



ORIGINAL ARTICLE

Effect of Aegle Marmelos Fruit Pulp Powder on Chronic Subclinical Inflammatory Status (Phase 3 clinical trial) of Type 2 Diabetic Patients

Murshida Aziz¹, Rupali Debnath², Tohfa - E-Ayub³, Fahmida Islam⁴, Farjana Aktar⁵, Safwanul Aman⁶

¹Assistant Professor, Department of Biochemistry, Ibrahim Medical College, Dhaka, Bangladesh; ²Associate Professor, Department of Biochemistry, North Bengal Medical College, Dhaka, Bangladesh; ³Lecturer, Department of Biochemistry, Ibrahim Medical College, Dhaka, Bangladesh; ⁴Lecturer, Department of Biochemistry, Ibrahim Medical College, Dhaka, Bangladesh; ⁵Assistant Professor, Department of Biochemistry, Ibrahim Medical College, Dhaka, Bangladesh; ⁶FCPS(Part-2) trainee, Internal Medicine, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh

[Received on: 1 November 2020; Accepted on: 20 December 2020; Published on: 1 January 2021]

Abstract

Background: The plant is reported to have multiple therapeutic properties such as chronic subclinical inflammation. **Objective:** In this present study *Aegle marmelos* was investigated for its anti-inflammatory effects on human T2DM subjects. **Methodology:** The present study was conducted at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh from July 2010 to June 2011 to explore the effect of *Aegle marmelos* unripe fruit pulp on chronic subclinical inflammatory status in T2DM patients as evident by hsCRP. It was an un-blinded clinical trial conducted under a cross-over design. The experiment was conducted under a crossover design and the effects were analyzed during the 0-21 as well as 28-49 days with 7 days wash out period. The data were then pooled and the baseline versus endpoint values was also compared. **Result:** The mean blood hsCRP values did not significantly differ between the intervention and control groups at any time points. No significant difference between the baseline and end point values regarding blood hsCRP. The effect on blood hsCRP was not significant in any of the analysis. **Conclusion:** This study reveals no changes in chronic subclinical inflammatory status of *Aegle marmelos* fruit pulp in T2DM patients. [Journal of Current and Advance Medical Research, January 2021;8(1):17-20]

Keywords: Aegle marmelos; type 2 diabetes mellitus; chronic inflammatory; hsCRP

Correspondence: Dr. Murshida Aziz, Assistant Professor, Department of Biochemistry, Ibrahim Medical College, Segunbagicha, Dhaka, Bangladesh; **Email:** murshidaaziz@gmail.com; **Cell no.:** +8801817573694

Cite this article as: Aziz M, Debnath R, Ayub TE, Islam F, Aktar F, Aman S. Effect of Aegle Marmelos Fruit Pulp Powder on Chronic Subclinical Inflammatory Status of Type 2 Diabetic Patients. J Curr Adv Med Res 2021;8(1):17-20

Funding: This study has been performed without any funding from outside else.

Conflict of Interest: There was no conflict of interest to any of the authors

Contributions to authors: Aziz M, Debnath R, Ayub TE were involved in literature collection. Data collection and analysis were done by Aziz M, Debnath R, Ayub TE. Aziz M was involved in manuscript writing & revision of the manuscript.

Copyright: ©2021. Aziz et al. Published by Journal of Current and Advance Medical Research. This article is published under the Creative Commons CC BY-NC License (<https://creativecommons.org/licenses/by-nc/4.0/>). This license permits use, distribution and reproduction in any medium, provided the original work is properly cited, and is not used for commercial purposes.

Introduction

Diabetes mellitus is associated with significant morbidity due to related microvascular and macrovascular complications and diminished quality of life¹. International Diabetes Federation estimated in 2015 that 75% of people with diabetes live in low and middle income countries². Type 2 diabetes mellitus occurs due to a progressive defect in insulin secretion on the background of insulin resistance³.

Low-grade inflammations appear to be major assaults on insulin sensitivity in insulin-responding tissues⁴ which is also known to precede the development of type 2 diabetes. This association suggests that hsCRP may be a useful marker in type 2 diabetes prediction⁵. CRP, a sensitive marker of inflammation is independently related to insulin sensitivity⁶. Conventionally, type 1 Diabetes Mellitus (T1DM) is treated with exogenous insulin and T2DM with synthetic oral hypoglycemic agents like sulphonylureas and biguanide⁷. A substantial proportion of T2DM also requires insulin. However, the existing therapeutic agents have considerable limitations in the management of this complex disorder and search for alternate agents are continuing all over the world.

Plants are sources of multiple compounds with nutritional and medicinal value and WHO has recognized the importance of natural products in the prevention and management of diseases. The plant kingdom has become a target for multinational drug companies and research institutes for the discovery of new biologically active compounds and potential drugs⁸. Among traditional medicinal plants, *Aegle marmelos* (Bael in Bengali) has enormous traditional uses against various diseases. Traditionally, various parts of the plant, *Aegle marmelos*, are used for the treatment of a variety of disorders. *Aegle marmelos* originated in India and is presently growing in most of the countries of Southeast Asia⁹. The bioactive compounds isolated from these fruits were marmelosin, luvangetin, auraptin, psoralen, marmelide and tannin. The plant is reported to have multiple therapeutic properties such as anti-inflammatory, antipyretic and analgesic¹⁰⁻¹¹. Aqueous fruit extract (250mg/kg, twice daily for one month) produce anti-hyperglycemic effect along with decreasing glycosylated hemoglobin level in STZ induced diabetic albino wistar rats¹².

Although a number of studies in STZ and alloxan induced diabetic rat model were conducted with *Aegle marmelos* but no study had yet been done on

human with T2DM. In the above context, the present study was undertaken to explore the anti-inflammatory effects of *Aegle marmelos* unripe fruit pulp powder in patients with T2DM.

Methodology

This study was conducted in Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh from July 2010 to June 2011. It was an un-blinded clinical trial conducted under a cross-over design. Previously diagnosed thirty T2DM cases were included in the study. T2DM cases were enrolled from the out-patient department of BIRDEM. The socio-demographic data of all the participants were recorded in a pre-designed questionnaire. They were advised to take their usual diet, to do normal physical activities and to continue their prescribed drugs during the experimental period. Patients unwilling to give consent, insulin dependent DM, pregnant or lactating women, patients with cardiovascular, renal, hepatic, endocrine, metabolic disorders were excluded.

Preparation of *A. marmelos* fruit pulp powder:

Unripe fruits of *Aegle marmelos* were collected from specific area of Chapainawabgonj. Fruit pulps of *Aegle marmelos* (FPAM) were dried in sunlight for 5 to 6 days, coarsely powdered by grinder machine and stored in a dry cool place.

Study procedure: The fasting blood samples of all the study participants were collected at day zero for estimation of hsCRP. They were then divided randomly into Group A and B each consisting of 15 cases. First, one group (Group A) was given 7 gram of *Aegle marmelos* fruit pulp powder in one glass of water daily before breakfast for 21 days while another group (Group B) was on their usual diet for 21 days. The dose of 7 gram fruit pulp powder was determined based on earlier study. The fasting blood samples of both groups were collected on 21st day. After 7 days of wash out period, the fasting blood samples were again collected on the 28th day from both the groups. The groups were then crossed over and the cases who consumed *Aegle marmelos* pulp drink in the first 21 days started their usual diet and the other cases started to consume the *Aegle marmelos* pulp drink for the next 21 days. The final fasting blood sampling was collected on 49th day from both the groups. All blood samples were preserved at -20^o C until analyzed. Statistical analysis was performed using SPSS (Statistical Package for Social Science) software for Windows version-16 (SPSS Inc.,

Chicago, Illinois, USA). The data were expressed as proportion and mean±SD (standard deviation) as appropriate. The statistical significance of differences between the values were assessed by paired or unpaired student's t test as appropriate. Correlation analysis between the parameters was done by using Pearson's Correlation test.

Results

A total number of 30 T2DM cases were included in the study. The mean age of the study participants was 51.5±12.0 years. There were 17 males and 13 females. Majority were urban residents (Table 1).

Table 1: Socio-Demographic Characteristics of the Study Subjects (n=30)

Variable	Frequency	Percent
Age in years (Mean±SD)	51.5 ±12.0	
WHR (Mean±SD)	0.91 ±0.08	
Gender		
Male	17	57.0
Female	13	43.0
Education		
Illiterate	2	6.7
Primary	8	27.0
Secondary	10	33.0
Graduate	10	33.0
Occupation		
Service	14	47.0
Business	6	20.0
Housewife	10	33.0
Residence		
Urban	27	90.0
Rural	3	10.0

Values were expressed as Mean±SD or numbers and percentages as appropriate. WHR=Waist Hip Ratio

Table 2 shows the hsCRP levels in T2DM cases after 21 days of daily drink of *A. marmelos* fruit pulp compared to those who did not receive the intervention. No significant difference was found between the hsCRP levels of the intervention group compared to control group after 21 days of fruit pulp administration.

Table 2: Effect of Aegle marmelos on high sensitivity C-reactive protein (hsCRP) in T2DM subjects during 0-21 days (n=15 in each group)

Group	hsCRP level (mg/l) at		P value
	0 day	21 day	
Group A	2.76 ± 2.40	3.83 ± 1.11	0.92
Group B	1.56 ± 1.23	1.37 ± 1.32	0.37
t/p value	1.7/0.09	-0.56/0.58	

Unpaired t-test was done; Group A= Intervention Group; Group B= Control group

Similarly, table 3 shows the effects of Aegle marmelos fruit pulp powder on hsCRP levels of T2DM cases during 28 to 49 days' time period after cross over and 7 days of wash period. No significant difference of subclinical inflammatory status was observed between the intervention and control groups after 21 days of Aegle marmelos drink at day 49. There was no significant change of hsCRP levels of all cases (Group A and B) at base point (day zero) versus end point (day 49).

Table 3: Effect of A. marmelos on high sensitivity C-Reactive protein in T2DM subjects during 28 to 49 days (n=15 in each group)

Group	hsCRP level (mg/l) at		P Value
	28 day	49 day	
Group A	1.50 ± 0.90	1.70 ± 1.20	0.54
Group B	2.53 ± 1.54	2.40 ± 1.80	0.48
t/p value	-0.06/0.95	1.18/0.25	

Comparison between intervention and control groups on day 28 and day 49 were done by unpaired t-test (n=15). Values were expressed as Mean±SD. P<0.05 was considered as statistically significant, n= number of subjects; Group A= Intervention Group; Group B= Control group

Discussion

Numbers of parts of Aegle marmelos have been studied for the anti-diabetic properties in diabetic rat models. The present one was probably the first study in which a part of the plant was tested on human for both anti-diabetic and anti-inflammatory properties. The part chosen was the unripe fruit pulp of *Aegle marmelos* as this was the commonest part consumed by people as drink and prescribed by the traditional healers in Bangladesh. Recently another trial was done in prediabetic people with the same part of *A. marmelos* used in our study¹². In fact, a few commercial preparations of the pulp are now available in Bangladesh market with wide spectrum of therapeutic claims including for diabetes. Testing the efficacy and safety of the fruit pulp has thus public health impact.

The experiment was conducted under a crossover design and the effects were analyzed for 0-21 as well as 28 to 49 days (with 7 days wash out period) intervention. The data were then pooled and the baseline versus endpoint values was also compared. The effect on hsCRP level was not significant in any of the analysis. Though, studies^{9, 11} in animal showed significant anti-inflammatory effects of different parts of *A. marmelos* plant, but the present study did not reveal any such anti-inflammatory effect of *Aegle marmelos* unripe fruit pulp in T2DM patients. However, the result did not indicate

conclusively that *Aegle marmelos* fruit pulp had no changes in chronic subclinical inflammatory status as we could not do a dose response curve.

Conclusion

Significant differences in hs-CRP were not found between end-point and baseline between the groups at any time point. *Aegle marmelos* fruit pulp seems to have no anti-inflammatory effect, which needs further investigation.

References

1. Rahman S, Rahman T, A.A. Ismail, A.R. Rashid. 2007. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. *Diabetes Obes Metab.*, 9 (6), pp.767–780
2. International Diabetes Federation 2015, Annual report, 7th edition.
3. ADA, 2016 ‘Diagnosis and classification of diabetes.’ *Diabetes Care*; 39(Suppl.1):S13 S22 | DOI: 10.2337/dc16-S0. http://care.diabetesjournals.org/content/diacare/39/Supplement_1/S13.full.pdf
4. Hotamisligil GS, 2006, ‘Inflammation and metabolic disorders.’ *Nature*; 444:860-7.
5. American Diabetes Association-Diabetes Care, 2015, ‘Diagnosis and classification of diabetes mellitus.’
6. Rosac C. The pathophysiological basis of efficacy and clinical experience with new oral antidiabetic agents. *J of Diabetes and its Complications* 2002;16:123-132
7. Evans WC. *Trease and Evan’s Pharmacology*. London, UK: WB Saunders,1996
8. Parmar C, Kaushal MK. In: *Wild Fruits*. Kalyani Publishers, New Delhi, India,1982; 1
9. Arul V, Miyazaki S, Dhananjayan R . Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos*. *J Ethnopharmacol* 2005; 96:159-165
10. Shankarananth V, Balakrishnan N, Suresh D, Sureshpandian G, Edurin E, Sheej E . Analgesic activity of methanol extract of *Aegle marmelos* leaves. *Fitoterapia* 2007; 78: 258-259
11. Kamalakkanan N, Prince PSM, Rajadurai M. Effect of *Aegle marmelos* fruits on normal and streptozotocin induced diabetes Wistar rats. *J Med Food* 2003; 6:93-95.
12. https://www.researchgate.net/publication/328570079_Effect_of_Aegle_marmelos_Fruit_Pulp_Powder_on_Glycemic_Lipidemic_Inflammatory_and_Kidney_Function_Status_among_Prediabetic_Subjects