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Antihyperglycemic Activity of Hydroalcoholic Extracts of Selective Medicinal Plants *Curcuma longa*, *Lavandula stoechas*, *Aegle marmelos,* and *Glycyrrhiza glabra* and Their Polyherbal Preparation in Alloxan-Induced Diabetic Mice

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Abstract

Background: Diabetes mellitus is a metabolic disorder associated with relative or absolute insulin deficiency or resistance, characterized by hyperglycemia. Modern prescriptions such as pioglitazone have better therapeutic potential, but its side effects and financial burden for developing countries have motivated the researchers to find alternative natural drugs to compete hyperglycemia in patients with diabetes. The present study was conducted to explore the therapeutic potential of selected medicinal plants for the treatment of diabetes as an alternative to allopathic medicines.

Method: In present study, hydroalcoholic extracts of *Curcuma longa*, *Lavandula stoechas*, *Aegle marmelos,* and *Glycyrrhiza glabra* and their polyherbal preparation (PHP) as compound drug were investigated for their antihyperglycemic potential in alloxan-induced diabetic mice. The study subjects (mice) were divided into different groups as normal control, diabetic control, pioglitazone treated (standard drug), test groups (plant extract treated 50, 100, and 150 mg/kg body weight), and PHP-treated group. Blood glucose concentration of all the study animals was determined by Glucose strip test. Qualitative phytochemical analysis of all the plant extracts was also performed following standard methods.

Result: It was investigated that treatment of alloxan-induced diabetic mice with hydroalcoholic extracts of studied medicinal plants showed significant ($P < .05$) effects on fasting blood glucose levels (from baseline to normal range) in a manner comparable to that of the reference drug, pioglitazone (1 mg/kg body weight intraperitoneal). The tested plant extracts significantly ($P < .05$) reduced the glucose concentration in blood of diabetes-induced mice in a dose-dependent manner.

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Conclusion: It could be concluded that studied medicinal plants have antihyperglycemic activity. The study findings favor the use of traditional herbal medicinal practices for the management of diabetes that might due to the presence of bioactive phytoconstituents in plants. However, larger studies are required to identify, isolate, and characterize the bioactive phytoconstituents responsible for antihyperglycemic activity of studied medicinal plants.

Keywords
metabolic disorder, antihyperglycemic activity, medicinal plants, polyherbal preparation, therapeutic use

Background

Diabetes mellitus (DM) is a rising epidemic metabolic disorder due to impaired discharge of insulin or insulin resistance at periphery results in chronic hyperglycemia. Diabetes mellitus leads to induction of micro- and macrovascular complications due to oxidative stress. Symptoms occur because of abnormality, especially in the metabolism of carbohydrates, lipids, and proteins. Numerous vital organs, including the eye, nervous systems, kidney, heart, blood, or vascular system, are adversely affected in result of long-term complications of diabetes. Diabetes mellitus may also lead to the development of dyslipidemia, obesity, hypertension, and the insulin resistance.[1,2] Therefore, diabetes is a major health problem that results to reduced life quality and increased morbidity.[2] Complications of diabetes and diabetic dyslipidemia are fundamentally connected and coexist in some patients.[3]

Approximately 10% cases of this metabolic disorder occur most often in the American population and 2 million people are affected by this disease in whole Europe and North America. Gestational DM (GDM) occurrence rate is 1% to 14% in all pregnancies in dissimilar populations. The prevalence rate of GDM is 2.1% to 21% of all pregnancies depending on the diagnostic test employed and different populations studied. Diabetic dyslipidemia accounts for 80% diabetic deaths only because of cardiovascular problems.[3]

In Baluchistan-Pakistan province, males (13.3%) and females (8.9%) both were affected by diabetes. In Khyber Pakhtunkhwa Pakistan, it is 9.2% in males and 11.60% in females, while in Sindh Pakistan province, DM is found as 16.2% and 11.77% in males and females, respectively; and 12.14% of male and 9.83% of female population in Punjab Pakistan suffer from DM. The prevalence of type 2 DM in urban areas is 14.81% and in rural areas is 10.34%. The overall prevalence of type 2 DM in Pakistan is 11.77%.[4]

It was reported that various factors including poor dietary habits and sedentary lifestyle led to deregulation of carbohydrates, lipids, and protein metabolism, resulting in DM. Conventionally, diabetes is controlled with 3 major strategies, including first, proper exercise in which the excessive glucose is utilized by the body tissues. Second, diabetes is controlled through diet by limiting the use of glucose-rich food. Third, oral hypoglycemic agents are used to maintain the glucose concentration at a certain level.[5] To minimize the complications, diabetes is also pharmacologically treated with oral hypoglycemic drugs along with insulin. Additionally, some drugs, such as lipid-lowering, antihypertensive, or antiplatelet drugs, are added with hypoglycemic agents. These drugs reduce cardiovascular complications and mortality.[2]

Numerous adverse events and even secondary malfunction rates are being faced due to the allopathic drugs. Countless patients are hospitalized due to drug-induced hypoglycemia. Therefore, World Health Organization recommends the medical scientists to focus their research consideration on safe and effective antidiabetic medicinal plants. Thus, current research project was designed to find out the plants as natural resources with potential therapeutic effect against hyperglycemia. Four medicinal plants, namely, *Curcuma longa*, *Lavandula stoechas*, *Aegle marmelos,* and *Glycyrrhiza glabra*, and their polyherbal preparation (PHP) were chosen to find out their antihyperglycemic action in experimental animals compared with standard hypoglycemic agent (pioglitazone).

Methods

Collection and Identification of Plant Materials

Roots of 4 selective medicinal plants, including *A marmelos*, *C longa*, *L stoechas*, and *G glabra*, were purchased from local market of Rawalpindi. The plant materials were identified and authenticated by the taxonomist at the Department of Botany, The University of Poonch, Rawalakot, Azad Jammu & Kashmir. Voucher specimens for each plant were deposited to the hebarium.

Extract Preparation

Collected plant materials, that is, roots, were washed thoroughly with distilled water, shade dried, and crushed into powdered form with the help of grinder. Powdered plant materials (100 g of each plant) were added to a stainless steel percolator containing extraction solution (ethanol:water, 70:30 [vol/vol]) at room temperature. The plant materials were dissolved in extraction solvent at a ratio of 1:10 (wt/vol) separately for each plant and placed for 72 hours at room temperature in a shaker. Then, the mixture was filtered using Whatman filter paper No. 1. The extraction was repeated 3 times and the filtrate was collected in a beaker. Then, the filtered extract was concentrated using rotary evaporator (Heidolph, model Laborata 4000, Schwabach, Germany) at 40^{o}C under vacuum.[6,7]

Table 1. Phytochemical Analysis of Studied Medicinal Plants.

S.N	Tests	Reagent	Color Appearance	Inference	References
1	Test for tannins	Extract + 2 mL $FeCl_3$	Blue and green color appear	Tannins present	8
2	Test for flavonoids	Extract + magnesium + HCl	Pink color appears	Flavonoids present	8
3	Test for saponins	Extract + 5 mL H_2O	Foams appear	Saponins present	8
4	Test for steroid	Extract + chloroform + H_2SO_4	Red color appears	Steroids present	9
5	Test for terpenoids (Salkowski test)	Extract + chloroform + H_2SO_4	Grayish color appears	Terpenoids present	8
6	Test for alkaloids	Extract +HCl + Wagner and Mayer reagent	Precipitate	Alkaloids present	10

Dose Selection and Preparation

For in vivo study on mice model, the extract solutions of each plant were prepared at concentrations of 50, 100, and 150mg/kg body weight of animals in 1 mL normal saline (pH 7.4). The prepared plant extract solutions at a dose of 0.1 mL/kg body weight were used for intraperitoneal (IP) injection into the experimental mice.

Animal Grouping and Experimental Design

The experimental animals used in this study were mice weighing 25 (2.5) g. They were given a standard diet and water ad libitum. The mice were acclimatized to handlers for 3 days before the start of the experiments. This study was approved from advance studies, research, and ethical committee for research, University of Poonch, Rawalakot, Azad Kashmir. Animals described as fasted were deprived of food for 16 hours before the start of bioassay. Animals had free access to water. The mice were divided into 5 groups: group I (n = 4; untreated mice) was given 0.1 mL/kg body weight normal saline; group II (n = 4; diabetic mice) was given 0.1 mL/kg body weight normal saline; group III (n = 4; diabetic mice) was given pioglitazone at a dose of 1 mg/kg body weight; and group IV (n = 48; diabetic mice) was given plant extracts at 3 dose levels (50, 100, and 150 mg/kg body weight), as 4 plant extracts were used so that group IV (n = 48) was further classified into 4 subgroups as group IV-A (n = 12), group IV-B (n = 12), group IV-C (n = 12), and group IV-D (n = 12) were given *A marmelos*, *C longa*, *L stoechas*, and *G glabra* extracts, respectively; and group V (n = 12; diabetic mice) was given PHP as compound drug, prepared by mixing equal (100 mg of each) amount of 4 plant extracts, at 3 dose levels (50, 100, and 150 mg/kg body weight).

Induction of Diabetes

Diabetes was induced by IP administration of alloxan monohydrate 150 mg/kg body weight after an overnight fasting for 16 hours (had access only to water), to make them more susceptible to develop diabetes. Alloxan monohydrate was dissolved in 0.9% NaCl solution and administrated to experimental mice. After 3 days of alloxan monohydrate induction, glucose level (GL) was monitored. Mice having blood GL of 200 mg/dL and above after 3 days were selected for the present study. The blood GL was first determined before treatment as 0 hour after induction of diabetes through alloxan administration. On the start of treatment according to experimental design, the blood was collected after 1, 2, 3, and 4 hours. The blood GL was determined after every blood collection. During the experiment, the animals continued to fast, but they were allowed free access to water. Blood GL was monitored using blood glucose test diagnostic strips (NIPRO Blood diagnostic strips). Blood glucose was determined by amputation of tail tip under mild anesthesia using NIPRO blood diagnostic strips. All experimental animals possessing GL less than 200 mg/dL were excluded from the current study (Sharma et al, 2003).

Phytochemical Screening

Phytochemical screening of the studied plant extracts was carried out using standard tests and procedures to know the presence of active principles. Standard screening tests were performed for different phytochemicals, including cardiac glycosides, reducing sugars, anthraquinones, terpenes, alkaloids, tannins, flavonoids, and saponins, as given in Table 1.

Statistical Analysis

The obtained data were subjected to statistical analysis for the determination of significance using analysis of variance. The analyzed data were presented as means (standard deviation [SD]). Statistical significance is expressed by *P* values less than .05.[6]

Results

Phytochemical screening of the hydroalcoholic extracts of *C longa*, *L stoechas*, *A marmelos*, and *G glabra* was performed. Phytochemical screening showed the presence of phytoconstituents such as carbohydrates, reducing sugar, tannins, phenols, flavonoids, saponins, glycosides, steroids, and alkaloids, as depicted in Table 2.

The animals were treated with the physiological saline, pioglitazone, and the plant extracts. The blood GL was first

Table 2. Phytochemistry of the Studied Medicinal Plant Extracts.

Class of Compounds	*Curcuma longa*	*Lavandula stoechas*	*Aegle marmelos*	*Glycyrrhiza glabra*
Alkaloids	+	+	+	−
Sterols	+	−	+	+
Terpenoids	+	+	−	+
Saponins	+	−	+	+
Flavonoids	+	+	+	−
Tannins	+	+	−	+

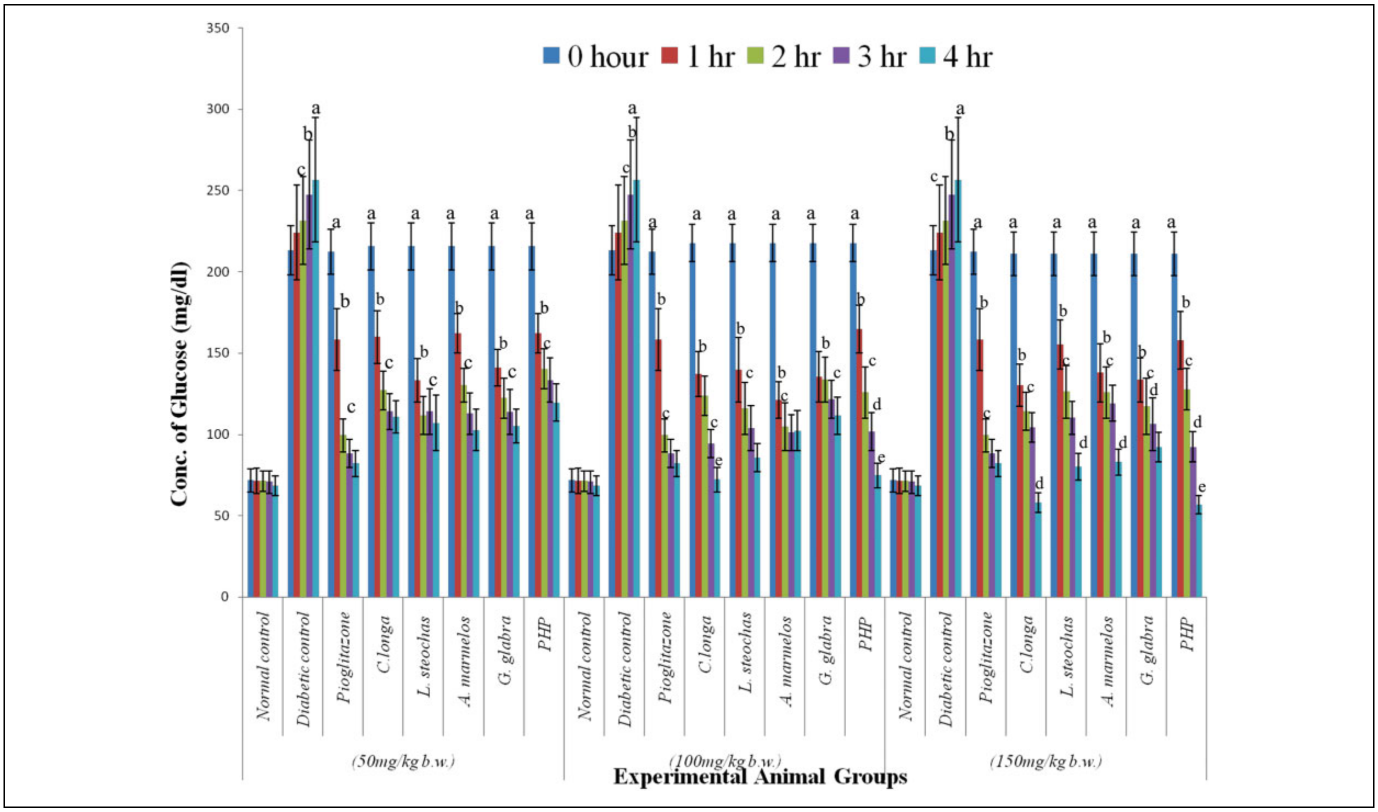

Figure 1. Multiple comparisons of mean (standard deviation) values of blood glucose concentrations at different time intervals before and after treatment in different study groups.

determined before treatment as 0 hour after induction of diabetes through alloxan administration. On the start of treatment according to experimental design, the blood was collected after 1, 2, 3, and 4 hours. The blood GL was determined after every blood collection. During the experiment, the animals continued to fast, but they were allowed free access to water. The results of all groups revealed that there was significant ($P < .05$) decrease in blood GLs of all treated groups in dose-dependent manner and that decrease in blood GLs comparable to standard antidiabetic drug is shown in Figure 1. Furthermore, it was found that PHP as compound drug is more antihyperglycemic than individual extracts at dose concentration of 150 mg/kg body weight as significant ($P < .05$) reduction in blood glucose concentration was observed as shown in Figure 1. Statistical analysis revealed that all medicinal plant extracts and their PHP have significant ($P < .05$) antihyperglycemic effect. The results showed no significant ($P > .05$) difference in blood glucose concentrations determined after particular time intervals with respect to same dose concentrations of each plant extract, PHP, and standard antidiabetic drug pioglitazone. But similar pattern of reduction in blood GL was observed in all treatment cases when determined at particular time intervals like from 0 to 4-hour collection (Figure 1).

Different alphabets on each bar within each group under study represent significant differences in blood GLs at different time intervals like 0 to 4 hours of sample collection for blood glucose measurement.

Discussion

Diabetes mellitus is the commonest disorder of pancreatic gland impairment that disturbs GL in the blood, leading to the complications such as kidney failure, diabetic neuropathy, eye diseases, and delayed wound healing.[11] It is estimated that DM causes complications that lead to death and placed on the level seventh for death-causing diseases (Trividi, 2004).[12] Nowadays, DM is considered as a serious worldwide health problem.[13] The occurrence of type 2 diabetes mellitus is growing more rapidly. The prevalence of non–insulin-dependent DM is increasing exponentially in Western countries.[14] Sulphonylurea, biguanides, thiazolidinediones, α-glucosidase inhibitors, and meglitanides are used in type 2 DM.[15]

Regardless of that, there are many antihyperglycemic drugs available in the market for the treatment of DM, but this metabolic disorder with its complications is serious socioeconomic burden both for developed and for developing countries. There are many plants that possess antihyperglycemic properties and these are used in the treatment of DM globally. More than 400 plant species having antihyperglycemic activity are reported, but research for new active antidiabetic plants is necessary because natural compounds have not well-reported side effects on the body. The majority of secondary metabolites used for the treatment of diabetes include carotenoids, flavonoids, terpenes, and glycosides. These secondary metabolites are commonly concerned for having antidiabetic activity.[16] Frequently, the plant's medicines are given for therapeutic purposes.[17,18] Alloxan is a chemical that is harmful to living body because it damages the pancreatic β cells. Alloxan decreases the secretion of insulin from pancreatic β cells, leading to extracellular hyperglycemia (Lachin et al., 2012). Numerous studies confirmed that many of the plant drugs effectively decrease the GL in alloxan-induced diabetic rats (Nammi et al., 2003).

The mean (SD) of blood GLs of mice after IP administration of various doses of studied plants extract at different time period are shown in Figure 1. Results revealed that administration of *C longa* at dose concentrations 50, 100, and 150 mg/kg body weight decreased the blood GL from above 200 mg/dL (baseline) to 110.75, 72.12, and 58.05 mg/dL after 4 hours of treatment, respectively (Figure 1). The given dose of *C longa* 150 mg/kg body weight to the normal mice created major ($P < .05$) decline at 4 hours as observed by Rai et alin their study on rat model.[19] They proposed that the *C longa at* dose of 200 mg/kg body weight declines the GL in blood of studied animals that become diabetic by the induction of streptozocin. Our outcome also demonstrates harmony with study findings of Mutalik et al who reported Dianex, the herbal formulation consisting of *C longa* and other plants when given at different doses (100-500 mg/kg body weight). Diane exhibited significant antihyperglycemic action in normal and diabetic rats that become diabetic by the induction of streptozocin. Our study findings were in agreement with previous study[20]; the authors proposed antihyperglycemic action of synthetic medicine "Rajanyamalakadi" exhibiting *C longa* in patients with diabetes for the duration of 3 months. Similarly *L stoechas* decreased the blood glucose to 107.05, 105.65, and 80.25 mg/dL after 4 hours of treatment, respectively. *Lavandula stoechas* exhibited hypoglycemic activity.[21] They reported that IP injection of *L stoechas* essential oils at concentration 50 mg/kg body weight protects the rat against alloxan-induced diabetes. Further, it was reported that *A marmelos* decreased the blood glucose to 102.75, 122.25, and 83.05 mg/dL after 4 hours of treatment, respectively. Sabu et al have explored that methanolic extract of *A marmelos* decreases blood GL in alloxan-induced diabetic mice.[22] It was also reported that *A marmelos* has capability to decrease blood GL constantly for the long time period.[23] The results are also coexist with the earlier study reported by Sharmila et al, in which they described the antihyperglycemic action of *A marmelos* in alloxan-induced diabetic mice.[24] Bhavani et al also reported analogous results of the antihyperglycemic action of ethanolic extract of *A marmelos* (Linn) in alloxan-induced diabetic mice.[25]

Glycyrrhiza glabra decreased the blood glucose from 214.8 (baseline) to 136.7, 124.5, 113.9, and 102.9 mg/dL after 4 hours of treatment, respectively. In the present study, *G glabra* exhibited hypoglycemic activity. Takii et al[26] have reported similar effects of *G glabra* on blood GL in mice after sucrose tolerance test. The most beneficial results were reported when PHP was given at 3 doses (50, 100, and 150 mg/kg body weight) to animals and it decreased the average blood GL of mice to 119.56, 74.67, and 56.63 mg/dL after 4 hours of treatment, respectively. At the dose of 150 mg/kg body weight, PHP exhibited significant effects ($P < .001$) at 4 hours. So it could be believed that there are definite chemical constituents that have synergistic effect in the form of compound drug that are responsible for decreasing blood GL. These chemical constituents might gradually start the synthesis of insulin and its release from the pancreatic β cells to target cells in alloxan-induced diabetic mice and act like insulin. These results are in accordance with the previously reported data that showed different medicinal plants and herbs decreased the blood GLs.

Conclusion

The current study concluded that hydroalcoholic extracts of *C longa*, *L stoechas*, *A marmelos*, *G glabra* and their PHP as compound drug explored the antihyperglycemic effect of medicinal plants. Further, it was also reported that PHP have more antidiabetic potential as compared to indiviual plant extract and this might be due to synergistic effect of all plant extracts when mixed to make compound drug. Basic screening of selected plants revealed the presence of different phytochemicals such as flavonoids, steroids, saponins, alkaloids, and terpenoids that might be responsible for hypoglycemic effects. These results have also recommended that the plants contain the active antihyperglycemic constituent. These active constituents might exert their effects on pancreatic β cell in normal healthy and diseased animals. However, more research is required to identify and isolate the phytochemical constituents responsible for antihyperglycemic effects of studied medicinal plants. So current investigations have affirmed and authenticated the therapeutic use of the studied plants (*C longa*, *L stoechas*, *A marmelos*, and *G glabra*) and PHP for the treatment of DM.

But further, exploration of the toxicity of long-term usage of these medicinal plants to handle diabetes is under consideration with different dosage combinations.

Authors' Note

This study was approved from advance studies, research, and ethical committee for research, University of Poonch, Rawalakot, Azad Kashmir. Hafiz Muhammad Asif is now affiliated with The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

Declaration of Conflicting Interests

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References

1. Kumar P, Clark M. Diabetes mellitus and other disorders of metabolism. *Clin Med*. 2002;2:1069-1071.
2. Sana T, Aslam B, Aslam N, et al. Therapeutic effect of atorvastatin on kidney functions and urinary excretion of glimepiride in healthy adult human male subjects. *Pak J Pharm Sci*. 2016;29(6 suppl):2321-2326.
3. Aziz A, Aslam B, Naz U, et al. Pharmacokinetic study of glimepiride alone and in combination with atorvastatin in healthy male volunteers. *Lat Am J Pharm*. 2017;36:151-156.
4. Meo SA, Zia I, Bukhari IA, Arain SA. Type 2 diabetes mellitus in Pakistan: current prevalence and future forecast. *J Pak Med Assoc*. 2016;66(12):1637-1642.
5. Mallick C, Chatterjee K, GuhaBiswas M, Ghosh D. Antihyperglycemic effects of separate and composite extract of root of *Musa paradisiacal* and leaf of *Coccinia indica* in streptozotocin-induced diabetic male albino rat. *Afr J Tradit Complement Altern Med*. 2007;4(3):362-371.
6. Quintão FJ, Tavares RS, Vieira-Filho SA, et al. Hydroalcoholic extracts of *Vellozia squamata*: study of its nanoemulsions for pharmaceutical or cosmetic applications. *Rev Bras Farmacogn*. 2013;23(1):101-107.
7. Riaz M, Shahid M, Jamil A, Saqib M. In vitro antioxidant potential of selected aphrodisiac medicinal plants. *J Biol Regul Homeost Agents*. 2017;31(2):419-424.
8. Khan FA, Hussain I, Farooq S, et al. Phytochemical screening of some Pakistanian medicinal plants. *Middle-East J Sci Res*. 2011; 8(3):575-578.
9. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *J Pharmacog Phytochem*. 2013;2(4):22-29.
10. Wadood A, Ghufran M, Jamal SB, et al. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem Anal Biochem* 2013;2:1-4.
11. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137-188.
12. Huang ES, Laiteerapong N, Liu JY, John PM, Moffet HH, Karter AJ. Rates of complications and mortality in older patients with diabetes mellitus: the diabetes and aging study. *JAMA Intern Med*. 2014;174(2):251-258.
13. Rato Q. Diabetes mellitus: a global health problem. *Rev Port Cardiol*. 2010;29(4):539-543.
14. Monesi L, Baviera M, Marzona I, et al. Prevalence, incidence and mortality of diagnosed diabetes: evidence from an Italian population-based study. *Diabet Med*. 2012;29(3):385-392.
15. Akram M.Diabetes mellitus type II: treatment strategies and options: a review. *J Diabetes Metab*. 2013;4:304.
16. Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012;2(4):320-330.
17. Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. *Evid Based Complement Alternat Med*. 2007;4(suppl 1): 37-40.
18. Khan V, Najmi AK, Akhtar M, Aqil M, Mujeeb M, Pillai KK. A pharmacological appraisal of medicinal plants with antidiabetic potential. *J Pharm Bioallied Sci*. 2012;4(1):27-42.
19. Rai P, Jaiswal D, Mehta S, Ra KD, Sharm B, Wata G. Effect of *Curcuma longa* freeze dried rhizome powder with milk in STZ induced diabetic rats. *Indian J Clin Biochem*. 2010;25(2): 175-181.
20. Faizal P, Suresh S, Kumar RS, Augusti KT. A study on the hypoglycemic and hypolipidemic effects of an ayurvedic drug Rajanyamalakadi in diabetic patients. *Indian J Clin Biochem*. 2009; 24(1):82-87.
21. Sebai H, Selmi S, Rtibi K, Souli A, Gharbi N, Sakly M. Lavender (*Lavandula stoechas* L.) essential oils attenuate hyperglycemia and protect against oxidative stress in alloxan-induced diabetic rats. *Lipids Health Dis*. 2013;12:189.
22. Sabu M, Kuttan R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. *Indian J Physiol Pharmacol*. 2004;48(1):81-88.
23. Arumugam S, Kavimani S, Kadalmani B, et al. Antidiabetic activity of leaf and callus extracts of *Aegle marmelos* in rabbit. *Science Asia*. 2008;34:317-321.
24. Upadhya S, Shanbhag KK, Suneetha G, Balachandra Naidu M, Upadhya S. A study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. *Indian J Physiol Pharmacol*. 2004;48(4):476-480.
25. Bhavani R, Rajeshkumar S. Anti-hyperglycemic activity of alcoholic leaf extract of *Aegle marmelos* (linn.) on alloxan induced diabetic rats. *Int J Pharma Sci Res*. 2014;5:56-62.
26. Takii H, Kometani T, Nishimura T, Nakae T, Okada S, Fushiki T. Antidiabetic effect of glycyrrhizin in genetically diabetic KK-Ay mice. *Biol Pharm Bull*. 2001;24(5):484-487.