Original Research Article

Flat Tummy Water Attenuates Lipid Profile and Serum Glucose of High-Fat Diet-Induced Obese Female Wister Rats

Abstract

Background

Flat tummy water (FTW) is herbal tonic preparation made from cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*) and is believed to be effective in the reduction of central/abdominal fat. While the potency and efficacy of FTW are hardly known, the present study presents a scientific investigation of its effect on high-fat-induced obese Wistar rat models.

Method

FTW was constituted using measured quantities of cut and infused cucumber, lemon and mint leaf while the individual plants were extracted and mixed to get an extract mixture (EM). Twenty-five (25) female Wistar rats were used for the study. They were divided into five (5) groups of five (5) animals each. Groups 1 & 2 served as the normal and negative controls and received standard rat chow/distilled water and high-fat diet/distilled water respectively. Groups 3, 4 & 5 served as the experimental groups. Group 3 received FTW *ad libitum* while groups 4 & 5 received 25 and 50mg/kg of extract mixtures (EM) respectively alongside the formulated high-fat diet. Lipid profile and serum glucose were determined using standard colourimetric methods. The body weight, naso-anal length, abdominal and thoracic circumferences (AC/TC) of the animals were measured before and after the experiment.

Result

There was a significantly reduced total cholesterol, triglycerides, low-density lipoproteins, very low-density lipoproteins and serum glucose and significantly increased high-density proteins among the obese animals treated with FTW and EM compared to the untreated obese negative control ($p<0.05$). The study also observed reduced body weight, AC/TC ratio and lee obesity index among treated obese animals when compared with the untreated negative control.

Conclusion

The present study shows that flat tummy water (FTW) as well as extracts mixtures (EM) from the component of FTW indeed caused a mild reduction in central abdominal obesity. FTW and EM showed potent hypolipidaemic, hypoglycaemic and anti-obesity activity. This study concludes that these observed effects could be due to the synergistic action of cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*).

Key Words: flat tummy water, lipid profile, serum glucose, AC/TC ratio, lee index

INTRODUCTION

The incidence of obesity has received global attention as a health burden and preceding factor in cases of terminal illnesses like diabetes, cancer, cardiovascular diseases and some neurological disorders [1, 2]. Over twenty (20) years ago, the World Health Organization (WHO) declared obesity a global epidemic with more than 39% of the adult population affected [3, 4]. Obesity results from the interplay between nature (genetic makeup) and nurture (high-energy diets and sedentary lifestyles) [5, 6]. In the face of the increased economic burden associated with the management of obesity, there has been an increase in the use of natural agents derived from plants, herbs and vegetables as potential solutions for achieving weight loss [7-9]. These preparations are used alone or in combination with other plants, herbs and vegetables. Some of the plants screened for their anti-obesity potential include *Amorphophallus koniac*, *Camellia Sinensis, Nigella sativa, Hibiscus, sabdariffa, Ginkgo biloba, Phaseolus vulgaris* and *Dioscorea bulbifera* [8, 10, 11]. The mechanism of action of some of these natural preparations includes improving lipid metabolism, appetite suppression and anti-lipase activity [7, 8, 11-13].

There are many other plant preparations which are yet to undergo scientific evaluation for their efficacy as anti-obesity agents. One such is the widely acclaimed "flat tummy water" (FTW). The origin of the FTW water is largely unknown and has continued to gain popularity in society, especially among the many health, wellness and fitness centres and online blogs. As the name suggests, it is said to be an indigenous herbal tonic preparation used specifically in the reduction of central/abdominal fat. Though a few variations on the composition of FTW water exist, it is typically composed of cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*). They are cut and soaked in water for about eight (hours) after which the aqueous mixture is regularly taken to attain a slimmer body shape, especially in the abdominal region [14, 15].

Cucumber (*Cucumis sativus*) is the most widely cultivated plant from the family of *Cucurbitaceae*. It is a cylindrical creeping fruit which is used in salads and also for medicinal purposes. The fruit is used in the folk remedy treatment of bronchitis, hepatitis, asthma and dyspepsia [16-19]. It has also been shown to possess hypolipidaemic, wound healing, anti-cancer, anti-diabetic and hypolipidaemic properties [19-23]. Lemon (*Citrus limon*) is a flowering plant from the family of Rutaceae. Lemon juice is consumed as a homemade drink as lemonade and cocktails. It is also used as a cleaning agent and as a preservative for baking. Folk medicinal applications include uses as an appetizer, treatment for a boil, throat and tonsillar abscesses, anthelmintic and remedy against drunkenness [24, 25]. Lemon has also been shown to possess antibacterial, hypolipidaemic, hepatoprotective and antioxidant properties [26-29]. Ginger (*Zingiber officinale*) is a herbaceous flowering plant commonly known by its root or rhizome. It belongs to the family Zingiberaceae and is mostly used as a spice and in folk medicine for the treatment of cough, rheumatism, nausea, asthma and loss of appetite [30, 31]. Mint (*Mentha piperita*) is a strongly scented glabrous perennial herb thought to be a natural hybrid between watermint (*Mentha aquatica*) and spearmint (*Mentha spicata*), all from the family of Lamiaceae [32, 33]. Their scented nature makes them applicable as a flavouring and cosmetic agent with varying uses in traditional medicine such uses in the treatment of headaches, fever, influenza and red eyes [33, 34]. Some of the pharmacological effects of the mint leaf include antibacterial, anti-nociceptive, antioxidant, hepato-protective and anti-carcinogenic activity [35-39].

With increasing testimonies highlighting the efficacy of FTW as an effective herbal tonic in reducing truncal adiposity, the present study, therefore, is aimed at evaluating the effect of flat tummy water on lipid profile, body weight and body lee index using female Wistar models. This study is an attempt to provide a scientific basis for the use of FTW.

MATERIALS AND METHODS

Plant Materials

Freshly harvested cucumber, lemon, ginger and mint leaf were sourced from the Fruit Garden Market, Port-Harcourt, Nigeria. The plants were identified and authenticated by a botanist at the Plant Science and Biotechnology Department of the University of Port Harcourt.

Preparation of Flat Tummy Water

The FTW was prepared according to the already existing formulation using 550g of cucumber, 130g of lemon, 10g of ginger and 15g of mint leaves [14, 15]. The plants were cut into and soaked in 6L of water for eight (8) hours. The mixture was refrigerated at 5ºC pending administration.

Preparation of Plant Extracts and Phytochemical Screening

Measured quantities of cucumber, lemon, ginger and mint leaves were constituted according to flat tummy formulation, sliced and air-dried for two (2) weeks. Individual grounded plant materials (1kg) were subjected to ethanolic extraction using standard methods. [40]. Phytochemical screening of each plant was performed by methods described by Trease and Evans [41]. The extracts were mixed and re-constituted to simulate the standard FTW composition.

Research Animals

Fifteen (15) female mice (20-30g) and twenty-five (25) female Wister rats (155 – 214g) were used for the study. They were sourced from the animal house of the Department of Human Physiology, Faculty of the Basic Medical Sciences University of Port Harcourt. They were allowed two (2) weeks of acclimatization under the natural light/dark cycle and access to standard rat chow and water *ad libitum*.

Determination of LD_{50} of Plant Extract Mixture (EM)

An acute toxicity test of the plant EM was carried out using fifteen (15) female mice according to the method described by Karber [42].

Induction of Obesity

A high-fat diet was formulated using a standard rat chow (80%) and rendered cow fat. The animals were allowed to feed on the formulated high-fat diet for seven (7) weeks. The length and weight of the animals were measured and Lee obesity index was calculated as follows:

$$Lee\ Obesity\ Index = \frac{\sqrt[3]{weight}(g)}{naso - anal\ lenght\ (cm)}$$

Abdominal and thoracic circumferences were also measured to obtain an abdominal/thoracic ratio (AC/TC ratio) as follows:

$$\text{AC/TC ratio} = \frac{Abdominal\ Circumference\ (cm)}{Thoracic\ Circumference\ (cm)}$$

After seven (7) weeks on a high-fat diet, animals with up to 35% weight increase or a Lee obesity of >0.30 were considered obese and selected for the study [43, 44].

Experimental Design

Thirty (25) female Wistar rats were used for the experiment. They were assigned to five (5) groups of five (5) animals each and treated as follows

Groups	Name	Treatment
1	Control	Normal rat chow + Distilled water
2	Negative Control	High-fat diet + Distilled water
3	FTW	High-fat diet + FTW (*ad libitum*)
4	Low Dose EM	High-fat diet + 25mg/kg EM
5	High Dose EM	High-fat diet + 50mg/kg EM

The oral administration of the FTW, plant extract mixture (EM) lasted for fourteen (14) days. Animals in group 3 (High-fat diet + FTW) were allowed to drink from the formulated FTW *ad libitum*.

Determination of food Intake

The quantity of rat chow left after twenty-four (24) hours of feeding were collected and measured three (3) times a week to determine food intake as follows

$$Food\ intake = \frac{Amount\ of\ chow\ given\ -\ Amount\ of\ chow\ left\ afer\ 24\ hours}{Number\ of\ animals\ in\ a\ cage}$$

Blood Collection and Analysis

The animals were anaesthetised by cervical dislocation following overnight fasting. The blood sample was collected by cardiac puncture and analysed for triglycerides (TG), total cholesterol (TC) and high-density lipoproteins (HDL) were determined using standard spectrophotometric methods while fasting blood glucose was measured using a standard glucometer (Accu-Chek, UK). Low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) are estimated using the standard formula [45, 46].

Statistical Analysis

The statistical analysis was done using IBM Statistical Product and Service Solutions (SPSS) version 25. The only-way ANOVA was used to determine the difference among the groups followed by Fisher's Least Significant Difference (LSD). A paired t-test was used to determine the difference in initial and final body weight, AC/TC ratio and Lee body index of the animals. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1: Qualitative Phytochemical Screening of ethanolic extracts of cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*).

Phytochemical	Test	Cucumber	Lemon	Ginger	Mint leaf
Alkaloids	Meyers	+	-	++	++
	Drangendorffs	+	++	++	++
	Hagers	+	+	++	++
Tannins	Ferric chloride	+	++	++	+
Saponin	Frothing	-	+	++	++
	Emulsion	-	+	-	++
Flavonoids	Shinola	+	+	++	++
	NaOH	+	+	++	++
Carbohydrate	Molisch	+	+	++	++
	Fehlings solution	+	+	++	++
Anthraquinones	Free anthraquinone	-	+	-	-
	Combined	+	+	+	-
Cardiac glycoside	Keller killiani	+	+	++	+
	Liebernzano	-	-	++	+
	Salkwoski	+	-	++	-
	Keddeksp	+	-	++	-
Cyanogenic glycoside		-	-	-	-
Fixed oil		-	++	++	++
Phenols		++	++	++	++

= Absent, + = Present, ++ = Largely Present

Qualitative Phytochemical Screening of ethanolic extracts of Cucumber, Lemon, Ginger and Mint leaf (Table 1) show the presence of alkaloids, tannins, flavonoids, carbohydrate and phenols among all the extracts with ginger and mint leaf having higher quantities of these phytochemicals. It was observed that saponin was not detected in cucumber extract while mint leaf extract did not contain any detectable anthraquinones. Cardiac glycosides were not found in any of the extracts

Table 2: Effect of flat tummy water (FTW) and extract mixture (EM) on lipid profile of high-fat diet obese Wistar rats

Groups	**TC (mmol/l)**	**TG (mmol/l)**	**HDL (mmol/l)**	**LDL (mmol/l)**	**VLDL (mmol/l)**	**Glucose (mmol/l)**
Control	1.86±0.51	0.96±0.80	0.52±0.42	0.90±0.41	0.44±0.04	6.12 ± 0.47
Negative control	2.94±0.86	1.57±0.10	0.39±0.18	1.52±0.86	0.71±0.05	9.58 ± 0.34
Positive control	2.14±0.51	1.28±0.45	0.45±0.37	1.11±0.40	0.58±0.02	6.76 ± 0.43
FTW	2.31[a]±0.14	1.16[a]±0.23	0.59[ab]±0.59	1.36[b]±0.13	0.53[a]±0.01	6.04[a] ± 0.56
25mg/kg EM	2.24[a]±0.14	1.27[a]±0.46	0.51[a]±0.37	1.35±0.46	0.58[a]±0.04	7.16[a] ± 0.42
50mg/kg EM	2.21[a]±0.15	1.21[a]±0.92	0.66[ab]±0.30	1.28[a]±0.36	0.55[a]±0.41	7.88[a] ± 0.34

mean±standard error of mean. significantly different compared to negative[a] and positive[b] control

Table 2 shows the effects of FTW and the ethanolic mixture of cucumber, lemon, ginger and mint leaf. Mean values of serum total cholesterol (TC), triglycerides, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and glucose were found to be significantly lower among the obese animals treated with FTW (Group 4) and the EMs (Groups 5&6) compared to the negative control ($p<0.05$). In the same way, mean values of serum high-density lipoproteins (HDL) among experimental groups (Groups 4, 5 & 6) were found to be significantly higher compared to the negative control (group 2) ($p<0.05$).

Table 3: Effect of flat tummy water (FTW) and extract mixture (EM) on Body weight, AC/TC ratio and Lee index of high-fat diet obese Wistar rats

Groups	Body Weight (kg)		AC/TC ratio		Lee Index	
	Initial	Final	Initial	Final	Initial	Final
Control	202.00±9.71	216.00±10.07	1.12±0.01	1.11±0.01	0.295±0.004	0.298±0.004
Negative control	155.00±4.17	168.00±6.23	1.09±0.01	1.08±0.02	0.305±0.003	0.308±0.002
Positive control	183.40 ±3.06	174.80±4.02	1.10±0.02	1.06±0.02	0.308±0.004	0.297±0.002
FTW	194.40 ±2.69	190.80 [a] ±3.56	1.13±0.01	1.07[a]±0.01	0.301±0.001	0.302±0.003
25mg/kg EM	201.20±3.38	199.00±3.69	1.14±0.11	1.08[a]±0.01	0.303±0.001	0.296±0.001
50mg/kg EM	214.20±8.08	212.00±6.16	1.13±0.31	1.03[a]±0.01	0.304±0.001	0.293[a]±0.002

mean±standard error of mean. significantly different compared to the initial ($P<0.05$)

The effect of FTW and EM on body weight, AC/TC ratio and Lee index of high-fat diet obese Wistar rats are shown in table 3 above. The results indicate that there was a significant reduction in the final body weight, AC/TC ratio of obese animals treated with FTW and EM compared to their initial ($P<0.05$). Only obese rats treated with 50mg/kg of EM had a significant reduction in their final Lee index compared to the initial ($P<0.05$).

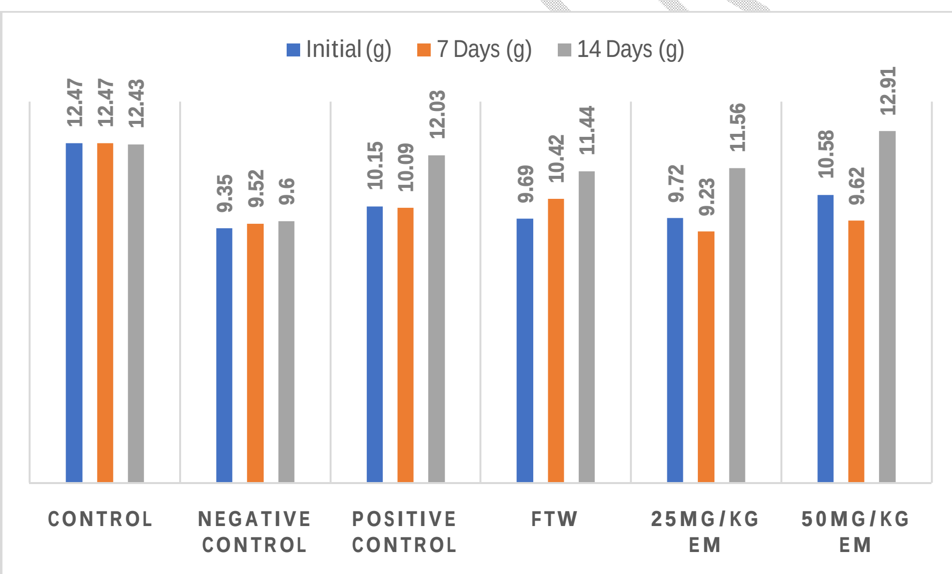

Figure 1: Effect of flat tummy water (FTW) and extract mixture (EM) on food consumption of high-fat diet obese Wistar rats

The effect of FTM and EM on the food consumption of high-fat diet obese Wistar rats is shown in Figure 1 above. The results indicate that there was a slight reduction in food consumption among animals treated with FTW and EMs after the first week of administration ($p<0.05$).

However, the food consumption significantly increased after the second week of administration compared to the initial food consumption ($p<0.05$)

DISCUSSION

Flat tummy water (FTW) is herbal tonic preparation made from cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*) and is believed to be effective in the reduction of central/abdominal fat. While the potency and efficacy of FTW are hardly known, the present study presents a scientific investigation of its effect on high-fat-induced obese Wistar rat models.

Effect on Lipid Profile

The result from the study shows a significantly reduced serum TC, LDL and VLDL among obese animals treated with FTW and extract mixture (EM) of cucumber, citrus lemon and ginger compared to the untreated animals (negative control) while serum HDL increased among FTW and EM treated animals compared to the negative control ($p<0.05$). This is attributable to the similarity of the individual phytochemical constituents of FTW such as flavonoids, tannins, alkaloids and saponins (Table 1). Flavonoids are known to impact lipid metabolism by reducing cholesterol synthesis or increasing LDL receptor expression [47, 48]. Also, saponins have been shown to suppress cholesterol absorption, increase lipid peroxidation and depress the rates of intestinal hepatic and intestinal cholesterol synthesis [48, 49]. *Cucumis sativus* has been demonstrated as a potent antihyperlipidemic agent in both human and animal studies [23, 50]. This is heavily linked to the linoleic acid and resin content of their seeds which have been severally demonstrated to lower LDL and TG [51, 52]. Similarly, *Citrus limon* is effective in attenuating lipid profiles due to its antioxidant nature and its ability to modulate lipid peroxidation [53-55]. Furthermore, *Mentha piperita* also possesses antihyperlipidemic activities as has been previously demonstrated [49, 56, 57]. This has been attributed to its constituent oils: menthofuran, menthol, menthyl acetate, neomenthol, menthone and isomenthone which are known to exhibit antioxidant and lipid peroxidation activity [56]. Hence, the improved lipid profile observed in this study suggests a synergistic antihyperlipidemic activity of the constituents of FTW and EM.

Effect on Serum Glucose

Similarly, data (Table 2) from the present study indicate that serum glucose was significantly reduced among obese animals treated with FTW and EM compared to the untreated animals (negative control) ($P<0.05$). The similar phytochemical constituents of FTW and EM suggest that they could have a glucose-lowering effect. *Cucumis sativus* extracts are effective in attenuating blood sugar levels among diabetic animals [58-60]. They are thought to increase glycolysis and peripheral insulin sensitivity [60, 61]. In the same way, *Citrus limon* has also been shown to be a potent antihyperglycaemic agent against diabetic animals [55, 62]. This is thought to be by improving glucose tolerance, reducing insulin resistance and restoring oxidant balance [62, 63]. Also, *Citrus limon* has been demonstrated to decrease blood glucose levels among diabetic animals [64, 65] by improving insulin secretion, and reducing lipid peroxidation and oxidative stress [56, 66]. These glucose-attenuating actions of FTW and EM can be due to the improved lipid metabolism as observed in this study. Randle's glucose-fatty acid cycle postulates that an increase in plasma triglycerides leads to an increase in free fatty acid (FFA), hence their oxidation tends to weaken glucose metabolism and insulin action, leading to hyperglycaemia. Therefore, the attenuation of FFA will lead t a reduction in plasma glucose [67]. Therefore, the improved serum glucose observed in this study suggests a synergistic antihyperglycaemic activity of the constituents of FTW and EM.

Effect on Body weight, AC/TC ratio, Lee Index and Food Intake

Data from the present study show that FTW significantly reduced the final body weight and AC/TC ratio of the obese animals while 50mg/kg of the EM significantly reduced the final Lee index of the obese animals (Table 3). The AC/TC ratio and the Lee Index serve as anthropometric obesity measurements for laboratory animals [44, 68]. A reduction in the AC/TC ratio suggests a decrease in central abdominal fat while a decrease in the Lee index a reduction in adipose tissue among the obese animals treated with FTW and EM. This goes further to show the effect of reduced plasma lipids as observed in this study (Table 2). There was a slight reduction in food intake for the obese animals treated with FTW and EM in the first week of the administration followed by a significant rise in food consumption within the last week of the experiment. This slight reduction in food intake appears to follow the second stage observed among high fat-induced obesity which is characterized by a reduction in food intake due to increased leptin production with retained central leptin sensitivity. The third stage involves increased food intake with a reduction in central leptin sensitivity [69].

CONCLUSION

The present study shows that flat tummy water (FTW) as well as mixtures of the extracts (EM) from the component of FTW indeed caused a mild reduction in central abdominal obesity. FTW and EM showed potent hypolipidaemic, hypoglycaemic and anti-obesity activity. This study concludes that these observed effects could be due to the synergistic action of cucumber (*Cucumis sativus*), lemon (*Citrus limon*), ginger (*Zingerber officinale*) and mint leaf (*Mentha piperita*).

Ethical Approval

The animals were treated according to the highest ethical standard as the experimental design and protocol were approved by the Centre of Research Management and Development (CRMD) of the University of Port Harcourt. (UPH/CEREMAD/REC/04)

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