STUDY OF INTERACTION OF AQUEOUS EXTRACT OF *PLEUROTUS PULMONARIUS* (FR.) QUEL-CHAMP WITH ROSIGLITAZONE IN ALLOXAN INDUCED DIABETIC MICE

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Summary

Mushrooms are low calorie food with very little fat, no starch and very low sugars, and so are the "delight of the diabetics". Recently, we have reported significant hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* (Family: Lentinaceae) (PP-aqu) on serum glucose levels and oral glucose tolerance test (OGTT) in alloxan induced diabetic mice at and more than 500 mg/kg, p.o. Herbal agents when given in combination with prescription medication may favorably alter pharmacodynamic activity of prescription medications. Hence, we have evaluated the antihyperglycemic effect of combination of PP-aqu (500 mg/kg, p.o.) with rosiglitazone (6 mg/kg, p.o.) on serum glucose levels and oral glucose tolerance test (OGTT) in alloxan induced diabetic mice. Our results from acute and sub-acute studies suggest that combination treatment of PP-aqu with rosiglitazone has synergistic antihyperglycemic effect than either drug alone. Concurrent drug regimen of PP-aqu and rosiglitazone may be considered for effective diabetes control.

Keywords: *Pleurotus pulmonarius* (Fr.) Quel-Champ, Rosiglitazone; Alloxan induced diabetic mice, Oral glucose tolerance test (OGTT)

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Introduction

Mushrooms are a group of fleshy macroscopic fungi, and have been valued throughout the world as both food and medicine for thousands of years (1, 2). Mushrooms are highly nutritive as they contain good quality proteins, vitamins and minerals (3, 4). Mushrooms are low calorie food with very little fat, no starch and very low sugars, and so are the "delight of the diabetics" (5). In adequate quantities they can serve as medicinal foods for diabetes. Earlier studies have reported insulin release and insulin like activity of some mushroom species like *Agaricus campestris* (6-8).

Among many varieties of mushrooms species, *Pleurotus pulmonarius* (Family: Lentinaceae) are characterized by a white spore print and commonly known as "oyster mushroom" (9). Recently, we have reported significant hypoglycemic activity of *Pleurotus pulmonarius* on serum glucose levels and oral glucose tolerance test (OGTT) in alloxan induced diabetic mice at dose levels of 500 mg/kg, p.o. and higher (10).

When used as directed and under the supervision of knowledgeable individuals most herbal remedies are safe. Herbal agents when given in combination with prescription medication may favorably alter pharmacokinetic (11) as well as pharmacodynamics (12-14) of prescription medications. However, the potential for adverse effects or intoxications certainly exists (15). To date, evidence relating to the herb-drug interaction in case of antidiabetic medicines is lacking and the understanding of the involved mechanisms is far from complete. Hence, we have evaluated the antihyperglycemic effect of aqueous extract of *Pleurotus pulmonarius* (PP-aqu) and its interaction with rosiglitazone on serum glucose levels and oral glucose tolerance test (OGTT) in alloxan induced diabetic mice.

Material and Methods

Drugs and chemicals

Pleurotus pulmonarius (Fr.) Quel.-Champ (Lentinaceae) was provided to us as a gift sample from Bajaj Orchard, Pvt. Ltd., Mumbai, India. It was authenticated by Dr. A. M. Mujumdar, Department of Botany, at Agharkar Research Institute, Pune and voucher specimen was deposited at that Institute. Rosiglitazone (Rosi) (USV. Pharma. Ltd., Mumbai, India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and d-glucose (S.D. Fine-Chem. Ltd, India) were purchased from respective companies.

Experimental animals

Swiss albino mice (25-30 g) were purchased from National Toxicology Centre, Pune, India. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55 % under 12-h light:12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) and water *ad libitum*. The experimental protocol was

approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India.

Preparation of aqueous extract of Pleurotus pulmonarius (PP-aqu)

Weighed quantity powder of air-dried *Pleurotus pulmonarius* was added to distilled water (1:15), boiled for 20 min on water bath, cooled to room temperature and filtered. The filtrate was dried on tray dryer at 70 ºC. (Yield - 24% w/w). The dry extract powder was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Induction of experimental diabetes

Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg/kg, i.v.) by the method described by Rao et al (16). After 48 hours, the animals showing serum glucose level above 200 mg/dl (diabetic) were selected for the study. All the animals were allowed free access to tap water and pellet diet.

Collection of blood and determination of serum glucose

Blood samples from the control and experimental mice were collected by orbital sinus puncture using heparinised capillary glass tubes. The blood samples so collected were analyzed for glucose levels by glucose oxidase peroxidase (GOD/POD) method as described earlier (17) and serum glucose levels were expressed as mg/dl.

Alloxan induced diabetic mice model (10)

The diabetic mice were divided into six groups of six animals each. The mice were administered orally with either vehicle (distilled water, 10 ml/kg), PP-aqu (500 mg/kg), Rosi (6 mg/kg), and the combination of PP-aqu (500 mg/kg) with Rosi (6 mg/kg). Acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after drug administration. Sub acute study involved repeated administration of drug for 14 days at prefixed time and serum glucose level was estimated on 7th and 14th day. Mean change in serum glucose level and standard error of Mean (SEM) were calculated. The mice were weighed daily during the study period of 14 days and their body weights were noted. From this data, mean change in body weight and SD were calculated. The mortality of mice was also noted during the study period and percentage mortality was calculated.

Oral glucose tolerance test (OGTT) in alloxan treated diabetic mice

Diabetic and non-fasted mice were divided into five groups of six animals each. They were administered orally with either vehicle (distilled water, 10 ml/kg), PP-aqu (500 mg/kg), Rosi (6 mg/kg), and the combination of PP-aqu (500 mg/kg) with Rosi (6 mg/kg). The mice of all the groups were loaded with d- glucose (2.5 gm/kg) solution after half an hour of drug administration. Serum glucose was estimated prior to drug

administration and at 30, 60 and 120 minutes after glucose loading and mean serum glucose levels were calculated.

Statistical analysis

The results are expressed as mean ± SEM. Comparison between the groups was made by Two-way analysis of variance (ANOVA) followed by *post hoc* Dunnett's test.

Results

PP-aqu (500 mg/kg, p.o.) and Rosi (6 mg/kg, p.o.) after acute administration in alloxan induced diabetic mice showed significant ($p < 0.01$) decrease in fasting serum glucose level as compared with their corresponding pre-treatment readings. However, as revealed by Figure 1, the effect of PP-aqu and combination treatment was strong ($P < 0.001$) even at 24 hrs. where anti-hyperglycemic effect of rosiglitazone was started diminishing ($P < 0.05$). After sub-acute administration, PP-aqu, rosi and combination treatment caused strong anti-hyperglycemic effect ($P < 0.001$) as compared with their pre-treatment glucose levels (Figure 2).

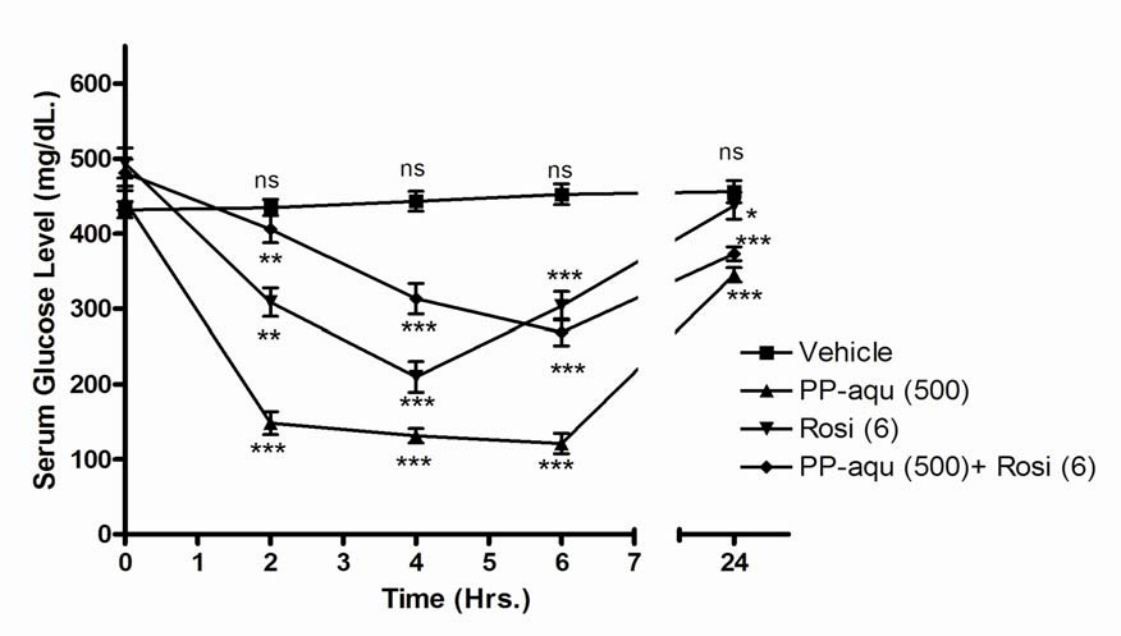

Figure 1: Effect of acute pretreatment of aqueous extract of *P. pulmonarius* (PP-aqu) and rosiglitazone on serum glucose level in alloxan induced diabetes in mice. n = 6, Data was analyzed by Two-way ANOVA followed by post hoc Dunnett's test. * $P < 0.05$), ** $P < 0.01$ and ** $P < 0.001$ as compared to pre-treatment readings of respective treatment group. All other values are non significant as compared with pre-treatment readings of respective treatment group.

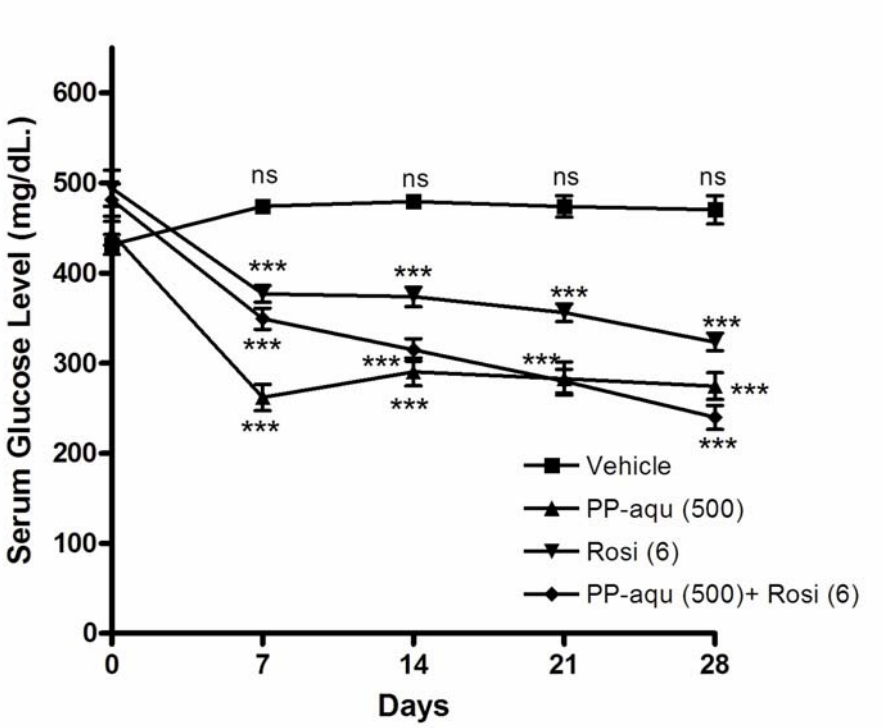

Figure 2: Effect of sub-acute pretreatment of aqueous extract of *P. pulmonarius* (PP-aqu) and rosiglitazone on serum glucose level in alloxan induced diabetes in mice. Data was analyzed by Two-way ANOVA followed by post hoc Dunnett's test. ns – non significant as compared to pre-treatment readings of respective treatment group. All other values are significant at $P < 0.001$ as compared with pre-treatment reading.

After alloxan treatment, mice lost their body weight significantly as reveled by vehicle control group (Figure 3). This loss of body weight was shown to be prevented by PP-aqu, or rosi treatment till day-14 but not on day-21 and day-28 (Figure 3). On the other hand, combination (PP-aqu + rosi) treatment prevented body weight loss of mice on day-21. On day-28, all treatment showed loss of body weight but combination group showed mild loss ($P < 0.05$) than *per se* treatment of either PP-aqu or rosi ($P < 0.001$).

Oral glucose load (2.5 g /kg) significantly increased serum glucose level ($P < 0.001$) as shown in Figure 4. After treatment with PP-aqu, rosi or their combination, serum glucose level was reduced significantly ($P < 0.001$). This decrease in serum glucose levels at 60 and 120 min was more after combination treatment (PP-aqu + rosiglitazone) than any of treatment alone (Figure 4).

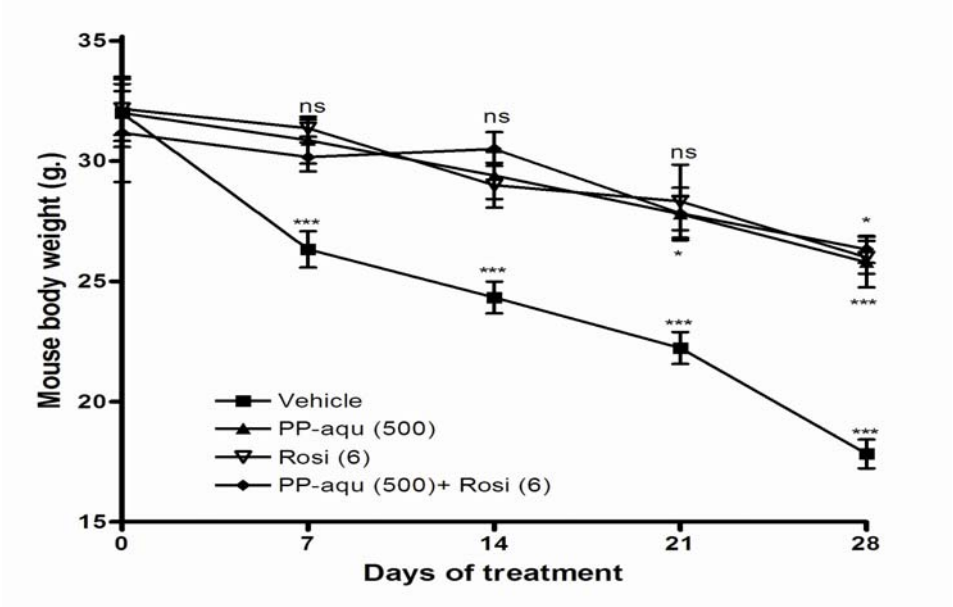

Figure 3: Effect of sub-acute pretreatment of aqueous extract of *P. pulmonarius* (PP-aqu) and rosiglitazone on body weights of alloxan induced diabetes in mice. Data was analyzed by Two-way ANOVA followed by post hoc Dunnett's test. ns – non significant * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared to pre-treatment readings of respective treatment group.

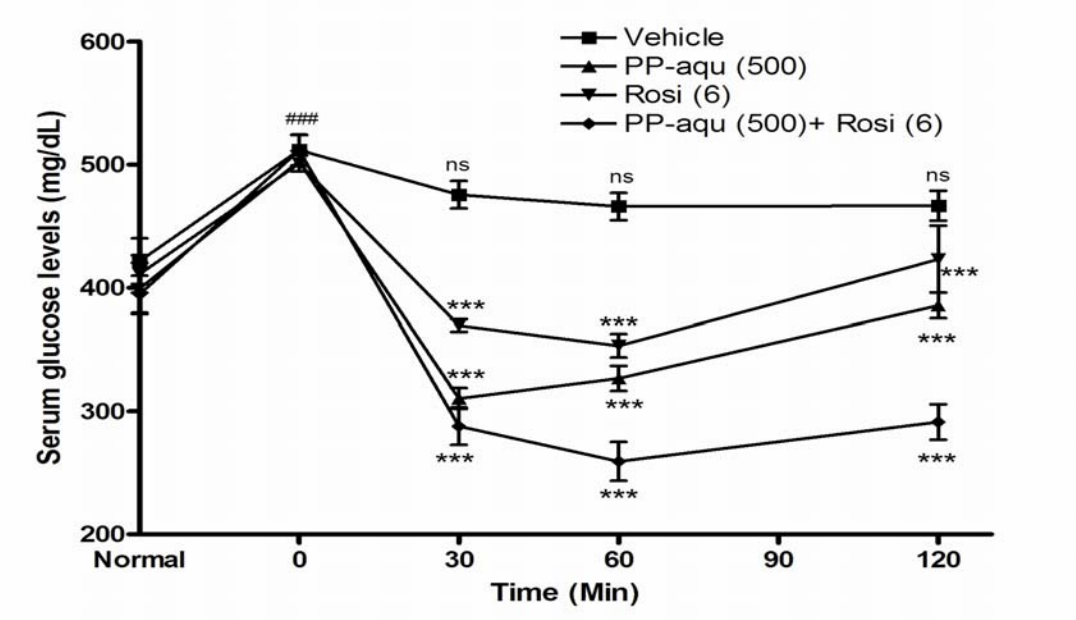

Figure 4: Effect of acute pretreatment of aqueous extract of *P. pulmonarius* (PP-aqu) and rosiglitazone on serum glucose level in oral glucose tolerance test (OGTT) in mice. Data was analyzed by Two-way ANOVA followed by post hoc Dunnett's test. ns – non significant $^{\#}$ $P < 0.05$ and $^{\#\#\#}$ $P < 0.001$as compared with reading of normal mice. * $P < 0.05$ and *** $P < .001$ as compared to pre-treatment readings (at 0 min) of respective treatment group.

Discussion

The results obtained are in support with our previous study on anti-hyperglycemic effect of PP-aqu (10). With increasing use of herbal medicinal products, adverse herb-drug interactions may be of significant public health consequence (18, 19). Moreover, nearly all herbal remedies contain multiple, biologically active constituents, and so interaction with conventional drugs is a important concern for medicinal use of herbs (20-22). In fact, the likelihood of herb-drug interactions is reported to be theoretically higher than drug-drug interactions (15). On the other hand, combination of herbal drugs (or isolated phytochemicals) are found to be beneficial in certain diseases (12) when given along with conventional drugs. Our results further suggested the use of PP-aqu as adjunct treatment with rosi for the effective control of type-2 diabetes.

Preliminary phytochemical analysis of the *P. pulmonarius* showed presence of proteins, minerals, vitamins and carbohydrates and are in line with earlier reports by Food and Agriculture Organization (23). Although, the active principle(s) in mushroom responsible for these effects remain to be elucidated but guanide (a phytochemical) has been detected in edible mushroom and has known hypoglycaemic effects (24). The ability of lectins isolated from other varieties of mushrooms (*A. camperstris*, *A. bispourus*) to enhance insulin release by isolated rat Islets of Langerhans has been documented by Ewart el al (6, 25)

The glitazones are known to enhance tissue sensitivity to insulin rather than stimulates insulin secretion. Rosiglitazone, a thiazolidinedione with a different side chain from those of troglitazone and pioglitazone, reduces plasma glucose levels and glucose production by acting primarily on peroxisome proliferator activated receptor gamma (PPAR-γ) (26) and increases glucose clearance in patients with type 2 diabetes mellitus (27). The combination of *P. pulmonarius* and rosiglitaznone may resulted in increasing the sensitivity of insulin and thus reduction in glucose levels are observed.

Conclusions

It is thus apparent that *P. pulmonarius* and rosiglitazone possesses significant synergistic antihyperglycemic activity. Addition of *P. pulmonarius* in diabetes regimen might improve the efficiency of rosiglitazone and appears to be a possible combination drug therapy for the treatment of diabetes mellitus.

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References

1. Wright T. Medicinal mushrooms. Nutraceuticals World. 2004:26- 9.

2. Tribe I, Tosco U. The world of mushrooms. London: Orbis Publishing; 1973.

3. Khanna P, Garcha HS. Pleurotus Mushroom- A source of Food Protein. Mushroom News Letter Tropics. 1984;4(3):9-14.

4. Flegg PB, Maw GA. Mushroom and their possible contribution to world protein needs. Mushroom Journal. 1976;48:396-405.

5. Bano Z. The Nutritive value of mushrooms. First Symposium on survey and cultivation of edible mushrooms of India; 1976; Regional Research Laboratory, Jammu: RRL, Jammu; 1976. p. 148-69.

6. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). J Endocrinol. 1998 May;157(2):259-66.

7. Talpur N, Echard B, Dadgar A, Aggarwal S, Zhuang C, Bagchi D, et al. Effects of Maitake mushroom fractions on blood pressure of Zucker fatty rats. Res Commun Mol Pathol Pharmacol. 2002;112(1-4):68-82.

8. Swanston-Flatt SK, Day C, Flatt PR, Gould BJ, Bailey CJ. Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetes Res. 1989 Feb;10(2):69-73.

9. Miles PG, Chang S-T. Mushroom Biology: Concise basics and current development. New York: World Scientific Publishing Company; 1997.

10. Badole SL, Shah SN, Patel NN, Thakurdesai PA, Bodhankar SL. Hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel.-Champ in alloxan induced diabetic mice. Pharmaceutical Biology. 2006;44(6):421-5.

11. Singh M, Varshneya C, Telang RS, Srivastava AK. Alteration of pharmacokinetics of oxytetracycline following oral administration of *Piper longum* in hens. J Vet Sci. 2005 Sep;6(3):197-200.

12. Kelly KM. Complementary and alternative medical therapies for children with cancer. Eur J Cancer. 2004 Sep;40(14):2041-6.

13. Lin YC, Bioteau AB, Ferrari LR, Berde CB. The use of herbs and complementary and alternative medicine in pediatric preoperative patients. J Clin Anesth. 2004 Feb;16(1):4-6.

14. Awang DV, Fugh-Berman A. Herbal interactions with cardiovascular drugs. J Cardiovasc Nurs. 2002 Jul;16(4):64-70.

15. Izzo AA. Herb-drug interactions: an overview of the clinical evidence. Fundam Clin Pharmacol. 2005 Feb;19(1):1-16.

16. Rao BK, Kesavulu MM, Giri R, Appa Rao C. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in alloxan-diabetic rats. J Ethnopharmacol. 1999;67(1):103-9.

17. Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum- graecum* leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol. 1997;58(3):149-55.

18. Coxeter PD, McLachlan AJ, Duke CC, Roufogalis BD. Herb-drug interactions: an evidence based approach. Curr Med Chem. 2004 Jun;11(11):1513-25.

19. Bressler R. Herb-drug interactions: interactions between kava and prescription medications. Geriatrics. 2005 Sep;60(9):24-5.

20. Poppenga RH. Herbal medicine: potential for intoxication and interactions with conventional drugs. Clin Tech Small Anim Pract. 2002;17(1):6-18.

21. Fugh-Berman A, Ernst E. Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmacol. 2001;52(5):587-95.

22. Woodward KN. The potential impact of the use of homeopathic and herbal remedies on monitoring the safety of prescription products. Hum Exp Toxicol. 2005 May;24(5):219-33.

23. Food and Agriculture Organization of the United Nations. Food composition table for use in Africa. Bethesda, Md.: US Dept.of Health, Education, and Welfare, Nutrition Program, and Food Consumption and Planning Branch, Food and Agriculture Organization of the United Nations; 1968.

24. Windholz M. The Merck index: an encyclopedia of chemicals, drugs and biologicals. 10th ed. New Jersey: Merck & Co.; 1983.

25. Ewart RB, Kornfeld S, Kipnis DM. Effect of lectins on hormone release from isolated rat islets of langerhans. Diabetes. 1975 Aug;24(8):705-14.

26. Doyle ME, Egan JM. Pharmacological agents that directly modulate insulin secretion. Pharmacol Rev. 2003 Mar;55(1):105-31.

27. Wagstaff AJ, Goa KL. Rosiglitazone: a review of its use in the management of type 2 diabetes mellitus. Drugs. 2002;62(12):1805-37.