



## Biochemical Evaluation of Some Compounds Used for Treatment of Obesity

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### Abstract

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The primary objective of this study was to assess the weight controlling properties of different compounds in their available products in Egyptian market used for the treatment of obesity in dietary obese female rates in comparison with orlistat as a reference FDA approved anti-obesity drug. Eighty female rats were allocated into 8 groups, ten rats per each group. Ten rats received normal palatable diet (NPD) for five months; the remaining rats received high fat diet (HFD) for three months to establish diet-induced obesity. Rats receiving HFD were divided equally into seven groups. One group returned to normal diet and the other groups returned to normal diet and treated with orlistat (10 mg/kg/day) or garcinia cambogia extract (400 mg/kg/day) or combination of garcinia cambogia extract (400 mg/kg/day) and chromium (28 µg/kg/day) or chromium (28 µg/kg/day) or chromium (80 µg/kg/day) or combination of garcinia cambogia extract (400 mg/kg/day) and chromium (80 µg/kg/day) for additional two months. Feeding with HFD induced a significant increase in body weight of rats as well as serum glucose, insulin resistance, serum lipids, leptin, adipose tissue index, atherosclerosis index and oxidative stress marker such as MDA and visfatin as compared to normal feeding group. These measurements were suppressed by orlistat, garcinia cambogia extract, chromium, combination of garcinia cambogia extract and chromium with different extend. Our data ensures the findings that garcinia cambogia, chromium and their combination possess weight controlling and insulin sensitizing properties. Combination of garcinia cambogia and chromium can be used as alternative to orlistat for treatment of obesity to overcome its adverse effect.

**Keywords:** Obesity, Anti-obesity drug, orlistat, Garcinia cambogia extract, Chromium, Insulin resistance.

### 1. Introduction

Obesity is a medical disorder characterized by the accumulation of extra body fat that can adversely impact health. Obesity is often identified when an individual's BMI exceeds 30 kg/m<sup>2</sup>, whereas the range of 25-30 kg/m<sup>2</sup> is classified as overweight

(Rahman et al., 2022). Obesity raises the risk of several diseases, especially cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, some forms of cancer, osteoarthritis and depression (Singh et al., 2020).

Obesity is mostly the result of overeating,

insufficient exercise and genetic predisposition. Some instances are largely caused by genetic factors, endocrine abnormalities, drugs, or mental health issues (Masood et al., 2023).

Obesity is emerging as a worldwide problem. Approximately 1.1 billion adults and 10% of the world's youth are either overweight or classified as obese. Obesity may be largely prevented by a mix of societal reforms and individual decisions. The primary therapies are modifications to food and exercise. Enhancing diet quality involves decreasing the consumption of energy-dense meals rich in fat or carbohydrates and boosting dietary fiber intake. Medications can be utilized in conjunction with an appropriate diet to either suppress appetite or inhibit fat absorption (Boccellino et al., 2020), (Payab et al., 2020).

Considering the vast scale of the obesity issue, supplementary medication offers an appealing answer. Only a small number of medications have been created for treating obesity, and the ones that have been authorized show poor effectiveness (Lin et al., 2020).

Five drugs with proven efficacy for extended use are orlistat, lorcaserin, liraglutide, phentermine-topiramate, and naltrexone-bupropion. Some drugs for obesity therapy can cause significant cardiovascular adverse effects, involving elevated systolic and diastolic blood pressure, increased heart rate, tachycardia, palpitations, and an increased risk of mood-related problems including sleeplessness. Generalized anxiety and panic disorders (Liu et al., 2020), (Marrelli et al., 2020).

In October 2010, sibutramine, a medication used to treat obesity, was withdrawn from the US market because of a clear rise in the risk of heart attacks and strokes. Following the discontinuation of sibutramine, the FDA has authorized orlistat, for long-term treatment of obesity (Gupta et al., 2020). Orlistat may cause adverse effects such as flatulence with discharge and greasy stool. Severe issues including fecal urgency, incontinence, and stomach discomfort may also arise (Shang et al., 2021), (Jin et al., 2023). The adverse effects of these agents highlighted a continued need for evaluation of different compounds used for the treatment of obesity to choose safe and effective medications with a positive impact on health-related quality of life.

Therefore, the present study was designed for evaluating the weight- controlling properties of

different compounds in their available products in Egyptian market used for the treatment of obesity in dietary obese female rates in comparison with orlistat as a reference FDA approved anti-obesity drug.

## 2. Materials and methods

### 2.1. Animals

Eighty female albino rats, gotten from the Egyptian Organization for Biological and Vaccines (Cairo, Egypt), were housed in standard stainless steel cages with free access to food and water. Initially rats body weight in the range of 145- 175 g. Rats were maintained under exact laboratory circumstances with normal light-dark cycle under controlled room temperature between 22-28°C.

### 2.2. Drugs

Orlistat: Orly capsule (batch No.: 2208660), labeled to contain 120 mg orlistat per capsule, manufactured by EVA Pharma for pharmaceuticals, Egypt. The content of capsules was dissolved in 5% gum solution and administrated orally in dose of 10 mg/kg/day (Othman et al., 2021).

Garcinia Cambogia: Garcinia capsule (batch No.:10106601), labeled to contain 750 mg Garcinia Cambogia fruit rind extract equivalent to 450 mg HCA per capsule, manufactured by Bluebonnet Nutrition Corporation, USA. The content of capsules was dissolved in 5% gum solution and administrated orally in dose of 400 mg/Kg/day (Gupta et al., 2021).

Chromium: Chromium Mepaco-Medifood capsule (batch No.: 550822), labeled to contain 200 mcg chromium picolinate equivalent to 24.85 mcg chromium per capsule, manufactured by Arab Company for Pharmaceuticals and Medicinal Plant, Egypt. The content of capsules was dissolved in 5% gum solution and administrated orally in dose of 28 and 80 mcg/kg/day.

Combination of Garcinia Cambogia and chromium: Chromax capsule (batch No.:2207614), labeled to contain 500 mg Garcinia Cambogia fruit extract equivalent to 250 mg HAC and 281.569 mcg chromium picolinate equivalent to 35 mcg chromium per capsule, manufactured by Horus for pharmaceutical industries (EVA group limited), Egypt. The content of capsules was dissolved in 5% gum solution and administrated orally in dose of 400 mg/Kg/day and 28 mcg/Kg/day for Garcinia Cambogia and chromium, respectively (as the same

concentration ratio of Chromax pharmaceutical product).

In addition a dose of 400 mg/Kg/day and 80 mcg/Kg/day for *Garcinia Cambogia* and chromium, respectively was administered orally.

### 2.3. Experimental design

Ten rats were received NPD for five months (group 8, NPD group); the remaining rats received HFD for three months to establish diet-induced obesity. Table 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 (Srinivasan et al, 2005). Rats receiving HFD were divided equally into seven groups, each group ten rats. One group returned to normal palatable diet for additional two months (group 7, HFD group) and the other groups returned to normal palatable diet and treated with the following anti-obesity agents for additional two months:

Group 1: treated with orlistat in a daily dose of 10 mg/kg.

Group 2: treated with *garcinia cambogia* in a daily dose of 400 mg/kg.

Group 3: treated with combination of *garcinia cambogia* and chromium in a daily dose of 400 mg/Kg and 28 µg /Kg, respectively.

Group 4: treated with chromium in a daily dose of 28 µg /Kg.

Group 5: treated with chromium in a daily dose of 80 µg /Kg.

Group 6: treated with combination of *garcinia cambogia* and chromium in a daily dose of 400 mg/Kg and 80 µg /Kg, respectively.

The changes in body weight were monitored every week. The animals were cared for in accordance with the principles and guidelines of the Canadian Council on care and use of experimental animals. All experimental procedures followed were in accordance with guidelines of the Institutional Animal Care and Use Committee.

### 2.4. Blood sampling and biochemical analysis

At the end of the experiment, rats were fasted overnight and blood samples were withdrawn from the orbital sinus of each rat to obtain the serum samples by centrifugation of the collected blood samples for 3 minutes at 3000 rpm which were used immediately for determination of fasting insulin level and fasting glucose level. The other serum samples were kept at -80°C for total cholesterol (TC), triacyl-glycerol (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein

cholesterol (HDL-C), leptin (LEP) and adiponectin (AD) analysis.

Serum insulin was determined using rat insulin ELISA kit (MBS281388, MyBioSource, USA). Serum glucose was determined using Sigma glucose assay kit (GAGO-20, USA). TC, HDL-C and LDL-C were measured using Biochain's colorimetric assay kit (USA). TG was determined using colorimetric enzymatic assay kit (5603-01, XpressBio life science products, USA). Serum LEP was measured by using rat leptin ELISA kit (MBS701500, MyBioSoutce, USA). Serum AD was determined using rat adiponectin Picokine ELISA kit (MBS177263, MyBiosource, USA) according to manufactures instructions. Additionally, atherosclerosis index was calculated by the following equation:

Atherosclerosis index = (serum TC- HDL-C) / HDL-C (Hua et al., 2009)

Fasting serum glucose level and fasting serum insulin level were used for determination of insulin resistance by estimation of the homeostasis model assessment of insulin resistance (HOMA-IR) index using the following formula described by Matthews et al (1985):

HOMA-IR index = [fasting serum insulin (µ U/ml) × fasting serum glucose (mM/L)]/22.5.

A high HOMA-IR index denotes low insulin sensitivity (Matthews et al., 1985).

To assess insulin sensitivity, another derived index was suggested, i.e., the revised quantitative insulin sensitivity check index (R-QUICKI) that estimated by the following formula (Katz et al., 2000):

R-QUICKI = [1/log fasting insulin (µ U/ml) + log fasting glucose (mg/dl)].

### 2.5. Adipose tissue sampling and oxidative stress biomarkers determination

At the end of the experiment, rats were anaesthetized with ether and sacrificed with cervical dislocation. Adipose tissue of all experimental groups were collected and kept at -80°C for determination of malondialdehyde (MDA), reduced glutathione (GSH), visfatin and visfatin (relative expression) by real-time quantitative polymerase chain reaction (qPCR).

MDA was determined by rat malondialdehyde ELISA kit (MBS268427, MyBioSource, USA). GSH was measured by rat reduced glutathione ELISA kit (E02G0367, ShangHai BlueGene Biotech Co., LTD). Visfatin was determined by rat visfatin ELISA kit (LS-F4384, LSBIO, an absolute biotech company, USA).

**Table 1: Composition of the high fat diet**

Ingredients	Amount (g/Kg)
Powdered NPD <sup>1</sup>	365
Lard <sup>2</sup>	310
Casein <sup>3</sup>	250
Cholesterol <sup>4</sup>	10
Vitamin and mineral mix <sup>5</sup>	60
DL-Methionine <sup>6</sup>	3
Yeast powder <sup>7</sup>	1
Sodium chloride <sup>8</sup>	1

<sup>1,7</sup> Purchased from local market.

<sup>2</sup> Isolated from slaughter house of the pig (Alexandria, Egypt).

<sup>3</sup> Difco (Becton Dickinson, France)

<sup>4</sup> Oxford Lab, Mumbai, India.

<sup>5,6</sup> Sigma-Aldrich, MO, USA.

<sup>8</sup> ADWIC Co., Cairo, Egypt.

**Table 2: Effect of the studied anti-obesity agents on body weight in the experimental groups of obesity model.**

Group n = 10	body weight(g) base line Mean ± SEM	body weight (g) final Mean ± SEM	% change in body weight (g) Mean ± SEM
Group (1) treated with orlistat in a daily dose of 10 mg/kg.	155.8± 4.3	226.0± 7.1	45.5± 2.3 <sup>*#</sup>
Group (2) treated with garcinia cambogia in a daily dose of 400 mg/kg	162.5±3.9	250.0±5.7	54.0±3.1 <sup>*#</sup>
Group (3) treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 28 mcg/Kg, respectively	163.6±4.5	242.5±6.4	48.0±3.9 <sup>*#</sup>
Group (4) treated with chromium in a daily dose of 28 mcg/Kg.	145.2±5.1	267.5±7.4	84.6±4.3 <sup>*#a</sup>
Group (5) treated with chromium in a daily dose of 80 mcg/Kg.	142.4±4.7	225.0±5.0	58.3±2.5 <sup>*#a</sup>
Group (6) treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 80 mcg/Kg, respectively	178.2±4.1	300.0±0.5	68.1±2.1 <sup>*#a</sup>
Group (7) HFD	153.2±5.2	363.3±7.5	137.4±5.4 <sup>*a</sup>
Group (8) NPD	168.7±4.9	225.0±6.7	33.1±2.5

\*Significantly different from NPD group at  $p \leq 0.05$ ,

# Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

## 2.6. Determination of body weight change percent, liver index and adipose tissue index

At the end of the experiment, all rats' body weight was measured and the percent of body weight change from starting of the experiment till the end were determined in all groups. Liver tissues and adipose tissues of all experimental groups were collected and used for calculation of liver index and adipose tissue index.

The following equation was used for calculation of liver index:

$$\text{Liver index} = (\text{liver weight} / \text{final body weight}) \times 100$$

The following equation was used for calculation of adipose tissue index:

$$\text{Adipose tissue index} = (\text{retroperitoneal adipose tissue weight} / \text{final body weight}) \times 100$$

## 2.7. Statistical analysis

The results are expressed as mean  $\pm$  SEM. The data was analyzed using Statistical Package of Social Sciences (SPSS) program version 16, Chicago, IL, USA. One-way analysis of variance, ANOVA, followed by Bonferroni's multiple comparisons test were employed for statistical analysis. A value of  $p \leq 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Effect of the studied anti-obesity agents on body weight gain in obesity model

In the current study, HFD group showed a significant increase in the percentage body weight gain as compared to NPD group ( $137.4 \pm 5.4$  vs  $33.1 \pm 2.5$  g %, respectively,  $p \leq 0.05$ ) (Table 2).

The percentage body weight elevation was significantly decrease in garcinia cambogia, chromium, their combination and orlistat treated groups as compared to the HFD group (Table 2, Figure 1) at,  $p \leq 0.05$ . Chromium induced dose-dependent decrease in percentage body weight gain as compared to HFD group. There is no significant difference in the % change of body weight for group treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 28 mcg/Kg, respectively, comparing with group treated with orlistat in a daily dose of 10 mg/kg.

### 3.2. Effect of the studied anti-obesity agents on liver index and adipose tissue index in obesity model

Liver and adipose tissue indices were significantly higher in HFD group as compared to NPD group ( $p \leq 0.05$ , Table 3). Orlistat (10 mg/Kg/day), low and high concentration of chromium (28, 80 mcg/Kg/day); and combination of garcinia cambogia and high concentration of chromium in a daily dose of 400 mg/Kg and 80 mcg/Kg, respectively could reduce the high liver index value. However, all the studied anti-obesity agents could reduce the high adipose tissue index ( $p \leq 0.05$ , Table 3, Figure 2). There is no significant difference in the adipose tissue index for groups treated with high concentration of chromium (80 mcg/Kg/day) either alone or in combination with garcinia cambogia in a daily dose of 400 mg/ Kg, comparing with group treated with orlistat in a daily dose of 10 mg/kg.

### 3.3. Effect of the studied anti-obesity agents on serum lipid profile and atherosclerosis index in obesity model

Serum lipid profile was significantly increased by feeding HFD. Table 4 shows that a significant increase in TC, TG and LDL-C and a decrease in HDL-C were detected in HFD group as compared to NPD group (Figure 3). Additionally a significant decreased in serum lipid profile and calculated atherosclerosis index were detected in the treated groups as compared to HFD group ( $p \leq 0.05$ , Table 4). There is no significant difference in serum lipid profile and atherosclerosis index for group treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 80 mcg/Kg, respectively, comparing with group treated with orlistat in a daily dose of 10 mg/kg.

### 3.4. Effect of the studied anti-obesity agents on fasting serum glucose, serum insulin, HOMA-IR and R-QUICKI in obesity model

Feeding with HFD resulted in significant hyperglycemia and hyperinsulinemia in rats as compared to rats fed with NPD ( $p \leq 0.05$ , Table 5). All groups of rats treated with all the studied anti-obesity agents showed significant reductions in serum glucose and insulin levels as compared to HFD group ( $p \leq 0.05$ , Table 5, Figure 4).

HOMA-IR index was significantly increased in HFD group as compared with NPD group. Treatment with all the studied anti-obesity agents

**Table 3: Effect of the studied anti-obesity agents on liver index and adipose tissue index in the experimental groups of obesity model.**

Group n = 10	Liver index Mean ±SEM	Adipose tissue index Mean ±SEM
Group (1)	2.2 ± 0.10 <sup>#</sup>	1.99±0.20 <sup>*#</sup>
Group (2)	2.7 ± 0.13 <sup>*a</sup>	2.72 ± 0.30 <sup>*#a</sup>
Group (3)	2.7 ± 0.12 <sup>*a</sup>	2.63 ± 0.20 <sup>*#a</sup>
Group (4)	2.4 ± 0.30 <sup>#a</sup>	2.78 ± 0.30 <sup>*#a</sup>
Group (5)	2.3 ± 0.20 <sup>#</sup>	2.16 ± 0.20 <sup>*#</sup>
Group (6)	2.3 ± 0.20 <sup>#</sup>	2.03 ± 0.20 <sup>*#</sup>
Group (7)	2.6 ± 0.10 <sup>*</sup>	3.64 ± 0.30 <sup>*</sup>
Group (8)	2.3 ± 0.20	1.40 ± 0.10

\*Significantly different from NPD group at  $p \leq 0.05$ .

<sup>#</sup>Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

**Table 4: Effect of the studied anti-obesity agents on lipid profile and atherosclerosis index in the experimental groups of obesity model.**

Group n = 10	TC (mg/dl) Mean ±SEM	TG (mg/dl) Mean ±SEM	LDL-C (mg/dl) Mean ±SEM	HDL-C (mg/dl) Mean ±SEM	Athero – sclerosis index Mean ±SEM
Group (1)	51.7± 2.5	43.3 ±1.9	23.0 ± 1.1	31.7 ± 1.1	0.63 ±0.04 <sup>#</sup>
Group (2)	70.3 ±3.1	78.3 ± 1.5	33.3 ±1.5	23.0 ± 1.0	2.06 ± 0.14 <sup>#</sup>
Group (3)	62.0 ±3.0	68.0 ± 2.5	30.0 ± 1.0	25.3 ±1.2	1.45 ±0.11 <sup>#</sup>
Group (4)	104.7 ±5.5 <sup>a</sup>	115.7 ± 3.1 <sup>a</sup>	57.7± 2.1 <sup>a</sup>	21.7 ±1.1 <sup>a</sup>	3.83 ± 0.12 <sup>#a</sup>
Group (5)	89.0 ±4.0	81.7 ± 3.5	41.7 ± 1.9	25.3 ± 0.6	2.51 ± 0.10 <sup>#</sup>
Group (6)	50.3 ±2.5	47.7 ±2.0	25.3 ± 1.0	27.7 ±0.9	0.82 ± 0.06 <sup>#</sup>
Group (7)	147.7±6.3 <sup>*</sup>	160.7 ± 6.1 <sup>*</sup>	85.7 ± 3.7 <sup>*</sup>	18.3±0.8 <sup>*</sup>	7.02± 0.51 <sup>*</sup>
Group (8)	51.6 ± 2.5	41.3 ± 1.7	16.7 ±0.8	33.0 ±1.2	0.56 ± 0.03

\*Significantly different from NPD group at  $p \leq 0.05$ .

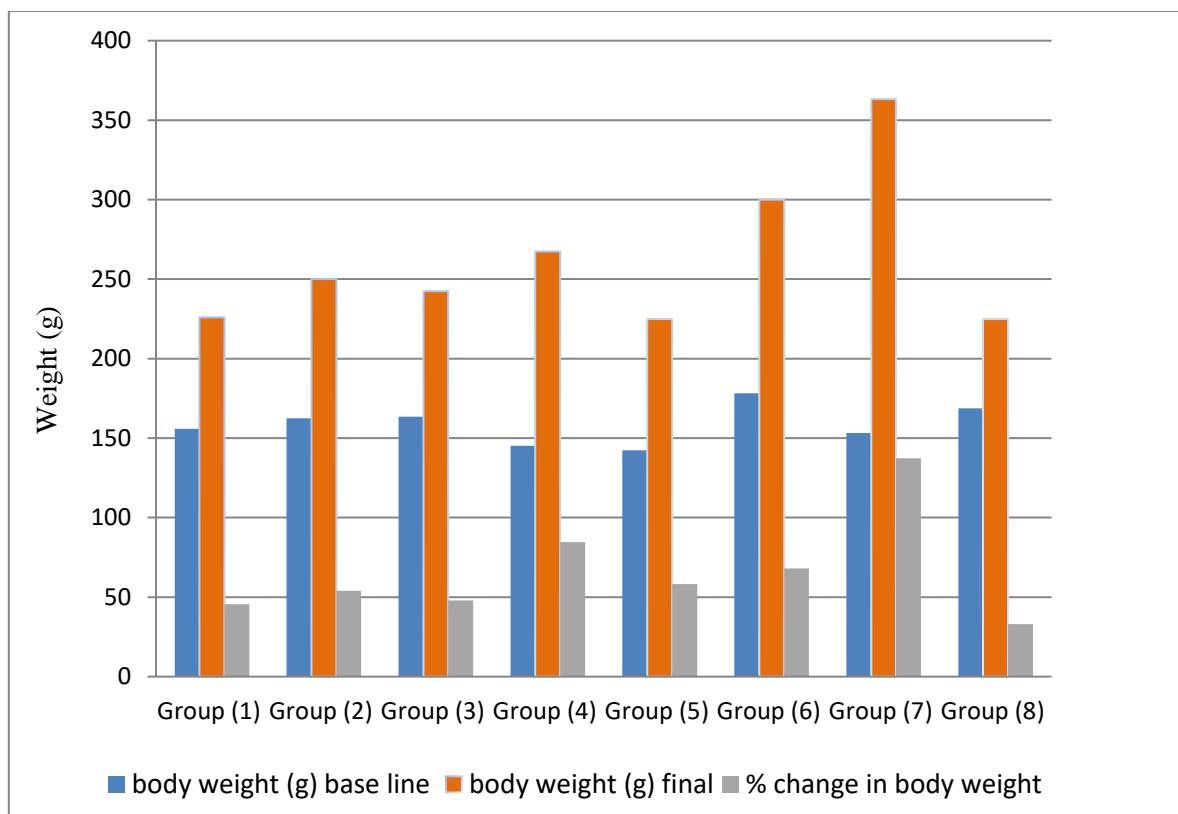
<sup>#</sup>Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

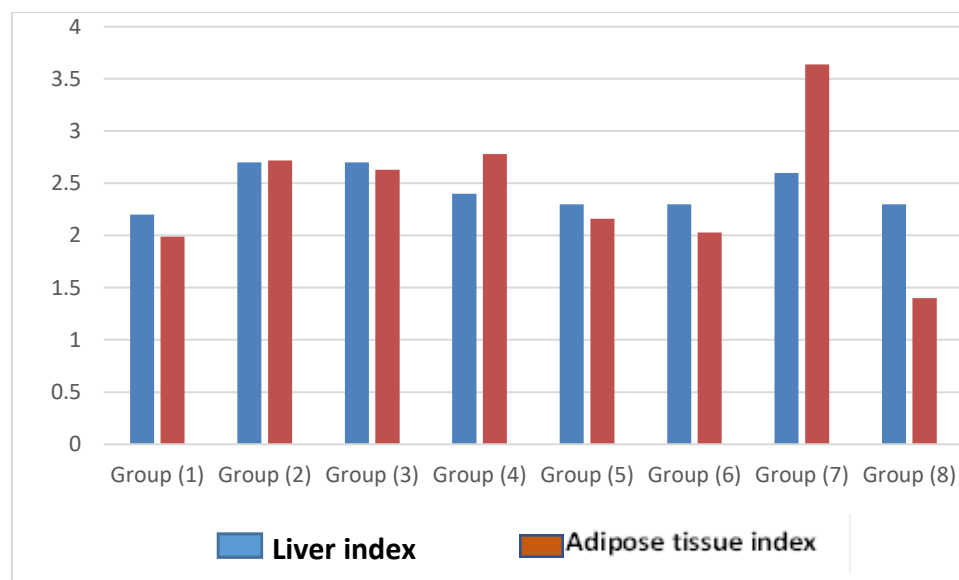
could decrease the calculated HOMA-IR index ( $p \leq 0.05$ , Table 5). On the other hand, R-QUICKI calculated in HFD group was significantly lower than the calculated value for NPD group. The lessened insulin sensitivity was improved after treatment with the studied anti-obesity agent ( $p \leq 0.05$ , Table 5, Figure 4).

### 3.5. Effect of the studied anti-obesity agents on serum leptin, serum adiponectin and adiponectin/leptin ratio in obesity model

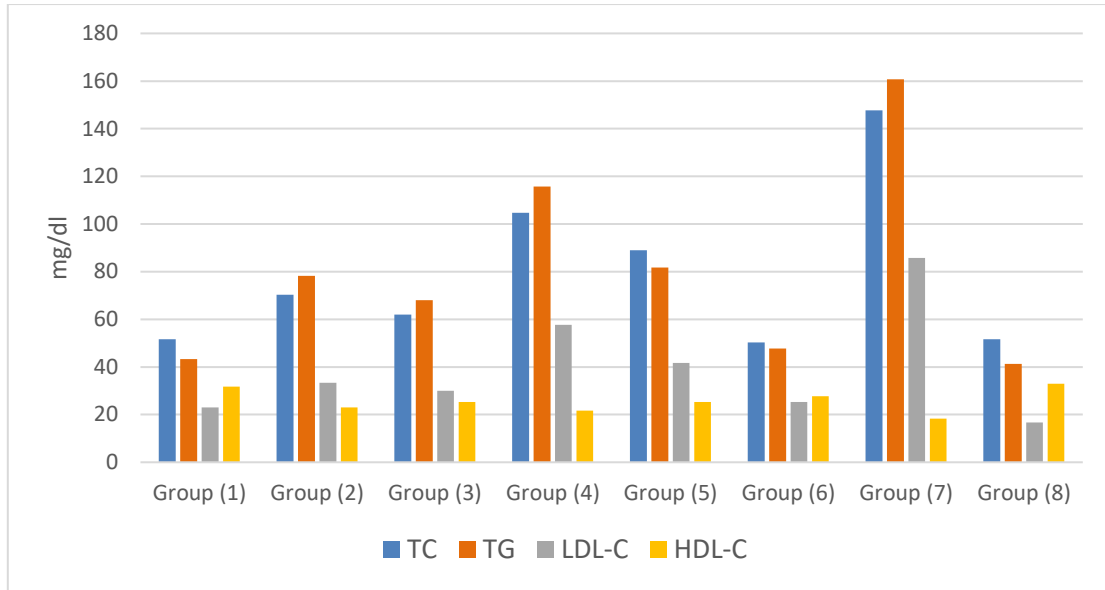
HFD group showed significant increase in serum leptin, decrease in serum adiponectin levels and decrease in adiponectin/leptin ratio as compared to NPD group ( $p \leq 0.05$ , Table 6). Treatment with the studied anti-obesity agents could decrease serum



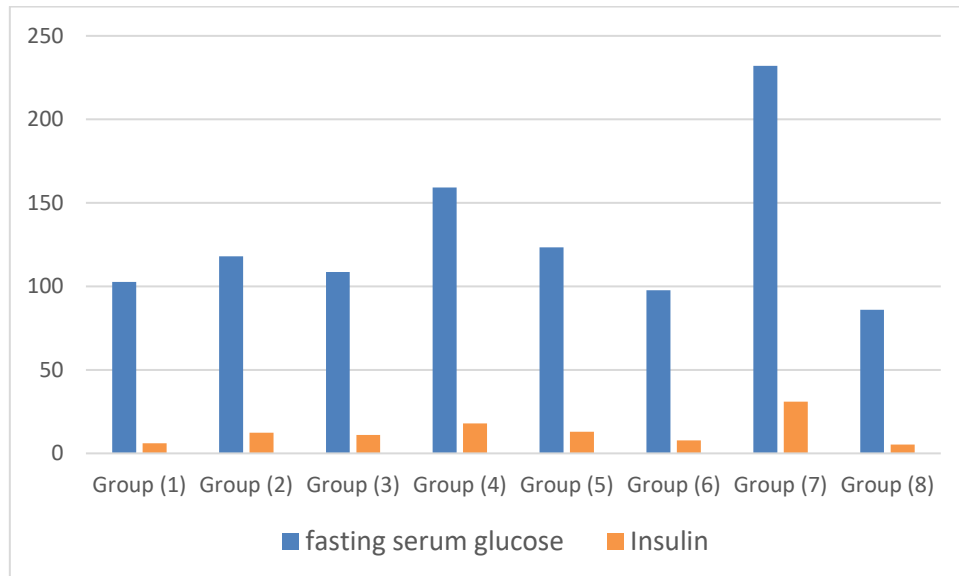
**Figure 1: Body weight (g) base line, body weight (g) final and % change in body weight for groups of the studied anti-obesity agents.**



**Figure 2: Liver index and Adipose tissue index for groups of the studied anti-obesity agents.**



**Figure 3: Lipid profile for groups of the studied anti-obesity agents.**



**Figure 4: Fasting serum glucose (mg/dl) and Insulin (µIU/ ml), for groups of the studied anti-obesity agents**

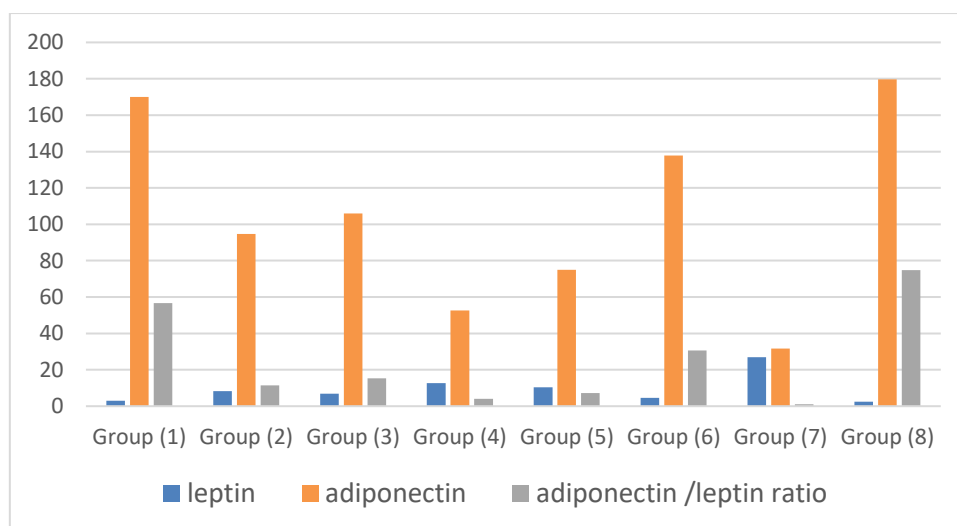
leptin, increase serum adiponectin levels and increase adiponectin/leptin ratio as compared to HFD group ( $p \leq 0.05$ , Table 6, Figure 5). The serum leptin level was highly decreased and the serum adiponectin level was highly increased for group treated with orlistat, and group treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 80 mcg/kg, respectively, as compared to HFD group.

**3.6. Effect of the studied anti-obesity agents on adipose tissue oxidative stress marker (MDA, GSH and visfatin levels) in obesity model**

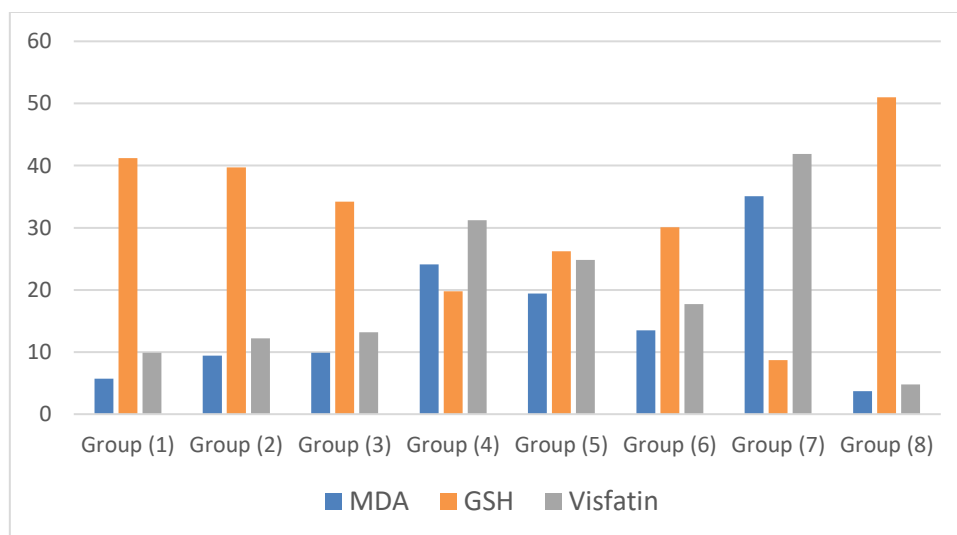
As shown in table 7, high fat diet feeding induced significant increase in MDA content and visfatin content; and decrease in GSH content in adipose tissue of HFD group in comparison with NPD group at  $p \leq 0.05$ . Treatment with all the studied anti-obesity agents could decrease MDA content and visfatin content and increase GSH content as compared to HFD group ( $p \leq 0.05$ , Table 7, Figure 6).

**3.7. Effect of the studied anti-obesity agents on adipose tissue oxidative stress marker (visfatin relative expression levels) using real time qPCR in obesity model**





**Figure 5: Leptin (ng/ml), Adiponectin (pg/ml) and Adiponectin/Leptin ratio, for groups of the studied anti-obesity agents**



**Figure 6: Adipose tissue oxidative stress marker (MDA (Nmol/g), GSH (pg/g) and visfatin (ng/g) levels) in obesity model**

HFD group showed significant increase in visfatin relative expression level of abdominal adipose tissue in comparison with NPD group at  $p \leq 0.05$  (Table 7). Treatment with the studied anti-obesity agents could decrease visfatin relative expression level of abdominal adipose tissue in comparison with HFD group at  $p \leq 0.05$ . Visfatin relative expression level of abdominal adipose tissue was highly decrease for group treated with combination of garcinia cambogia and chromium in a daily dose of 400 mg/Kg and 80 mcg/kg, respectively, as compared to HFD group.

#### 4. Discussion

The results of the current study showed that over a period of three months, exposure of rats to HFD led to increased weight gain, liver index, adipose tissue index, atherosclerosis index and hyperlipidemia as compared to NPD group. The hyperlipidemia was observed as an increasing of TC, LDL-C, TG and decreasing HDL-C. Consistent with our results, HFD has been shown to produce increase in the percentage body weight gain of rats, and hyperlipidemia (Abo-Elmatty & Zaitone, 2011).

**Table 5: Effect of the studied anti-obesity agents on fasting serum glucose (mg/dl), serum Insulin level, HOMA-IR and R-QUICKI in the experimental groups of obesity model.**

Group n =10	Fasting serum glucose (mg/dl) Mean ± SEM	Insulin μIU/ ml Mean ± SEM	HOMA-IR index Mean ± SEM	R-QUICKI Mean ± SEM
Group (1)	102.6±4.0	6.1±0.30	1.52±0.11 <sup>#</sup>	0.36± 3x10 <sup>-3</sup>
Group (2)	118.0±3.6	12.3±0.56	3.57±0.06 <sup>#</sup>	0.31±2x10 <sup>-3</sup>
Group (3)	108.6±2.5	11.0±0.28	2.96±0.15 <sup>#</sup>	0.32±2x10 <sup>-3</sup>
Group (4)	159.3±3.1 <sup>a</sup>	17.9±0.73 <sup>a</sup>	7.03±0.48 <sup>#a</sup>	0.29±2x10 <sup>-3</sup>
Group (5)	123.3±4.1	12.9±0.60	3.93±0.23 <sup>#</sup>	0.31±2x10 <sup>-3</sup>
Group (6)	97.6±4.0	7.7±0.36	1.85±0.06 <sup>#</sup>	0.35±3x10 <sup>-3</sup>
Group (7)	232.0±6.3 <sup>*</sup>	30.9±1.27 <sup>*</sup>	17.74±1.18 <sup>*</sup>	0.26±2x10 <sup>-3*</sup>
Group (8)	86.0±4.2	5.3±0.31	1.12±0.06	0.38±3x10 <sup>-3</sup>

\*Significantly different from NPD group at  $p \leq 0.05$ .

<sup>#</sup>Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

**Table 6: Effect of the studied anti-obesity agents on serum leptin (ng/ml), serum adiponectin (pg/ml) and adiponectin/leptin ratio in the experimental groups of obesity model.**

Group n = 10	leptin (ng/ml) Mean ±SEM	adiponectin (pg/ml) Mean ±SEM	adiponectin /leptin ratio Mean ±SEM
Group (1)	3.0 ± 0.12 <sup>#</sup>	170.0 ±7.7 <sup>#</sup>	56.6 ±2.6 <sup>#</sup>
Group (2)	8.2 ± 0.31	94.7 ± 4.8	11.5 ± 0.4
Group (3)	6.9 ± 0.35	105.9 ± 4.5	15.3 ± 0.7
Group (4)	12.7 ± 0.70 <sup>a</sup>	52.7 ± 2.4 <sup>a</sup>	4.1 ± 0.2 <sup>a</sup>
Group (5)	10.3 ±0.40	75.0 ±3.7	7.2 ± 0.4
Group (6)	4.5 ± 0.30	137.8 ±6.7	30.6 ± 1.5
Group (7)	26.9 ± 1.81 <sup>*</sup>	31.6 ±1.6 <sup>*</sup>	1.1 ±0.06 <sup>*</sup>
Group (8)	2.4 ± 0.14	179.7 ± 6.9	74.8 ± 3.0

\*Significantly different from NPD group at  $p \leq 0.05$ .

<sup>#</sup>Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

**Table 7: Effect of the studied anti-obesity agents on adipose tissue oxidative stress marker (MDA, GSH and visfatin levels) in the experimental groups of obesity model.**

Group n = 10	MDA (Nmol/g) Mean ± SEM	GSH (pg /g) Mean ± SEM	Visfatin (ng/g) Mean ± SEM	Visfatin (relative expression) by Real time qPCR
Group (1)	5.7±0.07 <sup>#</sup>	41.2±0.09 <sup>#</sup>	9.9±0.14 <sup>#</sup>	2.7 <sup>#</sup>
Group (2)	9.4±0.35	39.7±0.31	12.2±0.34	3.4
Group (3)	9.9±0.16	34.2±0.16	13.2±0.14	2.8
Group (4)	24.1±0.23 <sup>a</sup>	19.8±0.42 <sup>a</sup>	31.2 ±0.45 <sup>a</sup>	4.4 <sup>a</sup>
Group (5)	19.4±0.58	26.2±0.29	24.8±0.38	3.7
Group (6)	13.5±0.23	30.1±0.89	17.7±0.25	1.6
Group (7)	35.1±0.29 <sup>*</sup>	8.7±0.37 <sup>*</sup>	41.9±0.55 <sup>*</sup>	6.3 <sup>*</sup>
Group (8)	3.7±0.15	51.0±0.39	4.8±0.28	1.0

\*Significantly different from NPD group at  $p \leq 0.05$ .

<sup>#</sup>Significantly different from HFD group at  $p \leq 0.05$ .

<sup>a</sup> Significantly different from orlistat group (1) at  $p \leq 0.05$

Further, Levels of fasting serum glucose were greater in HFD group as compared to NPD group, indicating the impaired regulation of glucose homeostasis in addition to hyperinsulinemia and increasing of insulin resistance which was indicated by the significant elevation of the HOMA-IR index; and decreasing of insulin sensitivity which was indicated by significant decreasing of R-QUICKI. These results came on line with that reported by (Abo-Elmatty & Zaitone, 2011) who proved that obesity is positively correlated with hyperinsulinemia and hyperglycemia.

In the current study, serum leptin level was increase in rats fed HFD compared to those under NPD. Leptin is an adipocyte-derived hormone, which contributes to the homeostatic regulation of energy balance and metabolism through humoral and neural pathways. Leptin acts on the neurons in certain brain areas such as the hypothalamus, hippocampus, and brain stem to regulate food intake, thermogenesis, energy expenditure, and homeostasis of glucose/lipid metabolism. Leptin also reduces food intake and body weight through interactions with the central neural network, particularly in the hypothalamus (Campfield et al., 1995). In addition, central leptin administration suppressed daily food intake in rats (Wang et al.,

1997). The pathologically increased circulating leptin is a biomarker of leptin resistance, which is common in obese individuals. Leptin resistance is defined by a reduced sensitivity or a failure in response of the brain to leptin, showing a decrease in the ability of leptin to suppress appetite or enhance energy expenditure, which causes an increased food intake and finally leads to overweight, obesity, cardiovascular diseases, and other metabolic disorders (Liu, et al, 2018). It was suggested that there is a leptin-sensitive pathway and a leptin-insensitive (or less sensitive) pathway in the regulation of feeding (Caro et al., 1996). Another hypothesis argued that there is dissociation between the increase in both leptin store and adipose tissue weight (Selenscig et al., 2010).

The current study revealed that serum adiponectin level was decreased in rats fed HFD compared to those under NPD. In agreement, adiponectin is downregulated in a model of HFD feeding (Abo-Elmatty & Zaitone, 2011). Adiponectin is a circulating hormone secreted by adipose tissue which exerts protective effects against inflammation and can positively modulate the endocrine system, by enhancing insulin sensitivity in obese animals as well as in humans (Kayvan et al., 2021). Leptin and adiponectin are cytokines

produced excessively by adipocytes. Leptin is responsible for several cardiovascular diseases associated with obesity, while adiponectin is considered to be cardioprotective (**Ghantous et al., 2015**). With the expansion of adipose tissue, the expression and secretion of adiponectin decrease, which produces a drop in circulating concentrations. In parallel, expression and secretion of leptin increase, which triggers a boost in blood levels. Thus, as BMI increases with obesity, the adiponectin/leptin ratio decreases. Obesity and the metabolic syndrome (MS) are characterized by an increase in circulating leptin concentrations, in parallel to a decrease in blood levels of adiponectin. Consequently, the adiponectin/leptin ratio has been suggested as a maker of adipose tissue dysfunction. This emerging biomarker correlates with insulin resistance better than adiponectin or leptin alone, or even HOMA-IR index and is decreased with increasing number of metabolic risk factors having been proposed as a predictive marker for the metabolic syndrome. Moreover, the adiponectin/leptin ratio is negatively correlated with markers of low-grade chronic inflammation. In this sense, an increase in this ratio has been related with reduced atherosclerosis risk as well as with a decreased risk of some types of cancer (**Gema et al., 2018**).

The current study revealed that serum adiponectin/leptin ratio was decreased in rats fed HFD compared to those under NPD, that is indication for increasing number of metabolic risk factors with atherosclerosis risk and prediction of metabolic syndrome for rats fed HFD.

The obesity is an important factor for enhanced oxidative stress (**Warolin et al., 2013**). Oxidative stress plays an important role in the development of co-morbidities in obesity. Obesity-induced oxidative stress causes the development of various pathological events, including insulin resistance and diabetes, cardiovascular complications; sleep disorders, asthma, oncological problems, reproduction, rheumatological problems, and liver failure (**Prasenjit, Sushil, 2015**). The impact of obesity on oxidative stress markers such as MDA, GSH and visfatin was evaluated. The current study revealed that adipose tissue MDA level was increased in rats fed HFD compared to those under NPD. Our observation is in agreement with the literature support that obese subjects exhibit increased systemic oxidative stress and the concentration of serum MDA increases with increasing levels of BMI (**Sankhla et al., 2012**).

GSH is a powerful antioxidant. It protects mitochondria from oxidative and free radical damage. Mitochondria are the cells content that produce ATP used for energy, so GSH also helps with energy production and fat burning. Baylor College of Medicine research finds that increasing glutathione levels improves cellular fat burn by 104% and reduces liver fat by 63%. And in a Greek study, subjects with high GSH lost 60% more weight than those with low levels (**Lisa Maxbauer, 2024**). The current study revealed that adipose tissue GSH level was decreased in rats fed HFD compared to those under NPD.

Visfatin is an adipokine that is produced by the intra-abdominal adipose tissue, which simultaneously facilitates adipogenesis and has insulin-mimetic properties. Intra-abdominal obesity leads to increased visfatin production, which might simultaneously increase obesity and maintain insulin sensitivity in peripheral organs (**Jaswinder and Antonio, 2005**). Elevated plasma visfatin concentrations in obese subjects are reduced after weight loss (**Haider et al., 2006**). The current study revealed that adipose tissue visfatin level and its gene expression were increased in rats fed HFD compared to those under NPD.

The current results showed that orlistat mediated body weight loss in obese rats along with favorable effects on biochemical parameters, insulin sensitivity and oxidative stress marker. Orlistat decreases adipose tissue index, atherosclerosis index, TC, TG, LDL-C, fasting serum glucose, serum insulin, HOMA-IR, serum leptin, adipose tissue MDA, visfatin level and its gene expression, while increases HDL-C, R-QUICKI, serum adiponectin, adiponectin/leptin ratio and adipose tissue GSH in rats treated with orlistat (10 mg/Kg/day) compared to those under HFD. In accordance, many studies reported that treatment with orlistat, reduced weight among obese patients (**Tong et al., 2002**), decreasing visceral adipose tissue (**Smith et al., 2011**), improving glycaemic control and cardiovascular risk factor profile in overweight patients with type 2 diabetes (**Hanefeld & Sachse, 2002**), upregulating of adiponectin (**Ali Khan et al., 2017**) and improvement of insulin resistance (**Wasta et al., 2021**). Our findings are compatible with these observations. The meta-analysis of 9732 subjects shows favorable effects of orlistat on body weight, TC, LDL-C, and TG (**Sahebkar et al., 2017**). The main finding of another meta-analysis is that orlistat is effective in increasing of plasma concentrations of adiponectin

and decreasing those of leptin (**Derosa et al., 2016**). Additionally, significant reduction in body weight, BMI, lipid profile and visfatin was observed in the orlistat group in comparison with control group (**Derosa et al., 2012**). This observation may further support our findings. Clinically, the indicator of lipid peroxidation-MDA-falls markedly in association with weight loss with orlistat treatment (**Yesilbursa et al., 2005**).

Garcinia Cambogia is a fruit with a high content of hydroxycitric acid (HCA). HCA inhibits lipogenesis impairing hydrocarbon conversion in lipids. HCA produces the inhibition of ATP-citrate lipase, an enzyme that is required for the first step in lipogenesis process. HCA action also increments glycogen hepatic deposit, decrease appetite and reduces weight gain (**Karri et al.2019**).

Our findings demonstrate that treatment with garcinia cambogia extract produce a significant weight loss in obese rats with decreasing of adipose tissue index, Moreover, insulin resistance was diminished significantly as indicated by improving fasting serum glucose, HOMA-IR and R-QUICKI indices. In addition to improvement of lipid profile and decreasing of atherosclerosis index compared to those under HFD. These results seem to be compatible with those obtained by (**Raja et al., 2020**). The authors observed that garcinia cambogia has significant reduction effects on body weight as well as in lipid profiles including TC, TG, LDL-C and HDL-C in comparison to control group.

Also, our findings in line with (**El-Shaer et al., 2022**). The authors reported that feeding on garcinia cambogia powder and extract peels reversed the effect of obesity by decreasing of body weight, TC,TG, LDL-C, serum glucose and increasing HDL-C.

Similarly, our study finding in line with (**Chu et al., 2021**). The authors reported that garcinia cambogia supplementation significantly reduced body weight, body fat mass, body fat percentage in rats, and it thus has potential as a natural and safe plant extract dietary supplement.

In agreement with previous studies, the current findings showed that administration of garcinia cambogia in rats led to a significant decrease in the serum leptin level and increasing both of serum adiponectin level and adiponectin/leptin ratio compared to those under HFD. Our findings in consistent with (**Liu et al., 2015**). The authors demonstrated that garcinia cambogia extract could attenuated fat accumulation and body weight gain in rat model with high-fat diet induced obesity possibly through regulation of lipolysis gene

expression by affecting adiponectin-AMPK signaling pathway and increasing serum adiponectin level. Additionally, the Levels of serum insulin and leptin were lower in the mice treated with 3.3% Garcinia cambogia extract than in the control (**Hayamizu et al., 2003**).

In the current investigations, group of rats treated with garcinia cambogia showed increasing GSH, decreasing MDA, visfatin and its gene expression compared to those under HFD. Our results in agreement with (**Amin et al., 2011**). The authors reported that garcinia ameliorated the damaging effects of the HFD and decreased feed intake, MDA level and decreased oxidative stress. Consistently, some authors reported that the aqueous extract of garcinia cambogia increased GSH level and decreased MDA level in treated rats, demonstrating the garcinia cambogia plant potential as an antioxidant possibly due to the presence of quercetin, rutin, and kaempferol (**Rana et al., 2023**).

In addition, chromium may play an important role in decreasing insulin resistance and lipid abnormalities, as well as weight loss process in the body. Many studies examined the effects of chromium supplementation on the metabolic profiles. Earlier, it was reported that chromium supplementation significantly improved glucose homeostasis parameters and some lipids profiles (**Asbaghi et al., 2021**).

Our findings demonstrate that treatment with chromium produce a significant body weight loss, decreasing of adipose tissue index, atherosclerosis index, improvement of insulin resistance and lipid profile for obese rats compared to those under HFD. These results seem to be compatible with those obtained by (**Pala et al., 2020**). The authors reported that chromium picolinate improves various metabolic parameters in rats by decreasing blood glucose, total cholesterol and triglyceride in serum.

In the current investigations, a group of rats treated with chromium showed decreasing in the serum leptin level and increasing both of serum adiponectin level and adiponectin/leptin ratio compared to those under HFD. Our findings agreed with (**Sun et al., 2000**). The authors reported that the average body weight and the levels of leptin and insulin of group of rats treated with chromium were significantly lower than their corresponding control group without chromium. They concluded that chromium could cause weight loss and reduce the levels of insulin and leptin. Additionally, chromium may be effective in increasing insulin

sensitivity in diabetic rats and increasing adiponectin secretion (Abozaid et al., 2015).

The effect of chromium on oxidative stress marker currently investigated. A group of rats treated with chromium showed increasing GSH, decreasing MDA, visfatin and its gene expression compared to those under HFD. Our results came on line with the meta-analysis demonstrated that chromium supplementation increased GSH ([Amini et al., 2023](#)). Additionally, chromium picolinate reduces oxidative stress in rats by decreasing MDA levels in serum (Pala et al., 2020).

All the investigated effects of chromium were found to be dose-dependent response.

The current results showed that a combination of garcinia cambogia and chromium mediated additive body weight loss in obese rats along with additive favorable effects on biochemical parameters and insulin sensitivity. A combination of garcinia cambogia and chromium decreases adipose tissue index, atherosclerosis index, TC, TG, LDL-C, fasting serum glucose, serum insulin, HOMA-IR, serum leptin, adipose tissue MDA, visfatin level and its gene expression, while increases HDL-C, R-QUICKI, serum adiponectin, adiponectin/leptin ratio and adipose tissue GSH in rats treated with this combination compared to those under HFD. The combination of garcinia cambogia and chromium has no additive favorable effect on the studied oxidative stress markers including MDA, GSH and visfatin.

All the studied anti-obesity agents have limited or no favorable effect on liver index.

## 5. Conclusion

Garcinia cambogia and chromium picolinate possess weight controlling and insulin sensitizing properties in addition to improving lipid profile and reducing oxidative stress. Therefore, they may be used to reduce body weight and improve the metabolic status of overweight and obese patients. Evaluation of the studied anti-obesity agents was carried out relative to orlistat as a reference FDA approved anti-obesity drug to determine suitable therapeutic alternative agent to orlistat for overcoming its side effects. Depending on body weight loss, favorable effects on biochemical parameters and insulin sensitivity, the combination of garcinia cambogia and chromium can be used as the first therapeutic alternative agent to orlistat. This combination is more effective than garcinia cambogia or chromium as a mono therapeutic alternative.

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