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Full Length Research Paper

Comparative effects of peel extract from Nigerian grown citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet

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The effects of ethanolic extracts of five different citrus peels on mean body and liver weight and serum lipid content were investigated in albino rats. Six groups (n=8 each) were fed with a high fat diet for seven days *ad libitum* before oral daily administration of the peel extracts of orange (OR), lemon (LE), lime (LI), tangerine (TA), grapefruit (GR) and synergistic combination of equal ratios (w/w), that is, (SY), respectively at a dose of 500 mg / kg body weight for 14 days. The positive control group received only the high fat diet (HFD), while the negative control group received only a standard diet (STD). The body weights of the animals were monitored every two days and the animals were sacrificed after the 7th and 14th days of or following the administration of the extracts. All the parameters increased in the positive control group (HFD) compared to the negative control (STD) group. Body and liver weights decreased in all treated groups, as well as serum cholesterol and triglycerides, which decreased significantly in SY and GR groups, $p < 0.05$. All extracts contained mainly flavonoids and alkaloids while the grapefruit peel extract contained additional saponins that could contribute to the reduction in both body weight and serum lipid content. Conclusively, peel extract from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage obesity and associated pathologies.

Key words: Citrus peel, high fat diet, obesity, serum lipids, liver and body weight.

INTRODUCTION

Excessive body weight or obesity has in the last few decades become an emerging serious health concern throughout all cultures, especially when the diets tend towards western type. Obesity is generally associated with an increased risk of excessive fat – related metabolic and chronic diseases such as type two diabetes mellitus, hypertension and dyslipidemia (Bays et al., 2006). Excessive weight gain is also generally linked to the onset of cardiovascular disease, cancers fibroid, renal disease and psychosocial incapacity, amongst others (Abu-Abid et al., 2002; WHO, 2002; Hossain et al., 2007). There is also evidence that obesity is associated with increased morbidity and mortality (Huang et al., 2009). In order to reduce the prevalence of these excessive weight-related diseases, several measures, which include production of low fat diets, dietary restriction, use of

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leptin, induction of thermogenesis and liposuction have been tried out. Most recent studies on the treatment of overweight have focused on the potential role of plant constituents, including polyphenols found in citrus (Sindler, 2001; Murase et al., 2002; Aoki, 2007).

Citruses, belonging to the family *Rutaceae*, are one of the main fruit crops grown throughout the world. Citrus fruits have been used by man for centuries for agricultural, medicinal and herbal purposes (Tomar et al., 2013). Several pharmacological properties have been attributed to various members of the citrus species, ranging from anticancer, (Jacob et al., 2000; Silalahi, 2002; Enterazi et al., 2009) antimicrobial, (Nannapaneni et al., 2008; Tao et al., 2009; Dhanavade et al., 2011; Kumar et al., 2011; Lawal et al., 2012), antifungal, (Valezquez-Nunez et al., 2013) antityphoid, (Kumar et al., 2011), antioxidant, (Duda-Chodak and Tarko, 2007), anti-inflammatory, (Galati, 1994; Karaca et al., 2008), antiulcer, (Nagaraju et al., 2012), hypolipidemic (Khan et al., 2010), hepatoprotective (Karaca et al., 2008; Kangralkar et al., 2009) and antidiabetic, (Daniels, 2006; Parmar and Kar, 2007), among others. The peels of the citrus fruits, especially grapefruit and bitter orange, which are rich in flavonoid glycosides, polyphenols and volatile oils have been used in several cultures for weight control, amongst other pharmacological uses (Fujioka et al., 2006; Stohs and Shara, 2007). Previous studies have demonstrated the effects of these flavonoids on lipid and glucose metabolism in experimental animals and humans (Jung, 2004; Miwa, 2005), specifically on lipid catabolism, glucose transport, the insulin-receptor function, and peroxisome proliferator-activated receptors (PPARs) activation, all of which play essential roles in weight control (Shisheva, 1992; Liang, 2001; Kim, 2003; Lee, 2003).

Nigeria is richly blessed with an all year round availability of a number of citrus fruits, most of which form a huge economic asset to both rural dwellers who cultivate the fruits and the urban vendors. The most commonly sold citrus, sweet oranges (*C. sinensis*), are often sold in the peeled form, thus leaving huge amounts of peels as waste. As part of an ongoing search for local herbal drugs for weight control, this work investigated the comparative and synergistic effects of five locally grown citrus fruits, namely, sweet orange (*C. sinensis* L.), lemon, (*C. limon* L.), lime (*C. aurantifolia* L.), tangerine (*C. reticulate* L.) and grapefruit (*C. paradisi* L.) on mean liver and body weights and serum lipids in albino rats fed a high fat diet.

MATERIALS AND METHODS

Materials

Plants

Citrus species used were orange (*C. sinensis* L. (OR), lemon (*C. limon* L. (LE), grape (*C. paradise* L. (GR), tangerine (*C. reticulate* L. (TA) and lime (*C. aurantifolia* L. (LI) obtained from Eke Awka local market and private compounds in Awka, Anambra State. The samples were authenticated by Professor Clement Okeke, Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria..

Table 1. Percentage composition of experimental diets.

Component	STD	HFD
Carbohydrate	73	56
Protein	16	16
Palm oil	3	20
Crude fiber	5	5
Mineral salt[a]	2	2
Vitamins[b]	1	1

[a]The mineral mix above consisted of calcium (0.8 g), phosphorous (0.6 g), manganese (50 mg), zinc (30 mg), sodium (0.15 g). [b]The vitamins consisted of vitamin A (8000 iu), vitamin D3 (2400 iu), vitamin E (15 mg), vitamin B2 (4 mg), vitamin C (50 mg).

Chemicals: All chemicals used in this work are of analytical grade, and products of BDH, (Poole, England), Merck (Germany) and others. Kits by Randox, (UK) were used for the estimation of total serum cholesterol and triacylglycerol.

Animals

Male albino rats of about 6 weeks of age (weighing between 200 – 230 g) were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were left to acclimatize for one week under ambient conditions before the experiments.

The animals were handled in accordance with the guidelines of the Ethics Committee on Animal Research of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

Methods

Extraction and phytochemical analyses

Around 500 g of each peel were air dried, ground into coarse powder, extracted exhaustively with 70% ethanol using the soxhlet apparatus and concentrated *en vacuo* at 40°C. The yields of the extracts were calculated and phytochemical analysis was carried out on the citrus peel extracts according to the method of Harborne (2003).

Experimental procedure

Diets used consisted of corn flour, rice husk, crayfish, palm oil, multivitamins and mineral salts in the percentage combinations (w/w) as stipulated in Table 1:

Rats were divided into eight groups (n = 8 each). All groups, except

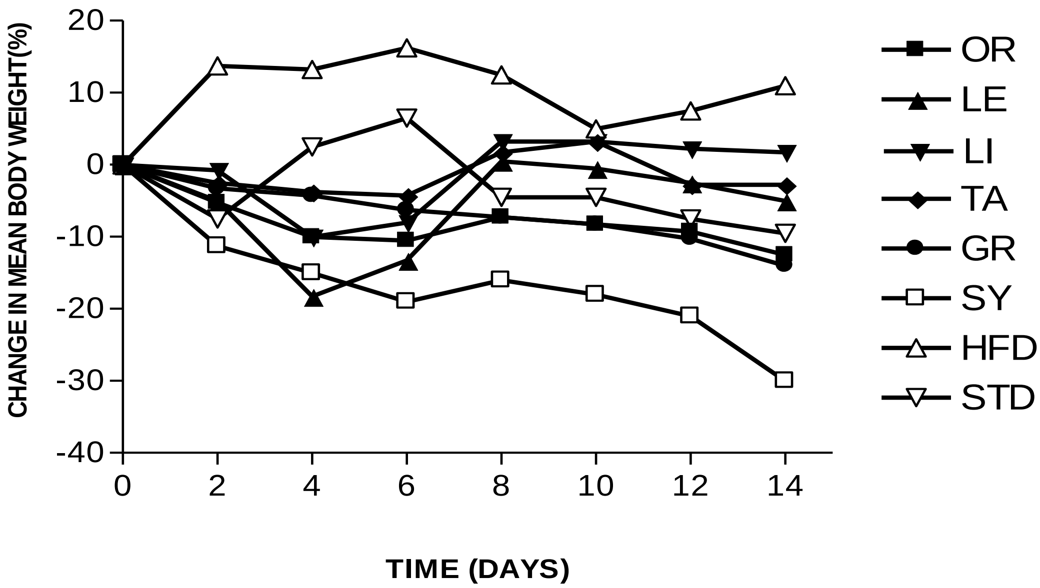

Figure 1. Percentage change from initial mean body weight with time.

the negative control, were initially allowed access to the high fat diet (HFD, Table 1) and water *ad libitum* for seven days. At the end of the 7 days the individual weights of the animals were taken and a daily oral dosage regimen of citrus peel extracts (500 mg/kg body weight of animal) was administered for 14 day as follows:

Group one was fed with HFD and orange peel extract (OR)
Group two was fed with HFD and lemon peel extract (LE)
Group three was fed with HFD and lime peel extract (LI)
Group four was fed with HFD and tangerine peel extract (TA)
Group five was fed with HFD and grapefruit peel extract (GR)
Group six was fed with HFD and synergistic (equal) combinations of the extracts (w/w) (SY)
Group seven was fed with HFD only (HFD; positive control)
Group eight was fed with standard diet only (STD, Table 1; negative control).

The weights of the animals were taken at 2 days interval after overnight fasting, the rats were sacrificed after the 7th and the 14th day of administration of citrus peel extract by cardiac puncture and the serum was collected. The total serum cholesterol and triacylglycerol were determined spectrophotometrically using Randox kits supplied by Randox, (UK). The liver weights at necropsy were also determined.

Statistical analyses

All statistical analyses were performed using Graph Pad Prism (version 4.0). A level of $p < 0.05$ was considered significant. Data were presented as mean ± SEM. The data were tested by ANOVA, followed by Bonferroni's pair-wise comparison test.

RESULTS AND DISCUSSIONS

There was high significant increase ($p < 0.05$) in the mean body weight, as expected, in the rats fed on a high fat diet (Figure 1). The body weight was, however, significantly reduced ($p < 0.05$) by concurrent administration of a daily oral dose of citrus peel (500 mg/kg body weight) for all test groups in the 1st week. The groups LE, LI and TA regained weight in the 2nd week, however, OR, GR and SY groups, significantly and consistently lost weight ($p < 0.05$). The relative liver weight increased significantly ($p < 0.05$) for the high fat fed group by the 14th day. The citrus peel extract caused a general non-significant decrease in liver weight by the 7th day when compared with the HFD group ($p > 0.05$), but there was no further significant loss except only for the OR group by the 14th day (Figure 2). The serum cholesterol levels decreased significantly in all the groups when compared with HFD group, by the 7th day, but the decrease was more in the GR, SY, and OR groups by the 14th day at $p < 0.05$ (Figure 3). Similarly, serum triglycerides also reduced significantly in all test groups, especially for SY, GR, OR and LE groups by the 14th day ($p < 0.05$). The least changes were observed for TA and LI groups (Figure 4).

There was also noticeable reduction of appetite and the animals were observed to show signs of tremor and exfoliation of fur for SY, LI, OR and GR groups in the 2nd week but not for LE and TA groups.

On a comparative basis, these results showed that grapefruits (GR) peel extract followed by orange (OR) were the most effective single remedies, while TA was the least effective for weight loss and for the reduction of cholesterol and triglycerides. A synergistic combination of all extracts was most beneficial.

Phytochemical analysis of the citrus peel extracts used in this work showed the presence of mainly flavonoids, alkaloids and additionally saponins in grapefruits (GR) (Table 2). The relative quantities and identities of the

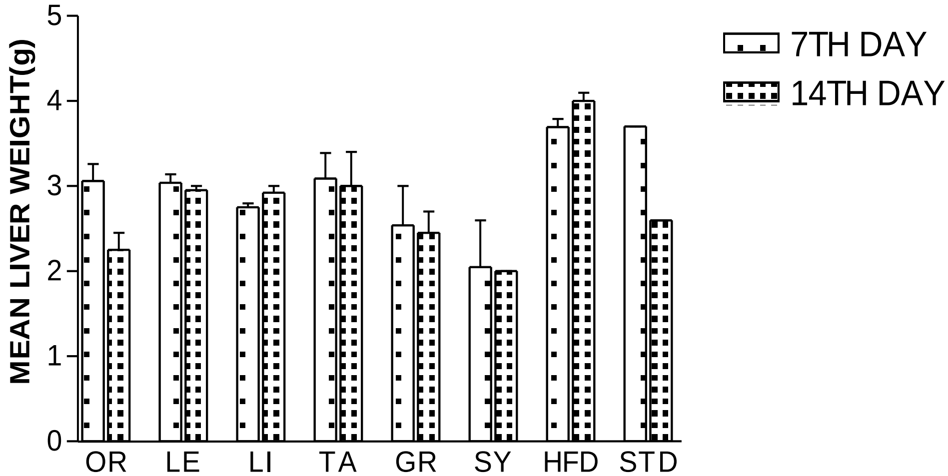

Figure 2. Mean liver weight after 7 and 14 days of administration of extracts.

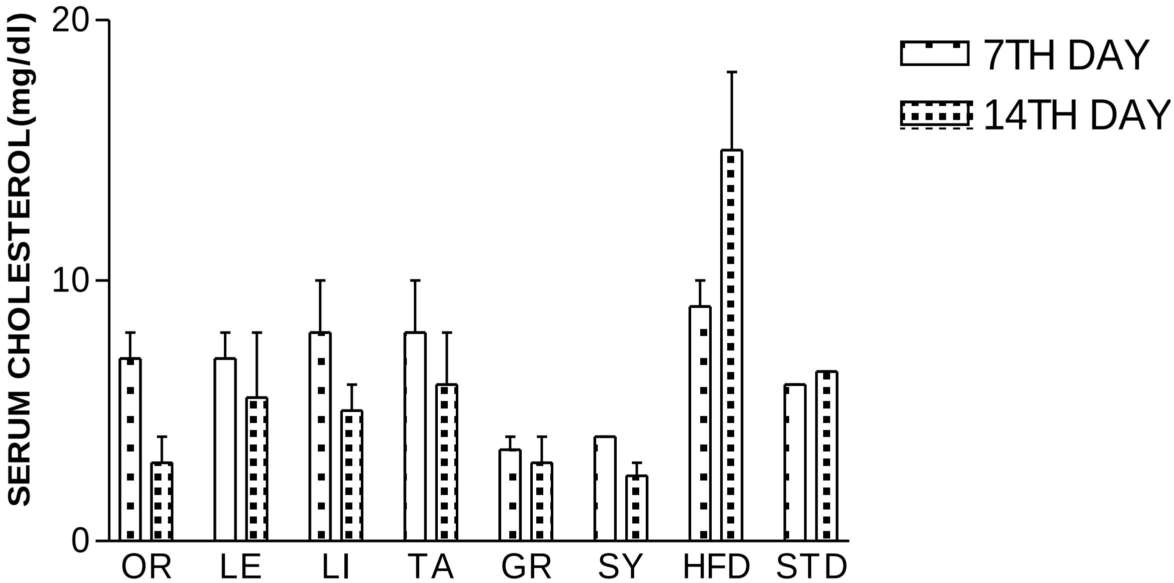

Figure 3. Serum cholesterol levels after days 7 and 14 of administration of extracts.

individual components were however not determined. Polymethoxylated flavones (PMFs - tangeretin, nobiletin, hesperidin, sinensten and naringin) found in the peels and in smaller in amounts in the juices of a variety of citrus fruits have been isolated from tangerine, orange, grapefruit (Rouseff and Ting, 1979). PMFs showed effects in reducing cholesterol (especially LDL cholesterol, by 30 to 40%, although treatment did not appear to have any effect on levels of HDL cholesterol) and to suppress appetite in previous animal studies, suggesting health benefits in cardiovascular health (Hakim and Harris, 2004; Kurowska and Manthey, 2004).

There was noticeable reduction of appetite for SY, LI, OR and GR group\s in the second week, observed by the increasing amount of leftover food per day (actual weight not determined). This may have been due to the presence of polyphenols, as well as pectin in the peel extracts. Pectin, though not soluble in pure alcohol, is extractible in the hydroacholic solution (70%, (v/v) used in this work. Pectin reduces appetite by swelling into a gel in the stomach to give a feeling of fullness for at least 4 h (Rayburn et al., 1998). Another mechanism for weight loss may include stimulation of β - 3 cell receptors, thus eliciting thermogenesis, leading to increased lipolysis and metabolic rate (Preuss et al., 2002). It has also been determined that PMFs also help reduce cortisol levels.

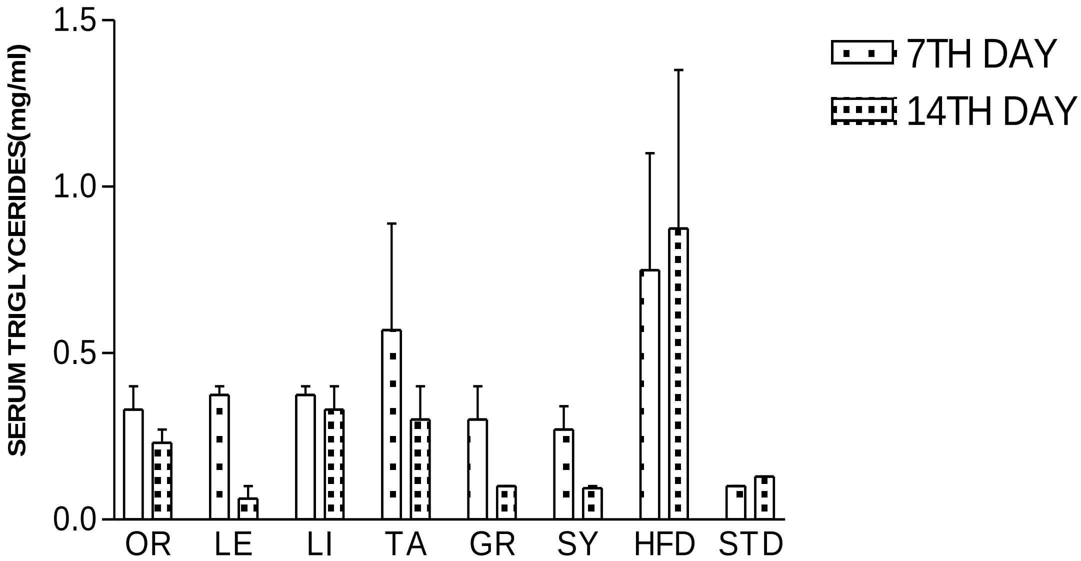

Figure 4. Serum triglycerides after days 7 and 14 of administration of extracts.

Table 2. Yield and phytochemical analyses of citrus peel extracts.

Sample yield (%)	Tannins	Saponins	Glycosides	Flavonoids	Alkaloids
OR (4.63%)	-	-	-	+	+
LE (4.52%)	-	-	-	+	+
LI (3.68%)	-	-	-	+	+
TA (3.50%)	-	-	-	+	+
GR (5.54%)	-	+	-	+	+

-Absent; + Present.

Cortisol is a stress hormone, higher levels of which have been linked to weight gain. The use of PMFs to reduce systemic and local cortisol concentrations (liver and adipose tissue), has been beneficial in promoting blood sugar control and weight loss (Talbot, 2009). Grapefruit peels have been reported to promote weight loss by reducing insulin spike after a meal, thus the body processes more food for use as energy and less is stored as fat. Grapefruit extract was the most effective single extract in this work.

The mechanisms by which cholesterol and triglycerides were reduced could be due to interaction of extract with bile acids thus preventing reabsorption of the bile acid, and therefore, cholesterol or by inhibition of β HMG CoA reductase and acyl CoA cholesterol acyl transferase (ACAT), thereby preventing de novo synthesis, or by increased lipase activity (Bok, 1999). Hesperidin and naringin, and their aglycones hesperetin and naringenin, have been reported to decrease plasma and hepatic cholesterol and triacylglycerol by inhibiting these hepatic enzymes in experimental animals (Lee, 1999; Lee, 2003; Kim, 2003). A study also demonstrated that hesperidin and naringin were beneficial for improving hyperlipidemia and hyperglycemia in type-2 diabetic animals by partly regulating the fatty acid and cholesterol metabolism and affecting the gene expression of glucose-regulating enzymes; they also markedly enhanced hepatic and adipocyte PPARγ protein expression (Jung et al., 2006). Furthermore, naringenin increased hepatic fatty acid oxidation through up-regulation of the gene expression of enzymes involved in peroxisomal β-oxidation and white adipose tissues in mice (Huong, 2006; Hamendra and Anand, 2007; Fukuchi et al., 2008).

Other positive effects of citrus extracts reported include antiviral, antiulcer, anticancer, antioxidant, diuretic, anti -allergy, antihypertensive, antimutagenic, relief of stomach upset, distension and asthma (Kim et al., 2000; Murakami et al., 2000; Kanaze et al., 2008).

Other constituents of citrus peels include essential oils which have lipolytic, antimicrobial, antioxidant and anti-inflammatory effects; (Hyang-Sook, 2006; Kanaze et al., 2008; Oliveira et al., 2014) and also vitamin C which contributes to effective digestion and weight loss by increasing acidity thereby increasing calcium assimilation and replacement of fat in cells.

No negative side effects were reported in previous work in the animals that were fed with PMFs. However, in this work, the animals showed signs of tremor and exfoliation of fur for SY, OR and GR groups, both in the second week. Synephrine, the major alkaloid of C. aurantium, similar in structure to epinephrine was reported to exhibit milder ephedrine-like effects, which range from CNS stimulation, energy boost, appetite suppression to increased fat metabolism without the cardiovascular side effects of nervousness, dry mouth and high blood pressure (Pellati et al., 2002). A number of adrenergic alkaloidal amines (synephrine, n-methyl tyramine, hordemine, octopamine and tyramine) have been reported in the Mediterranean citrus, *C. aurantium*, as major ingredients of dietary supplements for weight loss (Pellati et al., 2002). The citrus peels used in this work contained alkaloids, although not classified.

Results obtained in this work and other previous reports that assessed the effects of these citrus flavonoids on lipid and glucose metabolism have led to the conclusion that the extracts from peels of Nigerian grown citrus could prevent the development of obesity through the modulation of lipid and glucose metabolism, with grapefruit peels being the single most effective peels while synergistic effect gave best results. Grapefruit peels have been reported to contain the highest total phenolics and the highest total antioxidant activity, followed by sweet orange peels, while tangerine peels had the least (Li et al.,2006; Londono-Londono et al., 2010).

Conclusively, peels from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage weight problems and associated pathologies. However, despite the positive effects of citrus extracts in weight reduction in this work, there may be risk of cardiovascular toxicity, due to the possible presence of adrenergic amines such as synephrine, n-methyl tyramine etc, which may have epinephrine-like action. Dosage control may be required to reduce the adverse effects. It has, however, been reported that different extraction processes may result in different products with varying concentrations and ratios of PMFs. The extraction process can therefore be selected and modified as desired to shift the ratios of the component PMFs (Kawaii et al., 1999) to enhance the beneficial and reduce the untoward effects, although the soil composition may have an effect on the concentrations of constituents. However, additional studies are needed to validate these conclusions.

Conflict of interests

The authors did not declare any conflict of interest.

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