Preliminary Phytochemical Screening and Antidiabetic Activity of Extracts of Leaves of *Crinum Latifolium* Linn.

Original Research Article

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ABSTRACT

The aim of this study was to investigate the Antihyperglycemic activity of aqueous and ethanol extracts of leaves of *Crinum latifolium in Alloxan* (ALX) induced diabetic rats. Diabetes was confirmed after 5 days of single intraperitoneal injection of ALX (150 mg/kg) in albino Wistar rats. Aqueous and Ethanol extracts of leaves of *Crinum latifolium* (200 and 400 mg/kg) and *Glibenclamide* (10 mg/kg, p.o) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. Five days before the termination of the experiment, the Oral Glucose Tolerance Test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed glucose (2 g/kg) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation.

On the 15th day, overnight fasted rats were sacrificed and blood was collected for the determination of blood glucose .Aqueous and Ethanol extracts of leaves of *Crinum latifolium* at doses of 200 and 400 mg/kg showed significant reduction is blood glucose when compared to diabetic control group. We concluded that aqueous and Ethanol extracts of leaves of *Crinum latifolium* possess antihyperglycemic activity. The histopathology study of pancreas was also performed which showed hypoglycemic effect.

KEYWORDS: *Crinum latifolium* , Diabetes mellitus, Leaves, Ethanol and Aqueous extracts, Glibenclamide, Alloxan etc.

I. INTRODUCTION

Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytodrugs [1-5]. Some medicinal herbs have proven antidiabetic activity potential. Glibenclamide, also known as glyburide, is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfonamide antibiotics is a popular remedy for pancreatic diseases. .It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. Plants of the genus *Crinum* (*Amaryllidaceae*) are widely used in folk medicine in different tropical and subtropical regions around the world. The Indian species *Crinum latifolium* (L.), Fig. 1, was traditionally used to treat rheumatism, fistula, tumors, earaches, rubefacient, tubercle and whitlow. In Vietnamese and Chinese traditional medicine *Crinum latifolium* preparations are used until nowadays because of their antiviral and antitumor properties. Also reported the aqueous extract of *C. latifolium* showed immunomodulatory properties in human peripheral blood mononuclear cells. Extracts of *C. latifolium* slightly enhance neopterin production in unstimulated peripheral mononuclear cells.

Fig. 1: *Crinum latifolium* Leaves

I. MATERIAL AND METHODS [6-9]

Plant material

The leaves of *Crinum latifolium* were collected from the local area of Allahabad District, Uttar Pradesh, India in the month of May 2017 and authentified from the Botanical survey of India). Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the National Center for Laboratory Animal sciences, Hyderabad used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2^{o} C, 50± 5%, relative humidity), they were fed with standard rat pellet diet (Hindustan Lever Ltd; Mumbai, India) and were ad libitum. The Institutional Animal Ethics Committee approved the study. Remaining reagents were used as supplied by the manufacturer without further purification or investigation.

Preparation of plant extract

The leaves were dried under shade, powdered with a mechanical grinder and passed through a 40-mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. The solvents were removed from the extracts under reduced pressure by using a rotatotry vaccum evaporator. The percentage yields of extracts were noted.

Physicochemical evaluation [10-22]

Physicochemical studies was carried out using Ash values (Total ash value, Acid-insoluble ash value, Water soluble ash value), Extractive values (Alcohol soluble extractives, Water-soluble extractives), Loss on drying. Particle size of the microspheres was determine using optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer.

Phytochemical screening was perform using the process of successive solvent cold extraction method of leaves of *Crinum latifolium*, obtaining the extracts to use for to confirm the presence of various phytochemical using various identification tests.

Animals

Healthy, adult Albino Wistar rats (180-200gm) of either sex were used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2^{o}C, 50± 5%, relative humidity), they were fed with standard rat pellet diet and were ad libitum. The Institutional Animal Ethics Committee (SIP-IAEC-01-15-01) approved the study.

Acute toxicity study

Extract dose of crude drug were freshly prepared as a fine homogenized suspension in aqueous.

The rats have to be randomized into seven groups comprising of six animals in each groups as given below.

Group I : Normal control rats were given Tween 80 for 15 days.

Group II : Diabetic controls have been given 0.5%Tween 80 for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group III : Rats have been given Glibenclamide (10mg/kg, p.o.) for 15 days, 5days after alloxan (150mg/kg, i.p.) treatment.

Group IV : Test rats have been given ethanolic extract of *Crinum latifolium* (200mg/kg, p.o.) for 15 days, 5 days after alloxan ((150mg/kg, i.p.) treatment.

Group V : Test rats have been given ethanolic extract of *Crinum latifolium* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mm/kg, i.p.) treatment.

Group VI : Test rats have been given aqueous extract of *Crinum latifolium* (200mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) on 3rd day.

Group VII : Test rats have been given aqueous extract of *Crinum latifolium* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Oral Glucose Tolerance Test (OGTT)

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight (18 h) fasted rats were fed glucose (2 gm/kg) orally and blood was collected at 0, 30, 60 and 120 minute interval from orbital sinus for glucose estimation.

Assessment of oral glucose tolerance test

Blood samples were collected from tail puncturing of each rat at 0 minute, 30 minute, 60 minute and 120 minute and blood glucose was estimated by glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level.[75]

Assessment of Anti-diabetic activity

Blood samples were collected from tail puncturing of each rat at 0 day, 1st day, 10th day and 15th day and blood glucose was estimated by glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level.[75]

III. STATISTICAL ANALYSIS [21-24]

Oral glucose tolerance test

The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett's multiple comparison tests using Graph pad in stat 3.0 software.

Anti-diabetic activity

The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett's multiple comparison tests using Graph pad in stat 3.0 software.

IV. RESULT AND DISCUSSION

The percentage yield in different solvents is reported in Table 1. Physicochemical parameter data is reported in Table 2. The presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, Phenolic and tannins were determined and the results were presented in Table 3 & 4. Table 5 shows the effect of doses of Ethanol and aqueous extract of leaves on diabetic rats.

Table 6. shows the anti-hyperglycemic effect of ethanol and aqueous extracts of leaves at doses of 200 and 400mg/kg. Graphical representation is reported in Fig. 3 & 4, whereas histopathological studies is reported in fig. 2.

Table: 1 Percentage yield of various extracts.

S. No.	Extracts	Percentage yield (% w/w)
1.	Petroleum ether	0.05
2.	Chloroform	0.10
3.	Ethyl acetate	0.03
4.	Ethanol	0.16
5.	Aqueous	1.65

Table: 2 Physicochemical analysis of leaves powder

S.No.	Parameters	Percentage (% w/w)
1.	**Ash values** Total ash value Acid insoluble ash value Water soluble ash value	 25 9.0 18.0
2.	**Extractive values** Alcohol soluble extractive Water soluble extractive	 23 32
3.	**Loss on drying**	14.62

Table: 3 Phytochemical screening

Chemical constituents	Test	Result
Carbohydrate	Molisch's Reagent	-
Flavonoids	Shinoda Test	+
Phytosterols	Salkowski's test	+
Glycosides	Legal Test	+
Alkaloid	Dragondroff's Test	+
Tannin and Phenolic	Ferric chloride	+
Amino Acid	Ninhydrin Test	+
Saponin	Foam Test	+
Anthraquinone	Borntrager Test	-

Table : 4 Preliminary phytochemical studies of leaves extract

Phytoconstituents/ Extracts	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	-	+	-	+	+
Glycosides	-	+	+	+	+
Flavonoids	-	+	+	+	+
Steroids	-	-	-	+	-
Phenolics and Tannins	-	-	-	+	+
Fixed oils	+	-	-	-	-
(+) = Present, (-) = absent					

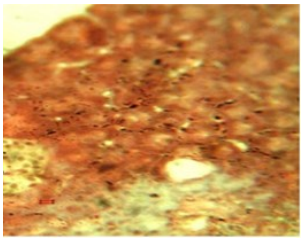
Table 5: Effect of ethanol and aqueous extract on OGTT of diabetic rats

Groups	Treatment / mg/kg	Blood glucose levels (mg/dl)			
		0 min	30 min	60 min	120 min
I	Normal control	89 ± 1.3	124 ± 1.2 (↑40.0%)	115 ± 1.4 (↑30.0%)	91 ± 0.9 (↑3.33%)
II	Glibenclamide, 10 mg/kg	232 ± 1.9	293 ± 2.26 (↑26.06%)	289 ± 1.5 (↑ 24.35%)	285.4 ± 2.6 (↑ 22.64%
III	Control, 0.5% Tween 80	177.2 ± 0.8	206.9 ± 1.6 (↑16.57%)	220 ± 1.02 (↑23.88%)	184.1 ± 0.8 (↑3.85%)
IV	Ethanol extract 200 mg/kg	164.3 ± 0.9	210.6 ± 1.09 (↑27.84%)a	237.9 ± 0.9 (↑44.25%)	194.1 ± 0.78 (↑17.91%)
V	Ethanol extract 400 mg/kg	181.3 ± 0.6	210.6 ± 1.05 (↑15.98%)a	246.3 ± 0.76 (↑35.46%)	187.9 ± 1.5 (↓3.6%)a
VI	Aqueous extract 200 mg/kg	207.1 ± 1.4	239.5 ± 0.6 (↑15.49%)	260.6 ± 0.56 (↑25.10%)	190 ± 0.96 (↓8.18%)
VII	Aqueous extract 400 mg/kg	193.5 ± 2.1	^{s}225.7 ± 0.76 (↑6.47%)a	234 ± 0.11 (↑20.71%)	178.1 ± 0.7 (↓12.77%)
Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01, *p<0.05 and ^{ns} p>0.05.					

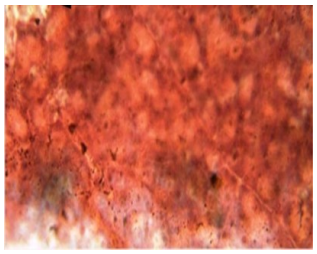
Table 6: Effect of ethanol and aqueous extracts on blood glucose level in alloxan induced

Group	Level of Blood sugar in Group (15 days) mg/dL (mean ± SD)				
	Initial	Day 1	Day 5	Day 10	Day 15
Normal control	68.76 ± 6.02	63.03 ± 9.31	64.68 ± 9.83	65.00 ± 7.39	**63.46 ± 5.86**
Glibenclamide,10 mg/kg	247.74± 8.83	260.26 ± 14.73	283.83 ± 4.76	307.18 ± 8.07	**311.26 ± 4.71**
Control, 0.5% Tween 80	248.83 ±8.38	250.47 ± 5.55	237.21 ± 8.40	202.36 ± 5.82	**190.01 ±5.78**
Ethanol extract 200 mg/kg	246.02 ±3.87	247.63 ± 7.83	219.22 ± 5.39	187.8 ± 8.20	**176.12 ± 9.28**
Ethanol extract 400 mg/kg	247.68 ± 8.83	254.06 ± 4.96	237.86 ± 8.82	212.21 ± 3.31	**180.83 ± 4.55**
Aqueous extract 200 mg/kg	249.82 ± 4.88	254.55 ± 5.55	231.43 ± 6.28	190.75 ± 4.87	**152.83 ± 10.22**
Aqueous extract 400 mg/kg	246.36 ± 3.48	249.15 ± 8.12	215.95 ± 4.50	188.08 ± 7.89	**178.19 ± 8.66**
Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01 and ns p>0.05.					

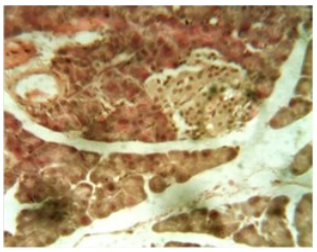
Fig. 2: Diagrammatic representation of histopathological studies



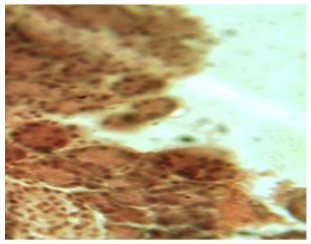
Damage islet of Langerhans



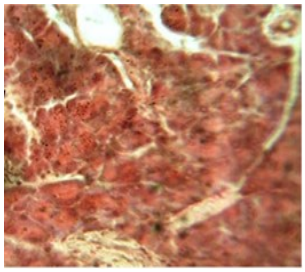
In ethanol Extract 200 mg/kg.



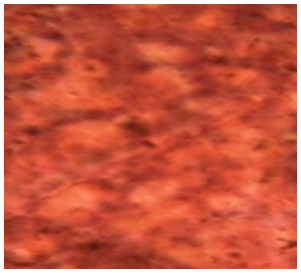
Normal Langerhans cells



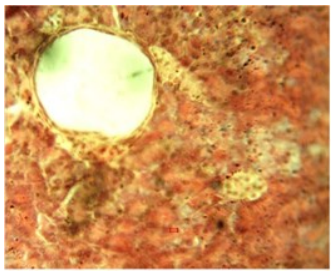
Globule with regenerated islets



In ethanol extract 400 mg/kg.



Aqueous extract 200 mg/kg.



Aqueous extract 400 mg/kg.

Fig. 3: Effect of ethanol and aqueous extract on OGTT of diabetic rats.

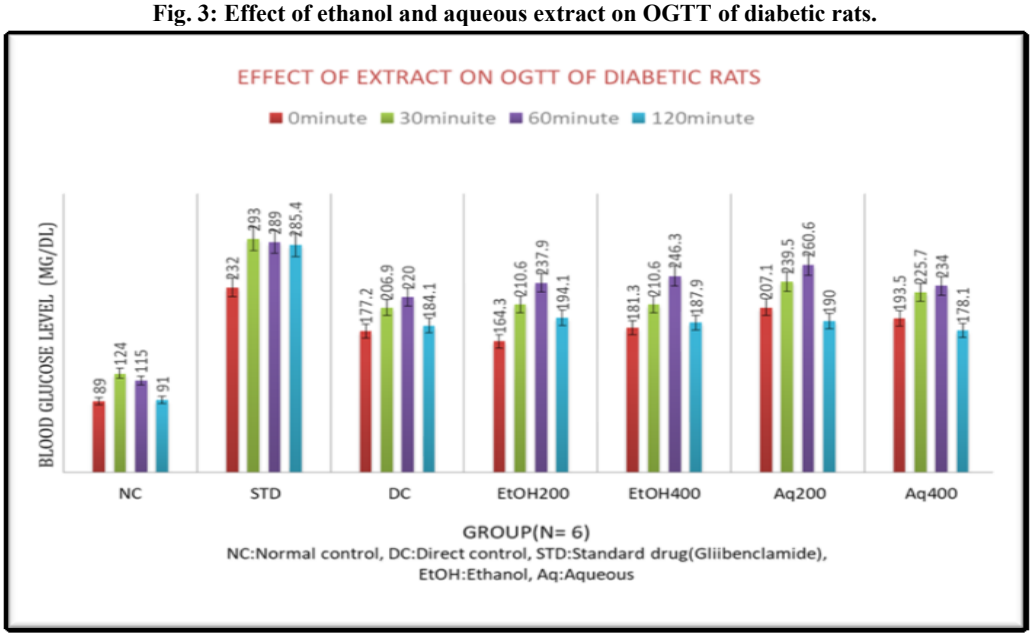
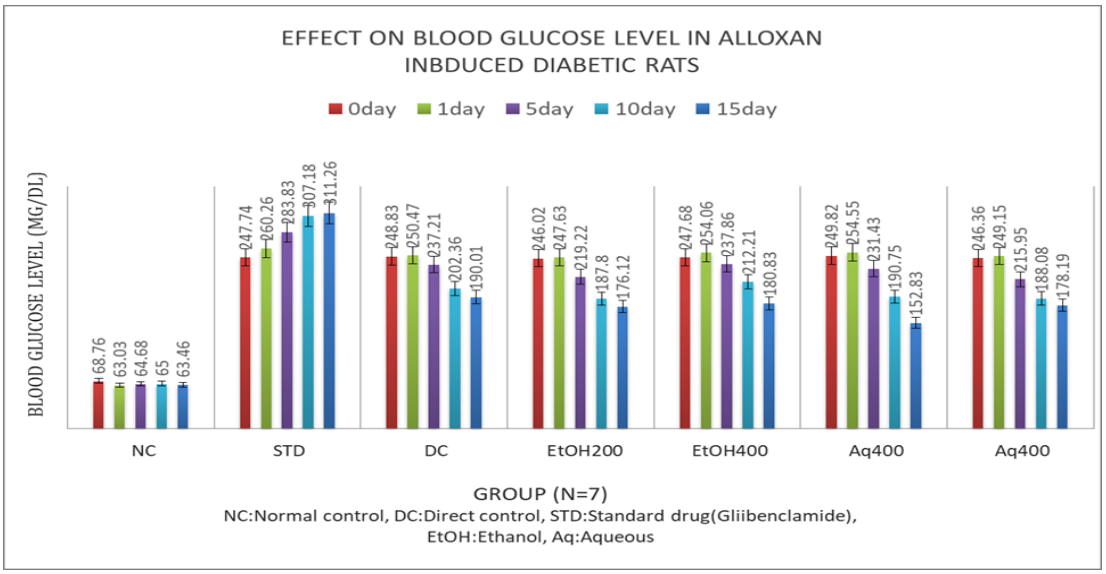


Fig. 4. Effect of ethanol and aqueous extract on blood glucose level in alloxan induced diabetic rats.



V. CONCLUSIONS

In the present study demonstrated that the ethanol and aqueous extracts of leaves of *Crinum latifolium* linn (200 and 400 mg/kg) showed significant antidiabetic activity, in which the level of 400 mg/kg showed potent antidiabetic activity than the dose level of 200 mg/kg. The antidiabetic activity may be attributed to the active principle present in the extracts, such as alkaloids, Terpenoids, flavonoids and tannins etc. The histopathological evidences showed that the treatment with the ethanol and aqueous extracts of leaves of *Crinum latifolium* linn effectively protected rats against alloxan induced diabetes. It provides a support for the traditional use in alloxan induced diabetes mellitus. Further studies should be conducted to determine the active compounds or principle that is responsible for the antidiabetic effects and to elucidate the mechanism are in progress.

REFERENCES

[1] **Kokate CK, Purohit AP, Gokhale SB.** Pharmacognosy 41st ed: Nirali Prakashan; 2008. 1.1-1.3.

[2] **Joy P, Thomas J, Mathew Samuel, Skaria Baby P.** Medicinal plants, Kerala agricultural university: Aromatic and Medicinal Plants Research Station; 1998. 1-211

[3] **Agrawal SS. 1st ed:** Drug Technology Universities Press (India) Pvt. Ltd Hyderabad; 2007. p. 578-582.

[4] **S Vipin Kumar, T Sanjeev, S Ajay, S Pravesh Kumar, S Anil, Seth G.L. A** Review on Hepatoprotective Activity of Medicinal Plants. International J Adv Res Pharm Bio Sci 2012;6:31-38.

[5] **Cai BC, Yang XW, Hattori M Namba T.** Processing of nux vomica (1): Four new alkaloids from the processed leaves of Strychnos nux-vomica. Shoyakugaku Zasshi. Thieme Planta Medica 1990;44(1):42-46.

[6] **Cai BC, Hattori M, Namba, T.** Processing of nux-vomica II: Changes in alkaloid composition of the leaves of Strychnos nux-vomica on traditional drug-processing. Chem Pharm Bull 1990;38 (5):1295-98.

[7] **Thomas, J.** Medicinal and aromatic plants research in India. In UNDP. Prccess training course on Indigineous medicinal and aromatic plants. Beijing, china; 1997. p. 17-27.

[8] **Hakim ZS, Banguru RA, Santani DD.** Potential antidiabetic agents from plant sources:Pharmacological aspects. Ind J natural products 1997;11(1):3-9.

[9] **Gansser A.** Geology of the Himalayas: Interscience,New York;1964. p. 289.

[10] **Cai BC, Yang XW, Hattori M Namba T.** Anaysis of spectral data for BC NMR of sixteen Strychnos alkaloids. Acta Pharma Sin 1994;29 (1): 44-48.

[11] **Patel PM, Patel KN, Goyal RK.** Development of HPTLC method for estimation of charatin in herbal formulations. Pharma magazine 2006;8:224-226.

[12] **Sharma PC, Yelne MB, Mehendale VV, Erande CM.** Cultivation of satavari (Asparagus racemosus Willd.). Bull. Med. Ethno. Bot. Res 1992;14:70-77.

[13] **Maiti R, Jana D, Das UK, Ghosh D.** Antidiabelic effect of aqueous extract of seed of tamarindus indica in streptozotocin induced diabetic rats. J Ethnopharmacol 2004; 92:85-91.

[14] **Wadkar KA, Magdum CS, Patil SS, Naikwade NS.** Antidiabetic potential and Indian medicinal plants. J Herbal Med Toxico 2008;2:45-50.

[15] **Welihinda J, Arvidson G, Gylfe E, Hellman B, Karlsson EA.** The insulin releasing activity of the topical plant Momordica charantia. Acta Biol Med Ger 1982;41:1229.

[16] **Hongxiang Hui, George Tang, Vay Liang W Go.** VLW Hypoglycemic herbs and their action mechanisms. Chin Med 2009;4:11-14.

[17] **Roman-Ramos R, Flores-Saenz JL, Alarcon-Aguilar FJ.** Antihyperglycemic effect of some edible plants. J Ethnopharmacol 1995;48:25-32.

[18] **Kumari K, Mathew BC, Augusti KT.** Antidiabetic and hypolipidaemic effects of S-methyl cysteinesulfoxide, isolated from Allium cepa Linn. Ind J Biochem Biophysics 1995;32:49-54.

[19] **Mathew PT, Augusti KT.** Hypoglycemic effects of onion, Allium cepa Linn, on diabetes mellitus-apreliminary report. Ind J Physiol Pharmacol 1975;19:213-217.

[20] **Grover JK, Yadav S, Vats V.** Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002;81:81-100.

[21] **Beckstrom-Sternberg SM, Duke JA, Wain KK.** The ethnobotany database. Beltsville, MD: Genome Informatics Group, National Agricultural Library. U.S., Department of Agriculture; 1994.

[22] **Usher G.** A Dictionary of Plants Used by Man. London: Constable and Company Ltd; 1974.

[23] **David G, Gardner, Dolores.** Shoback- Greenspan's basic & clinical endocrinology. 9th ed: McGraw-Hill Medical, New york; 2011. p. 17.

[24] **Ceriello A, Colgiuri S.** International Diabetes Federation guidelines for management of postmeal glucose: A review of recommendations. Diabet med Blackwell publishing ltd 2008;25(10):1151-1158.

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