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EXPERIMENTAL PAPER

Preliminary studies of Volten VR4® *Kaempferia parviflora* herb extracts on blood glucose levels in human type-2 diabetes mellitus and its mineral element analysis

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Summary

Introduction: Volten VR4® capsules containing herb extract of *Kaempferia parviflora* has been claimed to reduce blood glucose in patients with diabetes.

Objectives: This preliminary study is conducted to evaluate the efficacy of Volten VR4® on healthy individuals and type-2 diabetes mellitus volunteers. The extracts of 400 mg capsules of *Kaempferia parviflora* (KP) were used to measure the blood glucose level of 2-hour postprandial.

Methods: The healthy group consists of 15 young adults aged 20-30 with no history of serious diseases, while the diabetic group includes 12 individuals aged 35-75 diagnosed with type-2 diabetes mellitus. Data were validated through the Willcoxon and Friedman test statistics and error distribution. The investigation was continued to trace the capsules contents of elements using inductively coupled plasma optical emission spectrometry (ICP-OES) techniques.

Results: It has been shown that KP reducing blood sugar levels has been associated with flavonoids and methoxyflavones components. The result specifically showed that consuming VR4® capsules can significantly reduce blood glucose, either at the state of fasting or postprandially. In the study the content of mineral and heavy metal elements in VR4® capsules has been evaluated.

Conclusion: Volten VR4® *Kaempferia parviflora* extract is safe to be consumed at a single dose of 400 mg. The study also has shown that the participants are free from adverse reactions and hypoglycaemia.

Key words: ***Kaempferia parviflora, blood glucose, type-2 diabetes mellitus, flavonoids***

Słowa kluczowe: ***Kaempferia parviflora, stężenie glukozy we krwi, cukrzyca typu 2, flawonoidy***

INTRODUCTION

The prevalence of diabetes mellitus as a major health problem is reported to increase worldwide. The World Health Organisation (WHO) has predicted that number of diabetic patients will rise from 415 million in 2015 to 642 million in 2040. In Malaysia, the Ministry of Health reported that in 2019 around 4 million people have been diagnosed with type-2 diabetes mellitus and another 80,000 have been diagnosed with type-1 diabetes mellitus which is accumulated at 2% of total diabetes patients [1]. According to Hasan *et al.*, 64.9% of 230 patients with type 2 diabetes mellitus were using complementary and alternative medicines frequently. Their studies correlate with the standard on policy and guidelines of traditional and complementary medicine set by Malaysian Medical Council, Ministry of Health (MoH) [2]. Currently, the MoH has acknowledged eight plants as an alternative medicine to Malaysians diagnosed with diabetes mellitus. The herbal plants are *Orthosiphon stamineus, Centella asiatica, Andrographis paniculate, Momordica charantia, Ficus deltoidei, Morinda citrifolia, Gymnema sylvestre* and *Trigonella foenum-graecum* [3-6]. These medicinal plants are native to Southeast Asian countries including Malaysia that have tropical rainforests.

In 2004, Yenjai and his team have presented that *Kaempferia parviflora* (KP) contained bioactive flavonoids. They have found and described nine flavonoids in KP, a herbaceous plant belonging to the family *Zingiberaceae*, growing wildely in Loei, Phitsanulok and Phetchabun provinces in Northern Thailand [7, 8, 9]. At the same time, the *Zingiberaceae* family of ginger (zanjabil) has been mentioned in the Holy Quran. The ginger or *zanjabil* (in Arabic) has the potential to act as an antioxidant and anticancer due to the richness of its chemical compound known as zanjabil in Arabic [10]. Moreover, it was present along with gingerol compounds such zingerone, zerumbone, paradol, shogoal, terpenoids and ginger flavonoids [11]. Asamenew *et al.* in 2019 reported that the KP and ginger both have similar morphological characteristics of rhizome. They concluded that KP has a higher concentration of methoxyflavones applying advanced biosynthetic pathway methods [12].

It has been shown that KP, which is rich in flavonoids and methoxyflavone components, can be potentially identified as alternative medicine to control blood sugar [13]. The studies involving human samples testing the efficiency of KP and identifying specifics blood glucose will provide new data on the value of KP. KP has the potential to benefit from natural flavonoid substances that represent lead compounds with a diversity of molecular structure and biological activity, in relation to antidiabetic drug discovery process [14]. Diabetic wounds both in humans and animals have shown a deregulated inflammatory response as well as reduced neovascularisation, compared to non-diabetic subjects and control animals, as reported by Caskey and Liechty [15].

MATERIAL AND METHODS

Human subjects and methods

The study population consisted of two groups of volunteers. Group 1 consisted of 15 healthy young adult male and female volunteers aged 20-30 who received experimental products to see the effect on glucose in healthy individuals. Group 2 were patients with type 2 diabetes mellitus (T2DM) aged 35-75 who received the experimental products for five days. The recruited participants come from Selangor area, a central state on west coast of Malaysia that include Gombak, Ampang, Kajang and Bangi. The inclusion criteria were being a healthy individual measured by a health assessment questionnaire conducted by the investigators and assessment report by the authorised medical officer. All volunteers responded to the questionnaires that included history of hypertension, diabetes mellitus, heart disease, obesity, allergy and if they are on any medication or herbal supplement. On the other hand, exclusion criteria also include individuals with a history of major surgery, type 1 diabetes mellitus or any recent infection.

Blood glucose management

Blood glucose levels were monitored using an Accu-Chek Performa NC Model (Roche, Mannheim, Germany) blood glucose meter device and their Accu Chek test strips (Roche Diabetes Care, Australia). The reliability of the test kits has been calibrated and verified. The lancet of Accu Chek Safe-T Pro Plus with alcohol swabs were applied during the finger prick procedures.

The VR4® capsules were supplied by Volten Asiaemall Sdn Bhd located at the No.1 Jalan Tukul N15/5, Section 15, 40100 Shah Alam, Selangor, Malaysia. The 200 mg KP dry powder capsules were extracted from raw KP rhizome. The ground KP rhizome was extracted with deionised water at the temperature of 60°C, solvent to solid ratio of 10:1 (weight/weight). Thus, a 200 mg capsule is equivalent to 2,000 mg KP rhizome. The KP capsule shell is made from soft vegetable material. VR4® capsules product has been approved by the Ministry of Health Malaysia with registration number MAL20086115TC. The participants were scheduled to consume the capsule every night for five days after they have measured their baseline of blood glucose level three days prior to the consumption. The KP rhizome capsule extract contains active components of methoxyflavones phytochemical: 5,7-dimethoxyflavone (DMF), 5,7,4'-trimethoxyflavone (TMF), and 3,5,7,3',4'-pentamethoxyflavone (PMF) [16].

Procedure

This preliminary study has been approved by the Research Ethics Committee of University of Kuala Lumpur (reference number UNIKL REC/2020/004). The consent was obtained from all participants after discussing their recent conditions. Two stages of finger prick data collections from the participants

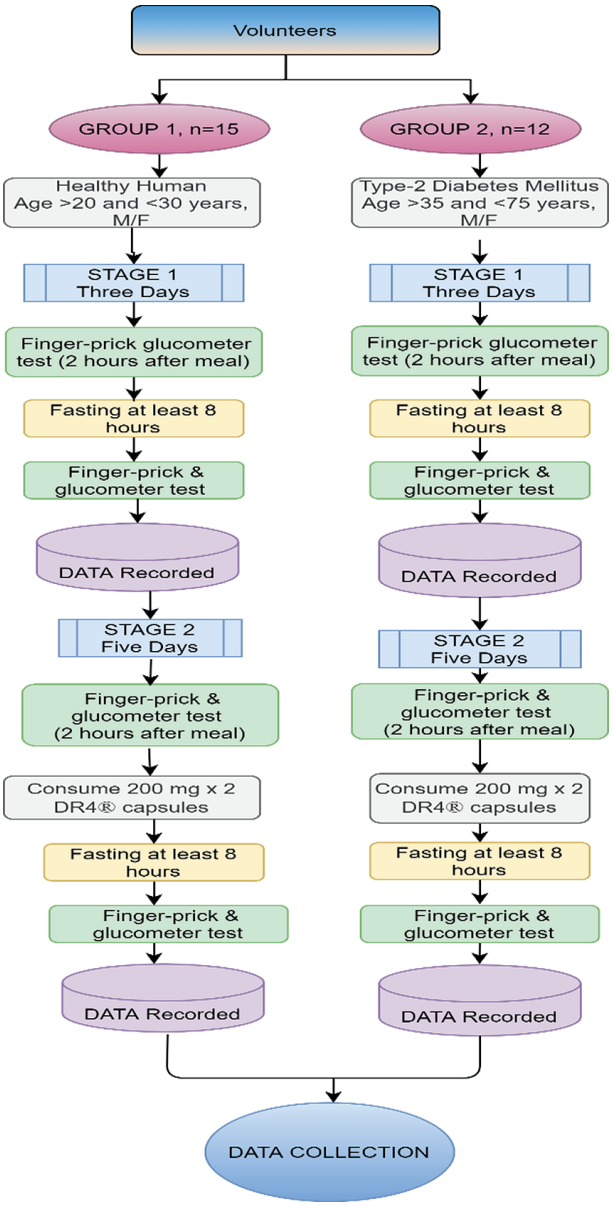

Figure 1.
Finger prick procedures

were obtained (fig. 1). Stage 1 procedures were run for 3 days of blood glucose meter reading as a baseline of the study. An average of 3 days of blood sugar was taken as baseline. Then, at the stage 2, the finger pricks were performed twice a day (fasting and 2 hours postprandial) while consuming the VR4® capsule 400 mg every night (ON) for five days. The postprandial blood glucose was taken before the consumption of the VR4® capsule. The healthy and T2DM participants' daily glucose readings were monitored for 3 days in advanced and 5 consecutive days when the KP is consumed. All volunteers started the finger prick at night 2 hours after meal and measured again after 8 hours of fasting in the morning. The data collected from Accu-Chek glucometer were recorded and plotted using OriginLab software.

ICP-MS experimental

The characterisation of VR4® capsules was performed using the inductively coupled plasma optical emission spectrometry (ICP-OES) method. The sample digestion and ICP-OES procedure were conducted at Material Characterization Laboratory (MCL), Department of Chemical, Environmental Engineering Faculty of Engineering, Universiti Putra, Malaysia. The ICP-OES Optima 7300 DV (Perkin Elmer, Inc. Shelton, USA) was calibrated and maintained as scheduled.

The digestion procedure of 5 mg VR4® capsule powder sample started with adding 5 ml of 65% HNO_3. The sample was mixed and boiled gently over a water bath until a clear solution was obtained. Then, the solution was added with another 65% of nitric acid (HNO_3) and additional heat until total digestion is achieved. The final solution was allowed to cool until it reaches room temperature of 26°C. The VR4® solution was filtered into a 100 ml volumetric flask using Whatman filter paper. Subsequently, the volumetric flask was added with deionized water and shaken for 15 minutes.

The ICP-OES analysis of targeted element and optical emission spectrometry parameters are presented in table 1. The use of multielement methods of ICP-OES instrument provides high efficacy, effective and less contamination procedure for determining the nutritive characterization of the KP extracts. The experiment was repeated for three times to get high precision and accuracy.

Table 1.

Optical spectrometry parameters

Elements	Detection wavelength [nm]	Sample concentrations unit [mg/kg]	Relative standard deviation [%]
Ag	328.068	0.000	0.0004
Al	396.153	0.012	0.0113
As	188.979	0.001	0.0059
B	249.677	0.042	0.0012
Ba	233.527	0.000	0.0002
Be	313.107	DL	0.0001
Bi	223.061	0.002	0.0033
Ca	317.933	0.237	0.0029
Cd	228.802	0.002	0.0002
Co	228.616	0.001	0.0003
Cr	267.716	DL	0.0004
Cu	327.393	0.001	0.0015
Fe	238.204	0.072	0.0015
Ga	417.206	0.005	0.0008
K	766.490	48.22	0.573
Li	670.784	0.020	0.0000
Mg	285.213	6.112	0.0435
Mn	257.610	0.290	0.0050
Mo	202.031	0.004	0.0002
Na	589.592	0.177	0.0023
Ni	231.604	0.000	0.0000
Pb	220.353	0.000	0.0014
Rb	780.026	0.027	0.0297
Se	196.026	0.064	0.0073
Sr	407.771	DL	0.0007
Te	214.281	0.002	0.0021
U	385.958	0.005	0.0015
V	290.880	0.001	0.0019
Zn	206.200	0.020	0.0015

*DL - detection limit

RESULTS

Study on blood glucose level

Data are presented as trends in blood glucose levels to fluctuate in high and low data. The primary outcome measure was blood glucose pattern and trend management. The data were analysed using the IBM SPSS Version 26 software. In healthy individual groups, consuming VR4® for five days did not cause an episode of hypoglycaemia or any adverse reaction. The product is well tolerated. As

the product is taken once a day, we did not see any significant drop in blood glucose among healthy individuals at fasting glucose after taking it for five days.

Table 2 shows a significant drop in postprandial blood glucose among healthy individuals after consuming VR4® for five days (5.93 mmol/l (5.7, 6.1) to 5.6 mmol/l (5.5, 5.8), $p<0.05$. The findings suggest the future benefit of VR4® administered to prevent diabetes, especially in those who have impaired glucose tolerance. However, a longer duration of consumption should be observed for its efficacy. There was no episode of hypoglycaemia recorded in the study period, as shown in table 3.

There were 12 subjects in this trial with the average age of 51 years (46.8, 58.5); the majority were male (10, 83.3%). Five (41.7%) of them were on diet control, four (33.3%) on insulin therapy and three (25%) on oral antidiabetic drugs.

Baseline fasting glucose was 11.97 mmol/l (10.4, 16.28), and baseline 2-hour postprandial was 16.37 mmol/l (12.08, 20.08). There was a significant downward trend in fasting glucose and 2-hour postprandial glucose after consuming 400 mg VR4® for 5 days (tab. 4). For fasting blood glucose, there was a significant reduction from (11.97 mmol/l (10.4, 16.28) to 8.25 mmol/l (8.25, 12.38), $p<0.01$ after five days. There was also a sig-

Table 2.

Baseline demographic data

Parameter	Healthy	Diabetes mellitus
Total (n)	15	12
Age, years (median, IQR)	22.0 (IQR 22.0,23.0)	51 (46.8, 58.5)
Gender (n, %)		
Female	9 (60)	2 (16.7)
Male	6 (40)	10 (83.3)
Diabetes (n, %)		
Nil	15 (100)	0
Diet control	0	5 (41.7)
Oral antidiabetic drugs	0	3 (25.0)
Insulin therapy	0	4 (33.3)
Baseline blood glucose (mmol), median, IQR		
Fasting	5.33 (IQR 5.13, 5.63)	11.97 (10.4, 16.28)
2-hours postprandial	5.93 (IQR 5.7, 6.1)	16.37 (12.08, 20.08)

Table 3.

Healthy individuals consuming 400 mg KP once a day for five days (n=15)

Parameter	Baseline (Day 0) mmol/L, (IQR)	Day 1	Day 2	Day 3	Day 4	Day 5	Friedman test (D0-D5)	Wilcoxon test (Day 0 versus Day 5)
Fasting	5.33 (5.13, 5.63)	5.2 (5.0, 5.4)	5.1 (4.9, 5.6)	5.1 (4.9, 5.3)	5.2 (5.0, 5.4)	5.3 (5.0, 5.4)	p=0.132	p=0.073
2-hours postprandial	5.93 (5.7, 6.1)	5.6 (5.4, 5.8)	5.8 (5.2, 5.9)	5.7 (5.4, 6.0)	5.8 (5.5, 5.9)	5.6 (5.5, 5.8)	p=0.350	p=0.041

Table 4.

Blood glucose profile of diabetes patients taking VR4® 400 mg for 5 days

Parameter	Baseline (Day 0) mmol, (IQR)	Day 1	Day 2	Day 3	Day 4	Day 5	Friedman test (D0-D5)	Wilcoxon test (Day 0 versus Day 5)
Fasting	11.97 (10.4, 16.28)	9.5 (8.5, 13.15)	9.5 (9.5, 11.85)	9.05 (8.05, 14.25)	8.80 (7.4, 13.95)	8.25 (8.25, 12.38)	p=0.01	P<0.01
2-hours post-prandial	16.37 (12.08, 20.08)	13.5 (11.58, 17.5)	12.5 (10.28, 20.93)	12.1 (11.4, 20.25)	12.05 (10.9, 19.35)	11.8 (11.8, 18.25)	p=0.01	P<0.01

nificant reduction in postprandial glucose from 16.37 mmol/l (12.08, 20.08) to 11.8 (11.8, 18.25), $p<0.01$, as shown in table 4. This result showed that consuming 400 mg VR4® once a day significantly reduced blood glucose, either fasting or postprandially. As the postprandial blood glucose was taken at 2 hours post-dinner and before consuming VR4®, this result showed that VR4® effectively reduces both fasting and postprandial blood glucose as an addition to existing diabetic treatment either patients on diet control, on oral antidiabetic drugs or on insulin therapy. As a 2-hour postprandial blood glucose was taken after 24 hours from the prior one, we can suggest that effect of VR4® capsule could have lasted for 24 hours, even though consumed only once a day.

Tables 5 and 6 show the data of finger prick collection from Group 1 and Group 2. Night reading

Table 5.

Finger-prick data collection of Group 1 of healthy volunteers with Accu-Chek glucometer [mmol/l]

Healthy volunteers	Normal caseline						Consumed VR4© capsule at night with 400 mg									
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 4		Day 5	
Sample	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM
Female	6.2	5.1	5.8	5.5	6.3	5.4	7.1	5.0	5.2	4.5	6.8	5.5	6.2	5.5	6.3	5.4
Female	4.7	4.5	5.5	5.3	5.6	5.2	4.9	4.7	5.4	5.1	5.7	5.3	5.8	5.2	5.5	5.1
Female	5.5	5.5	5.4	6.0	5.1	5.7	5.5	5.8	6.0	5.6	5.2	5.0	5.5	5.0	5.8	5.5
Female	4.8	5.0	5.4	4.9	5.3	5.0	5.5	5.3	5.2	5.4	4.4	5.1	5.0	5.1	5.0	4.9
Female	6.3	5.2	6.1	5.3	5.9	5.5	6.6	5.0	6.4	4.9	4.7	5.4	5.0	5.2	5.1	5.0
Female	5.4	4.8	6.7	5.0	6.2	5.6	5.4	4.9	5.8	4.8	5.6	4.9	5.7	4.8	6.0	5.2
Female	5.5	5.5	7.5	5.6	5.6	5.2	5.7	5.2	5.8	6.0	5.6	5.2	5.5	5.3	5.4	5.3
Female	6.5	4.5	6.3	5.5	6.1	5.8	5.2	5.1	5.2	5.7	5.9	4.7	5.8	4.9	5.5	5.0
Female	5.7	5.5	5.8	5.6	5.6	5.5	5.9	5.5	5.8	5.4	5.7	5.3	5.5	5.0	5.7	5.3
Male	6.0	5.6	5.8	5.7	5.9	5.7	5.8	5.4	5.9	4.7	5.8	4.5	5.9	4.9	5.7	5.0
Male	5.9	5.5	6.1	5.6	6.0	5.6	5.5	5.3	5.8	5.2	6.0	5.0	5.9	5.3	5.8	5.5
Male	5.5	5.2	5.8	5.5	6.5	5.3	5.6	5.0	6.4	4.9	5.5	5.0	5.8	5.3	5.5	5.2
Male	6.7	5.8	6.0	5.5	6.1	5.6	5.3	5.9	5.6	5.6	6.5	5.8	5.9	5.5	5.6	5.3
Male	6.3	6.0	5.9	5.5	5.4	5.6	5.7	5.4	5.0	5.0	6.0	4.9	5.8	5.5	5.6	5.4
Male	5.7	5.4	6.0	5.0	5.9	5.0	5.7	5.2	5.0	4.9	5.4	5.1	5.2	5.4	5.5	5.5

*AM - morning, PM - night

Table 6.

Finger-prick data collection of Group 2 of T2DM volunteers of Accu-Chek glucose meter [mmol/l]

Type-2 Diabetes Mellitus Volunteers		Baseline reading						Consumed VR4© capsule at night with 400 mg									
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8	
Sample	Type	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM
Male	OAD	13.2	11.5	12.9	11.6	13.5	11.8	14.1	9.3	12.1	8.7	11.1	8.5	10.1	7.9	11.3	8.1
Male	IT	23.0	16.0	24.0	17.3	25.0	18.4	17.1	13.7	25.1	12.3	25.6	16.9	16.3	12.9	19.1	12.5
Male	DC	20.0	17.1	21.0	16.9	19.0	18.1	22.3	16.4	26.7	15.9	21.5	15.5	20.3	14.5	19.8	14.1
Male	OAD	18.4	11.6	19.1	12.7	17.8	13.2	17.5	11.5	14.4	9.7	16.5	8.6	12.6	9.1	13.1	8.3
Male	IT	22.6	18.8	27.6	18.4	26.1	17.9	27.0	18.1	23.1	18.4	26.1	20.7	21.7	17.8	20.4	17.1
Male	DC	12.1	8.9	11.3	9.5	12.5	9.8	11.5	7.9	11.1	7.5	12.1	7.1	10.8	7.3	9.8	7.1
Male	DC	12.2	10.5	11.8	9.7	12.3	10.8	11.8	8.5	9.6	5.7	11.4	7.9	9.7	5.4	11.7	7.7
Male	DC	13.5	9.8	10.3	9.2	9.3	8.7	12.4	10.0	9.9	9.3	13.0	10.5	16.5	9.3	11.2	10.5
Male	IT	19.4	12.0	21.8	11.5	19.1	9.8	11.5	9.7	10.0	10.0	11.2	10.5	21.8	14.3	15.7	12.0
Male	DC	12.5	11.2	11.9	10.1	11.8	10.5	10.7	8.4	12.8	7.5	11.8	8.5	11.5	7.8	10.8	7.9
Female	IT	16.1	12.7	15.8	11.5	14.5	12.7	13.1	8.5	12.8	7.9	11.6	7.5	11.2	7.1	11.9	6.9
Female	OAD	19.9	12.2	15.7	13.9	16.2	14.1	13.9	8.9	12.2	10.5	12.1	9.5	11.3	8.5	11.5	8.2

*OAD - oral antidiabetic drugs, IT - insulin therapy, DC - diet control, AM - morning, PM - night

indicates PM (postprandial) and morning blood glucose reading indicates as AM (fasting). The T2DM was identified with three conditions of participants. OAD indicates oral antidiabetic drugs, IT indicates insulin therapy and DC indicates diet control without taking any medication or drugs.

Comparisons were made between healthy and T2DM of the actively consumed VR4® 400 mg capsules at each point using glucometer reading. Blood glucose measurements were compared. Comparisons were strictly planned prior to commencement of the study, only probabilities associated with planned comparisons were calculated and the reports and interpretation of results were measured to show a pattern of results commensurate with a genuine KP effect of VR4® capsule. However, after the group 1 consumed 400 mg capsules, the blood glucose readings became low that might indicate the KP has metabolic functions in the third and fourth day. Furthermore, as shown in table 3, the consumption of capsules from the first day for each participant significantly dropped after taking 400 mg capsules. The results that show the reduction of blood glucose using KP was consistent with results that have been reported by Azuma and Sripanidkulchai [17, 18].

Results of elemental analysis

The results of this study showed that the following elements: Ca, K, Mg, Mn, Na, Fe, Se, Zn, As, Cd, Pb, Cr, Co and Ni can be successfully retrieved from VR4® samples by using the ICP-OES method of analysis. The performance of the instrument indicates that there was a presence of a good source of nine mineral elements and six major heavy metal elements from the VR4® KP extraction. The determination of trace amounts of heavy metals in KP extract for arsenic (As) is 0.001 mg/kg, cadmium (Cd) is 0.002 mg/kg, chromium (Cr) is 0.005 mg/kg and cobalt (Co) is 0.001 mg/kg were observed through the performed procedure. There were none contain minerals and heavy metals for lead (Pb) and nickel (Ni).

DISCUSSION

Human study

The results of the potential antidiabetic effect of KP were compared with the healthy young adult participants (Group 1). KP exhibited anti-hyperglycaemic effects of natural flavonoids and methoxyflavones component. This study investigated the blood glucose management trend of the characteristic of supplementation of VR4® capsules in healthy individuals, T2DM including diet control, oral hypoglycaemic agent and insulin control. Group 1 has shown that the blood glucose declined after 3 days baseline recorded. The therapeutic Group 2 experimented with KP supplement that was consumed after 2 hours of their oral hypoglycaemic agent or insulin injection. Results similarly shown a decline after they have entered the 3-day baseline, respectively.

It has been demonstrated that both healthy and T2DM participants showed hypoglycaemic activity in this model. The study started finger-prick at Group 1 for n=15 and subsequently Group 2 for n=12 for 5 days. During the experimental period, blood glucose levels at the state of fasting in T2DM have decreased significantly. Group 1 has shown blood glucose reduced in Figure 2.

Hypoglycaemic activities of KP extract showed lower blood glucose records in all participants. The VR4® capsules were found to reduce the blood glucose level in humans. The flavonoids exhibited on T2DM have been studied comprehensively within the total process of carbohydrate metabolism, possessed with their acts in the complex indicating network of insulin action. Other effects include the decrease of G-6-Pase and phosphoenolpyruvate carboxykinase gene expression, thus restrain the gluconeogenesis or glycogenolysis [19].

Furthermore, the KP that contains methoxyflavones has significantly improved energy consumption through activation of Brown Adipose Tissue (BAT) and upregulation of uncoupling protein 1 (UCP1) expression. These show the reduction of plasma triglyceride and leptin levels and intra-abdominal fat accumulation. Thus, methoxyflavones component is found to exhibit the cellular metabolism regulating activity [20]. This evidence can be determined in healthy volunteers (fig. 3). The graph indicates that from day 3 to day 4, the level of blood glucose increases and decreases on the next day.

Recently, natural product screening has prevailed to achieve antidiabetic therapeutics in a T2DM model. The effect of *Orthosiphon stamineus* and *Morinda citrifolia* fruit extracts on insulin-sensitising and hypolipidemic activity have been investigated [21,22]. Similarly, this study has demonstrated that the administration of KP VR4© capsules can reduce high blood glucose levels.

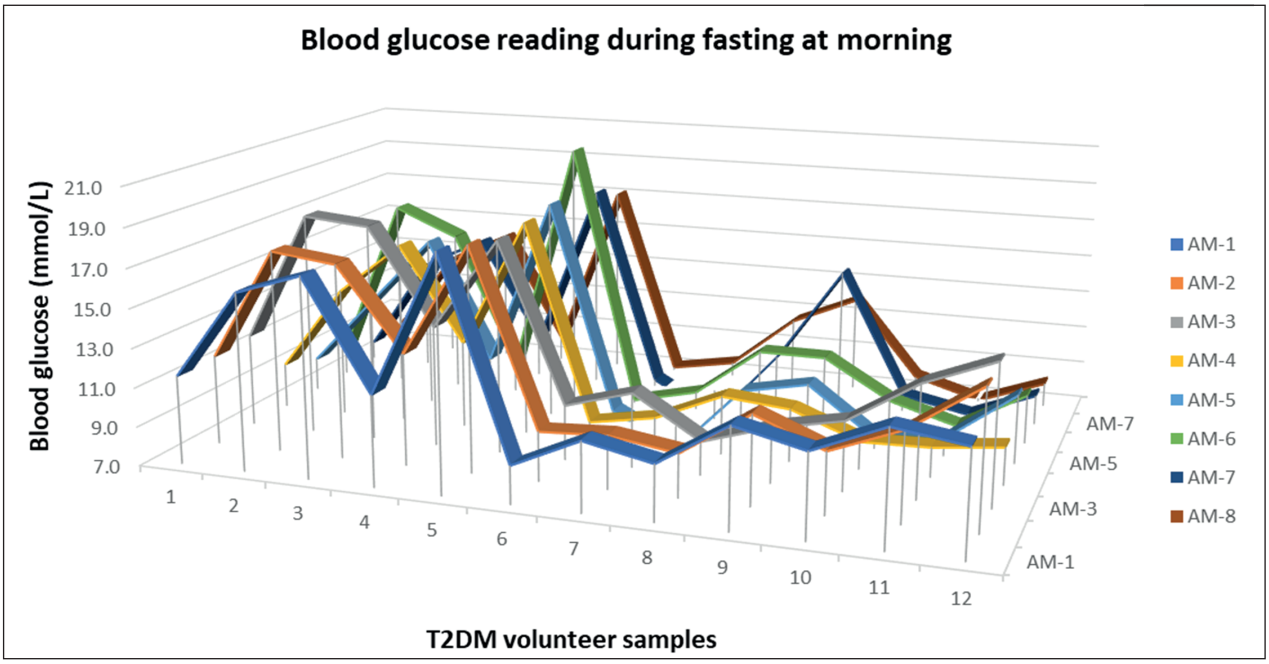

Figure 2.

Eight days of fasting blood glucose profile in type-2 diabetes mellitus patients taking VR4® (Group 2, n=12)

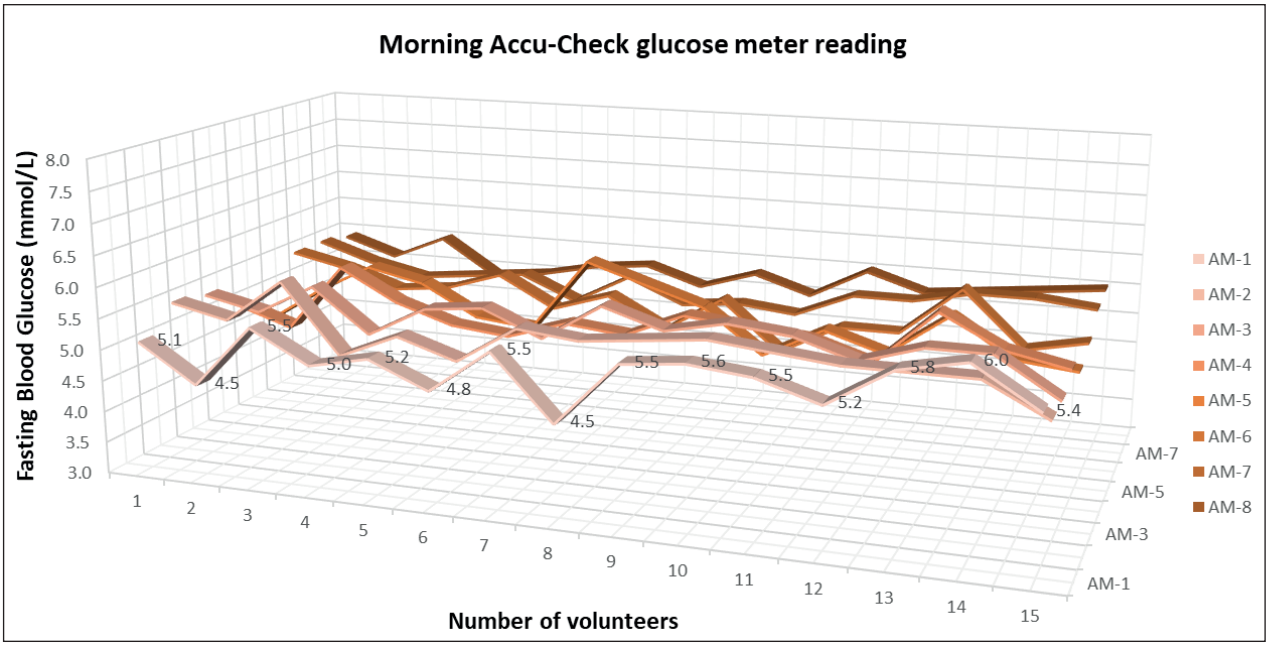

Figure 3.

Eight days of blood sugar profile for healthy individuals administered with VR4® (Group 1, n=15)

ICP-OES analysis

Medicinal plants and extract forms retrieved from these natural drugs are widely consumed as home remedies and raw material in the pharmaceutical industry. Sometimes, medical practitioners suggest herbal teas and herbal extracts as a supplementary type of treatment in common health problems that are usually caused by our modern way of living that can contribute to stress or insomnia.

In 1991, WHO has suggested herbal Good Agricultural and Collection Practices for assessing their inherent biological variant and complexity [23, 24]. Therefore, their safety, quality and efficacy can be

evaluated and characterized. Heavy metal elements can be contaminated from different plants causing serious health hazards such as adrenal dysfunction, damage to central nervous system, symptoms of chronic toxicity and other serious illnessess [25].

The macronutrients, micronutrients and toxic elements are present in VR4® capsule in digestive form or infusion. Figure 4 shows the mineral element of KP rhizome extracts with concentration parameters.

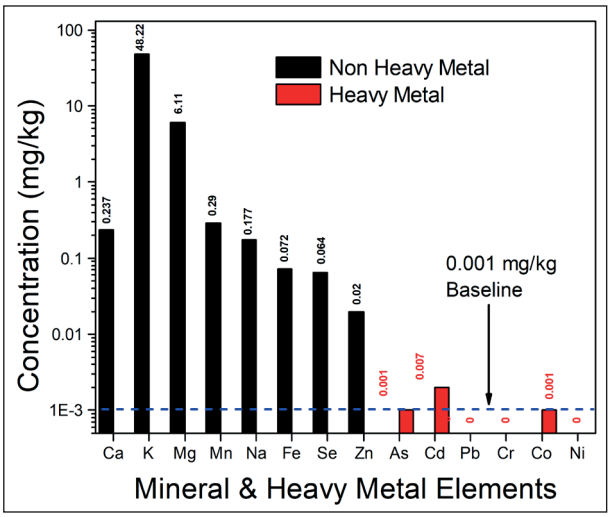

Figure 4.

VR4® capsule of mineral elements

The levels of cationic macroelements: K, Ca, Mg and Na may be easily digestible by the human body. The data has shown that potassium content in the KP extract was the highest at 48.22 mg/kg of concentration. After potassium, the highest concentration was detected for magnesium: 6.11 mg/kg. Potassium is important for the acid-base balance in human, inclusive of osmotic pressure and nerve impulse transmission. It participates in carbohydrate metabolism and a membrane transport correspondingly. Magnesium engages in the coordination of blood pressure, protein synthesis, muscle and nerve function. The WHO has recommended that daily intake of Mg for adults is approximately around 400 mg [26]. In human body, sodium is the most typical cation that is associated with water metabolism, the contraction of muscles structure and allows the transfer of carbon - dioxide to the lungs. Manganese is critical for osteogenesis or ossification, lipid metabolism, energy sources, and the synthesis of nucleotides. In this study, the obtained values for manganese were 0.29 mg/kg of concentration, respectively.

Zinc element relates to the group of microelements and it is crucial for the normal function of the brain and cell membrane, as well as for the treatment of the mental disorder. Moreover, it is vital for the metabolism of proteins, carbohydrates, prostate function and many other biological activities [27].

In order to demonstrate the validity of the method presented in this study, recovery service from the National Poison Centre at the University of Science Malaysia in Penang was used to detect four common heavy metal elements. Thus, the elements tracing that was performed at Advanced Toxicology Laboratory analysis has shown that As is 3.12 ppm, Cd is 0.20, Pb (lead) is 3.75 ppm and Hg is 0.06 ppm (fig. 5). The Flow Injection Mercury System was used to obtain the Hg test parameter and Graphite Furnace Absorption Spectroscopy was executed for As, Cd and Pb analyses. The results consistently indicate that heavy metals were far away from the minimum standard set by WHO, Malaysian Poisson Act 1952, Federal Drug Agency of United States of America, European Commission (Commission Regulation No. 629) and Natural Health Products Regulations (NHPR) of Canada.

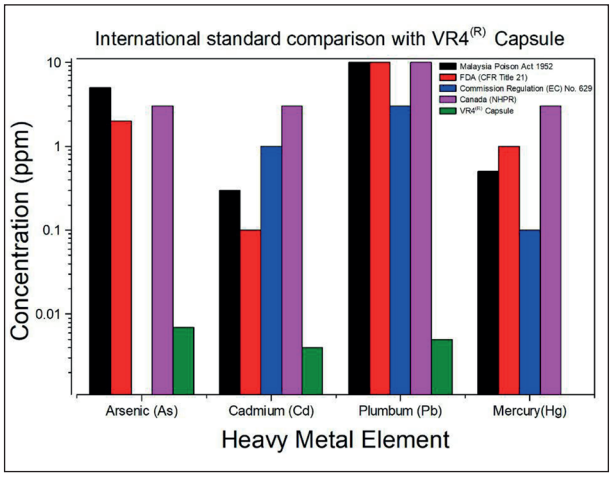

Figure 5.

VR4® capsule of heavy metal detection with international standard

CONCLUSIONS

In this study it was found that consumption of VR4® capsules at 400 mg once a day as additional treatment in diabetic patients has significantly reduced blood glucose without causing any adverse reaction or hypoglycaemia. In healthy individuals, it did not cause any hypoglycaemic events and helped reduce postprandial glucose level. The study has also demonstrated the degree of elements in VR4® capsules

evaluated, and their amounts of heavy metal are below the level of international standards and safe for consumers. The trace element contaminations using ICP-OES were also validated with Flow Injection Mercury System and Graphite Furnace Absorption Spectroscopy, respectively. Mineral elements present in the product have reached the WHO supplementary recommendation for daily intake. More research is needed to further establishment of KP rhizome as a potential complementary medicine that can benefit T2DM patients including a larger randomised clinical trial with longer duration of consumption should be carried out to determine the long-term efficacy, sustainability and significant outcomes concerning adverse effects of consumption of KP.

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