IJPSR (2021), Volume 12, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 May 2020; received in revised form, 02 September 2020; accepted, 23 September 2020; published 01 May 2021

NEGLIGIBLE EFFECT OF SOME COUNTERFEIT ANTI-OBESITY DRUGS

Maryam A. AL-Ghamdi [1], Etimad A. Huwait [1], Mohamed A. Abdelshakour [2], Ghada M. Hadad [3], Dina M. Abo-Elmatty [4] and Asmaa R. Abdel-Hamed [* 4]

Department of Biochemistry, Science Collage [1], King Abdulaziz University
Forensic Medicine Administration [2], Ministry of Justice, Egypt.
Department of Pharmaceutical Analytical Chemistry [3], Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.
Department of Biochemistry [4], Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.

Keywords:

Counterfeit drugs, Obesity, Orlistat

Correspondence to Author:
Asmaa R. Abdel-Hamed

Lecturer of Biochemistry, Faculty of Pharmacy, Suez Canal University, 41522 Ismailia, Egypt.

E-mail: asmaa.ramdan@pharm.suez.edu.eg

ABSTRACT: Objective: To evaluate the effect of some counterfeit anti-obesity drugs that are not licensed by the Ministry of Health. **Materials and Methods:** 100 male rats were divided into 10 groups. The first group received a normal diet for two months. The second group received a high-fat diet (HFD) for two months; the third group received HFD for one month then returned to a normal diet. The remaining seven groups received HFD for one month then treated with Orlistat, Turbo slim (dose 1), Turbo slim (dose 2), African mango (dose1), African mango (dose 2), Slim factor (dose 1), and Slim factor (dose 2) respectively for an additional one month. Bodyweight, glucose, insulin, leptin, adiponectin, MCP-1, reduced glutathione, MDA, lipid profile, kidney, and liver functions were measured. Adipose tissue index, atherosclerosis index, HOMA-IR, and QUICKI were calculated. **Results:** Feeding with HFD induced a significant increase in body weight as well as insulin resistance, serum lipids, serum leptin, plasma MCP-1, and tissue MDA and a significant decrease in serum adiponectin and tissue GSH as compared to the normal group. These measurements were significantly reversed after cessation of HFD. Treatment with orlistat significantly reduced serum TAG, insulin resistance, serum leptin, and plasma MCP-1 and significantly increased serum adiponectin and tissue GSH as compared to group ceased HFD. There is no notable changes observed in groups treated with the other three drugs as compared to group ceased HFD. **Conclusion:** Our data show that the three studied counterfeit drugs had a negligible anti-obesity effect in comparison with registered Orlistat.

INTRODUCTION: Counterfeit is a developing trend around the world, which threatens both safety of the patient as well as the confidence in the health systems designed to provide oversight and control. There was a high prevalence of spurious, falsely-labeled, fake, or counterfeit medicines that are deliberately and fraudulently produced, packaged and/or mislabeled.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(5).2603-12

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(5).2603-12

Counterfeits first identified as an issue in the mid-1980, ranging from random mixtures of harmful toxic substances to inactive and ineffective preparations. Some contain a declared, active ingredient and look so similar to the genuine product [1]. Obesity is a condition in which excess fat has accumulated to the extent that health is

adversely affected [2]. A person with Body mass index (BMI), which is the ratio of person's weight to the square of his/her height, is over 30 kg/m^2 is considered obese, and generally, people with BMI between 25–30 kg/m^2 are overweight [3]. Obesity is usually caused as a result of increased energy intake and decreased energy output due to poor nutritional habits, lack of physical activity, genetic predisposition, or medical reasons. Overweight and obesity are among the greatest public health challenges of the 21st century as they increase the risk of developing cardiovascular diseases, diabetes, musculoskeletal disorders, especially osteoarthritis, sleep apnoea, respiratory problems, and cancers of the breast, endometrium, colon, prostate, kidney, and gallbladder [4].

Behavioral modification such as diet and exercise should be included in all overweight and obesity management approaches. Drugs should be used only as adjunctive support to lifestyle change therapy. Currently, few drugs are approved for weight loss purposes, such as Orlistat, naltrexone/bupropion and liraglutide in both USA and EU, and lorcaserin and phentermine/topiramate in the USA for long-term treatment as well as phentermine and diethylpropion for short term use (3 months) in USA [5]. Drugs used for obesity management are usually expensive and should be administered under medical supervision as they can have serious adverse effects [6]. Hence, as the tendency of patients is to self-treat overweight and obesity, they are tempted to buy weight loss food supplements (FS) that are freely available in pharmacies, health food stores, media, and on the internet, and aggressively marketed with extreme claims such as quick and easy weight loss, totally safe, all-natural or 100% natural [7].

The Egyptian Ministry of Health and Population estimates that 10 percent of the pharmaceutical products sold in the country are counterfeit [1]. Investigators and inspectors found that counterfeit drugs are starting from pharmaceutical products used in organ transplants to those prescribed for disorders such as heart diseases, antipsychotics, obesity, erectile dysfunction, cancers, diabetes, and hyperprolactinemia [1]. Nowadays, there are lots of illegal products produced and sold online or in community pharmacies without being registered by the ministry of health. This gives us a great potential to search for counterfeit or suspected drugs as counterfeit, especially with the herbal origin. Three products (African mango®, Turbo slim®, and Slim factor®) from the market which is not registered by the ministry of health were chosen to conduct this study. *Irvingia gabonensis*, African mango seed extract, was the main ingredient labeled on packaging material with green coffee bean extract, *Garcinia cambogia*, and raspberry extract for the first brand, "African mango brand". *Garcinia cambogia* was labeled as the main constituent of the "Slim Factor brand," and finally, the brand Turbo slim was labeled to be 100% natural with no active ingredients on the package.

The literature survey on these herbal constituents revealed that an article had been published about the effect of African mango seeds on growth and lipid metabolism of young rats Wistar species [8] and its effect on overweight and obesity [9]. Green coffee seeds extracts has been cited in many articles as a weight loss supplement [10], also raspberry has been cited as anti-obesity [11].

A lot of scientific articles have been cited about *Garcinia cambogia* as a herbal medicine used for weight loss and appetite suppression [12-14], but it was noticed that few articles recorded a hepatotoxic effect of *Garcinia cambogia* [15]. This may call for the urgent need to raise awareness and health education of the seriousness of the usage of herbal products promoted by full validity and safety.

There was no literature about the counterfeit drugs used in the treatment of overweight and obesity in Egypt, so the aim of this work was to put a spot light on the counterfeit drugs in market especially that traded as food supplement which enter the country by illegal way and study the effect of these formulas on the metabolic parameters of rats.

MATERIALS AND METHODS:

Animals and Experimental Design: One hundred male albino rats purchased from the Egyptian Organization for Biological Products and Vaccines, Cairo, Egypt, were housed in standard cages and maintained under controlled room temperature (25 ± 3) and normal light-dark cycle with free access to food and water. Rats had an initial body weight of 160-165 g. Ten rats received normal palatable diet (NPD) for two months; the remaining 90 rats

received high-fat diet (HFD) for one month to establish diet-induced obesity; t**able 1** illustrates the formula of the HFD; it provides 17% energy as carbohydrates, 25% as protein, and 58% as fat as a percentage of total kcal/g [16].

TABLE 1: COMPOSITION OF THE HIGH-FAT DIET

Ingredients	Amount (g/kg)
Powdered NPD[1]	365
Lard[2]	310
Casein[3]	250
Cholesterol[4]	10
Vitamin and mineral mix[5]	60
DL-Methionine[6]	03
Yeast powder[7]	01
Sodium chloride[8]	01

Purchased from the market[1, 2, 7], Difco (Becton Dickinson [3], France), Oxford Lab [4], Mumbai, India [5, 6], Sigma-Aldrich, MO, USA [8]; ADWIC Co., Cairo, Egypt

Rats receiving HFD were divided equally into nine groups (10 rats on each group). First group continue on HFD, second group returned to normal diet and the remaining seven groups returned to normal diet and treated with orlistat (10 mg/kg/day, p.o.) [17], turbo slim (25 mg/kg/day, p.o.), turbo slim (50 mg/kg/day, p.o.), African mango (25 mg/kg/day, p.o.), African mango (50 mg/kg/day, p.o.), slim factor (25 mg/kg/day, p.o.) and slim factor (50 mg/kg/day, p.o.) respectively for an additional one month.

The changes in body weight were monitored weekly. Experimental animals were kept and used in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). All experimental protocols were approved by the Ethics Committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (IAEC approval number # 201805RA2).

Drugs: Orlistat (sigma pharmaceutical, Egypt) was used as a reference registered drug and administered orally. Turbo slim, African mango, and Slim factor (Purchased from a pharmacy, Ismailia, Egypt) were prepared using tween 80 and administered orally.

Blood Sampling and Biochemical Analysis: Blood samples were collected from the tail vein. Serum and plasma were separated and kept at –80 °C until performing the biochemical measurements. Serum insulin was determined using ultrasensitive rat insulin ELISA kit (Crystal Chem Inc., Downers Grove, IL 60515, USA) [18], serum leptin was measured by rat leptin ELISA kit (Crystal Chem Inc., USA) [19], serum adiponectin was detected by rat adiponectin ELISA kit (AdipoGen Inc. Korea) [20], serum glucose level was measured according to the method of Trinder [21] by the enzymatic colorimetric method (Biodiagnostic, Egypt), lipid profile including serum triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric methods according to Fossati and Prencipe [22], Allain *et al.,* [23] and Lopes-Virella *et al.,* [24] respectively using Biodiagnostic kits, Egypt, and low-density lipoprotein cholesterol (LDL-C) was calculated according to the method of Friedewald *et al.,* [25], liver enzymes including alanine aminotransferase (ALT) and aspartate amino-transferase (AST) were measured by enzymatic colorimetric method according to Reitman and Frankel [26] using Biodiagnostic kit (Egypt), and kidney function tests including creatinine was measured according to the method of Murray [27] and urea was measured according to the method of Kaplen [28] by enzymatic colorimetric methods using (Biodiagnostic kit, Giza, Egypt) according to manufacturer's instructions.

Plasma Monocyte chemoattractant protein-1 (MCP-1) was determined using rat MCP-1 ELISA kit (Biosource International, California, USA) [29] according to manufacturer's instructions.

Tissue reduced glutathione (GSH) contents were measured by a kinetic assay using a dithionitro-benzoic acid recycling method described by Ellman [30]. The absorbance was measured colorimetrically at 412 nm, using a Shimadzu (Tokyo, Japan) spectrophotometer. Tissue MDA level was assessed according to the spectrophotometric method of Ohkawa *et al.,* [31] based on the reaction with thiobarbituric acid using 1, 1, 3, 3-tetramethoxy-propane as standard. The color intensity was measured using a UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan). Additionally, adipose tissue index was calculated by the formula: adipose tissue index = (retroperitoneal adipose tissue weight/body weight) × 100 and atherosclerosis index was calculated by the formula: atherosclerosis index = (serum TC − HDL-C) / HDL-C [32]. Finally, two indirect indices were calculated. First, Homeostasis Model

Assessment-Insulin Resistance (HOMA-IR) was calculated by the equation: (Glucose × insulin) / 405, where serum fasting glucose concentration is given in mg/dl and serum fasting insulin level is given in µIU/ml, a high HOMA index denotes low insulin sensitivity [33]. Second, The Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated by the equation: 1/ (log Insulin + log glucose), where serum fasting glucose concentration is given in mg/dl and serum fasting insulin level is given in µIU/ml [34].

Statistical Analysis: Data were managed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL), version 17.0 software. The results were expressed as mean ± SEM. One-way analysis of variance, ANOVA, followed by Bonferroni's multiple comparisons test was employed for statistical analysis. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS:

Body Weight Gain and Adipose Tissue Index: Rats fed an HFD for 2 months showed a marked and significant increase in the total body weight (297.80 g ± 0.73) compared to the control group (193.80 g ± 0.83), **Table 2**. Cessation of HFD and returning to NPD for an additional one month significantly reduce body weight (266.20 ± 0.55). Treatment with orlistat (10 mg/kg/day, p.o.) slightly but not significantly reduce body weight compared to group ceased HFD (264.40 g ± 0.75). On the other hand, the six groups treated with other drugs did not show a significant decrease in total body weight compared to the group ceased HFD, **Table 2**.

TABLE 2: BODY WEIGHT IN THE EXPERIMENTAL GROUPS

Groups	Bodyweight (baseline)	Bodyweight (final)	weight (g)Δ
Feeding normal diet	165.80 ± 0.92	193.80 ± 0.83	28 ± 1.04
Feeding high fat diet	165.70 ± 0.79	297.80 ± 0.73	132.10 ± 0.95*****
Cessation of high fat diet	165.50 ± 0.98	266.20 ± 0.55	100.70 ± 1.36#
Treatment with orlistate (10 mg/kg)	164.50 ± 1.24	264.40 ± 0.75	99.90 ± 1.90
Treatment with turbo slim (25 mg/kg)	165.10 ± 0.99	278 ± 1.17	112.90 ± 1.52$
Treatment with turbo slim (50 mg/kg)	165 ± 0.94	277.20 ± 1.90	112.20 ± 2.02$
Treatment with African mango (25 mg/kg)	165.80 ± 1.1	278 ± 0.60	112.20 ± 0.93$
Treatment with African mango (50 mg/kg)	166.20 ± 0.74	274 ± 1.41	107.80 ± 1.55
Treatment with slim factor (25 mg/kg)	164.60 ± 1.18	283.40 ± 1.31	118.80 ± 1.56$
Treatment with slim factor (50 mg/kg)	165.20 ± 1.37	285.20 ± 2.28	120 ± 2.40$

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, * Significant difference from rats fed with a normal diet, # Significant difference from rats fed with the high-fat diet, $ Significant difference from rats ceased high-fat diet.

Adipose tissue index was significantly higher in HFD group (2.85 ± 0.03) as compared to NPD group (0.73 ± 0.07). The cessation of HFD significantly reduces the adipose tissue index to 1.73 ± 0.04. Treatment with Orlistat (10 mg/kg/day, p.o.) significantly reduce the high adipose tissue index value compared to group ceased of HFD (1.35 ± 0.02 *vs.* 1.73 ± 0.04) respectively. However, no significant differences were observed with the six groups treated with other drugs, **Table 3**.

TABLE 3: ADIPOSE TISSUE INDEX IN THE EXPERIMENTAL GROUPS

Groups	Adipose tissue index
Feeding normal diet	0.73 ± 0.07
Feeding a high-fat diet	2.85 ± 0.03*****
Cessation of a high-fat diet	1.73 ± 0.04#
Treatment with orlistate (10 mg/kg)	1.35 ± 0.02$
Treatment with turbo slim (25 mg/kg)	1.66 ± 0.02
Treatment with turbo slim (50 mg/kg)	1.64 ± 0.04
Treatment with African mango (25 mg/kg)	1.66 ± 0.03
Treatment with African mango (50 mg/kg)	1.66 ± 0.04
Treatment with slim factor (25 mg/kg)	1.65 ± 0.03
Treatment with slim factor (50mg/kg)	1.65 ± 0.04

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, *Significant difference from rats fed with a normal diet, # Significant difference from rats fed with high-fat diet, $ Significant difference from rats ceased high-fat diet.

TABLE 4: ATHEROGENIC INDEX IN THE EXPERIMENTAL GROUPS

Groups	Atherogenic index
Feeding normal diet	0.51 ± 0.09
Feeding a high-fat diet	5.38 ± 0.19*
Cessation of a high-fat diet	3.89 ± 0.09#
Treatment with orlistate (10 mg/kg)	3.82 ± 0.09
Treatment with turbo slim (25 mg/kg)	3.96 ± 0.10
Treatment with turbo slim (50 mg/kg)	3.95 ± 0.11
Treatment with African mango (25 mg/kg)	4.04 ± 0.15
Treatment with African mango (50 mg/kg)	3.87 ± 0.08
Treatment with slim factor (25 mg/kg)	3.92 ± 0.09
Treatment with slim factor (50 mg/kg)	3.96 ± 0.08

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, *Significant difference from rats fed with a normal diet, # Significant difference from rats fed with high-fat diet, $ Significant difference from rats ceased high-fat diet.

Atherogenic Index and Serum Lipid Profile: Atherogenic index was significantly increased by feeding HFD (5.38 ± 0.19) and significantly decreased upon return to NPD (3.89 ± 0.09). But the difference in the calculated atherogenic index between-group ceased HFD, and the seven treated groups were not significant in **Table 4**.

Serum lipid profile was significantly affected by feeding HFD; t**able 5** shows that a significant increase in TC, TG, and LDL-C and a significant decrease in HDL-C in HFD group as compared to NPD group, and this was significantly reversed by the cessation of the HFD as a significant decrease in TC, TG and LDL-C and a significant increase in HDL-C in the group returned to a normal diet.

Orlistat, turbo slim, and African mango significantly reduce the serum level of TAG as compared to group ceased HFD, while the slim factor was not significantly effective, in **Table 5**. No significant effect was observed in TC, LDL-C, and HDL-C levels in all treated groups compared to group ceased HFD, **Table 5**.

TABLE 5: SERUM LIPID PROFILE IN THE EXPERIMENTAL GROUPS

Groups	TAG (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Feeding normal diet	60.19 ± 4.17	53.14 ± 3.56	38.20 ± 0.42	30.50 ± 1.53
Feeding high fat diet	296.43 ± 5.14*	189.38 ± 2.56*	29.90 ± 0.87*	100.20 ± 2.90*
Cessation of high fat diet	219.67 ± 1.12#	161.94 ± 1.24#	33.20 ± 0.55#	84.81 ± 1.27#
Treatment with orlistate (10 mg/kg)	172.96 ± 2.03$	155.10 ± 0.55	32.30 ± 0.67	88.21 ± 0.44
Treatment with turbo slim (25 mg/kg)	190.79 ± 2.83$	161.14 ± 0.41	32.60 ± 0.64	90.38 ± 1.65
Treatment with turbo slim (50 mg/kg)	193.40 ± 2.77$	164.76 ± 1.12	33.40 ± 0.63	92.68 ± 1.84
Treatment with African mango (25 mg/kg)	189.89 ± 4.20$	160.09 ± 1.89	32 ± 0.87	90.11 ± 1.99
Treatment with African mango (50 mg/kg)	192.20 ± 3.99$	165.59 ± 1.36	34.10 ± 0.46	93.05 ± 1.51
Treatment with slim factor (25 mg/kg)	206.79 ± 3.49	161.95 ± 1.29	33 ± 0.60	87.59 ± 1.81
Treatment with slim factor (50 mg/kg)	211.16 ± 2.75	162.29 ± 1.95	32.80 ± 0.51	87.27 ± 2

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p value <0.05, n=10, *Significant difference from rats fed with normal diet, # Significant difference from rats fed with high fat diet, $ Significant difference from rats ceased high fat diet.

Glucose Homeostasis Traits: (Blood Glucose, Serum Insulin, HOMA-IR index and R QUICKI): Table 6 reveals that feeding with HFD resulted in significant hyperglycemia and hyperinsulinemia (246.69 ± 1.44, 24.94 ± 0.50) respectively as compared to group fed with NPD. Cessation of HFD for one month significantly reduce serum blood glucose and insulin levels (183.90 ± 1.14, 5.86 ± 0.13), respectively. Treatment with orlistat (10 mg/kg/day, p.o.) shows a significant reduction in serum glucose and insulin levels (151.55 ± 2.31, 4 ± 0.05), respectively, as compared to group ceased HFD. However, groups treated with turbo slim, African mango, and slim factor still showed high levels of both serum glucose and insulin levels compared to group ceased HFD. The calculated HOMA-IR index, which estimates the β-cell function and insulin resistance, was significantly increased in HFD group as compared with NPD group (15.20 ± 0.36). Cessation of HFD for one month significantly reduces the calculated HOMA-IR index (2.66 ± 0.06). A significant decrease in HOMA-IR index values was only observed in the group which received orlistat (10 mg/kg/day, p.o.) compared to the group ceased HFD (1.50 ± 0.03). Other treated groups still have a significant increase in the calculated HOMA-IR index values compared to group ceased HFD **Table 6**. On the other hand, R-QUICKI index, which estimates insulin sensitivity, was significantly reduced in HFD group compared to NPD group (0.26 ± 0.001 vs. 0.41 ± 0.01),

respectively. Cessation of HFD for one month significantly increased the calculated R-QUICKI index. A significant increase in R-QUICKI index values was only observed in the group which received orlistat (10 mg/kg/day, p.o.) compared to the group ceased HFD (0.33 ± 0.001). All other groups except groups received African mango (25 mg/kg/day, p.o.) show a significant but slightly decrease in R-QUICKI index values compared to group ceased HFD **Table 6**.

TABLE 6: FASTING SERUM LEVELS OF GLUCOSE, INSULIN, HOMA-IR INDEX, R-QUICKI, IN THE EXPERIMENTAL GROUPS

Groups	Glucose (mg/dl)	Insulin (uIU/ml)	HOMA-IR	QUICKI
Feeding normal diet	88.28 ± 3.75	3.05 ± 0.10	0.67 ± 0.04	0.41 ± 0.01
Feeding high fat diet	246.69 ± 1.44*	24.94 ± 0.50*	15.20 ± 0.36*	0.26 ± 0.001*
Cessation of high fat diet	183.90 ± 1.14#	5.86 ± 0.13#	2.66 ± 0.06#	0.33 ± 0.001#
Treatment with orlistate (10 mg/kg)	151.55 ± 2.31$	4 ± 0.05$	1.50 ± 0.03$	0.36 ± 0.001$
Treatment with turbo slim (25 mg/kg)	179.71 ± 1.22	12.86 ± 0.13$	5.70 ± 0.06$	0.31 ± 0.001$
Treatment with turbo slim (50mg/kg)	180.78 ± 1.49	11.94 ± 0.40$	5.33 ± 0.18$	0.30 ± 0.01$
Treatment with African mango (25 mg/kg)	190.46 ± 0.82	9.1 ± 0.16$	4.28 ± 0.07$	0.33 ± 0.001
Treatment with African mango (50 mg/kg)	189.46 ± 1.47	8.22 ± 0.11$	3.84 ± 0.03$	0.30 ± 0.001$
Treatment with slim factor (25 mg/kg)	198.26 ± 2.29$	6.98 ± 0.06$	3.42 ± 0.07$	0.32 ± 0.001$
Treatment with slim factor (50 mg/kg)	200.59 ± 2.40$	6.52 ± 0.04	3.23 ± 0.05	0.31 ± 0.001$

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, *Significant difference from rats fed with a normal diet, # Significant difference from rats fed with high-fat diet, $ Significant difference from rats ceased high-fat diet.

Serum Leptin, Adiponectin, and plasma MCP-1: Table 7 reveals that the HFD group has a significant increase in both serum leptin and plasma MCP-1 levels (226.09 ± 2.20, 8.81 ± 0.17) respectively, and a significant decrease in serum adiponectin levels (0.50 ± 0.01) as compared to NPD group. However, a significant decrease in both serum leptin and plasma MCP-1 levels (57.70 ± 0.36, 1.92 ±0.01), respectively and a significant increase in serum adiponectin (4.96 ± 0.03) was observed in the group ceased HFD compared to HFD group. Treatment with orlistat (10 mg/kg/day, p.o.) caused a significant decrease in serum leptin and plasma MCP-1 levels (45.30 ± 0.82, 1.36 ± 0.01), respectively, and a significant increase in serum adiponectin (6.86 ± 0.07) as compared to group ceased HFD. Further significant increase in serum leptin and plasma MCP-1 levels were observed in the remaining six groups compared to group ceased HFD; no changes in serum adiponectin levels were observed in these groups compared to group ceased HFD.

TABLE 7: LEPTIN, ADIPONECTIN AND MCP-1 IN THE EXPERIMENTAL GROUPS

Groups	Leptin (pg/ml)	Adiponectin (ng/ml)	MCP-1 (ng/ml)
Feeding normal diet	32.80 ± 0.70	8.81 ± 0.10	0.58 ± 0.01
Feeding high fat diet	226.09 ± 2.20*	0.50 ± 0.01*	8.81 ± 0.17*
Cessation of high fat diet	57.70 ± 0.36#	4.96 ± 0.03#	1.92 ± 0.01#
Treatment with orlistate (10 mg/kg)	45.30 ± 0.82$	6.86 ± 0.07$	1.36 ± 0.01$
Treatment with turbo slim (25 mg/kg)	108.16 ± 0.29$	4.72 ± 0.2	4.54 ± 0.10$
Treatment with turbo slim (50 mg/kg)	110.52 ± 1.10$	4.63 ± 0.09	4.84 ± 0.10$
Treatment with African mango (25 mg/kg)	96.20 ± 0.96$	4.83 ± 0.13	3.90 ± 0.05$
Treatment with African mango (50 mg/kg)	85.46 ± 0.47$	4.46 ± 0.11	3.56 ± 0.04$
Treatment with slim factor (25 mg/kg)	77.58 ± 0.29$	4.45 ± 0.11	3.06 ± 0.10$
Treatment with slim factor (50 mg/kg)	70.26 ± 0.58$	4.74 ± 0.12	2.92 ± 0.02$

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, *Significant difference from rats fed with a normal diet, # Significant difference from rats fed with high-fat diet, $ Significant difference from rats ceased high-fat diet

Malondialdehyde (MDA) and Glutathione Concentration: Table 8 illustrates that HFD group shows a significant increase in MDA levels (34.10 ± 0.12) and a significant decrease in reduced-GSH levels (24.82 ± 0.21) as compared to NPD group. The reverse was observed in the group ceased HFD, a significant decrease in MDA levels (24.39 ± 0.23), and a significant increase in reduced-GSH levels (39.62 ± 0.13). All treatments did not show significant changes in MDA levels as compared to group ceased HFD. Treatment with orlistat (10 mg/kg/day, p.o.) shows a significant increase in

reduced-GSH (43.12 ± 0.06) in comparison with group ceased HFD, while other treatments show a significant decrease in reduced-GSH in comparison with group ceased HFD.

TABLE 8: MALONDIALDEHYDE (MDA) AND GLUTATHIONE CONCENTRATION IN THE EXPERIMENTAL GROUPS

Groups	MDA (nmol/g tissue)	Reduced-GSH (µg/g tissue)
Feeding normal diet	16.03 ± 0.67	82.38 ± 0.54
Feeding high fat diet	34.10 ± 0.12*	24.82 ± 0.21*
Cessation of high fat diet	24.39 ± 0.23#	39.62 ± 0.13#
Treatment with orlistate (10 mg/kg)	24.49 ± 0.19	43.12 ± 0.06$
Treatment with turbo slim (25 mg/kg)	24.59 ± 0.18	25.05 ± 0.21$
Treatment with turbo slim (50 mg/kg)	24.64 ± 0.14	24.82 ± 0.23$
Treatment with African mango (25 mg/kg)	24.31 ± 0.14	24.33 ± 0.18$
Treatment with African mango (50 mg/kg)	24.66 ± 0.10	24.52 ± 0.21$
Treatment with slim factor (25 mg/kg)	24.40 ± 0.19	25.08 ± 0.09$
Treatment with slim factor (50 mg/kg)	24.41 ± 0.20	24.54 ± 0.20$

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10, *Significant difference from rats fed with a normal diet, # Significant difference from rats fed with high-fat diet, $ Significant difference from rats ceased high-fat diet.

Serum Creatinine and Urea: There is no significant difference was observed in serum creatinine and urea concentrations in all groups compared to NPD group **Table 9**.

TABLE 9: SERUM CREATININE AND UREA IN THE EXPERIMENTAL GROUPS

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Feeding normal diet	0.78 ± 0.02	24.61 ± 1.01
Feeding high fat diet	0.72 ± 0.02	22.67 ± 0.83
Cessation of high fat diet	0.79 ± 0.03	24.42 ± 0.94
Treatment with orlistate (10 mg/kg)	0.82 ± 0.02	22.04 ± 0.78
Treatment with turbo slim (25 mg/kg)	0.84 ± 0.02	21.79 ± 0.92
Treatment with turbo slim (50 mg/kg)	0.80 ± 0.03	23.81 ± 1.21
Treatment with African mango (25 mg/kg)	0.77 ± 0.02	25.19 ± 1.20
Treatment with African mango (50 mg/kg)	0.85 ± 0.02	22.36 ± 1.71
Treatment with slim factor (25 mg/kg)	0.84 ± 0.02	24.36 ± 0.87
Treatment with slim factor (50 mg/kg)	0.80 ± 0.01	22.91 ± 1.08

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10

TABLE 10: SERUM AST AND ALT IN THE EXPERIMENTAL GROUPS.

Groups	AST (U/l)	ALT (U/l)
Feeding normal diet	51 ± 1.93	16.10 ± 0.38
Feeding high fat diet	54.10 ± 0.89	15.50 ± 0.96
Cessation of high-fat diet	54 ± 2.24	17.60 ± 0.34
Treatment with orlistate (10 mg/kg)	58.80 ± 0.39	16 ± 0.47
Treatment with turbo slim (25 mg/kg)	50.40 ± 2.08	15.30 ± 0.63
Treatment with turbo slim (50 mg/kg)	54 ± 1.85	14.70 ± 0.76
Treatment with African mango (25 mg/kg)	48.20 ± 1.29	14.70 ± 0.58
Treatment with African mango (50 mg/kg)	51 ± 1.01	14.80 ± 0.57
Treatment with slim factor (25 mg/kg)	52 ± 0.87	15.50 ± 0.56
Treatment with slim factor (50 mg/kg)	56.20 ± 2.36	15.90 ± 0.67

Data are represented as mean ± SE and analyzed using ANOVA followed by Bonferroni's post-hoc test at p-value <0.05, n=10

Serum AST and ALT: Table 10 shows that no significant effect was observed in serum AST and ALT enzyme activity as compared to NPD group.

DISCUSSION: Our current study revealed that induction of obesity using HFD for two months resulting in increased body weight, adipose tissue index, and atherogenic index. Elevation of blood glucose and hyperinsulinemia, leading to insulin resistance, were also noticed. Compatible with our results, HFD has been shown to generate rapid weight gain in rodents, hyperglycemia, hypertriglyceridemia, hyper-cholesterolemia, and compensatory hyperinsulinemia together with reduced glucose disappearance rate [35]. Our

findings demonstrate that treatment with orlistat (10 mg/kg/day, p.o.) produced a loss in the weight of obese rats. Also, insulin resistance was reduced significantly as indicated by HOMA-IR and R-QUICK indices. These results seem to be compatible with those obtained by Karimi *et al.,* [36]

On the other hand, groups treated with different doses of turbo slim, African mango, and slim factor still have insulin resistance showing high levels of both serum glucose and insulin levels compared to group ceased HFD. However, there is a noticeable decrease in these levels when compared to the HFD group. This indicates that the reduction in the levels of glucose and insulin may be due to the cessation of HFD, not to the effect of these drugs, which emphasized that these drugs are counterfeits having no effect on decreasing insulin resistance. Leptin is exclusively produced by white adipose tissue, which acts as a global messenger to the central nervous system of systemic energy storage in order to control food intake and energy expenditure [19].

Adipocyte size is an important factor for the expression of leptin and its release into the blood. Therefore, and as we explained in our results, the reduced concentration of leptin in the group treated with orlistat versus that in the HFD group may be due to a higher proportion of small adipocytes, which decreases food intake and increases energy expenditure, and this was similar to the results of Karimi *et al.,* [36]

Another serological biomarker that decreased in obese individuals is adiponectin, a type of adipokine that is specifically produced by adipose tissue and regulates insulin sensitivity and tissue inflammation. Weight reduction reportedly leads to a significant increase in adiponectin level [37]. A high level of adiponectin increases insulin sensitivity, while a low adiponectin level contributes to insulin resistance in obesity and type 2 diabetes mellitus [38].

Several studies showed that probiotic supplementation might improve adiponectin secretion or expression [39]. Our results confirm the previous illustration as adiponectin levels significantly decreased in the HFD group, and after cessation of HFD, the levels return to increase again. An additional increase in adiponectin level after treatment with orlistat was observed, and this is similar to those founded by Karimi *et al.,* [36].

No change was observed in the other treated group compared to the group ceased HFD indicating that they have no biological effect on increasing the serum adiponectin levels even after the cessation of HFD.

MCP-1, a member of the C-C chemokine β subfamily, plays a diverse role in obesity [40]. It causes the recruitment of monocytes, and as such, may contribute to the initiation and maintenance of inflammatory reactions in the adipose tissues. MCP-1 has a direct angiogenic effect on endothelial cells [41] and thus may involve in the expansion and remodeling of the adipose tissue during the development of obesity. Therefore obesity causes low-grade chronic inflammation through enhanced adipose tissue-derived cytokines. The previous explanation was emphasized with our results, which revealed that feeding HFD causing an increase in plasma MCP-1 values, and this was in agreement with Mazur-Bialy *et al.,* [42] whom identified increased MCP-1 protein levels in obese mice. Moreover, the significant reduction in MCP-1 after treatment with orlistat (10 mg/kg/day, p.o.), which reduces fat absorption from the intestinal lumen by inhibiting lipase enzymes [43], also ensure the causal relationship between obesity and plasma MCP-1. Noticing that, treatment with different doses of turbo slim, African mango, and slim factor still showing higher plasma levels of MCP-1 compared to group ceased HFD and lower plasma levels of MCP-1 than the HFD group indicating that this reduction is not due to the effect of the drug and still emphasized our expectations that they are counterfeit drugs have no biological effect.

The observed decrease in tissue GSH levels and an increase in MDA levels are proofs of the oxidative stress caused by obesity. Adipose tissue is characterized by increased local, and systemic production of pro-inflammatory adipocytokines, which induce the production of reactive oxygen species and increased oxidative stress leads to important changes in adipose tissue that promotes a systemic low-grade inflammatory response with adverse effects throughout the body such as extensive tissue damage, reacting with membrane lipids, proteins and nucleic acids [44]. Treatment with different doses of turbo slim, African mango, and slim factor still have no effect on either the re-elevation of GSH levels or the re-reduction of

MDA levels comparing to the NPD group, and this still ensuring our expectations.

CONCLUSION: The present study aimed to investigate the effect of some drugs labeled as anti-obesity drugs and not licensed by the Ministry of Health. However, our results indicated that the three studied drugs turbo slim®, African mango®, and slim factor® with different doses from each one had a negligible anti-obesity effect in comparison with registered Orlistat ensuring that they are counterfeit drugs.

FUNDING: This work was funded by King Abdulaziz University.

ACKNOWLEDGEMENT: Authors, therefore, acknowledge King Abdulaziz University, Jeddah, for scientific research.

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest.

REFERENCES:

1. World Health Organization, Substandard and falsified medical products, 2018, January 31. https://www.who.int/en/news-room/fact-sheets/detail/substandard-and-falsified-medical-products. (Accessed November 13 2018).
2. World Health Organization, Obesity and overweight, 2018, February 16. (Accessed November 30 2018).
3. World Health Organization, Obesity: preventing and managing the global epidemic Report of a WHO Consultation (WHO Technical Report Series 894), WHO, 2000.
4. National Task Force on the Prevention and Treatment of Obesity, Overweight, obesity, and health risk. Archives of internal medicine 2000; 160:898-904.
5. Hachem R, Assemat G, Martins N, Balayssac S, Gilard V, Martino R and Malet-Martino M: Proton NMR for detection, identification and quantification of adulterants in 160 herbal food supplements marketed for weight loss. Journal of Pharmaceutical and Biomedical Analysis 2016; 124: 34-47.
6. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, Ryan DH and Still CD: Pharmacological management of obesity: an endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology and Metabolism 2015; 100: 342-62.
7. Vida RG, Fittler A, Somogyi-Végh A and Poór M: Dietary quercetin supplements: Assessment of online product informations and quantitation of quercetin in the products by high-performance liquid chromatography. Phytotherapy Research 2019; 33: 1912-20.
8. Nangue TJ, Womeni HM, Mbiapo FT, Fanni J and Michel L: Irvingia gabonensis fat: nutritional properties and effect of increasing amounts on the growth and lipid metabolism of young rats wistar sp. Lipids in Health and Disease 2011; 10: 43.
9. Martinez-Abundis E, Mendez-del Villar M, Perez-Rubio KG, Zuniga LY, Cortez-Navarrete M, Ramirez-Rodriguez A and Gonzalez-Ortiz M: Novel nutraceutic therapies for the treatment of metabolic syndrome. World Journal of Diabetes 2016; 7: 142-52.
10. Stohs SJ, Kaats GR and Preuss HG: Safety and Efficacy of Banaba-Moringa oleifera-Green Coffee Bean Extracts and Vitamin D3 in a Sustained Release Weight Management Supplement. Phytotherapy Research 2016; 30: 681-88.
11. Garcia-Mazcorro JF, Pedreschi R, Chew B, Dowd SE, Kawas JR and Noratto G: Dietary supplementation with raspberry extracts modifies the fecal microbiota in obese diabetic db/db mice. Journal of Microbiology and Biotechnology 2018; 28: 1247-59.
12. Haber SL, Awwad O, Phillips A, Park AE and Pham TM: Garcinia cambogia for weight loss. American Journal of Health System Pharmacy 2018; 75: 17-22.
13. Maia-Landim A, Ramirez JM, Lancho C, Poblador MS and Lancho JL: Long-term effects of *Garcinia cambogia* / Glucomannan on weight loss in people with obesity, PLIN4, FTO and Trp64Arg polymorphisms. BMC Complementary and Alternative Medicine 2018; 18: 26.
14. Yimam M, Jiao P, Hong M, Brownell L, Lee YC, Hyun EJ, Kim HJ, Kim TW, Nam JB, Kim MR and Jia Q: Evaluation of Natural Product Compositions for Appetite Suppression. Journal of Dietary Supplements 2018; 1-19.
15. Kothadia JP, Kaminski M, Samant H and Olivera-Martinez M: Hepatotoxicity associated with use of the weight loss supplement *Garcinia cambogia*: A Case Report and Review of the Literature. Case Reports in Hepatology 2018; (2018): 6483605.
16. Gheibi S, Kashfi K and Ghasemi A: A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomedicine & Pharmacotherapy 2017; 95: 605-13.
17. Cohen JB and Gadde KM: Weight loss medications in the treatment of obesity and hypertension. Current hypertension reports 2019; 21: 16.
18. Heeba GH, El-Deen RM, Abdel-latif RG and Khalifa MMA: Combined treatments with metformin and phosphodiesterase inhibitors alleviate non-alcoholic fatty liver disease in high-fat diet-fed rats: A comparative study. Canadian Journal of Physiology and Pharmacology 2020; 98: 498-05.
19. Pan WW and Myers Jr MG: Leptin and the maintenance of elevated body weight. Nature Reviews Neuroscience 2018; 19: 95-05.
20. Abdel-Sattar E, Mehanna ET, El-Ghaiesh SH, Mohammad HM, Elgendy HA and Zaitone SA: Pharmacological action of a Pregnane Glycoside, Russelioside B, in dietary obese rats: impact on weight gain and energy expenditure. Frontiers in pharmacology 2018; 9: 990.
21. Trinder P: Determination of blood glucose using 4-amino phenazone as oxygen acceptor. Journal of Clinical Pathology 1969; 22: 246.
22. Fossati P and Prencipe L: Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry 1982; 28: 2077-80.
23. Allain CC, Poon LS, Chan CS, Richmond W and Fu PC: Enzymatic determination of total serum cholesterol. Clinical Chemistry 1974; 20: 470-75.
24. Lopes-Virella MF, Stone P, Ellis S and Colwell JA: Cholesterol determination in high-density lipoproteins separated by three different methods. Clinical Chemistry 1977; 23: 882-84.
25. Friedewald WT, Levy RI and Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 1972; 18: 499-02.

26. Reitman S and Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 1957; 28: 56-63.
27. Murray RL: Creatinine, in: Kaplan LA and Pesce AJ (Eds.), Clinical chemistry, Mosby Co. C. V., St.Louis, Toronto, Princeton 1984: 1247-53.
28. Kaplen LA: Urea, in: Kaplan LA and Pesce AJ (Eds.), Clinical chemistry, Mosby Co. C. V., St. Louis, Toronto, Princeton 1984: 1257-60.
29. Hoffmann A, Ebert T, Klöting N, Kolb M, Gericke M, Jeromin F, Jessnitzer B, Lössner U, Burkhardt R, Stumvoll M, Fasshauer M and Kralisch S: Leptin decreases circulating inflammatory IL-6 and MCP-1 in mice. BioFactors 2019; 45: 43-48.
30. Ellman GL: Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 82: 70-77.
31. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979; 95: 351-58.
32. Tu L, Sun H, Tang M, Zhao J, Zhang Z, Sun X and He S: Red raspberry extract (*Rubus idaeus* L shrub) intake ameliorates hyperlipidemia in HFD-induced mice through PPAR signaling pathway. Food and Chemical Toxicology 2019; 133: 110796.
33. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-19.
34. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G and Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. The Journal of Clinical Endocrinology and Metabolism 2000; 85: 2402-10.
35. Eissa H, Boshra V, M El-Beltagi H, M Ghanam D and A Saad MA: Hypothalamic insulin-sensitizing effect of Exenatide in dietary induced rat model of obesity. Current Drug Therapy 2017; 12: 64-72.
36. Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, Khazaai H and Khodavandi A: The anti-obesity effects of Lactobacillus casei strain Shirota versus Orlistat on high fat diet-induced obese rats. Food & Nutrition Research 2015; 59: 29273.
37. Lobo TF, Torloni MR, Mattar R, Nakamura MU, Alexandre SM and Daher S: Adipokine levels in overweight women with early-onset gestational diabetes mellitus. Journal of Endocrinological Investigation 2019; 42: 149-56.
38. Banerjee A, Sharma D, Trivedi R and Singh J: Treatment of insulin resistance in obesity-associated type 2 diabetes mellitus through adiponectin gene therapy. International Journal of Pharmaceutics 2020; 583: 119357.
39. Liu Q, Liu Y, Li F, Gu Z, Liu M, Shao T, Zhang L, Zhou G, Pan C, He L, Cai J, Zhang X, Barve S, McClain CJ, Chen Y and Feng W: Probiotic culture supernatant improves metabolic function through FGF21-adiponectin pathway in mice. The Journal of Nutritional Biochemistry 2020; 75: 108256.
40. Engin AB: Adipocyte-Macrophage Cross-Talk in Obesity, Advances in Experimental Medicine and Biology 2017; 960: 327-43.
41. Tong X, Zeng H, Gu P, Wang K, Zhang H and LinX: Monocyte chemoattractant protein 1 promotes the proliferation, migration and differentiation potential of fibroblast like synoviocytes via the PI3K/P38 cellular signaling pathway. Molecular Medicine Reports 2020; 21:1623-1632.
42. Mazur-Bialy AI, Bilski J, Wojcik D, Brzozowski B, Surmiak M, Hubalewska-Mazgaj M, Chmura A, Magierowski M, Magierowska K, Mach T and Brzozowski T: Beneficial effect of voluntary exercise on experimental colitis in mice fed a high-fat diet: the role of irisin, adiponectin and proinflammatory biomarkers. Nutrients 2017; 9: 410.
43. Taltia A and Roy A: Orlistat-an anti-obesity drug-An overview. Journal of Advanced Pharmacy Education & Research 2017;| 7:190-193.
44. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E and Arrigo T: Oxidative stress in obesity: a critical component in human diseases. International Journal of Molecular Sciences 2015; 16: 378-00.

How to cite this article:
AL-Ghamdi MA, Huwait EA, Abdelshakour MA, Hadad GM, Abo-Elmatty DM and Abdel-Hamed AR: Negligible effect of some counterfeit anti-obesity drugs. Int J Pharm Sci & Res 2021; 12(5): 2603-12. doi: 10.13040/IJPSR.0975-8232.12(5).2603-12.