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A Study on Supplement Containing Moro (*Citrus Sinensis* (L.) Osbeck) Orange Extract of a Randomized Placebo-controlled Trial Part 2:

Analysis of Efficacy on BMI Reduction

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● **Abstract**

Objectives: The objective of this study is to examine how the ingestion of the Moro EX (referred to as "EX") containing Moro juice extract (Morosil®) contributes BMI reduction.
Methods: In this randomized, placebo-controlled, double-blind trial, 60 subjects with BMI ≥ 25, ≤ 35 were included. To evaluate this objective, body weight, BMI, waist and hip circumference were measured as the primary outcome. In addition, stratified analysis was carried out. The subjects with WHR (waist: hip circumference ratio or waist: hip ratio) ≤ 0.85 were targeted.
Results: With 60 subjects, body weight, BMI, waist and hip circumference of EX decreased significantly compared to Placebo after 12 weeks of ingestion. As the result of stratified analysis, the remaining 6 subjects, whose BMI < 30 as well as WHR ≤ 0.85, showed a significant difference between two groups after 12 weeks in all items of body weight, BMI, waist and hip circumference.
Conclusion: The present results suggest that daily ingestion of EX tablets containing Moro orange extract (Morosil®) can reduce body weight, BMI, waist and hip circumference.

Key Words: Moro, *Citrus sinensis* (L.) Osbeck, red orange, BMI, body weight, waist circumference, hip circumference

1. INTRODUCTION

Overweight and obesity have become important public health problems not only in affluent societies but also in developing countries[1]. In 2010, the International Obesity Task Force and the International Association for the Study of Obesity have estimated that 475 million are obese and approximately 1.0 billion adults are overweight [body mass index (BMI) 25- 29.9 kg/m^2]. Interventions on this prevalent health hazard mainly depend on recognizing the complications of obesity. In fact, obesity increases the risk for a wide range of chronic diseases such as type-2 diabetes, hypertension, coronary heart disease, and various cancers. Other comorbidities include gall- bladder disease, fatty liver, sleep apnea syndrome, and osteoarthritis with reduced quality of life and life expectancy. Conventional managements of overweight and obesity include low-fat diet, exercise, behavioral interventions, and pharmacological agents. Each intervention has some advantages but they also have significant limitations and adverse effects. Therefore, alternative remedies having a better safety profile in weight loss management have gained considerable attention in recent years[2)-6)].

Recently, *in vitro* and *in vivo* studies have investigated the health-related properties of red (or blood) orange (*Citrus sinensis* (L.) Osbeck) intake, especially of the Moro variety, on weight management[7)-9)]. Red oranges are pigmented sweet orange variety typical of eastern Sicily (Italy). The typical red coloration of the fruits is attributed to the presence of pigmented compounds called anthocyanins, not usually contained in blond sweet oranges and other citrus fruits. Moro is the most colorful variety, with deep red flesh ranging from orange-veined with ruby coloration, to vermilion, to vivid crimson and nearly black. From the results reported by Titta, et al. (2010), it was observed that Moro juice intake reduced significantly body weight gain induced by high fat diet in mice, almost abolishing it, with a reduction of the abdominal and inguinal fat mass by approximately 50%. Moreover, histological examination of the adipose tissue showed a marked reduction in the size of the adipocyte cells and lipid accumulation in mice treated with Moro juice[7)]. On the basis of these interesting results, a randomized, placebo, double- blinded clinical trial was carried out to evaluate the effects of Moro juice extract intake in decreasing body weight in human healthy subjects conducted by Cardile, et al. in 2014[10)].

The study of Cardile, et al. targeted healthy males and

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Table 1 The results of total subjects

Item	Time points	Values[1)]		P-value[2)]
		EX	Placebo	
Body weight (kg)	T0 (baseline)	77.80 ± 3.13	76.96 ± 3.37	
	T4 (12 weeks)	74.72 ± 3.09	76.55 ± 3.37	
	Variation	− 3.08 ± 0.24**	− 0.41 ± 0.05**	< 0.001##
BMI (Kg/m^2)	T0 (baseline)	27.91 ± 1.07	27.74 ± 1.09	
	T4 (12 weeks)	26.80 ± 1.05	27.59 ± 1.10	
	Variation	− 1.11 ± 0.09**	− 0.15 ± 0.02**	< 0.001##
Waist circumference (cm)	T0 (baseline)	96.05 ± 1.95	96.31 ± 2.04	
	T4 (12 weeks)	88.97 ± 1.92	95.51 ± 2.01	
	Variation	− 7.08 ± 1.33**	− 0.80 ± 0.15**	< 0.001##
Hip circumference (cm)	T0 (baseline)	110.07 ± 1.50	109.42 ± 1.69	
	T4 (12 weeks)	104.10 ± 1.72	108.73 ± 1.68	
	Variation	− 5.97 ± 0.97**	− 0.69 ± 0.12**	< 0.001##

Values are expressed as the mean ± SD.

1) ** $p<0.01$ against baseline.

2) ## $p<0.01$ between-group differences in change from baseline.

females with BMI between 25 and 35, which could include obese subjects. Therefore we reanalyzed only healthy subjects' data excluding obese subjects based upon the definition of "Metabolic Syndrome (MetS)" by World Health Organization (WHO). WHR (waist: hip circumference ratio or waist: hip ratio) played an important role in WHO criteria. According to that, people with BMI ≥ 30, or, people with WHR > 0.90 in males and WHR > 0.85 in females are deemed to be with MetS[11]. In contrast, The Japanese Society of Internal Medicine adopted the waist circumference ≥ 85cm in males and ≥ 90cm in females[12)13)]. In Japan, "waist circumference" means periumbilical, based on the umbilicus, as a contrasted with WHO based on the median between the bottom rib lower edge and the ilium upper edge[14)]. In the present study we analyzed the data excluding subjects with WHR > 0.85, because the trial was performed in Italy so that subjects would be Italian. The original authors shared their data with us.

2. METHODS

2.1. Trial design and subjects

A randomized, placebo-controlled, double-blind study was conducted. The study period was 12 weeks. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. Written informed consent was obtained from all subjects.

60 healthy subjects (aged 21-50 years old) participated in the present study. All of the subjects in this study were public volunteers. All subjects were randomized to Moro group (30) and Placebo group (30). Inclusion criteria was; BMI between 25-35 kg/m^2, not taking any drugs or dietary food supplement during the experimentation. Excluding criteria was; pregnancy, smokers, having a history of thyroid disease, cardiovascular disease, diabetes, using weight loss medications, laxative or diuretic, recent unexplained weight loss or gain, taking medications during the previous months (including vitamin and antioxidant supplement) or any significant dysfunctions during the previous months (including vitamin and antioxidant supplement) or any significant dysfunctions.

2.2. Description of test foods

The test food, "MoroEX" (referred to as "EX") is a tablet containing Moro juice extract (Morosil®) supplementation (1 tablet/day, containing 400 mg of Morosil®). Morosil® was analyzed by using HPLC and spectrophotometric system to identify the main class of compounds in it. Results obtained from the analysis of the chromatograms showed that the extract contained several citrus class compounds such as anthocyanins (1.5 mg), flavone glycosides (8 mg), hydroxycinnamic acids (3.2 mg), and ascorbic acids (17.2 mg as an antioxdant ingredient for the product). All data were conformed to the chemical composition reported in the technical data sheet of the product.

2.3. Experimental procedures

Subjects consumed a supplement of one tablet a day for 12 weeks. EX group received tablets which contained 400 mg of Morosil®, while placebo contained 400 mg of maltodextrin. During the clinical trial, subjects were evaluated at the beginning of the study (T0), after 2 weeks (T1), 4 weeks (T2), 8 weeks (T3) and 12 weeks (T4) of treatment. At each time point, subjects were monitored for several anthropomorphic parameters such as body weight and BMI; waist circumference and hip

Table 2　Subject demographics

Item	Unit	EX	Placebo	P-value
Subjects	−	4	2	−
Body weight *	kg	77.1 ± 3.6	82.4 ± 1.7	0.128
BMI *	kg/m^2	27.8 ± 1.5	28.7 ± 0.8	0.469
WHR *	ratio	0.838 ± 0.006	0.839 ± 0.009	0.879

mean ± SD

* No significant difference

Table 3　The results of stratified analysis

Item	Time points	Values [1)]		P-value [2)]
		EX	Placebo	
Body weight (kg)	T0 (baseline)	77.11 ± 3.57	82.42 ± 1.68	
	T4 (12 weeks)	74.09 ± 3.35	82.08 ± 1.65	
	Variation	− 3.03 ± 0.35**	− 0.34 ± 0.02*	< 0.001##
BMI (Kg/m^2)	T0 (baseline)	27.76 ± 1.48	28.69 ± 0.82	
	T4 (12 weeks)	26.67 ± 1.39	28.58 ± 0.81	
	Variation	− 1.09 ± 0.13**	− 0.12 ± 0.01*	< 0.001##
Waist circumference (cm)	T0 (baseline)	93.64 ± 0.96	93.85 ± 1.75	
	T4 (12 weeks)	87.16 ± 2.50	92.98 ± 1.44	
	Variation	− 6.48 ± 2.21**	− 0.87 ± 0.30	0.028#
Hip circumference (cm)	T0 (baseline)	111.69 ± 1.44	111.79 ± 0.86	
	T4 (12 weeks)	105.83 ± 1.69	111.16 ± 0.78	
	Variation	− 5.86 ± 0.65**	− 0.74 ± 0.08*	< 0.001##
WHR (ratio)	T0 (baseline)	0.838 ± 0.006	0.839 ± 0.009	
	T4 (12 weeks)	0.824 ± 0.020	0.837 ± 0.007	
	Variation	− 0.015 ± 0.019	− 0.002 ± 0.002	0.420

Values are expressed as the mean ± SD.

1) * $p<0.05$, ** $p<0.01$ against baseline.

2) # $p<0.05$, ## $p<0.01$ between-group differences in change from baseline.

circumference were monitored at the beginning and the end-point of the study. Vital signs such as blood pressure were measured to monitor the general health of the subjects and any side effects observed were recorded during each visit.

2.4. Data analysis

All data obtained was submitted to a statistical analysis and was expressed as mean ± standard deviation (SD). Changes in the same group were assessed using paired t-test, and Student's t-test was used for intergroup comparisons of changes from the baseline. Statistical analyses were performed using Statcel 4 (Yanai, 2015). The results were considered significant at the < 5% level in the two-sided test.

3. RESULTS

3.1. Total subjects

Table 1 shows the results of total subjects. After 12-weeks of ingestion, all items of body weight, BMI, waist and hip circumference illustrated a significant difference between Moro EX and Placebo. As for intragroup comparison, both groups differed significantly from the baseline in all items.

3.2. Stratified analysis

Excluding subjects with WHR > 0.85, stratified analysis was applied with the remaining 6 subjects (EX; 4, Placebo; 2). These demographics are shown in **Table 2**. There was no significant difference in body weight, BMI, and WHR between the two groups at the baseline. As well as this, there were no subjects with BMI > 30.0.

Table 3 depicts the results of the stratified analysis. Significant differences were observed between the two groups of changes in body weight, BMI, waist and hip circumference, other than WHR. With respect to the EX, body weight, BMI, waist and hip circumference differed significantly from the baseline, whereas the Placebo showed significant differences only in body weight, BMI, and hip circumference.

3.3. Adverse event

All other vital signs such as blood pressure were monitored and no adverse events were reported in both groups during the study.

4. DISCUSSION

We reanalyzed the study of Cardiles, et al. in 2014. They conducted a randomized, placebo-controlled, double-blind study to verify the effects of the "MoroEX" containing Moro (*Citrus sinensis* (L.) Osbeck) orange extract (Molosil®) in Italy. They found that Ex intake is able to induce a significant reduction in body weight, BMI, waist and hip circumference in comparison with the non-treated group.

To explore whether the intake of EX is effective for BMI reduction with non-obese subjects, we reanalyzed the data excluding obese subjects. The elimination was based upon not the definition of The Japanese Society of Internal Medicine but that of WHO because subjects should be Italian, so that subjects with WHR $>$ 0.85 were eliminated. As a result of the stratified analysis, it was observed that the ingestion of Ex reduce body weight, BMI, waist and hip circumference versus the placebo significantly.

Main findings

In this analysis, we evaluated the slimming effect of the tablet containing Molosil® by comparing body weight, BMI, waist and hip circumference.

Previous study carried out that intake of Moro juice able to affect fat accumulation. Salamone reported the main anti-steatotic effect is related to the promotion of lipolysis and lipid peroxidation by induction of PPAR-α and the inhibition of lipogenesis by the suppression of liver X receptor-α[8]. In addition, Titta elucidated that Moro juice intake can counteract the effect of high-fat diet on adipose tissue leading to marked reduction in the size of adipocyte cells and lipid accumulation, and that Moro juice could entirely revert the high fat-induced transcriptional reprogramming in adipocytes cells by means of high-throughput gene expression analysis of fat tissue[7]. The action on adipogenesis and fat accumulation has been demonstrated to be mediated by the regulation of oxidative stress and insulin signalling, leading to the decrease of the phosphorylation level of Akt (protein kinase) and insulin-induced reacting oxygen species production[7)8)]. In addition, previous reports have shown that anthocyanins, especially cyanidin-3 glucoside, from different fruits and vegetables are able to reduce body weight and visceral fat accumulation both in diet-induced and genetic models of obesity[15)16)].

Secondary Findings

During the 12-weeks of test period, no adverse events were reported, which indicated the safety of the ingestion of the test product.

General information

Since ancient times, Japanese have cultivated and consumed many kinds of citrus such as mandarin orange and citrus depressa. It seems that Morosil® as red orange juice is a food familiar to Japanese people.

On the other hand, obesity not only leads to diseases such as "lifestyle- related diseases", but also causes deterioration of the external appearance and may trigger serious degradation of QOL. For these reasons, it is considered that the combination of the improvement of daily eating and exercise habits and the ingestion of Morosil® can prevent obesity, improve external appearance and keep our body in good health.

Limitations

For this study we used the test product (a supplement of Moro EX) containing Morosil®, Moro juice extract, which include anthocyanins, hydroxycinnamic acids, flavone glycosides and ascorbic acid. Each ingredient reportedly has the functionality of improving lipid metabolism, so that the outcome of this study should be strongly related to these findings. However, the functional mechanism of these reports is mainly based upon the in-vitro settings or the research using rats, and the behavior in the human body is a matter of speculation. Accordingly this point should be further scrutinized in the future.

5. CONCLUSION

In conclusion, we found out that the ingestion of the tablet containing Morosil®, Moro juice extract for 12 weeks contributed to the weight reduction, BMI improvement, waist and hip circumference reduction for non-obese people. In addition, no safety-related matter occurred during 12-week test period.

CONFLICT OF INTEREST

All parts of this study to write the report was funded by Willfarm Co., Ltd. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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