**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Impact of Green Coffee Extract on Body Weight and Physiological Indicators of Metabolic State in Obese Male Rats

**Suzan S. A. Elpasty[1], Eman G. E. Helal [1*] Manal M. S. Mansoury [2], Ashraf M. M. Algendy [3]**

*[1] Department of Zoology, Faculty of Science, Al-Azhar University (Girls), Cairo, Egypt*
*[2] Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdul-Aziz University, Jeddah, Saudi Arabia*
*[3] Department of Medical Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*

**Abstract**

Obesity poses a significant health danger. The effects of green coffee extract (GCE) and turbo slim blue (TSB) on decreasing the body weight (BW) and their impact on various physiological indicators of metabolic state in obese rats were investigated in this study. Twenty-one male rats weighing 320-360 g were distributed into three groups. Rats were treated with the following for 4 weeks: distilled water (control), GCE (10 mg/kg), and TSB (10 mg kg). All measurements are percent (%) BW changes, pancreatic function markers (insulin, glucose, HOMA-IR), protein, and lipid indicators. Thyroid hormones, leptin, testosterone, liver, and renal function markers were also measured. Relative to the control group, GCE-treated rats significantly reduced the % of the change in BW, glucose, HOMA-IR, and leptin. GCE significantly increases T3 and T4. In addition, GCE-treated rats showed decreased serum lipids and increased serum testosterone levels. Biomarkers of protein metabolism, liver, and kidney function were unaffected by GCE. In contrast to GCE, the loss of BW induced by TSB is associated with a significant rise in liver and kidney function, which is a marker of toxicity. Therefore, GCE can be considered as a harmless and effective additional therapy to reduce BW.

**Keywords:** Obesity; green coffee; body weight; physiological indices.

## 1. Introduction

Globally, the epidemic of obesity is a worldwide public health concern. Obesity is linked to increased adiposity, which is well-known for being connected to an elevated risk of a variety of chronic disorders, including hypertension, cardiovascular disease, dyslipidemia, and insulin resistance [1-3]. In the previous two decades, obesity has become prevalent globally, which could be related to a sedentary lifestyle and increased intake of a high-caloric diet [4]. Obesity is associated with several morbidities and health disorders that can reduce human life quality, strain the healthcare system, and financial burden on the country [5]. A previous study revealed that lower 5-10 % of body weight resulted in significantly decreased lipid risk factors of cardiovascular diseases, type two diabetes, blood pressure, and inflammation [6-7].

To manage obesity, lifestyle modification is the first approach, including calorie restriction and increased physical activities. However, maintaining these efforts over time can be difficult, and the outcomes are frequently insufficient [8-9]. Pharmacotherapy for weight management has been recommended with lifestyle modification [10-11].

Several anti-obesity drugs have been developed with different mechanisms targeting diverse pathways and factors, while others have been removed owing to long-term severe adverse effects [12-13]. Most of these anti-obesity drugs are based on stimulating or blocking various enzymes and biomolecules involved in fat metabolism, decreasing food consumption or absorption, or increasing energy expenditure [14-15]. Turbo slim blue (TSB), an anti-obesity drug, contains chromium, an essential mineral involved in regulating carbohydrate and fat metabolism and decreasing body fats, which leads to

*Corresponding author e-mail: Eman G. E. Helal
**Receive Date:** 15 March 2022, **Revise Date:** 28 March 2022, **Accept Date:** 31 March 2022
DOI: 10.21608/EJCHEM.2022.126956.5660

weight loss [16]. Besides, a mixture of herbs such as *Garcinia cambogia* extract and *Gymnema* extract, which have been included in weight loss *via* inhibiting body fat biosynthesis, epididymal fat accumulation, and curbing appetite [17-19]. However, anti-obesity medications have numerous adverse side effects such as pulmonary hypertension, vascular heart disease, psychological effects, increased risk of stroke, and heart attack [20].

Green coffee (GC), unroasted coffee seeds, is high in bioactive phytochemicals, including chlorogenic acids (CGAs) and methylxanthines, which have a diversity of biochemical and physiological functions [21]. Green coffee extract (GCE) has potent antioxidant properties and has a positive preventive role against the risk of metabolic syndrome [22-23]. GCE showed hypoglycemic effect and inhibition of adipogenesis [24]. GC methanolic extract showed hepatoprotective, antioxidant, and anti-inflammatory impacts in hepatotoxic rats [25].

This research aimed to determine the efficacy of GCE comparable to TSB (an anti-obesity drug) on body weight and to detect their impact on various physiological markers that describe the metabolic state in obese adult male rats.

## 2. Material and Methods

### 2.1. Animals

Twenty-one adult obese male albino rats (320-360 g body weight (BW)) were acquired from the Nile Pharmaceutical Company's animal house, Cairo, Egypt. Rats were kept in well-aerated polypropylene cages in a room with controlled conditions for one week; the rats were freely allowed food and water. The experimental processes were done in conformity with the principles and guidelines of the Ethics Committee of the Faculty of Science, Al-Azhar University, Cairo, Egypt.

### 2.2. Drugs and chemicals

Turbo slim blue (TSB) capsules (400 mg) were purchased from Med Care for Pharma Clinic. Green coffee extract (GCE) capsules (800 mg GCE, 50% chlorogenic acid) were purchased from Seef Group Pharmacies.

### 2.3. Experimental design

All rats in this study were obese with an average BW of 340 ± 20g. They were divided into the following three equal groups (7 animals each):

Control: rats were ingested orally (p.o.) distilled water daily for four weeks.

TSB: rats were ingested p.o. TSB (10 mg / kg/day) for four weeks [26].

GCE: rats were ingested p.o. GCE (10 mg / kg/day) for four weeks [27].

### 2.4. Assessment of % change of the BW

BW for each rat was measured weekly, and the percent (%) change in BW was calculated after 2 and 4 weeks.

% Change of the BW = [(Final – Initial)/Initial] ×100

### 2.5. Samples collection

Blood samples were gathered from the retro-orbital sinus plexus after the end of the experiment under mild ether anesthesia. Serum was separated by centrifugation (5000 rpm) for 10 minutes, then kept frozen at -20°C until analysis.

### 2.6. Determination of pancreatic function biomarkers

Enzyme-linked immunosorbent assay (ELISA) kit (Abcam) was used for determining insulin levels and biochemical assay kit (Abcam) was used for determining glucose level as described in manufacturer's procedure. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated via a free online calculator (HOMA calculator, https://www.dtu.ox.ac.uk/homacalculator/download.php) [28].

### 2.7. Determination of serum triiodothyronine (T3), thyroxine (T4), and leptin

Triiodothyronine (T3) and thyroxine (T4) were estimated using ELISA kits (Abcam). Leptin was measured using ELISA kit (Bioassay technology laboratory) as the manufacturer's instructions.

### 2.8. Determination of protein biomarkers

Total protein and albumin levels were assessed by colorimetric assay kits (BioMerieux kits, France). Globulin level and albumin/globulin (A/G) ratio were calculated.

### 2.9. Determination of lipid biomarkers

Colorimetric assay kits (BioMerieux kits, France) were used for determination of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C). Equations were used to calculate low- and very low- density lipoprotein cholesterol (LDL-C and VLDL-C).

### 2.10. Determination of liver function biomarkers

Colorimetric assay kits (BioMerieux kits, France) were used for determining aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

### 2.11. Determination of renal function biomarkers

Renal function biomarkers were assessed through determination of serum creatinine and urea concentrations by using BioMerieux kits, France.

### 2.12. Determination of serum testosterone

ELISA Abcam kit was used for determination of testosterone level.

### 2.13. Statistical analysis

All the biochemical data were represented as mean ± SE. Results analysis was done using SPSS version 27 ($p \leq 0.05$ was considered statistical significance).

## 3. Results

### 3.1. GCE and TSB impacts on the percent change in BW in obese rats

Table 1 shows the percent change in BW in all experimental groups after 2 and 4 weeks. At 2 and 4 weeks, the percent change in BW in both the TSB and GCE groups was significantly lower ($p \leq 0.001$) than in the control group. Furthermore, when compared to the TSB-treated rats, the GCE-treated rats showed a significant reduction ($p \leq 0.01$) in percent change in BW after only 2 weeks.

**Table 1.** GCE and TSB impacts on the percent (%) change in BW in obese rats.

| Groups | % Change in BW | |
|---|---|---|
| | After 2 weeks | After 4 weeks |
| **Control** | 15.06 ± 0.89 | 50.26 ± 2.53 |
| **TSB** | 6.36 ± 0.95 $^{\#***}$ | - 0.24 ± 0.42 $^{\#***}$ |
| **GCE** | 1.91 ± 0.96 $^{\#***, \&**}$ | - 3.73 ± 0.75 $^{\#***}$ |

Values are mean ± SE (n=7). $^{\#}$Significant difference relative to the control; $^{\&}$Significant difference relative to the TSB. ($^{**}p \leq 0.01$, $^{***}p \leq 0.001$).

### 3.2. GCE and TSB impacts on pancreatic function biomarkers measured in obese rats

Table 2 shows all experimental groups' serum glucose, insulin, and HOMA-IR values. Administration of GCE and TSB significantly decreased the HOMA-IR value ($p \leq 0.01$ and $p \leq 0.05$, respectively) relative to the control group value. Only GCE administration significantly reduced ($p \leq 0.05$) serum glucose value relative to control value.

**Table 2.** GCE and TSB impacts on pancreatic function biomarkers measured in obese rats.

| Groups | Insulin (mU/L) | Glucose (mg/dl) | HOMA-IR |
|---|---|---|---|
| **Control** | 0.934 ± 0.017 | 100.40 ± 3.42 | 0.230 ± 0.009 |
| **TSB** | 0.916 ± 0.003 | 90.80± 3.92 | 0.204 ± 0.004 $^{\#*}$ |
| **GCE** | 0.899 ± 0.013 | 85.54 ± 3.98 $^{\#*}$ | 0.191 ± 0.008 $^{\#**}$ |

Values are mean ± SE (n=7). $^{\#}$Significant difference relative to the control. ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

### 3.3. GCE and TSB impacts on serum T3, T4, and leptin hormones measured in obese rats

Table 3 shows all experimental groups' serum T3, T4, and leptin hormone values. Administration of GCE or TSB produced significant increases in serum T3 and T4 values ($p \leq 0.05$ for both) relative to the control. Besides, leptin values in both the TSB and GCE groups were significantly lower ($p \leq 0.05$) than in the control group.

**Table 3.** GCE and TSB impacts on serum T3, T4, and leptin hormones measured in obese rats.

| Groups | T3 (ng/dl) | T4 (ng/dl) | Leptin (ng/dl) |
|---|---|---|---|
| **Control** | 47.94 ± 2.24 | 3.02 ± 0.03 | 1.90 ± 0.05 |
| **TSB** | 54.48±1.13 $^{\#*}$ | 3.39 ±0.13 $^{\#*}$ | 1.67 ± 0.11$^{\#*}$ |
| **GCE** | 53.60± 1.51 $^{\#*}$ | 3.36 ±0.14 $^{\#*}$ | 1.59 ± 0.04$^{\#*}$ |

Values are mean ± SE (n=7). $^{\#}$Significant difference relative to the control. ($^{*}p \leq 0.05$).

### 3.4. GCE and TSB impacts on serum protein biomarkers measured in obese rats

Table 4 shows all experimental groups' serum total proteins, albumin, and globulin values besides the A/G ratio values. Treatment with TSB significantly reduced ($p \leq 0.05$) serum albumin and A/G ratio relative to the control. No effect was noted on all these parameters in the GCE group.

### 3.5. GCE and TSB impacts on lipid biomarkers measured in obese rats

Figure 1 shows all experimental groups' serum TC, TG, HDL-C, LDL-C, and VLDL-C values. Treatment with TSB significantly reduced serum TC, TG, and LDL-C ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.01$, respectively) relative to the control. Administration of GCE produced significant decreases in serum TC, TG, LDL-C, and VLDL-C ($p \leq 0.01$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.05$, respectively) relative to the control. Furthermore, when relative to the TSB group, the GCE group showed a significant decrease ($p \leq 0.01$) in serum LDL-C.

### 3.6. GCE and TSB impacts on liver function biomarkers measured in obese rats

Figure 2 shows all experimental groups' serum AST and ALT values. Treatment with TSB significantly increased serum AST and ALT ($p \leq 0.01$ for both) relative to the control and GCE. Administration of GCE produced no effect on serum AST and ALT relative to the control group.

### 3.7. GCE and TSB effects on renal function biomarkers measured in obese rats

Figure 3 shows all experimental groups' serum urea and creatinine values. Treatment with TSB significantly increased serum creatinine relative to

the control and GCE ($p \leq 0.05$ and $p \leq 0.01$, respectively). Administration of TSB produced no effect on serum urea relative to the control. Administration of GCE had no impact on serum urea and creatinine relative to the control.

### 3.8. GCE and TSB impacts on serum testosterone measured in obese rats

Figure 4 shows all experimental groups' serum testosterone values. Treatment with GCE significantly increased serum testosterone relative to the control ($p \leq 0.05$). Ingestion of TSB produced no effect on serum testosterone relative to the control.

## 4. Discussion

Obesity is a serious medical condition, it increasingly affecting a large population worldwide. It is characterized by an excessive accumulation of adipose tissue, which can lead to a variety of health issues [29]. In recent years, advancements in the treatment of obese animals have been made, although the efficiency of these treatments is still debatable. This work aimed to elucidate the effects of GCE and TSB (anti-obesity drug) on body weight and some physiological biomarkers in obese male rats.

The present results denoted that there was a significant reduction in % change of the BW in rats administered GCE relative to the untreated obese rats after 2 and 4 weeks. Also, GCE produced a significant decrease in % change of the BW relative to TSB-treated rats after 2 weeks. In addition, administration of GCE induced significant hypolipidemic effects, significant decreases in glucose, HOMA-IR, and leptin hormone. Besides, GCE significantly increased serum thyroid hormones (T3 and T4) and testosterone. Ingestion of GCE has no side effects on protein metabolism. The levels of liver and renal function biomarkers in GCE group were comparable with the control levels. The obtained results agree with Hussein et al. [30] who demonstrated that treatment with GCE in high-fat diet (HFD)-induced obese rats significantly reduced BW than the untreated group. Another study showed that regular consumption of GCE for 60 days induced significant weight loss [31]. Song et al. [32] reported that consumption of GCE prevents fat accumulation in mice. The weight reduction effects of GCE may be attributed to the phytochemical compounds, especially chlorogenic acids (CGAs), which avoid weight gain and fat accumulation via increasing fat metabolism and inhibiting fat absorption, as well as reducing appetite [33-35]. Moreover, catechin, caffeine, and CGAs in GCE showed synergistic effects on enhancing fat expenditure [36-38].

The hypolipidemic effect of GCE agree with Hussein et al. [30] who reported that ingestion GCE ameliorated the HFD-caused hyperlipidemia in rats. The phytocompounds and CGAs in GCE have been proved to significantly improve lipid content and fat accumulation *via* activation of fat metabolism in the liver [34, 39]. CGAs in GCE down-regulation of liver X receptor α (LXRα), which regulate the syntheses of fatty acids and triglycerides *via* direct activation of the genes encoding lipogenic enzymes [40]. Also, hypolipidemic effects of GCE could be attributed to enhanced peroxisome proliferator-activated α (PPARα) expression, which speeds up fatty acid β-oxidation [40].

Additionally, Hussein et al. [30] revealed that GCE significantly decreased insulin and glucose levels in treated obese rats relative with the HFD-untreated group. A phytoconstituents of GCE, CGAs, inhibits glucose-6-phosphate translocase 1, which inhibits glucose absorption in the small intestine [41]. This explained the reducing glucose level induced by GCE. Leptin, the hormone which regulates food consumption and fatty acid oxidation, is secreted by the adipocytes. It is transported to the brain, which signals inhibited lipid accumulation in the adipose tissue [42]. Choi et al. [43] found that treatment with GCE reduces leptin hormone concentration in the treated group.

The enhanced in testosterone level in GCE treated rats agree with Wedick et al. [44] who reported that after 28 days, green coffee consumption significantly increased serum testosterone. It could be explained by its antioxidant effects [23].

In the present study ingestion of TSB induced significant weight reduction, hypolipidemia, and decrease in HOMA-IR in the treated rats relative to control rats. Levels of T3 and T4 were significantly higher in TSB-treated rats. However, TSB-treated rats induced significant decreases in serum albumin and A/G ratio, concurrent with significant increases in liver enzymes (AST and ALT) activities and creatinine levels relative to the control rats.

**Table 4.** GCE and TSB impacts on serum protein biomarkers measured in obese rats.

| Groups | Total Protein (g/dl) | Albumin (A) (g/dl) | Globulin (G) (g/dl) | A/G ratio |
|---|---|---|---|---|
| **Control** | 6.38 ± 0.09 | 3.14 ± 0.11 | 3.24 ± 0.06 | 0.97 ± 0.06 |
| **TSB** | 6.32 ± 0.06 | 2.78 ± 0.08$^{\#*}$ | 3.55 ± 0.15 | 0.78 ± 0.05$^{\#*}$ |
| **GCE** | 6.27 ± 0.04 | 2.86 ± 0.11 | 3.41 ± 0.10 | 0.84 ± 0.04 |

Values are mean ± SE (n=7). $^{\#}$Significant difference relative to the control. ($^{*}p \leq 0.05$).

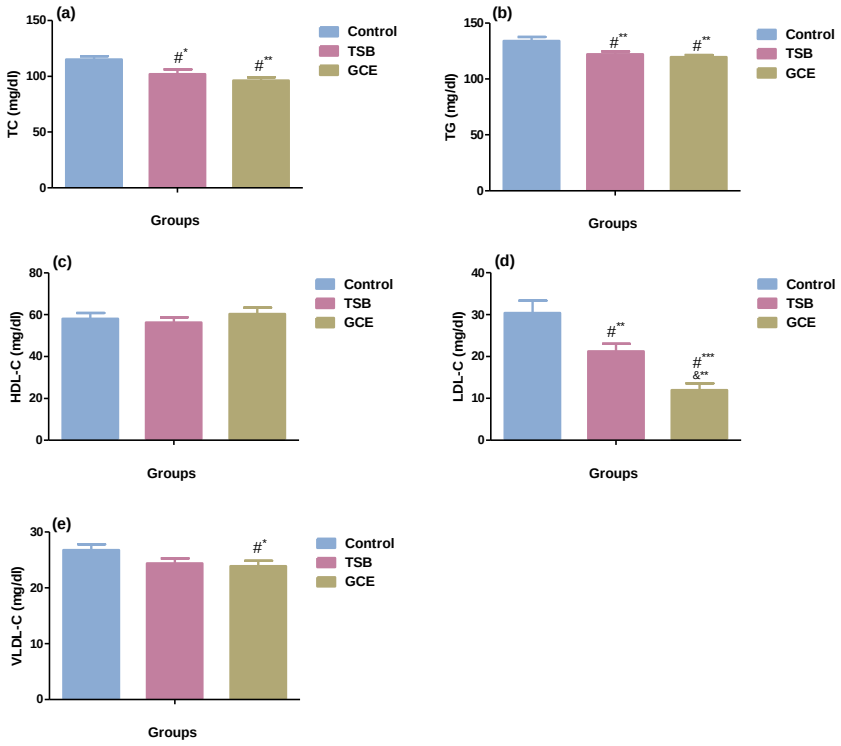


**Figure 1.** GCE and TSB impacts on lipid biomarkers measured in obese rats. (a) TC, (b) TG, (c) HDL-C, (d) LDL-C, and (e) VLDL-C. Values are mean ± SE (n=7). #Significant difference relative to the control; &Significant difference relative to the TSB. ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$).

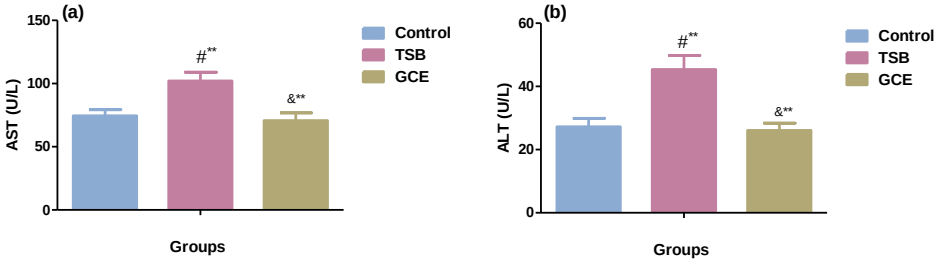


**Figure 2.** GCE and TSB impacts on liver function biomarkers measured in obese rats. (a) AST and (b) ALT. Values are mean ± SE (n=7). #Significant difference relative to the control; &Significant difference relative to the TSB. ($^{**}p \leq 0.01$).

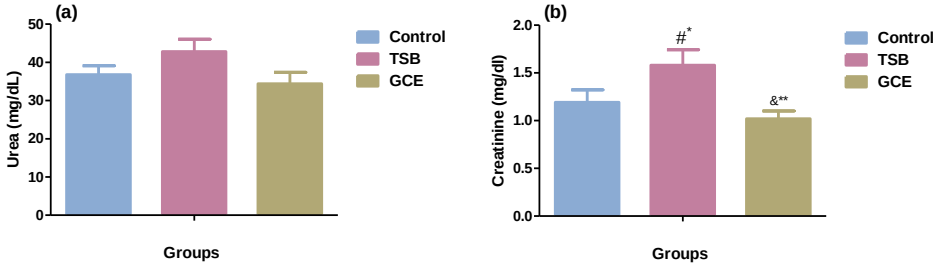


**Figure 3.** GCE and TSB impacts on renal function biomarkers measured in obese rats. (a)urea and (b) creatinine.. Values are mean ± SE (n=7). #Significant difference relative to the control; &Significant difference relative to the TSB. ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

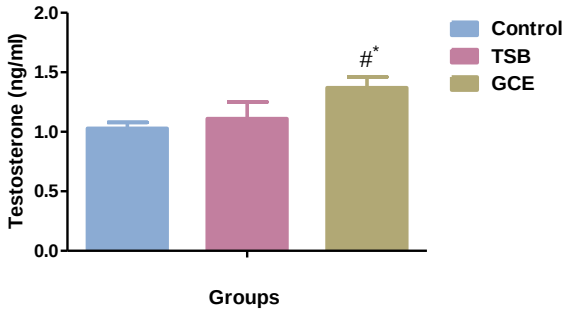


**Figure 4.** GCE and TSB effects on serum testosterone measured in obese rats. Values are mean ± SE (n=7). #Significant difference relative to the control. (*$p \leq 0.05$).

TSB, an anti-obesity drug, contains *Garcinia cambogia*. Studies revealed that *Garcinia cambogia* extracts and (−) hydroxycitric acid (HCA), the major component of TSB helps to restrict appetite by diminishing the desire to eat, resulting in a reduction in food consumption, reducing body fat gain, and promoting satiety by regulating serotonin levels [45-46]. *Garcinia cambogia* also caused fat dispersion, which aided lipase action on adipose tissue, suppressed body fat accumulation, inhibited cytoplasmic lipid accumulation, and regulated adipogenesis [18, 47].

TSB exhibited hypolipidemic effects through increased fat oxidation and declined de novo lipogenesis. Administration of *Garcinia cambogia*, the major component of TSB, decreased lipid levels in rats fed HFD by lowering lipogenesis and increasing lipid breakdown [48]. HCA is also a powerful inhibitor of adenosine triphosphate-citrate lyase, a catalyst for the conversion of citrate to acetyl-coenzyme A, which is involved in the synthesis of fatty acids, cholesterol, and triglycerides [49].

Thyroid hormones, which include T3 and T4, are important metabolic hormones, with T3 being the most functionally active. Thyroid hormone levels in the blood are linked to energy production. The significantly elevated thyroid hormones in TSB-treated obese rats in this study agree with Hornick et al. [50] and Smith et al. [51] they attributed the hyperthyroidism to the presence of HCA, which indicated the increase in energy expenditure.

Several anti-obesity medications can cause severe side actions, which are associated with the drug's mechanism of action [52]. The elevated liver and renal functions markers are indicators of liver and renal toxicity. The more severe the liver and renal damages the higher the release of the liver and renal function indicators. In this study, there were significant increases in serum renal functions (creatinine) and hepatic functions (AST and ALT) levels in TSB-treated obese rats relative to untreated obese rats. Consumption of the anti-obesity medicine (orlistat) caused severe renal injury [53-54]. These effects could be due to the toxic effect of chromium, as a component of TSB that can lead to disorder in renal function via a reduction in glomerular filtration rate followed by retention of creatinine in the blood [55]. The hepatotoxicity could be attributed to *Garcinia cambogia*, which was proved in several studies to cause hepatotoxicity [56-57].

## 5. Conclusion

According to the findings of this study, GCE effectively reduced BW in the obese rats which is comparable to the action of the anti-obesity drug, TSB. It also decreased serum lipids, glucose, insulin resistance, and leptin hormone. Opposite to TSB, GCE had no effect on liver and kidney functions. Therefore, GCE can be regarded as a safe and effective natural medicine that may help reduce BW.

## 6. Conflicts of interest

There are no conflicts to declare.

## 7. Acknowledgment

The authors' thanks and appreciation animal house, Faculty of Medicine, Al-Azhar University for technical support in the experimental study.

## 8. References

1- Drew, B., Dixon, A., and Dixon, J. 2007, "Obesity management, update on orlistat", Vasc Health Risk Manag., 3 (6), pp. 817–821.
2- Ebbert, J.O., and Jensen, M.D. 2013, "Fat depots, free fatty acids, and dyslipidemia", Nutri., 5(2), pp.498–508.
3- Mahmoud, R.H., and Elnour, W.A. 2013, "Comparative evaluation of the efficacy of ginger and orlistat on obesity management,

pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats", Eur Rev Med Pharmacol Sci., 17(1), pp. 75–83.

4- Chen, Y., Peng, Q., Yang, Y., Zheng, S., Wang, Y., and Lu, W. 2019, "The prevalence and increasing trends of overweight, general obesity, and abdominal obesity among Chinese adults: a repeated cross-sectional study", BMC Public Health, 19 (1), pp.1293.

5- Althumiri, N.A., Basyouni, M.H., AlMousa, N., AlJuwaysim, M.F., Almubark, R.A., BinDhim, N.F., Alkhamaali, Z., and Alqahtani, S.A. 2021, "Obesity in Saudi Arabia in 2020: Prevalence, distribution, and its current association with various health conditions", Healthcare (Basel), 9(3), pp. 311.

6- Padwel, R., and Majumdar, S. 2007, "Drug treatments for obesity: Orlistat sibutramine, and rimonabant", Lancet, 369 (9555), pp.71–77.

7- Ryan, D.H., and Yockey, S.R. 2017, "Weight loss and improvement in comorbidity: differences at 5%, 10%, 15%, and over", Curr Obes Rep., 6(2), pp. 187–194.

8- Curioni, C.C., and Lourenco, P.M. 2005, "Long-term weight loss after diet and exercise: a systematic review", Int J Obes., 29 (10), pp.1168–1174.

9- Thomas, A.W., Jena, S.T., and Meghan, L.B. 2020, "Lifestyle modification approaches for the treatment of obesity in adults", Am Psychol., 75(2), pp. 235–251.

10- Jensen, M.D., Ryan, D.H., Apovian, C.M., Ard, J.D., Comuzzie, A.G., and Donato, K.A. 2014, "AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society", Circulation, 129 (25 Suppl 2), pp.S102–S138.

11- Garvey, W.T., Mechanick, J.I., Brett, E.M., Garber, A.J., Hurley, D.L., and Jastreboff, A.M, 2016, "American association of clinical endocrinologists and American college of endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity", Endocr Pract., 22 (Suppl 3), pp.1 – 203.

12- Bhat, S.P., and Sharma, A. 2017, "Current drug targets in obesity pharmacotherapy- a review", Curr Drug Targets, 18 (8), pp.983 – 993.

13- Coulter, A.A., Rebello, C.J., and Greenway, F.L. 2018, "Centrally acting agents for obesity: past, present, and future", Drugs, 78 (11), pp.1113 – 1132.

14- Andrej, D., Biljana, G., Rok, D., Petra, K., and Julijana, K. 2010, "Nanosized particles of orlistat with enhanced *in vitro* dissolution rate and lipase inhibition", Int J Pharm., 396 (1-2), pp. 149 – 155.

15- Mariarosaria, B., and Stefania, D. 2020, "Anti-obesity effects of polyphenol intake: current status and future possibilities", Inter J Molecular Sci., 21(16), pp. 5642.

16- Anderson, R.A. 1998, "Effects of chromium on body composition and weight loss", Nutr Rev, 56(9), pp. 266–270.

17- Ohia, S.E., Opere, C.A., LeDay, A.M., Bagchi, M., Debasis Bagchi, D., and Stohs, S.J. 2002, "Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX)", Mol Cell Biochem, 238(1-2), pp. 89–103.

18- Saito, M., Nagata, J., and Takeuchi, M. 2005, "High dose of *Garcinia* is effective in suppressing fat accumulation in developing male Zucker obese rats but highly toxic to testes", Food Chem Toxicol., 43(3), pp. 411–419.

19- Kumar, V., Bhandari, U., Tripathi, C.D., and Geetika-Khanna, G. 2021, "Evaluation of antiobesity and cardioprotective effect of Gymnema sylvestre extract in murine model", Indian J Pharmacol., 44(5), pp. 607–613.

20- Daniel, H. B., and Luc, F.V. 2018, "Progress and challenges in anti-obesity pharmacotherapy", Lancet Diabetes Endocrinol., 6 (3), pp. 237–248.

21- Esquivel, P., and Jiménez, V.M. 2012, "Functional properties of coffee and coffee by-products", Food Res Int., 46 (2), pp. 488–495.

22- Priftis, A., Stagos, D., Konstantinopoulos, K., Tsitsimpikou, C., Spandidos, D.A., Tsatsakis, A.M., Tzatzarakis, M.N., and Kouretas, D. 2015, "Comparison of antioxidant activity between green and roasted coffee beans using molecular methods", Mol Med Reports, 12 (5), pp.7293–7302.

23- Sarria, B., Martinez-Lopez, S., Sierra-Cinos, J.L., Garcia-Diz, L., Mateos, R., and Bravo-Clemente, L. 2018, "Regularly consuming a green/roasted coffee blend reduces the risk of metabolic syndrome", Eur J Nutr., 57 (1), pp.269–278.

24- Budryn, G., Nebesny, E., Rachwał-Rosiak, D., Pałecz, B., Hodurek, P., Miśkiewicz, K., Oracz, J., and Żyżelewicz, D. 2014, "Inclusion complexes of β-cyclodextrin with chlorogenic acids from crude and purified aqueous extracts from green Robusta coffee beans (Caffea canephora L.)", Food Res Int., 61, pp.202–213.

25- El Rabey,H.A., Rezk,S.M., Sakran, M.I., Mohammed, G.M., Bahattab, O., Balgoon, M.J., Elbakry, M.A., and Bakry, N. 2021, "Green coffee methanolic extract and silymarin protect against CCl4-induced hepatotoxicity in albino male rats", BMC Complement Med Therap., 21 (1), pp.1–11.

26- Nair, A.B., and Jacob, S. 2016, "A simple practice guide for dose conversion between

animals and human", J Basic Clin Pharma, 7(2), pp. 27 – 31.
27- Ilmiawati, C., Fitri, F., Rofinda, Z.D., and Reza, M. 2020, "Green coffee extract modifies body weight, serum lipids and TNF-α in high-fat diet-induced obese rats", BMC Res Notes, 13, pp.208.
28- Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C.1985, "Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man", Diabetologia, 28 (7), pp. 412–419.
29- Sudeep, H.V., and Shyam, P. K. 2021, "Supplementation of green coffee bean extract in healthy overweight subjects increases lean mass/fat mass ratio: A randomized, double-blind clinical study", Sage Open Med., 9, 20503121211002590.
30- Hussein, M.M.A., Samy, M., Arisha, A.H., Saadeldin, I.M., and Alshammari, G.M. 2020, "Anti-obesity effects of individual or combination treatment with *Spirulina platensis* and green coffee bean aqueous extracts in high-fat diet-induced obese rats", Frontiers In Life Sci., 13 (1), pp. 328–338.
31- Dellalibera, O, Lemaire, B., and Lafay, S. 2006, "Svetol, green coffee extract, induces weight loss and increases the lean to fat mass ratio in volunteers with overweight problem", Rev Phytother., 4(4), pp. 194–197.
32- Song, S.J., Choi, S., and Park, T. 2014, "Decaffeinated green coffee bean extract attenuates diet-induced obesity and insulin resistance in mice", Evid Based Complement Alternat Med., 2014 ,718379.
33- Vinson, J.A., Burnham, B.R., and Nagendran, M.V. 2012, "Randomized, double blind, placebo-controlled, linear dose, crossover study to evaluate the efficacy and safety of a green coffee bean extract in overweight subject", Diabetes Metab Syndr Obese., 5, pp. 21–27.
34- Meng, S., Cao, J., Feng, Q., Peng, J., and Hu, Y. 2013, "Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review", Evid Based Complement Alternat Med., 2013:801457.
35- Meng, S.X., Liu, Q., Tang, Y.J., Wang, W.J., Zheng, Q.S., Tian, H.J., Yao, D.S., Liu, L., Peng, J.H., Zhao, Y., Hu, Y., and Feng, Q. 2016, "A recipe composed of Chinese herbal active components regulates hepatic lipid metabolism of NAFLD *in vivo* and *in vitro*", BioMed Res Int., 2016,1026852.
36- Post, S.M., de Roos, B., Vermeulen, M., Afman, L., Jong, M.C., Dahlmans, V.E.H., Havekes, L.M., Stellaard, F., Katan, M.B., and Princen, H.M.G. 2000, "Cafestol increases serum cholesterol levels in apolipoprotein E3-Leiden transgenic mice by suppression of bile acid synthesis", Arterioscler Thromb Vasc Biol., 20, pp. 1551 – 1556.
37- Zheng, G., Sayama, K., Okubo, T., Juneja, L.R., and Oguni, I. 2004, "Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice", *In Vivo*, 18(1), pp. 55 – 62.
38- Zheng, G.Y., Qiu, Q.F., Zhang, L.D., and Li, D. 2014, "Chlorogenic acid and caffeine in combination inhibit fat accumulation by regulating hepatic lipid metabolism-related enzymes in mice", Br J Nutr., 28(6), pp.1034–1040.
39- Shimoda, H., Seki, E., and Aitani, M. 2006, "Inhibitory effect of green coffee bean extract on fat accumulation and bodyweight gain in mice", BMC Complement Altern Med., 6,9.
40- Santana-Gálvez, J., Cisneros-Zevallos, L., and Jacobo-Velázquez, D.A. 2017, "Chlorogenic acid: recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome", Molecules, 22,E358.
41- Ma, Y., Gao, M., and Liu, D. 2015, "Chlorogenic acid improves high fat diet-induced hepatic steatosis and insulin resistance in mice", Pharm Res., 32 (4), pp. 1200–1209.
42- Arch, J.R. 2005, "Central regulation of energy balance: inputs, outputs and leptin resistance", Proc Nutr Soc., 64 (1), pp.39–46.
43- Choi, B.K., Park, S.B., Lee, D.R., Lee, H.J., Jin, Y.Y., Yang, S.H., and Suh, J.W. 2016, "Green coffee bean extract improves obesity by decreasing body fat in high-fat diet-induced obese mice", Asian Pac J Trop Med., 9 (7), pp.635–643.
44- Wedick, N. M., Mantzoros, C. S., Ding, E. L., Brennan, A. M., Rosner, B., Rimm, E. B., Hu, F. B., and Van Dam, R. M. 2012, "The effects of caffeinated and decaffeinated coffee on sex hormone-binding globulin and endogenous sex hormone levels: a randomized controlled trial", Nutr J., 11 (1), pp. 1 – 6.
45- Kim, Y.J., Choi, M.S., Park, Y.B., Kim, S.R., Lee, M.K., and Jung, U.J. 2013, "*Garcinia cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. World J Gastroenterol., 19(29), pp. 4689 – 4701.
46- Semwal, R. B., Semwal, D.K., Vermaak, I., and Viljoen, A. 2015, "A comprehensive scientific overview of *Garcinia cambogia*", Fitoterapia, 102, pp. 134–148.
47- Roy, S., Shah, H., Rink, C., Khanna, S., Bagchi, D., Bagchi, M., and Sen, C.K. 2007, "Transcriptome of primary adipocytes from an obese woman in response to novel hydroxy citric acid- based dietary supplement", DNA cell Biol., 26(9), pp. 627 – 639.

48- Asha, S.K., Anila, L., and Vijayalakshmi, N.R. 2001, "Flavonoids from *Garcinia cambogia* lower lipid levels in hypercholesterolemic rats", Food Chem., 72 (3), pp.289 – 294.
49- Marquez, F., Babio, N., Bullo, M., and Salas-Salvado, J. 2012, "Evaluation of the safety and efficacy of hydroxycitric acid or *Garcinia cambogia* extracts in humans", Crit Rev Food Sci Nutr., 52 (7), pp. 585–594.
50- Hornick, J.L., Van Eenaeme, C., Gerard, O., Dufrasne, I., and Istasse, L. 2000, "Mechanisms of reduced and compensatory growth", Domest Anim Endocrinol., 19 (2), pp. 121–132.
51- Smith, J.W., Evans, A.T., Costall, B., and Smythe, J.W. 2002, "Thyroid hormones, brain function and cognition: a brief review", Neurosci Biobehav Rev., 26 (1), pp.45–60.
52- Defo, P.B., Wankeu-Nya, M., Ngadjui, E., Fozin, G.R.B., Kemka, F.X., Kamanyi, A., and Watcho, P. 2017, "Palm oil diet induced obesity impairs male rat reproductive performance", Ann Reprod Med Treat., 2(2), pp. 1012.
53- Singh, A,, Sarkar, S.R., Gaber, L.W., and Perazella, M.A. 2007, "Acute oxalate nephropathy associated with orlistat, a gastrointestinal lipase inhibitor", Am J Kidney Dis., 49 (1), pp. 153–157.
54- Weir, M.A., Beyea, M.M., Gomes, T., Juurlink, D.N., Mamdani, M., Blake, P.G., Wald, R., and Garg, A.X. 2011, "Orlistat and acute kidney injury: an analysis of 953 patients", Arch Intern Med., 171 (7), pp. 703–704.
55- Tsung-Lin, T., Chin-Chi, K., Wen-Harn, P., Yu-The, C., Chiu-Ying, C., Trong-Neng, W., and Shu-Li, W. 2017, "The decline in kidney function with chromium exposure is exacerbated with co-exposure to lead and cadmium", Kidney Int., 92(3), pp.710 – 720.
56- Jiten, P.K., Monica, K., Hrishikesh, S., and Marco, O. 2018, "Hepatotoxicity associated with use of the weight loss supplement *Garcinia cambogia*: A case report and review of the literature", Case Reports Hepatol., 12, 6483605.
57- Rebecca, C.K., Tuesday, W., Andrew, S., Adyr, M., Maxwell, S., Jessica, N., and Jorge, R. 2016, "Acute liver failure associated with *Garcinia cambogia* use", Am Hepatol., 15(1), pp.123 – 126.