ANTIDIABETIC AND ANTI OXIDANT ACTIVITY OF METHANOL EXTRACT OF *CASERIA ELLIPTICA*

Sunanda Sabbithi[1], Sandeep kanna[2]

[1] Malla Reddy Institute of Pharmaceutical Sciences, Malla reddy viswa Vidyapeeth Suraram, Hyderabad- 500055, Research Scholar, Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur, Andhra Pradesh- 522510

[2] Chalapathi institute of Pharmaceutical Sciences, chalapathi Nagar, Lam, Guntur – 522034.Andhra Pradesh

Corresponding author
Mrs. Sunanda Sabbithi
Malla Reddy Institute of Pharmaceutical Sciences,
Malla reddy viswa vidyapeeth,Suraram
Hyderabad-500055

ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease that can cause significant complications and high mortality worldwide. Efforts are intensifying to find and develop new effective and potentially safe α-glucosidase inhibitors. Traditional methods are being replaced by newer techniques that are less complex and time-consuming. Nevertheless, both experimental and computational strategies in drug discovery and development are practical and complementary. Therefore, this study was conducted to investigate the anti oxidant and antidiabetic potential of methanol extract in vivo.. This study demonstrated the potential benefits of MECE plant extract and its phytochemicals, which could be studied to develop effective and safe anti diabetic drugs for the treatment of postprandial blood glucose levels in diabetic patients.

***Key words*:** Diabetes mellitus, *Casearia elliptica,* Anti oxidant, Postprandial blood glucose.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to insulin deficiency. The disease is characterized by a shift in fuel utilization from carbohydrates to lipids. Diabetes has been identified as a widespread disease that affects millions of people worldwide and causes chronic diseases in various body organs, including the cardiovascular, renal, and nervous systems, associated with diseases such as nerve damage, renal failure, and vascular diseases such as retinopathy [1]. The incidence of diabetes in all age groups was 2.8% in 2000 and is predicted to reach 4. 4% in 2030 [2]. Furthermore, prolonged hyperglycemia can generate more reactive oxygen species (ROS) in the body and increase their concentration in various body tissues, including soft tissues such as the liver, heart, and brain, which are particularly susceptible to ROS [3]. It has also been reported that overproduction of ROS significantly leads to diabetic complications, metabolic stress, and cell death [4,5]. In complementary and alternative medicine, medicinal plants have been and are currently used in the context of diabetes treatment [6,7,8]. In this context, several cross-sectional reports have demonstrated that the use of medicinal plants in diabetes is widespread, which may reflect patients' perceptions and the effectiveness of plants in disease treatment. For example, most people in Saudi Arabia use medicinal plants and also preferred to use medicinal plants for the treatment of diabetes and obesity [9]. Furthermore, reports have listed several medicinal plants used worldwide for the treatment of diabetes [10]. Several species of antidiabetic plants have been studied phytochemically, biologically, and clinically in many publications. [11] Pure natural substances obtained from these plants are used as promising candidates for the development of antidiabetic drugs.

Casearia elliptica is a genus of plants in the Salicaceae family, an annual deciduous tree. It is one of the most important medicinal plants. The leaves are elliptical, oblong or ovate, the flowers are greenish-yellow with dense crystals in the axils, and the fruits are elliptical capsules (3-4 mm) with scarlet pulp. Medicinal uses of Casearia elliptica: In folk medicine, the crushed bark is applied externally for dropsy and fever, the roots as a tonic for anemia, a decoction of the roots is used for diabetes, and the leaves are used for medicinal baths. A paste of the leaves is used as an anti-helminthic agent, the pulp has a diuretic effect, oil extracted from the seeds is applied to sprains, and leaves soaked in hot water applied to swellings promotes suppuration and expulsion of worms. It is also used to treat fever and malaria, having an antiprotozoal effect against

Plasmodium falciparum. [12, 13, 14] for potential drug discovery and development of safe and effective antidiabetic drugs from Casearia elliptica was studied. The aim of this study is to determine the potential use of Casearia elliptica extract and its phytochemicals as an alternative source of antidiabetic drugs.

MATERIALS AND METHODS

Plant material-collection and authentication:

The whole plant of *Casearia elliptica* was collected from native species growing in deciduous forests of Tirumala region, Andhra Pradesh, India. The whole plant material has been identified taxonomically and authenticated by Dr. S. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

Preparation of the extract:

The collected plant was washed thoroughly with water and dried in the shade. The dried leaves were ground well to coarse powder (500gms). Methanolic extract was obtained by extracting powder with methanol by Soxhlet extraction method for 72hr. After completion of the extraction the solvent was removed by rotary evaporator method. The methanolic extract was used for further study. The yield obtained from the above process was found to be 52.78% w/w. The extracts were preserved in a refrigerator. The Methanolic extract of *Casearia elliptica* was subjected to the following investigations [15].

4.2 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on *Casearia elliptica* extract for detection of phytoconstituents like carbohydrates, proteins, flavonoids, alkaloids, steroids and saponins [16, 17].

Alloxan induced model

Grouping of animals:

Male albino Wistar rats were divided into five groups each consisting of six animals as follows:

Group I- Administered vehicle serves as Normal control.

Group II- Administered Alloxan (120 mg/kg *s. c*) serves as diabetic control

Group III- Administered Reference Standard, (Glibenclamide 10 mg/kg, orally once daily)

Group IV- Diabetic rats treated with *Casearia elliptica* (250mg/kg *b. wt*), serves as treated group

Group V- Diabetic rats treated with *Casearia elliptica* (500mg/kg *b. wt*), serves as treated group

The male albino Wistar rats weighing (150-250gm) were fasted for overnight before challenging with single subcutaneous route (*s. c.)* of alloxan monohydrate, freshly prepared and injected within 5-min of preparation to prevent degradation at a dose of 120 mg/kg body weight after administration of alloxan monohydrate 5% glucose solution was given for 72 h to prevent hypoglycemic shock. Animals had access to feed and water. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation 72 h post alloxan monohydrate injection where in the animals were fasted again for 14h before blood collection from tail of animal. The rats with fasting serum glucose level of above 200mg/dl at 72h were considered as diabetic and are included in the study. Body weight and glucose levels were estimated on initial 0, 7^{th}, 14^{th} and 21^{st} day of treatment. On 21^{st} day, blood samples were collected from overnight fasted rats by retro orbital plexus under diethyl ether anesthesia for biochemical estimations and sacrificed for histopathological studies [18].

Parameters estimated:

Morphological parameter- Body weight, Biochemical parameters include Fasting blood glucose, Serum SGPT, Serum SGOT, Serum total cholesterol, Serum Triglycerides, Serum lipoproteins and in vivo antioxidant parameters like MDA by lipid peroxidation and catalase was performed

Statistical analysis

The data obtained from the present study were subjected to statistical analysis. All the results were expressed as Mean ± Standard Error (SEM). Data obtained from various groups was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Significant values were set accordingly.

RESULTS AND DISCUSSION

Preliminary phytoconstituents

The preliminary phytochemical analysis of methanolic extract of whole plant of *Casearia elliptica* willd revealed that the presence of carbohydrates, proteins, flavonoids, alkaloids, steroids and saponins which are listed in **Table 1**.

Table 1: Preliminary phytochemical screening

S. No.	TEST	Methanolic extract
1.	Carbohydrates	+++
2.	Proteins and Amino acids	+
3.	Tannins	–
4.	Flavonoids	++
5.	Alkaloids	++
6.	Steroids	+
7.	Glycosides	–
8.	Saponins	++
9.	Inulin	–

– indicates absent

\+ indicates Presence

++ indicates clarity

+++ indicates better response

Anti-diabetic activity:

Effect on Body weight in alloxan induced diabetic rats:

In groups treated with alloxan (G-II) (120 mg/kg, single dose) a significant decrease in the body weights of animals on the 3rd, 7th and 14th day (230.33±15.52, 221±4.09 and 204±9.57) was observed when compared to the 0th day body weight (247±11.8). This indicates that alloxan reduced the body weights persistently.

Methanolic extract (500 mg/kg) treated group showed significantly prevented reduction in body weights compared to Group-II and Group-IV. Although there was a marginal reduction in weight of animals on the 0, 7th, 14th and 21st day (231.33±7.06, 238.3±9.27 and 241.16±11.64) in these groups, compared to initial weight (242.33±13.92), the decreased reduction in body weight was significant when compared to diabetic control rats in alloxan induced model. The data is presented in **Figure 1.**

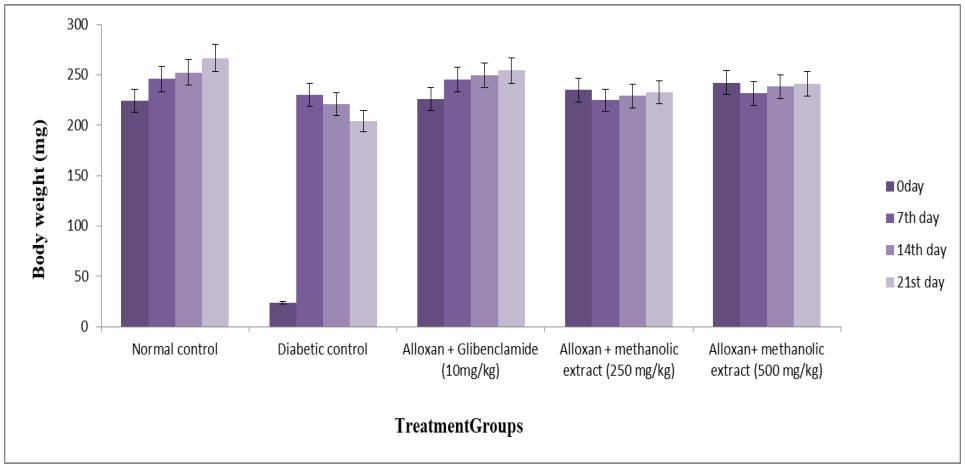

Figure 1: Effect of MECE on Body weights in Alloxan induced diabetic rats:

Effect on Serum Glucose levels in alloxan-induced diabetic rats:

In groups treated with alloxan (G-II) (120 mg/kg, single dose) a significant increase ($p<0.01$) in the serum glucose levels on the 0, 7^{th}, 14^{th} and 21^{st} day (454.83±6.49, 498.83±4.97 and 548.83±2.1) was observed when compared to the normal animals (G-I) respectively. This indicates that alloxan induces persistent diabetes mellitus.

In the group III, that received standard drug (glibenclamide, 10 mg/kg, p.o, once daily) there was significant decrease ($p<0.01$) in the serum glucose levels on the 0, 7^{th}, 14^{th} and 21^{st} (311±17.64, 255.5±5.88 and 120.66± 1.80) respectively when compared to the diabetic control group. In standard group, the serum glucose levels decreased significantly by day 21.

On administration of MECE (250mg/kg, p.o, once daily) there was a significant ($p<0.01$ & $p<0.005$) decrease in the serum glucose levels on 0, 7^{th}, 14^{th} and 21^{st} day (400±10.76, 374.16±7.42 and 190±1.75) when compared to diabetic control group (II) respectively. The other group receiving MECE (500mg/kg, p.o, once also showed a significant decrease ($p<0.01$) in serum glucose levels on 0, 7^{th}, 14^{th} and 21^{st} day (339.83±14.63, 330.5±5.92 and 166.16±3.06) when compared to diabetic control group. In both the groups (IV and V), thus the drug treatment restored the serum glucose levels almost nearer to standard drug values on day 21.

A dose related decrease in serum glucose levels was observed in the groups IV and V. This result suggests the anti-diabetic activity of *Casearia elliptica*. The effect of MECE on serum glucose levels is given in **Table 2**.

Table 2: Effect of MECE on Blood Glucose levels in Alloxan induced diabetic rats

S. No	Treatment	Blood Glucose levels (mg/dl)			
		0 day	7th day	14th day	21st day
1	Normal control	69.16 ±6.77	76.33 ±3.75**	83.83 ±1.79**	95.5 ±1.76**
2	Diabetic control	74.16 ±2.72	454.83 ±6.49	498.83 ±4.97	548.83 ±2.109
3	Alloxan + Glibenclamide (10mg/kg)	74.5 ±5.06	311 ±17.69**	255.5 ±5.88**	120.66 ±1.80**
4	Alloxan + methanolic extract (250 mg/kg)	66.33 ±2.57	400 ±10.76*	374.16 ±7.42**	190 ±1.75**
5	Alloxan+ methanolic extract (500 mg/kg)	74 ±3.46	339.83 ±14.63**	330.5 ±5.92**	166.16 ±3.06**

Values are expressed as Mean ± SEM; n=6

*P < 0.05 and ** P < 0.01 Vs Diabetic control

Statistical analysis is done by ANOVA followed by Dunnett's t-test

antioxidant parameters

In the present study, various antioxidant parameters were assessed in the pancreas of alloxan induced diabetic rats at the end of the study on 15th day.

Estimation of Malondialdehyde by Lipid peroxidation:

Rats treated with only alloxan (G-II) had MDA levels of (1.86±0.20 µmoles/gm tissue) when measured on day 21. This was significantly higher ($p<0.01$) when compared to MDA levels in normal group (I) (0.25±0.06µmoles/gm tissue).

Diabetic rats treated with standard drug (Glibenclamide, 10mg/kg, p.o, once daily) had MDA level of (0.28±0.06 µmoles/gm tissue) when measured on day 15. This was significantly lower ($p<0.01$) when compared to the diabetic control group (II).

The groups treated with different doses of MECE (250mg/kg & 500mg/kg p.o, once daily) also exhibited a significant decrease ($p<0.01$) in the MDA levels when compared to the diabetic control (G-II) respectively. The levels of group (V) were almost similar to those observed with the standard group (III). The results are given in **Table 5.**

Estimation of Catalase

A significant decrease in the levels of catalase was observed in the diabetic Control group (2.13±0.26 µmoles/gm tissue) when compared to the normal group (I) (3.27±0.27 µmoles/gm tissue). The group (III) receiving standard drug had significant ($p<0.01$) increase in the catalase levels (3.26±0.24 µmoles/gm tissue) when compared to the diabetic control group (II).

The groups treated with different doses (250mg/kg & 500mg/kg p.o, once daily) of MECE also exhibited a significant increase ($p<0.01$) in the catalase levels (3.10±0.24 and 3.20±0.29 µmoles/gm tissue) when compared to the diabetic control group (II) respectively. The results are given in **Table 5.**

Table 5: Effect of MECE on MDA levels in Alloxan induced diabetic rats on 21st day

Groups	Treatment	Lipid Peroxidase levels	Catalase levels
1	Normal control	0.25±0.06**	3.27±0.26*
2	Diabetic control	1.86±0.20	2.13±0.25

3	Alloxan + Glibenclamide (10mg/kg)	0.28±0.06**	3.26±0.23*
4	Alloxan + methanolic extract (250 mg/kg)	1.16±0.05**	3.10±0.24*
5	Alloxan+ methanolic extract (500 mg/kg)	0.89±0.13**	3.24±0.28**

Discussion

Diabetes mellitus is a group of metabolic disorders with the common manifestations, hyperglycemia. The prevalence of diabetes throughout the world has increased dramatically during the last few decades affecting nearly 10% of the world population[178]. Diabetes mellitus relates to the development of micro and macro vascular complications, which contribute greatly to the morbidity and mortality associated with the disease. There is a high level of treatment failures, unpleasant side effects and enormous cost associated with oral anti-diabetic drugs generating an urgent need and desire for alternate treatments [23].

Recent studies have clearly demonstrated the importance of medicinal plants in treatment of diabetes. Indian traditional medicine is one of the richest medicinal systems among those available in the world. Long before the use of insulin, since the time of Charka and Sushruta (400 BC), indigenous remedies have been used for the treatment of diabetes mellitus. Development of phytomedicine is relatively inexpensive and less time consuming. It is more suitable to our economic conditions compare to allopathic type of drug development [24].

In the present study, administration of alloxan has a destructive effect on the beta cells of the pancreas. Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of langerhans, there by inducing hyperglycaemia. Alloxan has been shown to induce free radical production and cause tissue injury [25].

In the present study, the plant drug used is *Casearia elliptica.* The phytochemical screening on MECE reveals that extract contained various pharmacologically active compounds such as carbohydrates, proteins, steroids, alkaloids, flavonoids and tannins.

In our study the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control groups as compared with normal animals at the end of the 21st day experimental period.

Our investigations indicate the efficiency methanol extract in maintenance of blood glucose level in both alloxan and alloxan induced diabetic rats.

Administration of methanolic extract of *Casearia elliptica* to diabetic rats showed a significant decrease in levels of blood glucose in both alloxan and alloxan induced diabetic rats. The methanolic extract of whole of *Casearia elliptica* exhibited dose dependent anti-diabetic property. The anti-diabetic effect of it at the dose of 500 mg/kg is equally effective with glibenclamide 10 mg/kg and alloxan 250mg/kg.

The possible mechanisms of anti-diabetic activity of the methanolic extract of whole plant *Casearia elliptica* is that it may contain some bio molecule(s) that may sensitize the insulin receptor to insulin or stimulate remnant the β-cell of Islets of Langerhans in pancreas in alloxan and these natural compounds could act separately or synergistically to cause the hypoglycemic effect. The Flavonoids and Saponins of the extract may be classified as direct hypoglycemic agents, by checking hyperglycemia due to alloxan and alloxan induced diabetes.

The present investigation reveals that methanolic extract of *Casearia elliptica* has shown significant hypoglycemic action in alloxan and alloxan induced diabetic rats and effect was found to be almost equally effective with glibenclamide and metformin.

Oxidative stress occurs at an early stage in diabetes, leading to the appearance of complications. Hyperglycemia aggravates endothelial ROS generation by a variety of mechanisms such as activation of protein kinase-C isoform, increased formation of advanced glycation end products and increased glucose flux through aldose reductase pathways. These are some of the known biochemical mechanisms of hyperglycemia-induced tissue/organ damage.

In present investigation, oxidative stress induced by alloxan has been shown to damage pancreatic β-cells and produce hyperglycemia in rats. Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidants and thus protecting tissue/organ damage from oxidative stress. In the present study, we estimated both enzymatic and non-enzymatic antioxidants in pancreas *in vivo*.

Catalase is an enzymatic antioxidant. Catalase is a haemprotein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protects the cell from oxidative damage produced by highly reactive hydroxyl radicals. In our study, the reduced activity of catalase in pancreas is observed during diabetes may result in deleterious effects due to accumulation of superoxide anion radicals and hydrogen

peroxide. The activity of catalase was increased in alloxan induced diabetic rats treated with metformin and MECE indicating that they both have antioxidant property.

Malondialdehyde (MDA) is an end product of lipid peroxidation, a non-enzymatic anti-oxidant, present in less concentration scavenges hydroxyl free radicals. In our study, increase in MDA is observed in diabetic rats than in group treated with metformin and MECE. A dose dependant response was observed. High dose of MECE possessed almost similar antioxidant property like standard drug.

From these results, it is proved that MECE has an anti-diabetic and anti-oxidant activity. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as therapeutic target in diabetes research.

Conclusion

further investigations of Methanolic extract showed a noticeable drop of blood glucose level related to respective doses of MECE. Therefore, based on the results obtained from the antihyperglycemic study it is possible to say that *MECE* can be used as a substitute supplement for the management of DM.

REFERENCES

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37(Supplement_1): S81–90.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047–53.
3. Matsumoto N, Omagari D, Ushikoshi-Nakayama R, Yamazaki T, Inoue H, Saito I. Hyperglycemia induces generation of reactive oxygen species and accelerates apoptotic cell death in salivary gland cells. Pathobiology. 2021;88(3):234–41.
4. Choudhury S, Ghosh S, Gupta P, Mukherjee S, Chattopadhyay S. Inflammation-induced ROS generation causes pancreatic cell death through modulation of Nrf2/NF-κB and SAPK/JNK pathway. Free Radic Res. 2015;49(11):1371–83.
5. Mohammed HA, Sulaiman GM, Anwar SS, Tawfeeq AT, Khan RA, Mohammed SAA, et al. Quercetin against MCF7 and CAL51 breast cancer cell lines: apoptosis, gene expression and cytotoxicity of nano-quercetin. Nanomedicine. 2021;16(22):1937–61.
6. Otoom SA, Al-Safi SA, Kerem ZK, Alkofahi A. The use of medicinal herbs by diabetic Jordanian patients. J Herb Pharmacother. 2006;6(2):31–41.

7. Jouad H, Haloui M, Rhiouani H, El Hilaly J, Eddouks M. Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). J Ethnopharmacol. 2001;77(2–3):175–82.
8. Al-Rowais NA. Herbal medicine in the treatment of diabetes mellitus. Saudi Med J. 2002;23(11):1327–31.
9. Balwan WK, Saba N, Zargar JI. Burden of diabetes and role of medicinal plants in its treatment. Saudi J Med Pharm Sci. 2022;8(7):355–61.
10. Dalar A. Plant taxa used in the treatment of diabetes in Van Province, Turkey. Int J Second Metab. 2018;5(3):171–85.
11. Bouyahya A, El Omari N, Elmenyiy N, Guaouguaou F-E, Balahbib A, Belmehdi O, et al. Moroccan antidiabetic medicinal plants: Ethnobotanical studies, phytochemical bioactive compounds, preclinical investigations, toxicological validations and clinical evidences; challenges, guidance and perspectives for future management of diabetes worldw. Trends Food Sci Technol. 2021; 115:147–254.
12. Rerup, CC. (1970): Drugs producing diabetes through damage of insulin secreting cells. Pharmacol Rev., 22: 485-520.
13. Szkudelski, T. (2001): The mechanism of Alloxan and Streptozotocin Action in beta cells of the rat pancreas. Physiol Res., 50: 536-546.
14. Jennifer J. Bedford, Susan Weggery, Lithium-induced Nephrogenic Diabetes Insipidus: Renal Effects of Amiloride. clin J Soc Nephrol, 2008 September, 3 (5): 1324-1331.
15. Saad SY, Najjar TA. Effects of STZ-induced diabetes and its treatment with vanadyl sulphate on cyclosporine A-induced nephrotoxicity in rats. Arch Toxicol. 2005 Sep; 79(9):493-9. Epub 2005 Jun 7.
16. Kokate CK. Practical Pharmacognosy. Delhi: Vallabh Prakashan. 1994; 4th ed.
17. Khandelwal KR. Practical Pharmacognosy, Pune: Nirali Prakashan. 2004; 11th ed: p 149-56.
18. S Badami, S Mahendran and V Maithili. Evaluation of antidiabetic effect of embelin from Embelia ribes in alloxan induced diabetes in rats. Biomedicine & Preventive Nutrition. 2011; 1: 25-31.
19. (Opo F, Rahman Mm, Ahammad F, Ahmed I, Bhuiyan Ma, Asiri Am. 2021. Structure

based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. Scientific Reports 11(1): 4049.).

20. (Pettersen Ef, Goddard Td, Huang Cc, Couch Gs, Greenblatt Dm, Meng Ec, Ferrin Te. 2004. Ucsf Chimera – a visualization system for exploratory research and analysis. J Comput Chem 25: 1605–1612.
21. (Shapovalov Mv, Dunbrack Rl. 2011. A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. Structure 19(6): 844–858.).
22. Vina [Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. **2009**, 31, 455–461]
23. "A closer look at ayurvedic medicine". Focus on Complementary and Alternative Medicine (Bethesda, Maryland: National Center for Complementary and Alternative Medicine (NCCAM), U.S. National Institutes of Health (NIH)) XII (4) Amr dr Pharmacy., 148: 46-52.
24. Raut NA, Gaikwad NA. Antidiabetic activity of hydro-ethanolic extract of Cyperus rotundusin alloxan induced diabetes in rats. Fitoterapia, 2006; (77): 585-88.
25. Szkudelski T. The Mechanism of alloxan and alloxan action in B cells of the rat pancreas. J. Ethnopharmacol 2005; 99: 199- 202.