Middle East Journal of Applied Sciences
ISSN 2077-4613
Volume : 09 | Issue :01 |Jan.-Mar.| 2019
Pages: 117-124

Citrus reticulata peel extract: an Anti-obesity therapy for Adolescents

Iman H. Kamel, Ph.D., Josline Y. Salib, Ph.D., Sayed A. El-Toumy, Ph.D.[2], Amina H. Awad, Ph.D.[1], and Mohammed K. Elmenabbawy, Ph.D.[1]

[1]*Child Health Department,* [2]*Chemistry of Tannins Department, Medical Division, 33 El Buhouth St., (Former El-Tahrir St.) 12622, Dokki, Giza, Egypt.*

Received: 10 Feb. 2019 / Accepted 14 Mar. 2019 / Publication date: 20 Mar. 2019

ABSTRACT

The potential benefits of the water extract of *Citrus reticulate* peel were assessed on obesity and fat reduction in adolescent. Clinical trials were done on double-blind, placebo- controlled study, 40 obese adolescent subjects/each group randomized from both sex with age vary between 12 and 18 years old. Group A participants received an 800 mg of dry extract daily and group B received placebo. Both groups received three meals (2000 kcal/day) throughout the study. The primary outcome in group A was reduction in Body mass index (BMI), Body fat percentage (BF %) and in waist circumference (WC). Secondary outcomes were significant reduction in BMI, BF% and WC, as well as better lipid profile criteria. Statistically high significant reductions in BMI by 5.74 kg/m^2 (P<0.001), WC by 11.33 cm (P <0.001), body fat by 4.24 % (P = 0.006), total cholesterol (TC) by 35.56 mg/dl (P = 0.008) and triglyceride (TG) by 24.66 mg/dl (P <0.001) were observed in the group A compared to the group B as study conclusion. Many of the beneficial effects in management of obesity attributed to the presence of polyphenols and flavonoids constituents of the extract.

Keywords: Citrus peel extract, anti-obesity management, adolescents.

Introduction

Available studies in Eastern Mediterranean countries indicate that obesity has reached an alarming level among both children and adults. Among Egyptians above the age of 15 there are more overweight and obese females than males. According to WHO's statistics, an estimated 76% of females are said to be overweight or obese, in comparison with approximately 64.5% of Egyptian males in this age group. Indicators reveal that the rate of obesity in Egypt has risen markedly over the past 30 years (Ghalli *et al.,* 2008).

Obesity is a social health problem that causes the development of various metabolic disorders such as diabetes mellitus (DM), hypertension, cardiovascular diseases, and inflammation-related pathologies (Devendra *et al.,* 2004). While genes are important in determining people susceptibility to weight gain, energy balance is determined by calorie intake and physical activity. Thus societal changes and worldwide nutrition transition are driving the obesity epidemic. Economic growth, modernization, urbanization and globalization of food markets are just some of the forces though to underlie the epidemic (WHO, 2004).

Although numerous commercial drugs are available for treating obesity, their treatment are based on limiting the absorption of food, suppressing appetite and reducing food intake, and/or altering metabolism or increasing energy expenditure. Unfortunately many of these drugs are unavailable to a large number of patients besides it causes adverse effects (Daniels *et al.,* 2012; Brenot *et al.,* 1993). The utilization of medicinal plants and their phytochemicals for treating obesity is not only a priority for developing safer alternatives to pharmaceuticals but less costing as well. A number of studies have demonstrated the potential health benefits of natural flavonoids against obesity (Havsteen, 2002; Prasain *et al.,* 2010; Kumar *et al.,* 2013; Cook and Samman, 1996; Rice-evans *et al.,* 1995). Flavonoids are aromatic secondary plant metabolites, which have been recognized as important due to their physiological, (Buslig and Manthey, 2002) pharmacological role, (Manach and Donovan, 2004) and their health benefits (Hooper and Cassidy, 2006).

Citrus fruits are primarily used in juice and related industries, and the production method leaves significant amounts of by-products such as peel, pulp, and seeds. Recent studies credit the Citrus by the

Corresponding Author: Iman H Kamel, Child Health Department, Medical Division, 33 El Buhouth St., (Former El-Tahrir St.) 12622, Dokki, Giza, Egypt. Email: imankamelh@gmail.com

most available flavonoids and polyphenols dietary sources that have different pharmacological activities, (Ghasemi *et al.,* 2009; Nakajima *et al.,* 2014) yet still much to be explored.

Thus the present work was undertaken to study the anti-obesity potential of the *Citrus reticulate* peel extract on adolescent subjects as well as their lipid profile changes and body fat percentage.

Methods

Patient Eligibility

This study was carried out at the Out-Patient Clinic of the National Research Center between April to May 2016. Written consents were obtained from the parents to enroll their children in the study. All the procedures used in this study were by the guidelines of the Helsinki Declaration on Human Experimentations. The study was approved by local ethics committee of the National Research Centre (No: 16433).

Materials

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR: Joel spectrometer (Kyoto, Japan) in DMSO-d6, UV: Shimadzu spectrophotometer model UV-240 (Kyoto, Japan), ESI-MS: on a Finnigan MAT 4600 spectrometer (Thermo-Finnigan, USA), Column Chromatography: Sephadex LH-20 (Fluka, UK), Paper Chromatography was carried out on Whatman No.1 and 3MM paper (Sigma Aldrich, USA) using the following solvent systems: (1) BAW (nBuOH/AcOH/H2O, 4:1:5 (upper layer)); (2) H_2O; and (3) AcOH/H2O (15:85). Diagnostic biochemical kits were purchased from Sigma (St. Louis, MO, USA).

Plant Material

The fruits of *Citrus reticulate* were collected from Cairo market, Egypt during December 2015 - February 2016. The peel was then collected and authentication was performed by Dr. M. El-Gebali, former researcher of botany at the National Research Centre. A voucher specimen (C_16) is deposited in the National Research Centre Herbarium for future references.

Extraction and isolation

The fresh fruits peel (10 kg) were washed in running tap water in laboratory, vigorously swirled by the blender then soaked in sterile distilled water, warmed at 40 °C and then filtered (using Whatmann No.1 filter paper) into another sterile Container. Extraction processes was repeatedly done. The combined water extracts were concentrated under pressure and then the crude extract was placed in lipholization to give a dry residue (2.5 kg). The peel extract was prepared by dissolving 8 g solid materials in 200 ml of distilled water to have a dose of 20 ml (800 mg) per day for ten days. The containers were covered in order to keep all active elements and stored at 4 °C when not in use.

Part of the resulting extract (500 g) was fractionated by applying onto a Sephadex LH-20 column (500 g, 40 X 1000 mm) using an eluting system of ethanol followed by different percentage of ethanol/water giving rise to four collective fractions. Further chromatographic separations using Sephadex LH-20 column and Whatmann 3 MM preparative paper led to the identification of six flavonoids and phenolic compounds namely; hesperidin, querectagetin, naringin, acacetin, rutin, and quercetin (Harborne, 1986).

Study Design

The study was conducted on 80 obese adolescents (12 -18 years) 40 females and 40 males. All obese adolescents attended and were followed at the Out-Patient Clinic of the National Research Centre from April to May 2016. They had neither received any other weight control measures nor had any medical and/or drug history within the last 3 months before their participation in the study. Obesity was defined according to Cole *et al.* (2000)*,* in children with BMI > 95th percentile for age and sex[1] (BMI Egyptian growth reference chart percentile was used).

These subjects were divided randomly into 2 groups:

Group A: were instructed to receive 20 ml of the extract once a day, 30 minutes before breakfast or dinner, followed by a balanced low caloric diet for 8 weeks.

Group B: instructed to receive 20 ml of placebo once a day, 30 minutes before breakfast or dinner, followed by a balanced low caloric diet for 8 weeks, the placebo syrup each containing 200 ml of excipient identical in appearance to the extract.

The attendances undergo evaluation at the baseline and subsequent two follow-up visits, one after one month and the other after two months (0, 1, & 2). All patients of both groups were subjected to history taking, thorough clinical examination and Anthropometric evaluation. The height, weight and waist circumference were measured following the recommendations of the International Biological Program. The height was measured to the nearest 0.1 cm using a Holtain portable anthropometer, and the weight was determined to the nearest 0.01 kg using a Seca Balance Scale, with the subject wearing minimal clothing and no shoes, where the Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). Waist circumference (WC) was measured at the level midway between the lower rib margin and the iliac crest; using non-stretchable plastic tape to the nearest 0.1 cm. Circumference was taken with the subjects standing upright and breathing normally, with their face directed forward and shoulders relaxed at the end of expiration.

Body Composition

Each participant was also examined by the TANITA Body Composition Analyzer. As specified by the manufacturer, the unit was calibrated before testing. The participant stood on the foot board of the device, while he was holding the 2 handles carefully; each by one hand at the same time. By using his sex, age, weight and height approximated to the nearest unit, the basal metabolic rate (BMR), body fat percentage (BF %), muscle mass percentage (M%) and body water percentage (W%) were derived.

Sample Preparation

Blood samples were collected from the subjects to evaluate their lipid profiles through measuring the serum total cholesterol (TC), low density lipoprotein cholesterol (LDL) and triglycerides (TG) by the colorimetric enzyme assays.

Statistical analysis

All data was converted and manipulated by using SPSS software program version 18.0. Mean and standard deviation was calculated as regarding quantitative data in terms of BMI, WC, BF%, M%, W%, BMR, TC, LDL and TG. Qualitative data as sex was presented by number and percent. Comparisons between the both groups were done. The quantitative data was compared and t test was applied while Chi square was calculated among groups as regards qualitative data. p value was established to determine the statistically significant difference between the two groups. The difference between the two groups was considered statistically significant when $p<0.05$, and considered highly statistically significant when $p<0.01$. Graph was done using Excel 2016 to represent the comparison between the different measures among the group A along the treatment course.

Results

Extraction and Isolation

Phytochemical screening of the water extract of the *Citrus reticulate* peel after subjecting it to Sephadex LH-20 column chromatography and Whatmann 3 MM preparative paper, yielded 6 active flavonoide constituents (Harborne, 1986; Markham *et al.,* 1982) namely; hesperidin (40 mg), querectagetin (35 mg), naringin (28 mg), acacetin (20 mg), rutin (25 mg), and quercetin (26 mg), (Figure 1). The structure of the isolated compounds was established through chromatography as well as conventional chemical and spectroscopic method analysis (Harborne and Baxter, 1999).

Baseline Characteristics of the Study

Adolescents Obese' parameters are summarized in (Table 1). The patients' profiles as regard to age, sex, BMI, WC, were similar between group A (n=40) and group B (n=40) groups.

Reduction in BMI & WC

Tables (2&3) summarize the physical and biological changes through the study for obese adolescents supplemented with the *Citrus reticulate* peel extract (group A) compared to the group B.

Group A experienced a statistically significant greater reduction in their BMI and WC at 4 & 8 weeks of supplementation compared with subjects consuming placebo group B (P < 0.001). Group A experienced a net reduction of 3.24 and 5.74 kg/m^2 in BMI at 4 & 8 weeks, respectively.

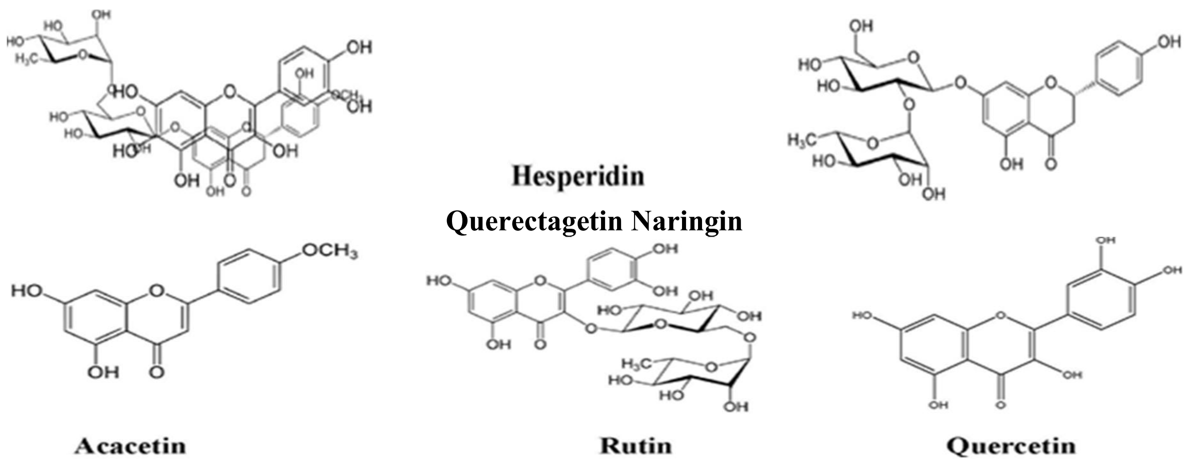

Fig. 1: Structures of the isolated flavonoid compounds

Table 1: Comparison between obese groups at the beginning of the study
At the beginning

	A N=40	BN=40	t	P
Age	15.43±3.0	16.18±2.7	1.173	0.244
Sex			X^2	
Male	20 (50%)	20 (50%)	1.0	1.0
Female	20 (50%)	20 (50%)		
BMI (kg/m^2)	32.48±1.7	32.63±1.9	0.372	0.711
WC (cm)	83.03±5.5	82.05±6.3	0.373	0.465
BF%	38.85±3.5	39.83±3.3	1.291	0.200
M%	29.08±0.9	29.13±0.8	0.266	0.791
Water%	38.45±2.4	38.88±2.4	0.786	0.434
BMR	1344.4±98.1	1344.6±109.2	0.006	0.995
TC (mg/dL)	213.4±25.2	218.48±30.2	0.815	0.417
LDL (mg/dL)	119.5±23.8	122.48±25.4	0.54	0.591
TG (mg/dL)	142.38±11.3	143.25± 11.7	0.339	0.735

BMI, Body mass index; WC, waist circumference; BF%, Body fat percentage; M%, muscle mass percentage; W%, body water percentage; BMR, basal metabolic rate; TC, total cholesterol; LDL, low density lipoprotein cholesterol; TG, triglyceride.

Table 2: Comparison between obese groups after one month of the study
After one month

	A N=40	B N=40	t	P
BMI (kg/m^2)	29.16±2.3	31.2±2.4	3.885	<0.001**
WC (cm)	75.05±5.8	79.3±6.2	3.15	0.002**
BF%	36.43±4.1	38.19±3.5	2.062	0.043*
M%	31.66±1.1	30.33±0.9	5.888	<0.001**
Water%	40.83±2.4	40.2±2.4	1.171	0.245
BMR	1363.1±96.2	1352.2±109.4	0.471	0.639
TC (mg/dL)	187.01±26.2	211.05±32.5	3.638	<0.001**
LDL (mg/dL)	107.55±25.4	118.20±25.1	1.056	0.294
TG (mg/dL)	126.24±13.6	136.57±11.4	3.682	<0.001**

* Statistically significant difference p<0.05 "statistically highly significant difference p<0.01

Table 3: Comparison between obese groups after two month of the study

After two month	A N=40	B N=40	t	P
BMI (kg/m^2)	26.74±2.4	29.83±2.3	5.849	<0.001**
WC (cm)	71.1±5.8	77.31±6.1	4.696	<0.001**
BF%	34.61±4.3	37.02±3.4	2.8	0.006**
M%	33.11±1.3	31.03± 1.0	8.156	<0.001**
Water%	41.94±3.7	42.3±5.6	0.342	0.733
BMR	1379.8±96.5	1376.3±115.2	0.144	0.886
TC (mg/dL)	177.84±29.9	197.8±35.2	2.734	0.008**
LDL (mg/dL)	101.57±26.5	111.46±25.5	1.95	0.055
TG (mg/dL)	117.72±13.1	140.2±11.8	8.044	<0.001**

*Statistically highly significant difference p<0.01

A similar significant decrease in WC was observed in group A resulted in reduction (P < 0.001) of 7.98 and 11.93 cm, respectively, at 4 & 8 weeks. This corresponds to a 9.6 and 14.36 % reduction in waist size verses the baseline parameters. It was also found that the net impact of the *Citrus reticulate* peel extract on waist circumference was statistically significant as early as 4 weeks of supplementation (reduction = 7.98 cm, P = 0.002).

Reduction in TC & TG

Analysis of the obese adolescent baseline blood biochemical and anthropomorphic profile indicates that many of the therapeutic trial participants in both groups had high total cholesterol and high triglycerides values also large waist circumferences. Fortunately, the *Citrus reticulate* peel extract supplementation significantly decreased the total cholesterol levels by 12.36 and 16.67 % in group A compared to group B which decreased by 3.4 % and 9.46 % whereby P <0.001 & P=0.008 respectively, at 4 & 8 weeks. A significant reduction in triglycerides levels by 11.33 and 17.3 %, respectively in group A compared to group B by (P <0.001) was observed throughout the study as shown in (Table 2 & 3). Also satisfactory non significant LDL reduction after 2 months was observed in group A compared to group B as shown in (Table 3).

Modulation of Biochemical Parameters of Fat Metabolism

Huge reduction in BF% (10.91 %), TC (16.67%) and triglycerides (17.3%) was observed in group A at the end of the study compared to baseline, (Figure 2). Meanwhile the BMR wasn't significantly increased (2.56%) which suggested the *Citrus reticulate* peel extract to be a well-tolerated and effective ingredient for weight management.

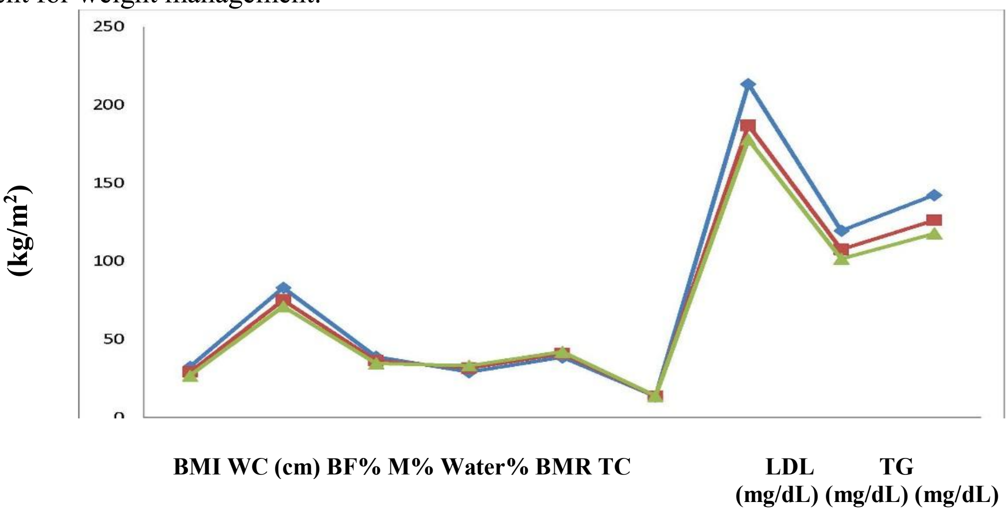

Fig. 2: Comparison between different parameters among group A participants along the treatment course

Adverse Events

During the 8 weeks study period, there were no major adverse events reported. However, one adverse event such as diarrhea was reported by two obese adolescents in group A.

Discussion

Adipose tissue performs many functions in the body, being considered an endocrine organ due to substances secreted, called adipokines, yet excess of adipose tissue is called obesity and is associated with a state of chronic subclinical inflammation.

Plant-based foods contain significant amounts of bioactive compounds, which provide desirable health benefits beyond basic nutrition. Various studies assured that consumption of vegetable and fruits based diet has positive impact on the human health. Interestingly, studies showed that abundant natural extracts have been explored for their obesity potential treatment, among which, the Citrus's polyphenols could assist in the management of obesity, since they cause a reduction in adipocyte differentiation, lipid content in the cell and adipocyte apoptosis (Nakajima *et al.,* 2014).

Therefore the aim of the study was to evaluate the efficacy of the *Citrus reticulate* peel extract in developing healthy weight loss whereby, the results in (Table 2) showed that after one month of study there were significant statistical differences between group A and group B according to BMI, WC, M% and TC by $P < 0.05$, while there were no statistically significant differences between them in the W% and BMR. Moreover, after two months, as shown in (Table 3), there were highly significant and promising statistical differences between group A and group B according to BMI, WC, BF%, M%, TC and TG parameters by $p < 0.01$. Also, there were significant differences with mean statistical $p < 0.05$ in W % and satisfactory non-significant reduction of LDL after two months between both groups (Table 3).

The above results indicated that the *Citrus reticulate* peel extract, in combination with a standard diet of (2000 kcal/day, group A) revealed a remarkable reduction in body mass index and body fat percentage by 17.67 % and 10.9 %, respectively as shown in (Table 3, Figure 2) in obese adolescents for 8 weeks which strongly indicated its efficacy for weight management. These findings were assured by the lipid profile criteria resulted whereby beneficial reduction to normal occurred in the total cholesterol and triglycerides levels in group A compared with the placebo alone in group B thus supporting our hypothesis that the supplementation of the Citrus peel extract could improve the lipid profile as it decreases the fatty mass of adipocytes by increasing break-down of the intracellular lipid depot (Janssen *et al.,* 2004).

One of the most significant parameters was the decrease of WC by 11.93 cm in group A compared to 4.74 cm to group B ensuring the extract anti-obesity potential in the adolescent obese. Thus the reduced levels of serum blood lipid profiles reflect improved status of fat metabolism and reduced stored fat (Paek and Hong, 2006). These observations are very important as it proves that the *Citrus reticulate* peel extract may be more effective in reducing visceral over subcutaneous fat tissue, thereby allowing for possible body reshaping and improvements in metabolic parameters (Rehman *et al.,* 2011; Fox *et al.,* 2007). Furthermore, the body metabolic rate wasn't significantly increased (2.56% only) which reveals that the reduction mechanism of the body fat is not due to increase in the metabolic rate and not due to energy expenditure but rather prove that the *Citrus reticulate* peel extract may inhibit the formation of fat cells and intra-cellular fat accumulation and/or by impairing differentiation of pre-adipocyets to mature adipocytes (Hasani-Ranjbar *et al.,* 2008).

It was thus entrenched that the quality of life parameters were improved for subjects ingesting the extract supplement. It has also been reported that peel extracts contain large amounts of flavonoids, such as hesperidin and narnginin which are linked to the anti- obesity health benefits (Abdollahi, 2009; Abbasi *et al.,* 2015). Studies on the mechanisms for the antioxidant, anti-diabetic, cardioprotective, neuroprotective and anticancer activities of the peels have established the interaction with some key enzymes relevant to the management of such diseases. The phenolic compounds and volatile compounds which have been linked with the biological activities of the peels have been characterized. This study looks at the bioactive compounds of Citrus peels and the mechanisms for the biological activities of the peels (Ademosun *et al.,* 2018).

Conclusion

In countries with developing economies, the problem of obesity is emerging at a time when under nutrition remains a significant problem. Strategies that take account of both these important nutritional problems will need to be developed, particularly obesity in children and adolescents. Here comes the importance of natural dietary extracts such as Citrus peel extract which is a cheap source of nutraceuticals and safe dietary means for the management of obesity.

Funding
No funding was provided for this work.

Disclosure
All authors declare no conflicts of interest.

References

Abbasi, H., A.R. Seidavi, W. Liu, et al., 2015. Investigation on the effect of different levels of dried sweet orange *(Citrus sinensis)* pulp on performance, carcass characteristics and physiological and biochemical parameters in broiler chicken. Saudi J. Biol. Sci., 22(2):139-146.

Abdollahi M., 2009. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. World J. Gastroenterol.,15(25):3073-3085.

Ademosun, A.O., G. Oboh, T.A. Olasehinde, et al., 2018. From folk medicine to functional food: a review on the bioactive components and pharmacological properties of Citrus peels. *Orient* Pharm. Exp. Med., Doi:10.1007/s13596-017-0292-8.

Brenot, F., P. Herve and P. Petitpretz *et al.,* 1993. Primary pulmonary hypertension and fenfluramine use. Br. Heart J.,70(6):537-541.

Buslig, B.S. and J.A. Manthey, 2002. Flavonoids in cell function. New York (NY): Kluwer Academic/Plenum Publishers.

Cole, T.J., M.C. Bellizzi and K.M. Flegal, et al., 2000. Establishing a standard definition for child overweight and obesity worldwide, international survey. Br. Med. J., 320(7244):1240-1245.

Cook, N. and S. Samman, 1996. Flavonoids - chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem., 7:66-76.

Daniels, S.R., D.K. Arnett and R.H. Eckel, et al., 1999. Overweight in children and adolescents: pathophysiology, consequences, prevention and treatment. Circulation. 111:1999-2012.

Devendra, D., E. Liu and G.S. Eisenbarth, 2004. Type 1 diabetes: Recent developments. Br. Med. J. 328(7442):750-754.

Fox, C.S., J.M. Massaro, U. Hoffmann, et al., 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation, 116:39-48.

Ghalli, I., N. Salah, F. Hussien, et al., 2008. Egyptian growth curves for infants, children and adolescents. In: Sartorio A, Buckler JMH, Marazzi N, eds. *Crecere nel mondo.* Italy: Ferring Publisher.

Ghasemi, K., Y. Ghasemi and M.A. Ebrahimzadeh, 2009. Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues. PJPS, 22:277-281.

Harborne, J.B. and H. Baxter, 1999. The Handbook of Natural Flavonoids. 2nd ed. New York (NY): John Wiley & Sons, Inc.,

Harborne, J.B., 1993. The Flavonoid Advances in Research since 1986. London: Chapman and Hall, 1993.

Hasani-Ranjbar, S., B. Larijani and M. Abdollahi, 2008. A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch. Med. Sci., 4(3):285-292.

Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacol. Ther., 96:67-202.

Hooper, L. and A. Cassidy, 2006. A review of the health care potential of bioactive compounds. J. Sci. Food Agric., 86:1805-1813.

Janssen, I., P.T. Katzmarzyk and R. Ross, 2004. Waist circumference and not body mass index explains obesity-related health risk. Am. J. Clin. Nutr., 79:379-384.

Kumar, S., A. Gupta and A.K. Pandey, 2013. *Calotropisprocera* root extract has the capability to combat free radical mediated damage. ISRN Pharmacol., ID 691372:8 pages.

Manach, C. and J.L. Donovan, 2004. Farmacokinetics and metabolism of dietary flavonoids in humans. Free Radic. Res., 38:771-785.

Markham, K.R., V.M. Chari and T.J. Mabry, 1982. Carbon-13NMR spectroscopy of flavonoids. In: Harborne JB, Mabry TJ, eds. *The Flavonoid Advances in Research.* 1st ed. London: Chapman and Hall, P:19-134.

Nakajima, V.M., G.A. Macedo and J.A. Macedo, 2014. Citrus bioactive phenolics: Role in the obesity treatment. Food Sci. Tech., 59:1205-1212.

Paek, K.W. and Y.M. Hong, 2006. Health behavior factors affecting waist circumference as an indicator of abdominal obesity. J. Prev. Med. Pub. Health, 39:59-66.

Prasain, J., S. Carlson and J. Wyss, 2010. Flavonoids and age-related disease: Risk, benefits and critical windows. Maturitas, 66:163-171.

Rehman, R.U., K. Usmanghani, H. Nazar, et al., 2011. Clinical study on the management of obesity with certain natural medicine. J. Med. Plants Res., 5:4094-4098.

Rice-evans, C.A., N.J. Miller and P.G. Bolwell, *et al.,* 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res., 22:375-383.

World Health Organization, 2004. Global strategy on diet, physical activity and health. WHO.