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Original Article

A study on Anti-diabetic effects of ethanolic extract of Pomelo (Citrus Maxima Linn) and Glibenclamide on blood glucose level of diabetic rats

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Abstract:

Background: Diabetes mellitus is a one of the major public health problem adversely affecting health and socio-economic status of people at both national and global level.

Objective: To evaluate the effects of ethanolic extract of Pomelo (Citrus maxima linn) fruit juice on blood glucose level in alloxan induced diabetic rats.

Methods: This experimental study was carried out in two parts on 30 albino rats. In experiment-I; 12 rats were divided into 2 groups; each comprising of 6 rats. Group-I: Normal non-diabetic rats are fed with normal feed only and Group-II: Normal non-diabetic rat were fed with normal feed with Citrus maxima extract for 21 days. In experiment-II, 18 rats were divide into three groups; Group III (diabetic control), Group IV (experimental group) and Group V (standard drug group). To induce diabetic, these 18 rats were injected alloxan 120 mg/kg body weight intraperitoneally. Then group III and IV were treated with the Citrus maxima ethanol extract and Glibenclamide respectivelyfrom day1 to day 21.

Results: Blood glucose level of group IV decreased significantly ($p<0.05$) from 12.10-13.30 to 7.00-7.50 mmol/l. Blood glucose level of group V also decreased significantly ($p<0.05$) from 12.50-13.50 to 6.50 to7.90 mmol/l.Difference of blood glucose results between group IV and V was not statistically significant.

Conclusion: The ethanolic extract of Citrus maxima has shown significant activity like Glibenclamideto reduce blood glucose in hyperglycemic rats.

Keywords: Diabetes Mellitus, Citrus maxima, Ethanolic extract, Glibenclamide

Introduction

Diabetes mellitus, a metabolic endocrine disorder, has become a common global health problem and one of the leading causes of death and disability.Diabetes was one of the four priority non-communicable diseases (NCDs) targeted by world leaders in the 2011.[1] Epidemiological studies on urbanization and aging influences have shown the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% increase in 2000 and 4.4% in 2030.[2] The IDF diabetic atlas 10th edition reveals that approximately 537 million adults (20-79) years were living with diabetes and this number is predicted to rise to 643millions by 2030 and 783 million by 2045.[3] USD 10 billion was spent on healthcare for people with diabetes in 2021— the second lowest expenditure of all IDF regions.[4] In Bangladesh, there were 8.4 million adults living with diabetes in 2019, and projected to almost double (15.0 million) by 2045.Studies, including a systematic review and meta-analysis, and national survey reports showed that the prevalence of diabetes among adults has increased substantially in Bangladesh, from ~5% in 2001 to ~14% in 2017.[5-8]

According to WHO (1999) Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyper[1]glycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[4]

Patient complains of symptoms suggesting of diabetes is diagnosed by test urine for glucose and ketones, measure random or fasting venous blood glucose and testing of level of glycosylated hemoglobin (HbA1c). Diagnosis is confirmed by fasting blood glucose ≥ 7.0mmol/l (120mg/dl) or plasma glucose ≥ 11.1mmol/l (200mg/dl) two hours after a 75g oral glucose load in glucose tolerance test. HbA1c of 6.5% is recommended as the cut point of diagnosis.[9-11] Diabetes is an incurable disease. The goal of diabetes management is to maintain the level of blood glucose; this goal should be achieved through an anti-diabetic agent (either natural or chemical) that will be effective, affordable, and available and with less side effects. Type-1 diabetes requires absolute insulin therapy, whereas Type-2 can be treated with oral anti-diabetic drugs or Insulin or as a combination of both with life style modification.[1,12] Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drug continues because the existing synthetic drugs have several limitations. For a developing country like Bangladesh herbal plants may be the most attractive target for their availability, low cost and safety margin.[13]

Citrus maxima, the pomelo in rutaceae (Citrus family), is known as batabilebu in Bengali is the biggest citrus fruit. Like other citrus fruits, pomelos also are rich in vitamin C and contain polyphenol, proteins and polysaccharide. In the field of polyphenol compound, It contains flavonine, narginin and hesperidine.[13-16] Citrus maxima cited for its various medicinal properties, especially analgesic, anti-inflammatory, antibacterial, antioxidant, hepatoprotective, antidiabetic, Anti-depressant, anti-tumor and antihyperlipeadimic properties.[13-20]

Materials and Method

Place and Period of Study: This experimental (animal) study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with institution of Nutrition and Food Science (INFS) from July 2016 to June 2017. Total 30 male Swiss albino rats were taken for the study.

Materials

Animals: A total number of 30 healthy Swiss albino male rats were purchased from the animal resource division of ICDDRB, Mohakhali Dhaka. The age of the rats was between 8-10 weeks weighing about 150 to 170 grams. They were kept in metallic cage (1 rat /cage) in the animal house of Institute of Nutrition and Food science in a well-ventilated room and a temperature of about 26-28°C. the animal room was maintained under a constant 12-hours light: 12- hours dark cycle. They were allowed to feed standard rat food pellets and drink water ad libitum, except during the day of blood sampling when animals were kept an overnight fasting.21

Drugs:

a) Alloxan Monohydrate (Sigma Chemical Co.USA) was purchased. Alloxan Monohydrate was administered by single intraperitoneal (i.p) injection at a dose of 120 mg/kg body weight.[22]

b) Glibenclamide: as Diabenol (Square pharmaceutical Pvt Ltd), which was purchase from Lazz pharma, Dhaka. It was then crushed into powder and dissolved in water given at a dose of 10 mg/kg body weight.[23]

Medicinal plant: Citrus maxima linn (pomelo) batabilebu was purchased from local market.

Procedure of obtaining ethanolic extract of Citrus maxima linn (pomelo): Pomelo was brought from local market, the pulp was collected by peeling off and washed thoroughly with water. By using a commercial blender fruit juice was made (600mg). 600 mg of citrus fruit juice was soaked in 300ml ethanol alcohol with continuous shaking (40 rpm) at 25°C for three days and filtered. The organic extract was evaporated under vacuum to obtain a semisolid residue (4g). The extract was kept in refrigerator.[24]

Experimental dose: Ethanolic extract of pomelo with a dose of 200 mg/kg body weight was given to Group II and Group IV rats with laboratory diet and water for 21 days. The dose was selected from Parixitet al.[23] Pomelo extract was weighed according to group wise body weight accurately by electric analyzer. Extract was then dissolved in 2.9 ml of water. The liquid form of extract was administered through a micropipette.

Design of experiment:

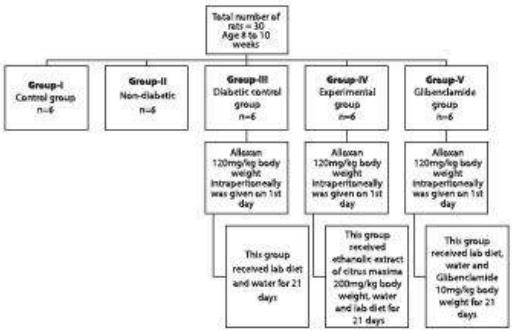
Group-I (Normal non-diabetic rat normal feed only): In this group, the rats were given normal feed and water for 21 days and fasting blood was estimated on day 1, 4, 7, 21.

Group-II (Normal nondiabetic rat normal feed with Citrus maxima extract): rats were given normal feed with Citrus maxima extract (200mg/kg) for 21 days and fasting blood glucose was estimated day 1, 4, 7 and 21 of the experiment.

Group-III (Alloxan induced diabetic control group): Alloxan 120 mg/kg body weight was administered intraperitoneally for induction of diabetes at day 1.[25] After peritoneal injection rats were given standard food and water. Fasting blood glucose level was estimated onday 1 (before alloxan), on day 4 (after alloxan to confirm induction of diabetes mellitus) and on day 7, 14, 21 of the experiment.

Group-IV (Diabetic rat normal feed with Citrus maxima extract): The rats were administered ethanolic extract of Citrus maxima 200mg/kg body weight orally along with standard food and water for 21 days.

Group-V (Diabetic rat normal feed with Glibenclamide): In this group, 10mg/kg body weight Glibenclamide was given orally with normal feed for 21days of the experiment.



Sacrifice of the Animals and collection of blood: Blood sample were collected via tail vein by aseptically cutting the tip of tail with a sharp sterile blade after an overnight fast for measurement of fasting blood glucose levels. All the animals were sacrificed under light chloroform anesthesia after completion of treatment. Blood was collected in epindroff and kept in standing position till clotting of blood had occurred. The blood sample were centrifuged for 15 minutes in a tabletop clinical centrifuge at 3000 rpm for serum separation, the serum was then used for biochemical analysis.

Determination of blood glucose level: Estimation of serum glucose concentration by using an oxidase and peroxidase (GOD-POD) method.[26]

Statistical Analysis: The results are given as Mean ± SD for the six independently performed experiments. Unpaired student "t" test was used to see the level of significance. p-value <0.05 was considered statistically significant.

Result

Table-I shows average weight of rats of different group. Each group consists of six rats.

Table-I: Weight of the subjects in different groups (n=30)

Parameters	Groups				
	I (n=6)	II (n=6)	III (n=6)	IV (n=6)	V (n=6)
Weight (gm)	155.0±8.3 (150-170)	156.6±10.3 (140-170)	158.3±7.5 (150-170)	158.3±9.8 (150-170)	158.3±9.8 (140-160)

Table-II: Mean blood glucose of the subjects in different groups at different followups (n=30)

Experimental animal group (n=6)	Serum blood glucose level (mmol/L)				
	Day-01	Day-04	Day-07	Day-14	Day-21
Group-I	4.47 ± 0.48 (4.10 -5.40)	5.43±0.20 (5.20-5.70)	4.93±0.61 (4.10-5.60)	5.37±0.20 (5.10-5.60)	5.50±0.16 (5.30-5.70)
Group-II	4.20 ± 0.14 (4.00 -4.40)	5.18±0.27 (4.70-5.50)	5.25±0.33 (4.70-5.70)	5.42±0.27 (5.10-5.80)	5.61±0.27 (5.20-5.90)
Group-III	4.28 ± 0.54 (3.50 -4.90)	13.20±0.24 (12.80-13.50)	13.35±0.33 (13.00-13.80)	13.47±0.32 (13.00-13.90)	13.58±0.36 (13.10-14.10)
Group-IV	3.97 ± 0.23 (3.70-4.20)	12.67±0.46 (12.10-13.30)	9.85±0.30 (9.50-10.30)	8.58±0.36 (8.10-9.10)	7.20±0.18 (7.00-7.5)
Group-V	5.82 ± 2.55 (3.90-9.10)	12.97±0.37 (12.50-13.50)	10.02±0.29 (9.50-10.30)	8.95±0.46 (8.30-9.70)	7.00±0.48 (6.50-7.90)

Unpaired t-test:

Groups	P-values				
	Day-01	Day-04	Day-07	Day-14	Day-21
I vs II	0.22	0.098	0.288**	0.722**	0.391
I vs III	0.578	1.000	0.092^{ns}	0.341*	<0.001***
I vs IV	0.045	<0.001***	<0.001***	<0.001***	<0.001***
I vs V	0.232	<0.001***	<0.001***	<0.001***	<0.001***
II vs III	0.721	<0.001***	<0.001***	<0.001***	<0.001***
II vs IV	0.063	<0.001***	<0.001***	<0.001***	<0.001***
II vs V	0.232	<0.001***	<0.001***	<0.001***	<0.001**
III vs IV	0.216	0.031	<0.001***	<0.001***	<0.001***
III vs V	0.181	0.220	<0.001***	<0.001***	<0.001***
IV vs V	0.108	0.242	0.349	0.157	0.363

Results are expressed as mean ± SD. Unpaired t test was performed to compare between groups. The test of significance was calculated &p value < 0.05 was accepted as level of significance.

n = number of subjects; ns = not significant; */**/*** = significant

Group I: Normal feed only (Control group)

Group II: Normal feed+ ethanol extract of Citrus maxima (Non-diabetic group)

Group III: Alloxan + normal feed (Diabetic group)

Group IV: Diabetic rats + ethanol extract of Citrus maxima (Experimental group)

Group V: Diabetic rats + Glibenclamide (Standard group)

Table-III: Effect of ethanolic extract of Citus maxima on fasting blood glucose level in non diabetic rats

Group	No of rats (n)	Fasting blood glucose level (mmol/L in mean±SD)	p-value
Group-I (Normal feed rats group)	6	5.50±0.20	0.391
Group-II Rats feed on ethanolic extract ov Citrus maxima	6	5.61±0.27	

p-value is not significant (>0.05)

Comparison between fasting blood glucose level of Group-II with that of normal control Group I was done by unpaired student's t-test. There is a non significant difference (p>0.05) between Group I and Group-II.

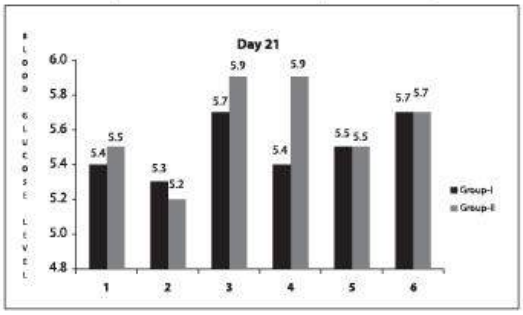


Fig-I: Bar graph showing fasting blood glucose level of Group-I (normal feed rats group) and Group-II (ethanolic extract of Citrus maxima feed rats group)

Table-IV: Administration of alloxan on blood glucose level of adult rats

Group	No of rats (n)	Fasting blood glucose level (mmol/L)	p-value
Group-I (Normal feed rats group)	6	5.50±0.20	*0.001
Group-III (Alloxan-induced diabetic Rats)	6	13.58±0.36	

*p-value is highly significant (<0.01)

Significant at p<0.001 level in unpaired student's t-test of significance of difference when compared with the control.

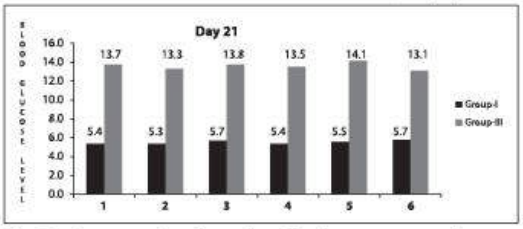


Fig-II: Bar graph showing fasting serum glucose level in Group-I (normal feed rats group) and Group-III (Alloxan-induced rats group)

Table-V: Effect of ethanolic extract of Citrus maxima on fasting blood glucose levels of alloxan induced hyperglycemic rats

Group	No of rats (n)	Duration of tratment (day 4-21)	Fasting blood glucose level (mmol/L in mean±SD)	p-value
Group-III (Alloxan-induced Rats group)	6		13.58±0.36	0.001
Group IV (Alloxan induced diabetic rats fed on ethanol extract of CM)	6		7.20±0.16***	

***highly significant (<0.001)

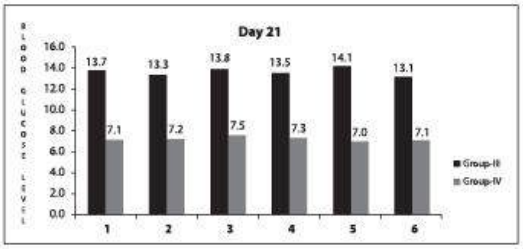


Fig-III: Bar graph showing the blood glucose level in Group-III (alloxan-induced rats group) and Group-IV (alloxan-induced diabetic rats treated with ethanolic extract of Citrus maxima) on day 21

Table-VI: Effect of Glibenclamide on fasting blood glucose level of alloxan induced hyperglycemic rats

Group (Mean±SD)	No of rats (n)	Duration of tratment	Fasting blood glucose level (mmol/L in mean±SD)	p-value
Group-III (Alloxan –induced rats group)	6	(4-21days)	13.58±0.36	0.001
Group-V (Alloxan induced Diabetic rats treated with Glibenclamide)	6	(4-21days)	7.00 ±0.48 ***	

***highly significant (<0.01)

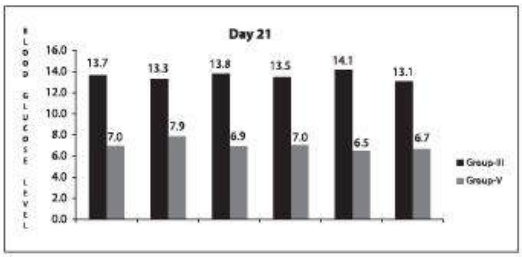


Fig-IV: Bar graph showing the blood glucose level in Group-III (alloxan-induced rats group) and Group-V (alloxan-induced diabetic rats treated with Glibenclamide) on day 21

Table-VII: Comparison of fasting blood glucose level of rats with the ethanolic extract of Citrus maxima treated rats and the Glibenclamide treated hyperglycemic rats

Group of	No of rats (n)	Duration of tratment	Fasting blood glucose level (mmol/L in mean±SD)	p-value
Group-IV (Alloxan–induced diabetic rats Treated with extract of CM)	6	(day 4-21)	7.2±0.18	0.363
Group-V (Alloxan induced diabetic rats treated with Glibenclamide)	6	(day 4-21)	7.00 ±0.48	

ns : non-significant

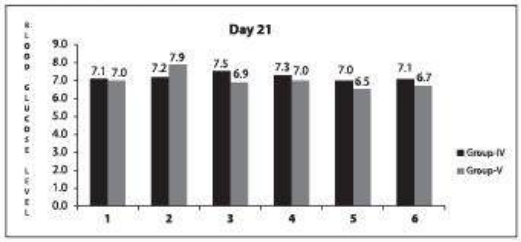


Fig-V: Bar graph showing the blood glucose level in Group-IV (ethanolic extract Citrus maxima treated rats group) and Group-V (alloxan-induced diabetic rats treated with Glibenclamide) on day 21

Discussion

In recent, the world is facing an unprecedented increase in the incidence of diabetes mellitus. Most of the commonly used anti-diabetic agents have some significant side effects and are not cost effective. An increasing interest in herbal and complementary medicine has led to a search for effective natural therapies that have significant effect on blood glucose level.[27] Herbal treatment can be a safe and cost-effective way to combat diabetes.

This research work has been conducted on the basis of the prospect mentioned above. The mentioned parameter was also tested in non-diabetic as well as experimentally induced diabetic rats after 21 days of treatment, the glucose lowering effect of the extract of Citrus maxima was compared with a standard drug, Glibeclamide.

In the present study, diabetes was induced by alloxan monohydrate. The dose and route of administration of alloxan was selected from.[22] The blood glucose levels in animals were measured 72 hours after administration of alloxan which was done according to experiment of Shamim et al.[28] In this study, intraperitoneally (i.p) administration of single dose of alloxan (120mg/Kg), increased blood glucose level significantly ($p<0.001$).The mean ± SD of blood glucose level in rats of Group III was 13.20±0.24 mmol/L and in Group I it was 5.43±0.20 on day 4; in day 21, the mean ± SD of Group III was 13.58±36 mmol/L and 5.50±0.16 mmol/L in Group I. similar observation was reported by number of researchers .Shamim et al.[28] observed that blood glucose level was increasing after 72 hours of intraperitoneal injection of freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg body weight in Swiss alibino rats. Etuk et al.[29] observed that the condition of diabetes after 48 hours of intraperitonel administration of freshly prepared alloxan monohydrate at a dose of 150mg/Kg b.w. In that study, the rise of blood glucose level in experimental rats was also highly significant as $p<0.001$. so it may be concluded that alloxan is a potent hyperglycemic agent in rats.[29]

The study was divided into two parts: Experiment-I and Experiment-II. Experiment-I includes Group I and II. Experiment-II includes Group III, IV and V. blood was collected from Group I and II on day 1, 7, 14 and 21of experiment. Similar experimental design was found in other studies as experiment design-I and experiment design-II.[28,30]

The dose of ethanolic extract of Citrus maxima (200mg/Kg body weight) used in this study was selected based on the dose used in the research done by Bhandurge et al.[23] & Kharjul et al.[24]

The experiment-I of the present study has demonstrated that normal rats group's serum glucose levels was 5.50±0.16 mmol/L (Group I- received only

laboratory diet for 21 days) and the same in group of non-diabetic rats treated with ethanol extract of Citrus maxima juice (Group II) at 200 mg /kg b.w for 21 days; the serum glucose concentrations were 5.61±0.27 mmol/L (mean ± SD), there was no statistically significant difference ($p>0.05$) in the mean value of blood glucose level between two groups. Sriparna K et al.31 also found similar result in her research. So, it may be concluded that Citrus maxima ethanolic extract has no effect on blood glucose level of non-diabetic rats.

In the experiment-II, the effect of ethanolic extract of Citrus maxima was observed in alloxan induced hyperglycemic rats and compared it with Glibenclamide. Decrease in the mean value of blood glucose level was observed in the experimental hyperglycemic group when treated with ethanolic extract of Citrus maxima at a dose of 200mg/Kg (Group IV) and compared with Glibenclamide after 21 days. Diabetes was induced in all groups first by administration of single dose i.palloaxan (120mg/Kg).

The mean ± SD of serum glucose concentration in ethanolic extract of Citrus maxima treated group (Group IV) was 7.20±0.18 mmol/L and in Group III was 13.58±0.36 mmol/L. The mean reduction of serum glucose concentration in Group IV compared to Group III was statistically significant ($p<0.001$). Therefore,the findings of this study are in a well agreement with the fidings of other researchers.[22,23,31,32]

So it may be concluded that ethanolic extract of Citrus maxima has glucose lowering effect in experimentally induced hyperglycemic rats.

The exact mechanism of ethanolic extract of Citrus maxima in reduction of blood glucose level is not well understood. Kim Y et al.[33] suggested in their research that polyphenols isolated from Citrus maxima produced antididiabetic effect by inhibiting the α-amylase and α-glucosidase activities. Polyphenols also enhanced insulin-mediated glucose uptake, a glucose transporter 4-mediated process. This compound inhibited cytokine-induced β-cell damage through suppression of nuclear kappaB (NF-κB) activation in rat pancreatic cells (RINmF5 cells). They observed that the polyphenols also helped to maintain the liver glucose homeostasis.[33]

Guocong et al.[34] evaluated the chemical composition and α-amylase and α-glucosidase enzyme inhibitory effect of crude polysaccharide of citrus maxima endodermis. In his study, it was found that citrus maxima reduced the blood glucose level by inhibiting these two enzymes.

Another study showed that it reduced blood glucose level in mice by activating the PPARα and GLUT4 pathway.[35]

Natarin C et al.[37] investigated the protective effects of pomelo against fructose mediated protein oxidation and glycation. They found that pomelo reduced the blood glucose level and chronic complications of diabetic mellitus due accumulation of AGEs (advanced glycation end products). The polyphenols and flavonoids present in polmelo were responsible for inhibition of the glycation, Sugar-mediated non-enzymatic protein glycation and oxidation.

In the last experimental part, the mean ± SD of serum glucose concentration in Glibenclamide treated group (Group V) was 7.00±0.48 mmol/L and in Group III was 13.58±0.36 mmol/L. The mean reduction of serum glucose concentration in Group V compared to Group III was statistically significant ($p<0.001$). Result is shown in table-VI. So, the Glibenclamide significantly reduces the serum glucose level. Similar observation was made by Bhandurge P et al.[23] who used Glibenclamide at a dose of 10 mg/kg body weight in alloxan induced diabetic rats and found the effect of the drug statistically significant.

The mean ± SD of serum glucose concentration in Glibenclamide treated rats (7.00±0.18) was compared to ethanolic extract of Citrus maxima treated rats (7.20±0.18 mmol/L). The both group was effective for decreasing blood glucose level. But the mean reduction of glucose in Group IV compared to Group V was not statistically significant ($p>0.05$). These result were similar with the results of other studies.[31,32] So, it may be suggested that the glucose lowering effect of ethanol extract of Citrus maxima is almost nearly effective to that of Glibenclamide.[31]

From all above results, it was observed that the ethanolic extract of Citrus maxima has blood glucose lowering effect in alloxan induced hyperglycemic rats as like Glibenclamide but it has no effect on blood glucose level in non-diabetic rats. The result is suggestive of ethanolic extract of Citrus maxima as a useful glucose lowering agent in the treatment of diabetes mellitus. Due to time constrain the following parameters could not be taken in the present study ; 2 hours after blood glucose , plasma insulin level, HbA1c, liver glycogen level and free radicals in the tissues after

treatment with ethanol extract of Citrus maxima. Different extract of Citrus maxima was not used. Despite of all these limitations, interpretation of the results obtained in this study was made carefully and cautiously.

Conclusion

The observations and results of this study provide information that Citrus maxima ethanolic extract have glucose lowering effect at a dose of 200mg/Kg body weight in experimental diabetic rats. Thus, it provides a rationale for its use in development of new drug, required for treatment and prevention of diabetes mellitus. However, if these experimental data are endorsed in the clinical trials in future, Citrus maxima may be considered as a natural alternate or adjuvant remedy for type 2 diabetes mellitus.

References

1. WHO: diabetes in South East Asia region, 2016 available athttp://apps.who.int/iris/bitstream/10665/204979/1/Vol5%20Issu1.pdf.
2. Wild S, Rogelic G, Green a, Sincree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes care 2004; 27 (5): 1047-53.
3. IDF atlas 10th edition available at https://diabetesatlas.org/

4. WHO Global report on diabetes available at http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf

5 .https://www.idf.org/our-network/regions-members/south-east-asia/diabetes-in-sea.html

6. Akter S, Rahman MM, Abeb SK, Sultanac P. Prevalence of diabetes and prediabetes and their risk factors among Bangladeshi adults: a nationwide survey. Bull World Health Organ. 2014;92:204–13A.
7. National Institute of Population Research and Training (NIPORT) Ma, and ICF International,. Bangladesh Demographic andHealth Survey 2011. Dhaka, Bangladesh and Calverton, Maryland, USA:NIPORT, Mitra and Associates, and ICF International 2013.
8. Research NIoP, Training - NIPORT, Health Mo, Family Welfare, ICF. Bangladesh Demographic and Health Survey 2017-18. Dhaka, Bangladesh: NIPORT/ICF; 2020.
9. WHO/. Report of a WHO Consultation, definition, diagnosis and classification of Diabetes Mellitus and its complications. Department of noncommunicable diseases Surveillience. Geneva :1999:p 1-23. Available at http:/www.staff.ncl.ac.uk/Philip.home/who_dmc.htm.
10. WHO/IDF consultation: Defination and diagnosis of diabetes meliitus and intent hyperglycemia, 2006: 21-29.
11. Frier BM, Fisher M. Davidson's Principles and practice of medicine. 21st ed. 2011. p. 806.
12. Saquib N, Saquib J, Ahmed T, Khanam MA, Cullen MR. Cardiovascular diseases and type 2 diabetes in Bangladesh: a systematic review and meta-analysis of studies between 1995 and 2010. BMC public health. 2012; 12: 434.
13. Antidiabetic Potential of Medicinal Plants and Their Active Components Biomolecules. 2019 Oct; 9(10): 551; Published online 2019 Sep 30. doi: 10.3390/biom9100551;PMCID: PMC6843349; PMID: 31575072
14. Vijaylakshmi P, Radha R. An overview of Citrus maxima. The Journal of Pharmacology 2015; 4(5): 263-267.
15. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicines: India's Opportunity. Current Science 2004; 86(1): 37-41.
16. Mabberley DJ. A classification for edible Citrus (Rutaceae). Telopea 1997; 7: 167-72
17. Okwu DE. Citrus fruits: a rich source of phytochemicals and their roles in human health. Int J Chem Sci. 2008; 6(2): 451-471
18. Cordenonsi LM, Sponciado RM, Camapanharo SC, Garcia CV, Ralfin RP, Schapoval EES. Study of flavonoids present in Pomelo (Citrus maxima) by DSC UV-VIS, IR, 1H, and 13C NMR and MS. Drug Anal. Res. 2017; 01: 31-37.
19. Feng B, Pei Y. Study on the coumarins from Citrus grandis. Shenyang Yaoke Daxue Xuebao 2000; 17(4): 253-255.
20. Feng BM, Shah Y, Pei YH, Hua HM, Li W. Structure determination of the constituents from Citrus gandis Osbeck. In China Journal of Chinese Meteria Medica 2001; 26(11): 764-765.
21. Kim K, Kim H, Kwon J, Lee S, Kong H, Im SA, et al. Hypoglycemic & hypolipidemic effects of

processed Aloe vera gel of non-insulin dependent diabetes mellitus.

Phytomedicine 2009, available at www.scien cedirrect.com.

22. Qureshi SA, Hasnain SN. Hyperglycemic and antidiabetic activities of aqueous leaf extracts of Azadiractaindica (Neem) in alloxan-induced diabetic rats. Proceedings of ISSBP symposium of biochemistry & Biophysics 1997; 2: 267-270.
23. Bhandurge P, Rajarajeshwari N, Alagawadi KR, Agrawal S. Antidiabetic and hyperlipaemic effects of citrus maxima linn fruits on alloxan-induced diabetic rats. International Journal of Drug development and research 2010; 2(2): 273-278.
24. Khajrul M, Gali V, Khajrul A. Antidiabetic potential of ethanolic extract of Citrus maxima fruit peel and Anvilleagarcinii. Int. Journal of pharmaceuticals Innovations 2014; 4.
25. Chouglae AD, Panaskar SN, Gurao PM, Arvindekar AU. Optimization of Alloxan dose is essential to induce stable diabetes for prolong period. Asian Journal of biochemistry 2007; 2(6): 402-408.
26. Trinder P. Determination of glucose by enzymatic colorimetric method. Ann. Clin. Biochem. 1969; 6: 24.
27. Diabetes Action Research and Education Foundation 2010 available at http://www.diabe tes action.org./site/pageServer?pagename=complem entary_7_10 accessed on 20-4-17
28. Qureshi SA, Asad W, Sultana V. The Effect of Phyllantusemblica Linn on Type - II Diabetes, Triglycerides and Liver - Specific Enzyme. Pakistan Journal of Nutrition 2009; 8(2): 125-128.
29. Etuk EU et al. Animals models for studying diabetes mellitus. Agriculture and Biology Journal of North America 2010; 1(2): 130-134.
30. Kim SH, Hyun SH, Choung SY. Anti-Diabetic Effect of Cinnamon Extract on Blood Glucose in db/db Mice. Journal of Ethnopharmacology 2006; 104: 119-123.
31. Kundusen S, Gupta M, Majumder UK, Haldar PK, Saha P, Battachariya S et al. Antihyperglycemic effect and antioxidant properties of Citrus maxima leaf in streptozocin- induced diabetic rats. Diabetologia Croatica 2011; 40(4): 113-120.
32. Abdul MMT, Ashok S, Karunakar H, Sayed A, Shabaraya AR. Evaluation of antidiabetic activity of Citrus maxima stem berk on streptozocin induced diabetic rats. Int. Journal of Pharmaceuticals and chemical sciences 2014; 3(3): 647-650.
33. Kim Y, Keogh JB, Cliftoin PM. Polyphenol and glycemic control. Nutrients 2016; 8(1): 17.
34. Guocong L. Chemical compositions, A-Glucosidase and A-amylase inhibitory activities of crude polysaccharide from the endodermis of Shaddock (Citrus maxima). Archives of Biological Sciences 2012; 64(1): 10.2298/ABS12010712.
35. Xu GH, Liu DH, Chen JC, Ye XQ, Ma Y, Shi J. Juice components and antioxidant capacity of Citrus varieties cultivated in China. Food Chemistry 2008; 106(2): 545–551.
36. Yang XH, Zhang GX, Cui P. Gc/Ms analysis of the chemical constituents of Pomelo peel volatile oil. Wuhan Huagong Cueyuan Xuebao 2001; 23(2): 13-15.
37. Caengprasath N, Ngamukote S, Makynen K, Adisakwattana S. The protective effect of pomelo extract [Citrus grandis] against fructose mediated protein oxidation and glycation. EXCLI Journal 2013; 12: 491-502.