Effectiveness of *Brassica juncea* (mustard green) leaf decoction as an adjunct in the treatment of type 2 diabetes mellitus among Filipinos: a randomized clinical trial

Samantha Pauline G. Chio, Ma. Elaine D. Chua, Monica B. Coralde, Raphael Carlos P. Covar, Mariah Sandrine M. Dating, Janica Crissel Y. Francisco, Kryzta Kate V. Gabay, Bianca Marguerite DG. Gatbonton, Jose Jonathan F. Giron, Herald Jervy D. Go, Clarissa Isabel B. Hernandez, Tsung-Jen Hou, Monaflor Abigail G. Ignacio, Anna Victoria F. Ilagan, Remigio Jay-Ar Z. Butacan IV, MD[a]

Abstract

Introduction This study aimed to determine the effectiveness of mustard green leaf decoction as an adjunct to drug treatment in controlling blood glucose among Filipinos with type 2 diabetes mellitus.

Methods Participants were randomly assigned to receive mustard green decoction or a placebo solution for eight weeks on top of their oral anti-hyperglycemic medication. Fasting blood sugar and complete blood counts were determined at baseline, Week 4 and Week 8, and compared within and across the two groups.

Results There was a decreasing trend in the blood sugar level in the mustard green group while the opposite was noted in the placebo group. The mean FBS levels of the mustard green group were significantly lower than that of the placebo group at the Week 8 determination (6.10 vs 8.69 mmol/L, p = 0.004). The decrease in blood sugar level on the eighth week in the mustard green group was significant compared with the baseline level (p = 0.008).

Conclusion This study has demonstrated that the intake of *Brassica juncea* decoction can significantly decrease blood sugar levels among type 2 diabetics compared to metformin alone.

Keywords: Mustard green, *Brassica juncea*

Diabetes is one of the leading causes of morbidity among older adults. In the Philippines, it is estimated that 1 in 5 Filipinos is diabetic.[1] In a survey published by the Food and Nutrition Research Institute of the Philippines, the prevalence of high fasting blood sugar (FBS) based on the World Health Organization criteria of fasting blood glucose >125mg/dL for individuals older than 20 years old was 5.4%, which showed a 0.6% increase compared with a similar study in 2008.[1] Those living in the urban areas, and who were in the 60 to 69 years age bracket in both sexes showed the highest prevalence and was found among the richest in the wealth index. This tells us that there is an alarming upward trend in diabetes not only in the Philippines but also worldwide.[2]

Correspondence:
Remigio Jay-Ar Z. Butacan IV, MD, Department of Preventive and Community Medicine, College of Medicine, University of the East Ramon Magsaysay Memorial Medical Center Inc., 64 Aurora Boulevard, Barangay Doña Imelda, Quezon City, PH 1113; E-mail: rzbutacan@uerm.edu.ph

[a]Department of Preventive and Community Medicine, College of Medicine, University of the East Ramon Magsaysay Memorial Medical Center Inc., Quezon City, PH

The Organization of Diabetes Care in the Philippines has conducted lay fora in several settings wherein screening, education, and management are delivered. An example would be at the level of the barangay health center, the health worker must deliver diabetes self-management education, blood pressure monitoring, weight/BMI monitoring. At the level of the RHU/City or Provincial Health Office the diabetes clubs are established to encourage patients to learn from other patients. At all levels of the health care system, education and training are being given so health care workers will have the skills and materials needed for screening, management, and education.[3]

Currently, management of diabetic patients consists of blood sugar control, home blood sugar testing, HbA1C testing, cardiovascular risk control which includes lifestyle modifications, and intake of oral anti-hyperglycemic agent such as metformin, and insulin for advanced diabetes management.[4] In the data obtained from IMS Health in 2007 gathered from the retail sector indicate that the total consumption of oral hypoglycemic agents in the Philippines in 2007 was 114 million defined daily doses (DDD) which doubled from the 2000 figure of 50 million DDD. Four major oral anti-hyperglycemic agents are being taken by Filipinos (metformin, gliclazide, glimeprimide, and glibenclamide) which comprises 84% of DDD, while the consumption of insulin increased from 5 million DDD in 2000 to 12 million DDD in 2007.[5]

Since diabetes mellitus affects Filipinos across socio-demographic backgrounds, many have tried, and some are still constantly trying to find cheaper and more effective medications. *Brassica juncea* or mustard green is a widely known plant used as an oil source, and a green vegetable that has a lot of medicinal potential. The leaf extracts of *Brassica juncea* have been reported to exhibit antioxidant, anti-nociceptive, and anti-hyperglycemic properties both in vitro and in vivo.[4] The anti-hyperglycemic effect may be due to isothiocyanate, glycoside sinigrin, protein and fixed oil. These substances have been shown to increase activity of glycogen synthase and decrease glycogenolysis and gluconeogenesis, resulting in a decrease of glycogen phosphorylase and gluconeogenic enzymes.[13]

Different hematologic effects have been reported with the intake of *Brassica juncea*. One study showed that the administration of the seed extract may cause anemia, leukocytosis, and thrombocytopenia.[12] The main component of the seed extract that causes anemia, and leukocytosis is isothiocyanate, which is both present in the seed and leaf extracts.[12,15] Despite these side effects, *Brassica juncea* is considered to be generally safe by the Department of Health and has many beneficial properties such as antibacterial and antioxidant, and helps improve blood circulation.

According to the World Health Organization, the Philippines ranks 15th for diabetes prevalence. Considering the proportion at risk for diabetes mellitus, the researchers focused on the hypoglycemic properties of mustard green in lowering blood sugar levels for those with FBS ≥ 7.0 mmol/L.[7] The main objective of the study was to determine the effectiveness of mustard green leaf decoction as an adjunct to drug treatment in controlling blood glucose among Filipinos with type 2 diabetes mellitus. The specific objectives of the study were to determine the changes on the fasting blood sugar levels (mmol/L) of those taking mustard green leaf decoction as treatment adjunct compared to placebo and to measure the hematologic changes with regards to WBC, RBC, and platelets and its possible hematologic side effects. This study can benefit not only patients but also other practicing doctors as they could use this study in the future to further research on mustard green and its effects on diabetes, and they can also include this in managing their patients as this has the potential to be an adjunct in managing patients with type 2 diabetes mellitus.

Methods

The study was a randomized, double-blinded, clinical trial. A measured set of *Brassica juncea* decoction or a placebo solution consisting of FDA-approved food coloring were given to the participants to be taken for 8 weeks. Fasting blood sugar and CBC with platelet count were checked every 4 weeks and compared.

The study participants were diabetic residents of Taytay, Rizal identified from a list of patients obtained from the barangay health centers. The potential participants were identified, contacted, and those who replied favorably were screened. They were selected by purposive sampling. The participants were 40 to 59 years, had type 2 diabetes mellitus with FBS range of 7.0-12.0 mmol/L living in Taytay, Rizal, and diagnosed at least one year prior to the study.[7,10] Recent FBS results not

exceeding three months were used for the initial screening. In addition, potential participants must only be taking metformin 500 mg once a day as maintenance medicine. Those who were pregnant and/or lactating, had other chronic diseases, and/or had a history of stroke or myocardial infarction were excluded. Those who were anemic, and/or thrombocytopenic were also excluded from the study. Additional participants referred by the barangay health workers were also screened to meet the required sample size. The computed sample size for each group was 10 with level of significance set at 95% with 80% power.[9] Two additional participants per group were included to account for possible dropouts.

Those who were qualified were randomly allocated to either *Brassica juncea* decoction (experimental) or placebo (control) group using an online randomizer. Each participant was assigned a numerical code known only by the logistician. After obtaining informed consent, participants were asked to complete a profile sheet. Baseline CBC with platelet and FBS were taken before they were started on the decoction or placebo. The researchers were blinded accordingly: the logistician who was the only person who had access to everything and knew of the participants' assignment. The participants did not know which group they were in.

Mustard green (*Brassica juncea*) was procured from Balintawak Organic Market and from Good Foods Community. It was then verified by the Botany Division of the National Museum of the Philippines. The authenticated fresh leaves were carefully washed under running tap water and any foreign matter was completely removed. They were kept in a dry, well-ventilated place, protected from light and dried at room temperature for 7 days.[8] Once dried, they were carefully weighed and placed in a closed container for storage. The decoction was prepared weekly for eight weeks with a measurement of 2g/250 mL. A total of 348 grams dry weight was used to decoct 48 liters of mustard green decoction. Eight grams of dried leaves were placed in a stock pot containing 1 liter of water and heated until it boiled. The solution was cooled at room temperature. After cooling, it was strained and then transferred to clean 1L bottles, coded and kept refrigerated overnight at 4 °C.

The placebo decoction was prepared weekly as well. One liter of placebo decoction was prepared using 1 liter of distilled water mixed with 0.5 mL of FD & C Yellow No. 6, 0.1 mL of FD & C Yellow No. 5 and Blue No. 1 and 0.1 mL of FD & C Red No. 40 and Red No. 3. Each clear bottle was then coded and refrigerated overnight at 4°C.

The experimental group was given mustard green decoction while the control group was given placebo for eight weeks. They were given 4000 mL of mustard green decoction or placebo placed in four pieces of one-liter bottles that were refilled at each weekly visit. The participants were given a measuring cup for uniform intake. Participants were instructed to take approximately one cup (250 mL) of the assigned liquid after breakfast and dinner daily for eight weeks.[11]

FBS levels and CBC with platelet of both groups were measured after every 4 weeks. Common symptoms of possible side-effects were explained to the participants. They were instructed to immediately inform the researchers if any side effects were noted such as anemia, hypoglycemia, allergic reactions. Blood specimens such as CBC with platelets were analyzed using a Samsung LABGEOHC10 Hematology Analyzer at Evergreen Medical and Diagnostic Center, Inc.[12]

The data were recorded in MS Excel 2013 and constantly checked for any inconsistencies then analyzed using SPSS v. 23. The researchers used a paired t-test for comparing means within groups. An independent t-test was used in comparing means between treatment and control groups. Chi square was utilized to analyze proportions and distributions. ANOVA was also utilized to compare mean FBS levels of both groups within different points in the study (baseline, 4 weeks and 8 weeks).

This study was approved by the Ethics Review Committee of the UERMMMCI before it was implemented. Informed consent was obtained for every participant before the start of the assigned treatment. All participants were told of all the risks and benefits that they may encounter in the study. They were informed that there is a 50% chance of not receiving the mustard green decoction. Whatever information they would hand over to the researchers will be kept confidential. No personal information was published. Their identities were concealed, and each participant was given a corresponding unique code. The information was only accessible by the researchers and only used for the study.

Results

From the list of diabetic patients provided, 50 individuals were invited to join the study and 24 were enlisted. There were two dropouts each in the mustard green and placebo groups. The mustard green group was significantly older (p = 0.038), had more females (75.0% vs 58.3%) and more married participants (83.3% vs 58.3%), though the differences were not significant, as seen in Table 1. All the participants in the control group were non-smokers.

As seen in Table 2, the baseline FBS levels in the mustard green and placebo groups were comparable. There was a decreasing trend in the mustard green group while the opposite was noted in the placebo group. The mean FBS levels of the mustard green group were significantly lower than that of the placebo group at the eighth week determination (6.10 vs 8.69 mmol/L, p = 0.004). The decrease in blood sugar level on the eighth week in the mustard green group was significant compared with the baseline level (p = 0.008).

A decrease in the RBC count and increases in the WBC and platelet counts were seen in the mustard green group but the changes were not significant, as shown in Table 3. All values were still within normal limits. Increases in all three parameters were noted in the control group (Table 4), with all values within normal limits.

Discussion

The results of this study showed that mustard green, taken as an adjunct to metformin, was able to provide better blood glucose control than metformin alone. The significant decrease in the blood sugar levels of diabetic patients in this study, according to

Table 1. Sociodemographic profile of 24 participants.

Sociodemographic Information	Mustard Green Frequency (%)		Control Frequency (%)	p-value
Age (yr) [mean ± SD]	56.1 ± 4.44		50.00 ± 8.22	0.038
Sex		n (%)		0.386
Female	9 (75.0)		7 (58.3)	
Male	3 (25.0)		5 (41.7)	
Civil Status				0.178
Single	2 (16.7)		5 (41.7)	
Married	10 (83.3)		7 (58.3)	
Occupation				0.638
Self-employed	5 (41.7)		3 (25.0)	
Employed	4 (33.3)		6 (50.0)	
Housewife/husband	3 (25.0)		3 (25.0)	
Highest educational attainment				0.549
Attended elementary	1 (8.3)		0	
Elementary graduate	1 (8.3)		1 (8.3)	
Attended high school	2 (16.7)		0	
High school graduate	4 (33.3)		4 (33.3)	
Attended college	1 (8.3)		1 (8.3)	
College graduate	3 (25.0)		6 (50.0)	
Smoking				0.028
Yes	8 (83.3)		0	
No	4 (33.3)		12 (100.0)	
Coffee drinking (cups/day)				0.537
1	6 (50.0)		3 (25.0)	
2	4 (33.3)		2 (16.7)	
3	1 (8.3)		1 (8.3)	
None	1 (8.3)		2 (16.7)	

Table 2. Mean fasting blood sugar (mmol/L) per testing period for mustard green and placebo groups.

	Mustard green (Mean ± SD)	Control (Mean ± SD)	p-value (between groups)
Baseline (n=12)	8.32 ± 1.87	8.24 ± 1.90	0.918
Four weeks (n=10)	7.77 ± 2.03	8.54 ± 0.05	0.388
Eight weeks (n=10)	6.10 ± 0.65	8.69 ± 2.14	0.004
P-value (within groups)	0.008	0.638	

Table 3. Comparison of eighth week vs baseline complete blood count of mustard green group.

	Baseline (Mean ± SD)	Eight weeks (Mean ± SD)	p-value
RBC (x 10^9/L)	5.03 ± 0.28	4.96 ± 0.42	0.501
WBC (x 10^9/L)	8.12 ± 1.33	8.86 ± 1.37	0.050
Platelets (x 10^9/L)	256.80 ± 74.67	298.20 ± 60.95	0.077

Table 4. Comparison of eighth week vs baseline complete blood count of control group.

	Baseline (Mean ± SD)	Eight weeks (Mean ± SD)	p-value
RBC (x 10^9/L)	5.06 ± 0.38	5.37 ± 0.32	0.007
WBC (x 10^9/L)	6.61 ± 1.44	7.20 ± 0.96	0.234
Platelets (x 10^9/L)	306 ± 52.09	319 ± 54.88	0.259

previous studies, may be due to isothiocyanate, glycoside sinigrin, protein and fixed oil.[13] Another reason may be the increased secretion of insulin from the pancreas that leads to increased uptake of glucose or may be due to the inhibition of glucose from the gut to the bloodstream.[9] In terms of hematologic effects, it is stated that the causes of leukocytosis are isothiocyanate and nitric oxide which affect the bone marrow.[14] Other side effects such as anemia and thrombocytopenia were not seen in any of the participants, most probably because of the limited intake of the decoction. One particular study stated that intake of mustard green for 60 days will not have any adverse effects.

Type 2 diabetes mellitus has one of the highest prevalences among the non-communicable diseases in the Philippines. This study has demonstrated that the intake of *Brassica juncea* decoction can significantly decrease blood sugar levels type 2 diabetics compared to metformin alone. This study focused on determining the effect of mustard green as an adjunct therapy on lowering the blood sugar of the participants diagnosed with type 2 diabetes mellitus and maintained on metformin alone. The results of this study may not apply to diabetic patients with other comorbidities or those taking other medications. Measurement of changes in blood sugar levels was limited to the use of FBS. The results of the study add to the evidence of the potential of mustard green as an effective adjunct therapy for type 2 diabetes mellitus. Further investigation into its beneficial effects under different circumstances can help in establishing mustard green as a viable alternative in supplementing diabetes therapy. It is of benefit if the specific active component of mustard green can be identified as it can result in the development of new diabetes drugs in the future. For the patients, the positive results can be encouraging as mustard green is readily available in the local market and can provide affordable dietary supplements for those trying to control their blood sugar levels.

Acknowledgements

The researchers would like to thank all barangay health workers in Taytay for helping gather the participants, as well as the diabetic patients who participated in their study.

References

1. Magtubo CA. Philippines' diabetes profile. MIMS Today [Internet]. 2016 Sep 26 [cited 2017 Nov 26]. Available from: https:/ /today.mims.com/philippines-diabetes-profile
2. Tan G. Diabetes care in the Philippines [Internet]. 2015 Nov-Dec; 81:(6), 863-9. Available from: http:// www.annals ofglobalhealth.org/article/S2214-9996(15) 01264-3/abstract
3. UNITE for Diabetes Philippines. Compendium of Philippine Medicine [Internet]. 2014 [cited 2017 Nov 26]. Available from: http://endo-society.org.ph/v5/wp-content/uploads/2013/06/Diabetes-United-for-Diabetes-Phil.pdf
4. David MK. Patient education: diabetes mellitus type 2: treatment (beyond the basics) [Internet]. Diabetes mellitus type 2: Treatment. UptoDate; 2017 [cited 2017 Nov 26]. Available from: https://www.upto date.com / contents/diabetes-mellitus-type-2-treatment-beyond-the-basics
5. Higuchi M. Costs, availability and affordability of diabetes care in the Philippines [Internet]. Foundation of Advanced Studies on International Development; 2009 [cited 2017 Nov 26]. Available from: http:// citeseerx.ist.psu.edu/viewdoc/down load? doi=10.1.1. 176.6958&rep=rep1&type=pdf
6. ADA Diabetes Management Guidelines A1C Diagnosis [Internet]. National Diabetes Education Initiative. 2016 [cited 2017 Jan 12]. Available from: http://www.ndei.org/ADA-2013-Guidelines-Criteria-Diabetes-Diagnosis.aspx.html
7. Vinagre AS, Della Santa Rubio A, Rönnau O, Pereira SF, et al. Anti-diabetic effects of *Campomanesia xanthocarpa* (berg) leaf decoction. Braz J Pharmaceut Sci 2010 Apr-Jun; 46(2): 2.
8. Rahmatullah M, Shefa TF, Hasan L, Hossain T, Ahmed S, Mamun AA, Islam R, Rahman S, Chowdhury MH. A Study on antinociceptive and anti-hyperglycemic activity of methanol extract of Brassica juncea (L.) Czern. leaves in mice. Adv Nat Appl Sci 2010; 4(3): 221-5.
9. Diagnosing diabetes and learning about prediabetes [Internet]. Arlington VA: American Diabetes Association; c1995-2016. Available from: http:// www.diabe tes.org/diabetes-basics/diagnosis.
10. Kitalong C, Nogueira RC, Benichou J, et al. "DAK", a traditional decoction in Palau, as adjuvant for patients with insufficient control of diabetes mellitus type II. J Ethnopharmacol [Internet]. 2017 Jun [cited 2017 Oct 12]; 205: 116-22. Available from: https:// www.antenna.ch/ wp-content/uploads/2017/04/ kitalong-et-al-017.pdf
11. Mishra SB, Rao CV, Ojha SK, Vijayakumar M, Verma A, Alok S. An analytical review of plants for anti-diabetic activity with their phytoconstituent and mechanism of action. Int J Pharmaceut Sci Res 2009; 715(3): 29-46.
12. Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard green): mechanism of action. Indian J Biochem Biophys 1995; 32(2): 106-8.
13. Ufelle SA, Ukaefiofo EO, Naboh EE, et al. The effects of crude methanol seed extract of *Brassica juncea* on haematological parameters in Wistar rats. Br J Pharmacol Toxicol 2011; 2(3): 123-6.
14. Singh U, Singh S, Kochhar A. Therapeutic potential of antidiabetic nutraceuticals. Phytopharmacology 2012; 2(1): 144-69.