ANTI-HYPERGLYCAEMIC EFFECT OF MODIFIED TMF-17 ON TYPE 2 DIABETES MELLITUS PATIENTS

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INTRODUCTION

- Diabetes mellitus is an important public health problem. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades[1].
- The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014.

- World health organization estimated that, in the year 2000, 171 million people worldwide had diabetes, and it expected to double by the year 2030[1].
- In Myanmar, diabetes is one of the six major diseases of morbidity. The international diabetes federation (IDF) has estimated the national prevalence of diabetes in Myanmar as 6.5% adults (aged 20-79 years)[2].

- The WHO estimates that more than 80% of the world's population rely either solely or largely on traditional remedies for health care[3].
- Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost[4].

- In Myanmar Traditional Medicine, Tin-Tin-Wynn *et al.*, (2010) indicated that TMF-28 has blood glucose lowering effect on type 2 diabetes mellitus patients[5].
- Win-Myint (2012) also approved that combination of TMF-27 and TMF-35 has anti-hyperglycaemic effect on type 2 diabetes mellitus patients[6].

- TMF-17 has been used by Myanmar traditional medicine practitioners for a long time without adverse effects.
- In Myanmar traditional medicine, hot, bitter and pungent plants and medicines are considered as to have more effective for diabetes. TMF-17 has hot, bitter and pungent tastes[7].

- In 2012, Khin-Moe-Aung *et al.*, studied the blood glucose lowering effect of modified TMF-17 on adrenalin induced diabetes rats[8].
- Although TMF-17 is a popular drug used in traditional medicine clinics and hospitals, there is no clinical study for its anti-hyperglycaemic effect.
- TMF-17 is composed of sixteen plant ingredients, three chemicals and one animal product.

- Two chemical ingredients of TMF-17, camphor and nagi camphor have some side effects such as respiratory and chest problems, lip dryness, rashes, eczema, hepatotoxicity and seizure[9].
- For the long-term used of diabetes patients, these two chemicals were removed in this study.
- Therefore, this research was carried out to determine the anti-hyperglycemic effect of modified TMF-17 on type 2 diabetes mellitus patients.

OBJECTIVES

General objective

- To determine the anti-hyperglycaemic effect of modified TMF-17 on type 2 diabetes mellitus patients

Specific objectives

1. To prepare modified TMF-17
2. To determine the physicochemical and phytochemical properties of modified TMF-17
3. To determine the quality control of modified TMF-17 tablet form
4. To determine the daily FBS and 2HPP blood glucose level and to compare the FBS and 2HPP blood glucose level of type 2 diabetes mellitus patients before and after intervention

MATERIALS AND METHODS

A hospital based clinical trial was carried out in 100 Bedded Traditional Medicine Teaching Hospital, Mandalay from 1st July 2017 to 30th June 2018.

Materials

- Ingredients of modified TMF-17
- Powder making machine
- Granulator
- Tableting machine

- Friability tester
- Hardness tester
- Disintegration tester
- Electronic balance
- 3cc sterilized syringes and needles
- Spirit, swab and plaster
- Collection tubes
- Blood pressure measurement cuff
- Glucometer

Methods

Preparation of modified TMF-17 drug

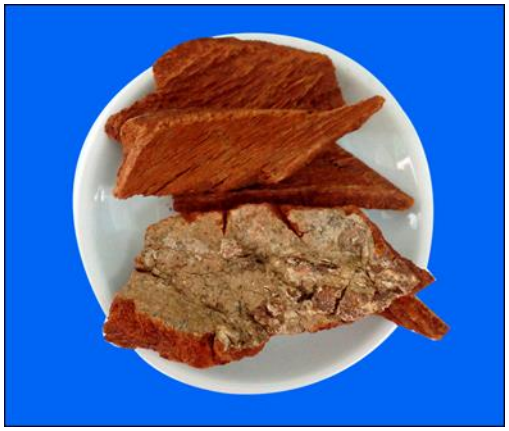
No	Myanmar Name	Scientific or Botanical Name	Weight			Percentage
			Kyat	*Pe*	Gram	
	Plant ingredients					
1.	Zardeikpho thee	*Mysterica fragrans* Houtt.	1		16 g	5.56 %
2.	Lay-hnyin	*Syzygium aromaticum* (L.) Merr. & Perry	1		16 g	5.56 %
3.	Kyasu	*Terminalia citrina* (Gaertn.)Roxb.	1		16 g	5.56 %
4.	Pannu	*Saussurea affinis* Spreng.	1		16 g	5.56 %
5.	Pan ma	*Anneslea fragrans* Wall.	1		16 g	5.56 %
6.	Kattara-thinche	*Terminalia chebula* Retz.	1		16 g	5.56 %
7.	Nantha-ni	*Pterocarpus santalinus* L.	1		16 g	5.56 %
8.	Nantha-hpyu	*Santalum album* L.	1		16 g	5.56 %

No	Myanmar Name	Scientific or Botanical Name	Weight			Percentage
			Kyat	*Pe*	Gram	
	Plant ingredients					
9.	Pharlarngei	*Elettria cardamomum* (L.) Maton	1		16 g	5.56 %
10.	Kat-pho	*Myrica nagi* Thumb.	1		16 g	5.56 %
11.	New-gyo	*Glycyrrhiza glabra* L.	1		16 g	5.56 %
12.	Saung-may-ga	*Picrorhiza kurroa* Royle.	1		16 g	5.56 %
13.	Hsay-pazun-doke	*Plectranthus amboinicus* (Lour.) Spreng.	1		16 g	5.56 %
14.	Padonma-kyar wutsan	*Nelumbo nucifera* Gaertn.	1		16 g	5.56 %
15.	Kharthee-san	*Nelumbo nucifera* Gaertn.	1		16 g	5.56 %
16.	Gangaw wutsan	*Mesua ferrea* L.	1		16 g	5.56 %
	Chemical					
17.	Theindaw	Natural sodium chloride	1		16 g	5.56 %
	Animal product					
18.	Kyew thwe	*Bubalus bubalis* L.	1		16 g	5.56 %
		Total	18		288g	100 %

Ingredients of Modified TMF-17

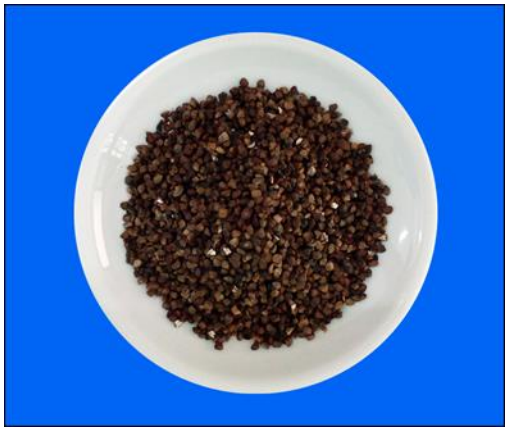







Ingredients of Modified TMF-17

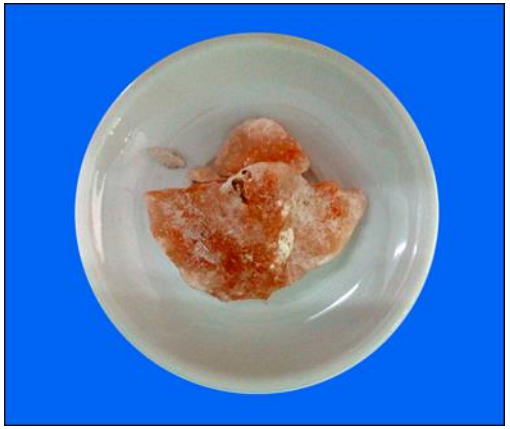





Ingredients of Modified TMF-17








- The ingredients of modified TMF-17 were collected from crude drug market.
- The plant materials and animal product were carefully washed to remove dust and any foreign matters and were air dried.
- The ingredients were powdered with an electric grinder.

Physicochemical properties of modified TMF-17

- The physicochemical properties of modified TMF-17 were analyzed by the methods of WHO (2011)[10].

Preliminary phytochemical analysis of modified TMF-17

- The preliminary phytochemical constituents of modified TMF-17 was carried out at Research Division, University of Traditional Medicine, Mandalay by the methods of Harbone (1998)[11] and Ramman (2006)[12].

Preparation of tablet of modified TMF-17

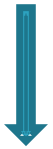
- To develop the dried powder of modified TMF-17 as the tablet dosage form, the granulator and the tableting machine (ZP 33 B rotary tablet press machine) were used.
- The tablet formulation was developed by wet granulation technique using tapioca starch and made into 300 mg tablets in average weight at traditional medicine factory, Mandalay.
- The tablets were kept in a tightly closed bottle at the room temperature until used for testing.

Evaluation of Tablet Dosage Form

- In determining the quality of tablet dosage form, modified TMF-17 tablets were prepared into the average weight of 300 mg per tablet.
- It was needed to test uniformity of weight test, disintegration test, hardness, friability, thickness and diameter of the tablets.
- These tests were analyzed at the Department of Pharmaceutics, University of Pharmacy, Mandalay.

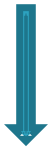
Flow diagram of preparing of modified TMF-17 tablet dosage form

The raw ingredients of modified TMF-17



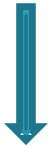
Making powder

Powder of modified TMF-17



Tableting

Tablets of modified TMF-17



Evaluation of tablets of modified TMF-17

Qualified modified TMF-17 to administer the participants

Modified TMF-17 powder and tablets

Determination of the effect of modified TMF-17 on blood glucose level on type 2 diabetes mellitus patients

- The participants were chosen by the inclusion and exclusion criteria. Informed consent was given to all the participants before the study.
- After washing out for 72 hours, the patients were administered 7 tablets (300 mg/tablet) three times per day before the meal.
- The patients were advised to follow the dietary control during the intervention.

- All patients were checked baseline ECG, urea and creatinine.
- The FBS and 2HPP were examined on day 0 at the laboratory of Clinical Pathology Department of MGH.
- The study period was completed on 21 days and the daily assessments of the FBS and 2HPP blood glucose level on all patients were done by using glucometer to prevent the hypoglycaemic conditions.

Inclusion Criteria

- Subjects were selected according to WHO definition.
- Type 2 diabetes mellitus patients with symptoms of hyperglycemia
- Age - between 40-60 years
- Sex - both male and female
- FBS - ≥ 126 mg/dL (7.0 mmol/L)[13]
- 2HPP - ≥ 200 mg/dL (11.1 mmol/L)[13]

Exclusion Criteria

1. Patients with complicated type 2 diabetes mellitus
2. Diabetes known history of more than 10 years[14]
3. Patients who are receiving corticosteroid therapy
4. Pregnant or lactating mother

Statistical Analysis

- Data were analyzed by SPSS version 21.
- For statistical analysis, paired t test was applied for comparison of blood glucose levels before and after treatment.

FINDINGS AND DISCUSSIONS

- The aim of this study was to determine the anti-hyperglycaemic effect of modified TMF-17.
- Modified TMF-17 was the removal of camphor and nagi camphor from the original TMF-17 and *Bubalus bubalis* was substituted for *Rhinocerous unicornis*.

- The formulation was modified not only formula but also tablet dosage. The modified formula can improve patient's compliance and tablet dosage form.
- It was to under taken the anti-hyperglycaemic effect of modified TMF-17 on 22 numbers of patients with type 2 diabetes mellitus admitted to 100 bedded Traditional Medicine Teaching Hospital, Mandalay.

- Ash values are helpful in determining the quality and purity of the crude drugs in powder form.
- Quantity determined percentage of total ash in this study was 7.85%.
- Acid insoluble and water soluble ashes are the part of total ash content and contained 2.1% and 95.9% respectively.

- The acceptable pH levels set for the medicinal plants range from 4.0% - 7.5%[15].
- The pH values of the test drug are 1% pH solution (5.03%) and 10% herbal solution (4.83%) respectively.
- Therefore, modified TMF-17 was used as phytomedicine for the treatment of diabetes mellitus.

- Moisture is one of the major factors responsible for the deterioration of drugs and herbal formulations.
- The moisture promotes the degradation process caused by enzymes, development of micro organisms, oxidation and hydrolysis reactions.
- In this study, the moisture content of the test drug was 12.176% which is deemed to be good as water content in herbal drugs should not be greater than 14% [16].

- On assessing the extractable matters, modified TMF-17 was soluble in water, methanol, ethanol, ethyl acetate and petroleum ether (21.60%, 22.40%, 12.8%, 4% and 2.4% respectively).
- Therefore, modified TMF-17 was more soluble in methanol.

- In modified TMF-17, the rich presence of alkaloids, flavonoids, glycosides, terpenoids, polyphenols, phenolic compound and tannins were found.
- Carbohydrates were a little present.
- The rich constituents can give more effects than normal constituents.

- Saponin, amino acids, starch, cyanogenetic and cardiac glycosides were absent.
- On assessing acid (or) base (or) neutral, it was acid in nature. According to these findings, modified TMF-17 may also have anti-oxidant, anti-inflammatory, anti-cancer and anti-ulcer activities.

- In evaluation of the tablets, random tablets were tested for uniformity of weight and (Mean ± SD) was 300.2 ± 6.4 mg.
- All tablets were disintegrated in 22.94 ± 4.9 min (Mean ± SD).
- Mean ± SD of hardness was 20.81 ± 2.5 (N), the mean friability (Mean ± SD) was 0.56 ± 01%.
- The mean thickness (Mean ± SD) was 5.24 ± 0.3 mm and the mean diameter (Mean ± SD) was 10.60 ± 0.5 mm.

- On reviewing the outcome measures, the FBS level was 235.86 ± 55.5 mg/dL (Mean ± SD) and serum glucose level of 2HPP was 370.22 ± 66.5 (Mean ± SD) mg/dL before the intervention of modified TMF-17 tablets on day-0.
- During the intervention of modified TMF-17 tablets on day-7, 14.76% of mean FBS level and 16.18% of mean 2HPP level was reduced.

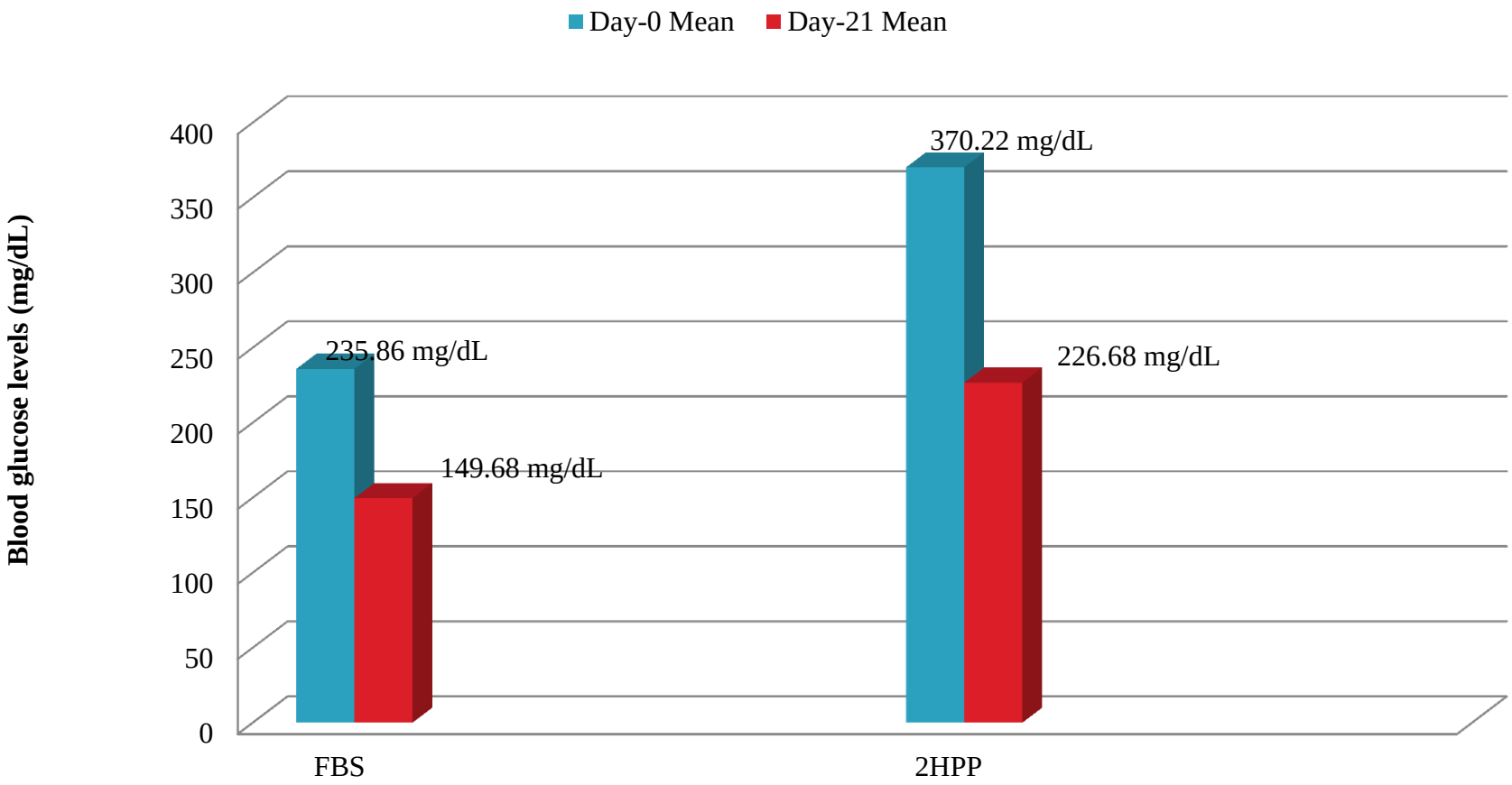
- During the intervention of modified TMF-17 tablets on day-14, 26.49% of mean FBS level and 27.69% of mean 2HPP level was reduced.
- After the intervention of modified TMF-17 tablets on day-21, the FBS level (Mean ± SD) was significantly reduced from 235.86 ± 55.5 to 149.68 ± 21.9 mg/dL (p<0.0001) and 36.53% of mean FBS level was reduced.

- In 2HPP, the mean blood glucose level (Mean ± SD) was also decreased from 370.22 ± 66.5 to 226.68 ± 63.3 mg/dL (p<0.0001) and 38.77% of mean 2HPP level was reduced.
- Therefore, the serum glucose level in both FBS and 2HPP was significantly reduced after intervention in comparison with before.

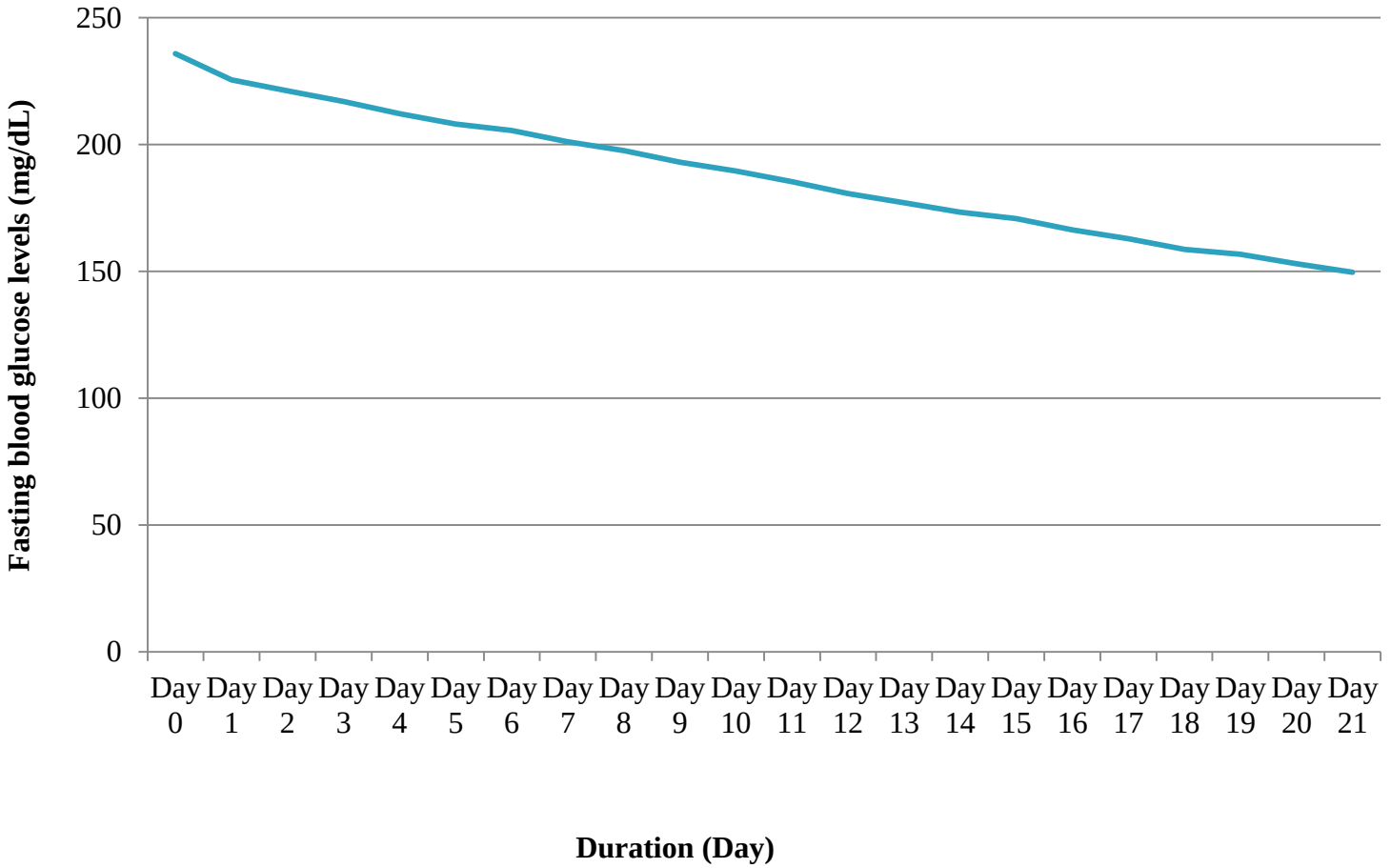
Comparison of mean FBS and 2HPP blood glucose levels before the intervention (at day-0) and after the intervention (at day-21)

No	Blood Sugar Level (mg/dL)	Day-0	Day-21	*p* value	Percent relief (%)
		Mean ± SD	Mean ± SD		
1	FBS	235.86 ± 55.5	149.68 ± 21.9	<0.0001	36.53%
2	2HPP	370.22 ± 66.5	226.68 ± 63.3	<0.0001	38.77%

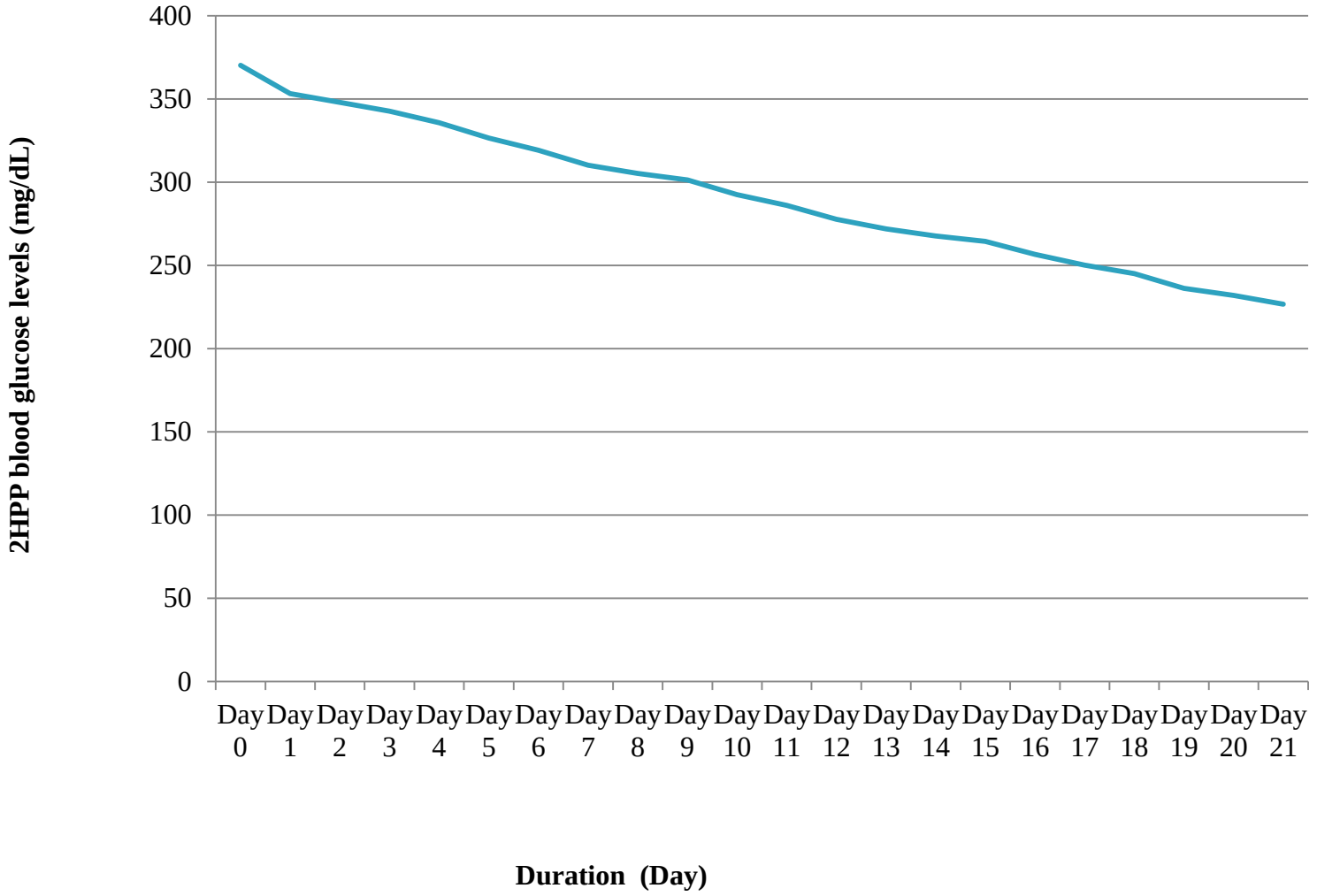
Comparison of mean FBS and 2HPP blood glucose levels before the intervention (at day-0) and after the intervention (at day-21)



Reduction of fasting blood glucose levels in all patients



Reduction of 2HPP blood glucose levels in all patients



- In this study, modified TMF-17 reduced the blood glucose level ($p<0.0001$) in type 2 diabetes mellitus patients.
- Therefore, it was clinically evidenced that modified TMF-17 could reduce significantly the serum glucose level in type 2 diabetes mellitus patients.

CONCLUSION AND SUGGESTIONS

- The purpose of this study was to prove that traditional medicine formulations are also effective in curing the diseases.
- It is important for diabetes patients to monitor their blood glucose concentrations which may obviously fluctuate from time to time due to various factors such as daily activities, mental status, diet components and environment changes.

- In 2015, Adnan *et al*. studied that the mean difference of 0.84 mmol/L between the venous and capillary blood glucose levels were not significant at glucose levels near normal[17].
- In 2015, Patel & Patel also mentioned that capillary blood glucose estimation by glucometer is a better alternative to venous plasma glucose estimation for diagnosis[18].

- Therefore, venous blood glucose levels before the intervention of modified TMF-17 was compared with the capillary blood glucose levels of patients after the intervention of modified TMF-17.
- Quality control of tablet such as uniformity of weight test, disintegration test, hardness and friability test, diameter and thickness of the tablets are complied the reference standard by followed in quality control of the good manufacturing products.

- In conclusion, this study shows that modified TMF-17 has significant anti-hyperglycaemic effect on type 2 diabetes mellitus patients and there are no any adverse effects on the patients during this study.
- And, it can also provide a traditional medicine formula which can be used safely and easily available alternative way for the management of type 2 diabetes mellitus patients.

Suggestions

- A large clinical trial and or long term study should be carried out to evaluate the serial changes of anti-hyperglycemic activity of modified TMF-17.
- The extract of modified TMF-17 should be formulated.
- Some possible traditional medicine formulations for systematic study of respective disease should be done with appropriate sample size.

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