclamide. *Boerhaavia diffusa* is considered to act mainly by increasing insulin secretion from pancreatic β-cells. Most of the studies conducted to evaluate anti-hyperglycemic activities of these plants were of relatively short duration. Moreover, to our knowledge none of the studies tried to took over the trend of plasma glucose reduction over time. In this study, we tried to address this issue by monitoring the plasma glucose level every two weeks for duration of 10 weeks so that the anti-hyperglycemic effect of these drugs could be evaluated for a longer duration of treatment.

Our study showed that the test drugs *Boerhaavia diffusa* extract is capable of decreasing plasma glucose level in type-2 diabetic rats. These reductions in plasma glucose levels were consistent throughout the 10 weeks duration of therapy. There was a significant reduction in plasma glucose level 4 weeks onwards with both low and high doses of *Boerhaavia diffusa*. This reduction in plasma glucose level was started after 4 weeks of therapy in both doses, reduction in next 2 weeks was greater than earlier but the decrease in plasma glucose levels remain consistent during the remaining duration of study in both low dose and high dose groups.

These findings were quite similar to those of standard drug group in which the significant reduction in plasma glucose level achieved in first 4 weeks of therapy and this reduction remain consistent throughout the study period. Similarity in the pattern of both test drugs and standard drug in reducing plasma glucose levels can lead to a clue regarding some similarity in the mechanism of action of both the drugs. These results were consistent with other studies which were done earlier[7,8,9,10]. Based on the previous studies[7,11,12] following mechanisms of action can be proposed to explain this anti-hyperglycemic activity of *Boerhaavia diffusa* extract-

1. **Alteration in activity of enzymes of glucose metabolism:**
   Enzymes of glucose metabolism are markedly altered in experimental Diabetes and persistent hyperglycemia is a major contributor to such metabolic alterations. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate[7,13]. The decrease in activity of hexokinase can cause decreased glycolysis and decreased utilization of glucose for energy production, resulting in hyperglycemia.

2. **Increased scavenging of reactive oxygen species (anti-oxidant effect):**

In pancreatic β-cells, oxidative glucose metabolism will always lead to production of reactive oxygen species, normally detoxified by catalase and superoxide dismutase. Hyperglycemia leads to a large amount of reactive oxygen species in β-cells, with subsequent damage to cellular components[14, 15, 16, 17].

3. **Immunomodulatory activity:** TNF-α enhances adipocyte lipolysis, which further increases free fatty acids and also exerts its own direct negative effect on insulin signalling pathway[18]. In a study methanolic extract of *Boerhaavia diffusa* significantly inhibited TNF-α in human peripheral blood mononuclear cells in a very low dose of 10 µl/ml[19]. This immunomodulatory activity of *Boerhaavia diffusa* may also account for alteration in insulin signalling pathway and resultant anti-hyperglycemic effect.

These effects of *Boerhaavia diffusa* justify future investigation into their role in Diabetes and its complications in spite of the less effect which they have shown as compared to Glibenclamide. There was one important limitation of our study that we did not measure the serum insulin level. Inclusion of serum insulin measurements can provide a better picture regarding their anti-diabetic activity and their mechanism of action behind the activity in any future study. In our study we have seen that *Boerhaavia diffusa* possess anti-hyperglycemic activity, but the mechanism of their anti-hyperglycemic activity is not yet well elucidated. As our study revealed that *Boerhaavia diffusa* produce dose and time dependent anti-hyperglycemic effect. Future studies can be done taking different doses and durations of study in account. In our study we used commercially available crude extracts of *Boerhaavia diffusa*. So the active ingredients of these plants which are responsible for their anti-hyperglycemic activity are not clear. Future studies can be carried out taking active principles of these plants to establish better picture of their anti-hyperglycemic activity producing agent.

## CONCLUSION

The present study was carried out in albino rats of either sex, weighing 100-150 g. The aim of the study was to evaluate the anti-hyperglycemic activity of herb namely *Boerhaavia diffusa* and to compare it in high fat diet and streptozocin induced diabetic rats. The following conclusions was drawn after the completion of study-

- *Boerhaavia diffusa* was found to possess anti-hyperglycemic activity. It produced lower plasma glucose values as compared to diabetic control group.
- Both low and high doses of *Boerhaavia diffusa* consistently improved plasma glucose level from fourth week to tenth week, suggesting a time dependent effect.
- Our standard drug glibenclamide showed better anti-hyperglycemic effect than *Boerhaavia diffusa* at all the doses which we used.

- On comparing the maximum mean % reduction in fasting plasma glucose levels of all the groups, the order of their anti-hyperglycemic effect is – Glibenclamide >*Boerhaavia diffusa* (200 mg/kg/day) > *Boerhaavia diffusa* (100 mg/kg/day)

Our study suggests that both *Boerhaavia diffusa* possess anti-hyperglycemic activity. More studies are needed to elucidate their optimum dose and exact mechanism of action.

**REFERENCES**


1. Jain S, Saraf S. Type 2 diabetes mellitus-its global prevalence and therapeutic strategies. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2010(4):48-56.
2. Report of a WHO/IDF consultation. Definition and diagnosis of Diabetes Mellitus and intermediate hyperglycemia. World health organization. Geneva, Switzerland: 2006; p.17-29
3. Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, et al. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010;87(3):293-301
4. Pouwer F, Geelhoed-Duijvestijn PH, Tack CJ, Bazelmans E, Beekman AJ, Heine RJ, et al. Prevalence of comorbid depression is high in out-patients with Type 1 or Type 2 diabetes mellitus. Results from three out-patient clinics in the Netherlands. Diabetic medicine: a journal of the British Diabetic Association. 2010;27(2):217-24.
5. Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. Diabetes research and clinical practice. 2010; 87(1):15-9.
6. Powers AC, D'Alessio DA. Endocrine Pancreas and Pharmacotherapy of Diabetes Mellitus and Hypoglycemia. In: Brunton L, Chabner B, Knollman B, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw Hill; 2011. p. 1237-73
7. Pari L, AmarnathSatheesh M. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. Journal of ethnopharmacology. 2004; 91(1):109-13.
8. Chude MA, Orisakwe OE, Afonne OJ, Gamaniel KS, Vongtau OH, Obi E. Hypoglycemic effect of the aqueous extract of *Boerhaavia diffusa* leaves. Indian Journal of Pharmacology. 2001; 33:215-6.

9. Murti K, Lambole V, Panchal M, Kumar U. Antidiabetic and antihyperlipidemic activity of roots of *Boerhaavia diffusa* on streptozocin induced diabetic rats. Pharmacologyonline. 2011;1(1):15-21
10. Nalamolu RK, Boini KM, Nammi S. Effect of chronic administration of *Boerhaavia diffusa* Linn. leaf extract on experimental diabetes in rats. Tropical Journal of Pharmaceutical Research. 2004;3(1):305-309
11. Pereira DM, Faria J, Gaspar L, Valentao P, de Pinho PG, Andrade PB. Boerhaavia diffusa: metabolite profiling of a medicinal plant from Nyctaginaceae. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association. 2009; 47(8):2142-9.
12. Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of Boerhaavia diffusa Linn against acetaminophen-induced liver damage in rats. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association. 2010;48(8-9):2200-5
13. Laakso M, Malkki M, Deeb SS. Amino acid substitutions in hexokinase II among patients with NIDDM. Diabetes. 1995; 44(3):330-4.
14. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone badly, and the glutathione connection. Diabetes. 2003; 52(3):581-7.
15. DeFronzo RA, Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. The Journal of clinical investigation. 1985; 76(1):149-55.
16. Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes. 1999; 48(5):1198-214.
17. Grill V. A comparison of brain glucose metabolism in diabetes as measured by positron emission tomography or by arteriovenous techniques. Annals of medicine. 1990; 22(3):171-6.
18. Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. Trends in endocrinology and metabolism: TEM. 2000;11(6):212-7
19. Mehrotra. Immunomodulation by ethanolic extract of *Boerhaavia diffusa* roots. International immunopharmacology. 2002; 2(7):987-96.