

Efficacy of goldenberry extract in chelated iron overload induced by obesity: Novel safety concept for the treatment of iron overloads diseases

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ABSTRACT

Dysregulation of any step implicated in iron (Fe) metabolism in obesity may cause Fe overload. Excess Fe may cause toxic oxygen damage by generating oxygen-free radicals through the Fenton reaction. Furthermore, the three Fe chelating drugs deferiprone, deferoxamine, and deferasirox have many side effects which may limit their use. Therefore, this study intended to treat obesity-induced Fe overload with methanolic extract of goldenberry (GB) fruit with husk on obese rats. Overall, obese rats fed the GB extract showed lower levels of cholesterol, triacylglycerol, total low-density lipoprotein cholesterol, and higher levels of high-density lipoprotein cholesterol than obese rats, along with an improvement in anthropometric characteristics of obese rats. In addition, GB supplementation improved all parameters of Fe status in blood plasma and the trace elements homeostasis in adipose tissues, proving that the GB has a substantial ferric reducing property. GB enhanced the increase in hepcidin concentration in blood plasma and hepcidin gene expression in adipose tissues. In conclusion, there is an ascendant assessment of the role of GB as a natural Fe chelator that inhibits oxidative stress, which plays an essential role in recovering the conformational structure of hemoglobin as a macromolecule.

1. INTRODUCTION

Obesity is a multisystem disease that elevates the risk of the most common non-communicable chronic diseases of the 21st century [1,2]. Being overweight (OW), which encompasses a wide range of excess body fat, has emerged as a significant public health issue [3]. The influence of comorbidities grows in parallel with obesity [4]. The WHO reported that obesity and OW are significantly higher in the Eastern Mediterranean Region, which includes Egypt. The prevalence of OW and obesity in this region ranges from 74% to 86% among females and 69% to 77% among males [5]. A definite problem in Egypt is that the disparity in obesity is twice as high for females as for males [6,7].

Iron (Fe) is a vital mineral that plays a meaningful role in cellular respiration, DNA synthesis, and cell proliferation [8]. Wenzel *et al.* were considered the first team to detect lower serum Fe values in obese patients compared to the control group [9]. Some authors noted a decrease in serum Fe levels and transferrin saturation in the OW group compared to the control group [10]. Furthermore, Manios *et al.* stated that ferritin levels are elevated in obese children (9–13 years old) [11]. Other scientists demonstrated lower Fe levels and transferrin saturation

and higher hepcidin levels for the obese group compared to the control [12]. The recent rise in obesity-related chronic inflammatory conditions, along with the involvement of hepcidin, has been of considerable value [13]. Obesity boosts the levels of the Fe regulating hormone hepcidin, which can lead to a misdistribution of Fe in the body, which may play a role in metabolic disorders [14] where hepcidin controls the expression of ferroportin (Fe transporter) in target cells [15].

The choice of successful obesity treatment depends on an accurate diagnosis. There are various approaches to dealing with obese patients in treatment, such as nutritional system, sport and exercise medicine, abscission solution, lifestyle change, pharmacotherapy drug, acupuncture, and herbal medicine [16].

Deferiprone, deferoxamine, and deferasirox drugs treated Fe overload in different diseases such as thalassemia and hemochromatosis. Deferiprone has common side effects: Agranulocytosis, neutropenia, musculoskeletal and joint pains, gastrointestinal complaints, and zinc deficiency. Deferoxamine side effects are bluish fingernails, lips, skin blurred vision or other vision problems, convulsions (seizures), difficulty breathing or rapid breathing, fast heartbeat, hearing problems, and redness of the skin. Deferasirox has limitations related to dose, toxicity, cost, and ineffective removal of excess Fe from the heart [17]. Eventually, goldenberry (GB) is a natural product and safe as a Fe chelator, and has no side effects.

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New plants extracts of medicinal herbs have been identified to show their efficacy and role in weight loss [18]. GB (*Physalis peruviana* L.), also known as Harankash in Egypt, is a remarkable fruit with many medicinal and culinary applications. Puente *et al.* (2011) reported that the presence of several specific phytosterols: “Campesterol, β -sitosterol, and stigmasterol” lowers cholesterol levels in the blood, as they are found in high concentrations in oils derived from the fruit of *P. peruviana* L., offering them with antihypercholesterolemic properties and antioxidant. Moreover, high amounts of polyphenols as well as Vitamins A and C contribute to the antioxidant activity associated with this finding [19].

Our current study was conducted to verify the efficacy of GB (*P. peruviana*) fruit with husk extract as a novel nature’s safe dietary supplement in relieving obesity-induced Fe overload. The obtained data suggests that this extract can be useful in treatment of chelated Fe overload caused by many illness such as thalassemia, obesity and other Fe chelator chemical drugs such as deferoxamine which have potential side effects.

2. MATERIALS AND METHODS

2.1. Plant Material

P. peruviana were collected from the market in Cairo, Egypt, during its season on February/March 2020. The identification of the plants was confirmed by “Herbarium of the Department of Botany, Faculty of Science, Cairo University. The voucher specimens (G3) were kept in the National Research Centre Herbarium, Cairo, Egypt.”

2.2. Preparation of Extract

The fresh fruits with their husk (5 kg) were washed in running tap water in the laboratory, vigorously swirled by the blender, then soaked in 70% MeOH, warmed at 40°C, and filtered (using Whatman No.1 filter paper) into another sterile container. Extraction processes were repeatedly done thrice at 40°C; then, the resulting liquid was collected, filtered, and reduced through evaporation under vacuum by a rotary evaporator (Heidolph, Germany) at 45°C and then the crude extract was placed in lyophilization to give a dry residue (60 g).

2.3. Phytochemical Screening

The concentrated methanolic extract of *P. peruviana* fruit was applied to a polyamide column and eluted with water followed by different ratios of water/methanol to give rise to five fractions, which were further purified by a series of fractionations on a Sephadex LH-20 column and preparative paper chromatography to afford five phenolic acids, namely, gallic acid, pHydroxy benzoic acid, caffeine, o-Coumaric acid, and cinnamic acid together with six flavonoids identified as the three aglycone naringenin, kaempferol, and apigenin with their glucosides derivatives, respectively. The structures of the isolated compounds have been established by conventional methods of chemical and physical analyses and were confirmed by ¹H- and ¹³C-NMR spectroscopy.

2.4. Reagents

Folin–Ciocalteu and AlCl₃ reagents were supplied by Sigma (St. Louis, MO) and were used as they were received.

2.5. Estimation of Total Phenolics and Flavonoid Contents

The total phenolic content (TPC) of *P. peruviana* fresh fruit with husk was determined using Folin–Ciocalteu colorimetric assay described by Saboo *et al.* [20] with minor modification, using gallic acid as

a standard. The results were calculated using the standard curve of gallic acid with known concentrations (2.5–50 $\mu\text{g mL}^{-1}$). The phenolic content was expressed as mg gallic acid equivalent (GAE)/g plant extract. Both sections’ total flavonoids content (TFC) was measured using AlCl₃ colorimetric assay [21]. The total flavonoids concentration of the extract was determined according to the reported procedure [22]. TFC was expressed as mg rutin equivalent (RE)/g plant extract.

2.6. Animal Care and Treatments

Thirty-two adults female Wistar rats weighing 150–170 g (National Research Center, Animal house, Egypt) were stayed at stable room temperature (25°C) with a 12 h light/dark cycle and free access to food and water. The animals were left a week for adaptation. Eight rats were kept as control, and others were given a high-fat diet (HFD) and water from the tap with 25% sucrose for 12 weeks to develop obesity. HFD contains carbohydrate 42.3%, protein 17%, fat 22.50%, fiber 3%, 2%, minerals 5%, and moisture 10%. Normal rats were fed free standard chow pellets. The animal was handled following the recommendations of the National Institute of Health Guide for Care and Use of Laboratory Animals. The Research Ethics Committee of the National Research Centre, Egypt, approved the study protocol (approval number 19161).

The rats were separated into four groups:

Group-I: Normal control rats served were fed a free standard chow diet.

Group-II: Rats were given a HFD and water from the tap with 30% sucrose for 12 weeks to develop an obesity model.

Group-III: Obese rats treated orally with a low dose of 200 mg/kg bodyweight (b.w) of GB extract for 8 weeks.

Group-IV: Obese rats treated orally with a high dose of 400 mg/kg b.w of GB extract for 8 weeks.

2.7. Anthropometric Measures

The b.w. (g) and nasal-anal length (NAL) (cm) of rats in the normal control group and experimental groups were assessed at the beginning of the experiment, as well as after 2, 4, 6, and 8 weeks. The circumference of the waist was measured. The body mass index (BMI) (g/cm^2) was calculated according to the formula: “BMI = b.w. (g)/NAL (cm^2)” Obesity was defined as a BMI of 0.68 g/cm^2 or above, as previously reported by Novelli *et al.* [23].

2.8. Samples Collection

After the therapy period, the rats of all groups were fasted for about 10 h. Blood samples were collected from a retro-orbital vein under local anesthesia by phenobarbital, and separated plasma was stored in Eppendorf tubes at –30°C for biochemical analysis.

Immediately after blood sampling, animals were sacrificed by cervical dislocation and the adipose tissue was collected to measure biochemical parameters and trace elements.

2.9. Lipid Profile Determination

The lipid profile was evaluated calorimetrically using serum determination kits from “Salucea Company, The Netherlands.” The lipid profile included total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

2.10. Adiponectin and Leptin Determination

Rat adiponectin and leptin were quantified by enzyme-linked immunosorbent assay (ELISA) in plasma using “SinoGeneClon Biotech Co. Kit, China.”

2.11. Fe Profile Measurement

Plasma Fe and total Fe-binding capacity (TIBC) concentrations were determined quantitatively by Quimica Clínica Aplicada S.A. Kit [24,25]. Ferritin and transferrin receptor (TFR) were quantified in plasma by Sunlong Biotech Co. Kit.

2.12. Trace Element Analysis

Table 1 shows the operating conditions for the determination of Fe, Cu, and Zn ions using the Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES, with Agilent Vapor Generation Accessory VGA 77. All adipose tissue samples were digested with nitric acid before metal determination since it is an appropriate matrix for consistent recovery of metals suitable with the analytical technique (APHA, 2017) [26]. The Agilent 5100 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) with SVDV was used for all heavy metal studies. A blank and three or more Merck Company standards were used to create an intensity calibration curve for each set of measurements (Germany). External reference standards from Merck, as well as standard reference material for trace elements in water and a quality control sample from the National Institute of Standards and Technology, were used to ensure the accuracy and precision of the instrument reading.

2.13. Rat Hepcidin Concentration (ELISA)

Plasma hepcidin concentration was determined quantitatively by Elabscience Biotechnology Kit from China.

2.14. Hepcidin Gene (HAMP) Expression

2.14.1. RNA isolation

RNA was extracted from the adipose tissue (using 50 mg) by QIAamp RNA Blood Mini kit (CAT#52304 QIAGEN). The concentration of RNA was determined using a nanophotometer and was reverse transcribed. The RNA content was obtained by measuring the A260/A280 ratio, which should be between 1.8 and 2.0.

2.14.2. Reverse transcription

cDNA was generated by the QuantiTect Reverse Transcription kit (CAT# 205311 QIAGEN). The manufacturer's instructions were followed for setting up and running the reactions. Until used in RT-qPCR, samples were stored at -20°C . QuantiNova SYBR Green PCR kit (CAT#208052 QIAGEN) and QuantiTect primer assay

(CAT#249900 QIAGEN) were used to perform qRT-PCR gene expression investigation.

2.14.3. RT-qPCR

To determine fold change in gene expression, the fluorescence signals were analyzed with the Applied Biosystems Sequence Detection Software, and CT (cycle threshold), ΔCT , and $\Delta\Delta\text{CT}$ were computed using the $2^{-\Delta\Delta\text{CT}}$ technique. The rate of change in HAMP expression was determined using the GAPDH gene as a reference gene in this approach (housekeeping gene) [27].

2.15. Oscillator Strength

Hemoglobin oscillator strength was determined using a Cary UV/VIS dual-beam spectrophotometer (model 100 UV-VIS) made by Agilent Technologies, Australia, at room temperature (at $25 \pm 1^{\circ}\text{C}$). The extinction coefficient was measured at wavelengths ranging from 310 to 700 nm, and oscillator strength per molar heme was computed using the formulae below:

$$f = \frac{2.303MC}{N\pi e^2} n_o \left\{ \frac{3}{n_o^2 + 2} \right\}^2 \int \epsilon dv = 1.44 \times 10^{-19} \int \epsilon dv$$

Where, M is the mass of electron in gram; C is the velocity of light in cm/s; N = Avogadro's number; e = the charge of the electron in e.s.u; n_o = the index of refraction of the solvent (water); ν = the frequency of light in s^{-1} , and ϵ = the molar extinction coefficient obtained based on Briggsian logarithms [28].

2.16. Statistical Analysis

Values were stated as mean \pm S.E., and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by "Tukey-Kramer multiple comparisons test" by "GraphPad Prism version 8.0.2. For Windows, GraphPad Software, San Diego, California USA, www.graphpad.com." The level of statistical significance was at $P < 0.05$.

3. RESULTS

3.1. Total Phenolics and Flavonoid Contents

The TPC and flavonoids contents (TFC) of *P. peruviana* fruit with husk extract are demonstrated in Table 2. TPC was calculated as GAE/g plant extract, and TFC was calculated as RE/g of dried extract.

3.2. Anthropometric Measures of Obese Rats

Figure 1 depicts the time-dependent alterations in b.w. and BMI in the four groups throughout 2, 4, 6, and 8 weeks. At the start of GB extract supplementation, there was a significant increase in b.w. and BMI in obese, low dose of GB (200 mg/kg b.w), and high dose (400 mg/kg b.w) of GB rats compared to the normal control group. In the 4–8 weeks period marked by observation of the opposite, there was a significant decrease in BMI of low dose of GB and high dose of GB rats compared to obese rats. Figure 2 shows the advanced changes in naso-anal length and waist in the four groups over the 2, 4, 6, and 8 weeks. This means that the BMI of the two GB administrated groups (low and high dose of GB) showed decreases in BMI values after cessation of the experiment (after 8 weeks GB oral administration) compared to the obese group, which showed an increase in BMI values by a highly significant difference ($P < 0.05$).

Table 1: The operating conditions for the determination of iron, zinc, and copper ions using ICP-OES.

Operating conditions	Fe	Zn	Cu
Wave length	238.204	213.857	327.395
Intensity	41,015	42,500.9	53,785
Radio frequency (RF) plasma torches power kW	1.2	1.2	1.2
Nebulizer flow (L/min)	0.7	0.7	0.7
Plasma flow (L/min)	12	12	12
Viewing mode	Axial	Axial	Axial
Background correction	Fitted	Fitted	Fitted
Correlation coefficient	0.98	0.98	0.98
Calibration error	10%	10%	10%
Calibration fit	Rational	Rational	Rational

3.3. Effects of GB Supplementation on Lipid Profiles of Obese Rats

Table 3 shows the lipid profile of obese rats that took the GB supplement. This study observed a substantial increase in plasma TG levels and TC ($P < 0.05$) in obese rats compared to the normal control group. GB supplementation notably reduced the levels of plasma TC and TG in obese rats ($P < 0.05$), as shown in Table 3, along with significantly lowering the level of HDL cholesterol in obese rats ($P < 0.05$) compared to the normal control group. GB supplementation groups significantly improved HDL compared to obese rats ($P < 0.05$). The level of LDL cholesterol was significantly increased ($P < 0.05$) in obese rats compared to the normal control group. GB supplementation at a low or high dose significantly decreased the plasma LDL cholesterol level in obese rats ($P < 0.05$).

Table 2: Total phenolics and flavonoids contents in *Physalis peruviana* fruit with husk extract.

<i>Physalis peruviana</i> fruit with husk extract	Concentration (M±SE)
Total phenolics content (mg GAE/g of dried extract)	135.8±4.502
Total flavonoids content (mg RE/g of dried extract)	45.7±3.302

Value is presented as the mean (M) of triplicates±standard error (S.E.)

3.4. Effect of GB Supplementation on Adiponectin and Leptin Hormones of Obese Rats

Adiponectin and leptin for the obese, obese + low dose of GB extract, and obese + high dose of GB extract groups as compared to control group illustrated in Figure 3. Adiponectin was significantly decreased in HFD rats compared with control rats ($P < 0.05$). Otherwise, leptin was significantly increased in obese rats in contrast to the normal control group. Differently, GB supplementation at low or high doses significantly increased adiponectin and decreased leptin levels compared to obese rats ($P < 0.05$).

3.5. Effect of GB Supplementation on Fe Overload of Obese Rats

The present results attained that the studied obese rats had a highly significant decrement ($P < 0.05$) in serum Fe and transferrin saturation levels as well as highly significant increases in ferritin, TIBC, and sTfR levels compared to the normal control group [Table 4]. Furthermore, the hepcidin hormone in plasma was significantly increased in obese rats compared with control rats. Administration of low or high dose of GB significantly improves hepcidin hormone compared with obese rats.

3.6. Effect of GB Supplementation on Trace Elements in Adipose Tissue of Obese Rats

Table 5 demonstrates the alterations in trace elements in adipose tissue of control and obese rats. A significant overload and elevation in Fe

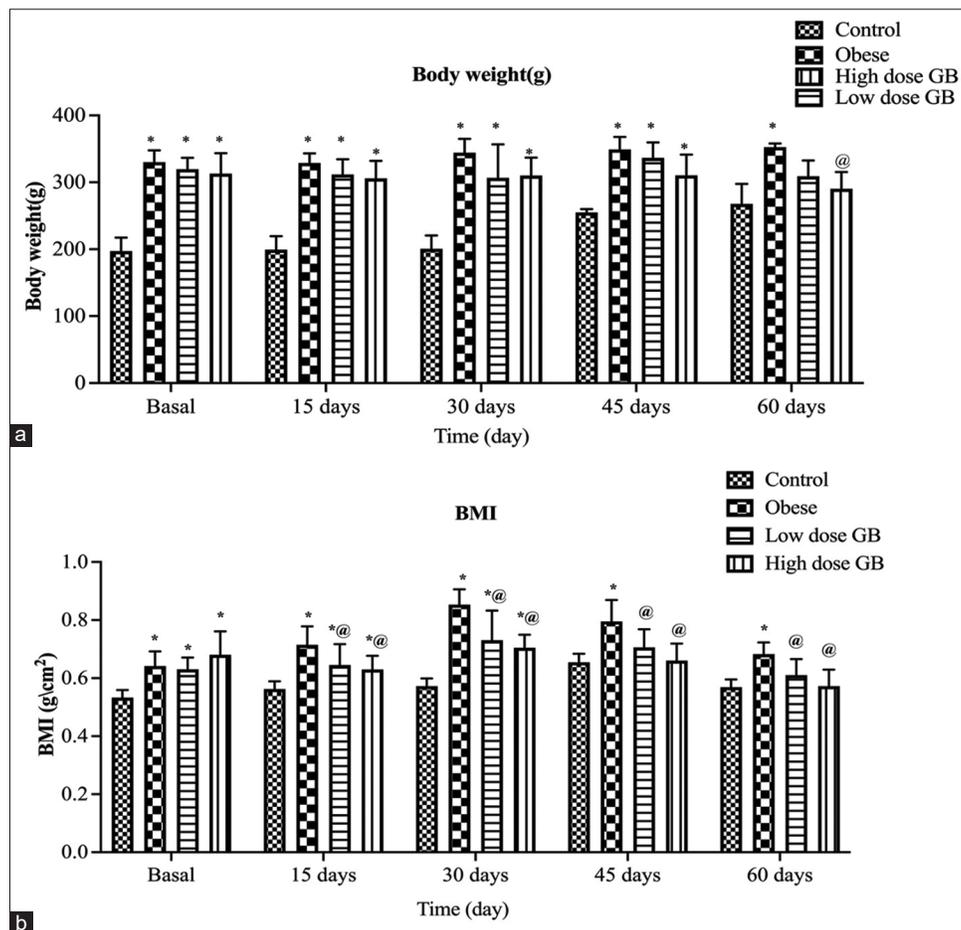


Figure 1: Body weight variance over time (a) and BMI over time (b). Each bar represents the mean ± SE. Statistical analysis was conducted using one-way ANOVA followed by Tukey-Kramer multiple comparisons test for the respective time period. (*vs. control group and @vs. obese group) at $P < 0.05$.

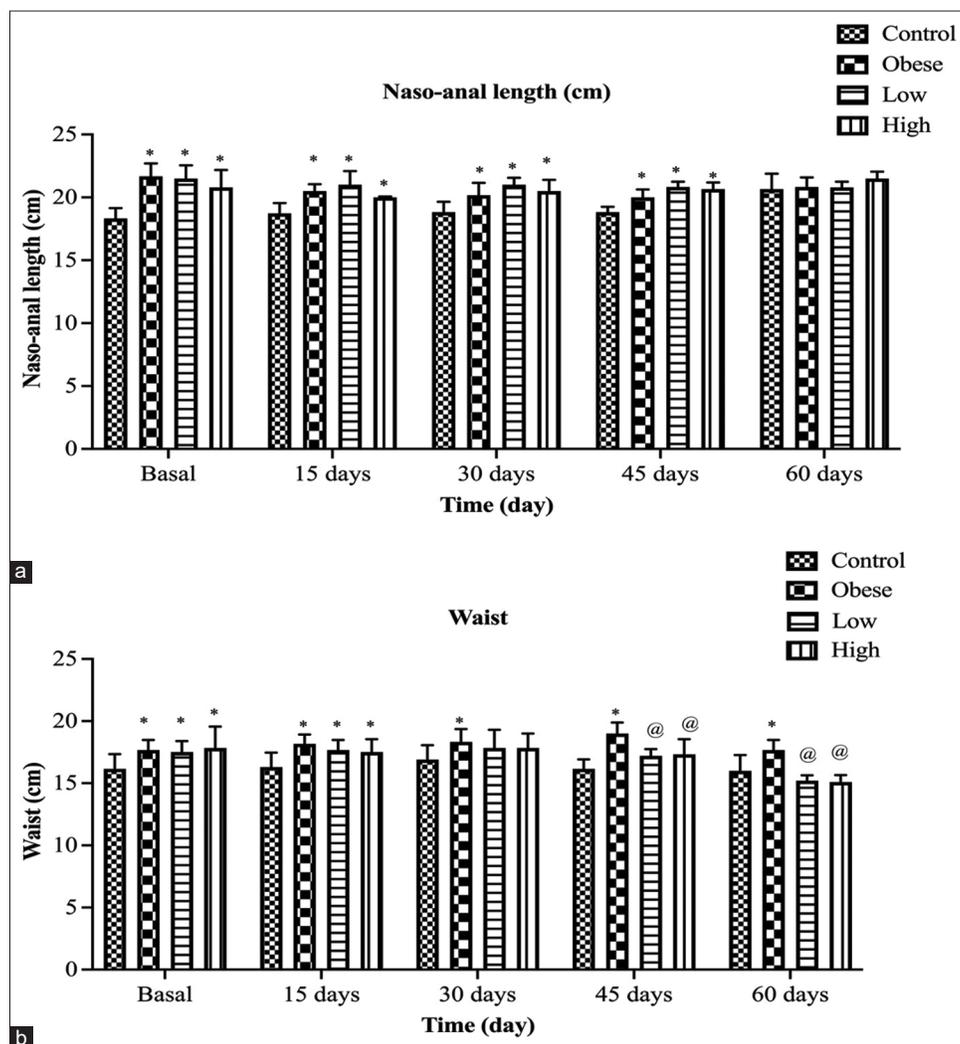


Figure 2: Variation of the length of naso-anal with time (a) and waist with time (b). Each bar represents the mean±SE. Statistical analysis was conducted using one-way ANOVA followed by Tukey-Kramer multiple comparisons test for the respective time period. (*vs. control group and @vs. obese group) at $P < 0.05$.

Table 3: Lipid profile parameters of obese, obese+ low dose of GB extract, and obese+ high dose of GB extract groups compared to control. Each reading represents the mean±SE.

Parameter groups	Total cholesterol	Triglyceride	HDL	LDL
Control	59.8±1.02	51.4±5.16	41.75±1.44	215.20±3.71
Obese	94.2±3.17*	154±19.31*	4.50±0.65*	244.40±5.19*
Obese+low dose of GB extract	59±0.63@	47.4±5.27@	30.50±8.18@	199.00±3.33@
Obese+high dose of GB extract	61.2±1.39@	43.6±7.55@	42.75±3.09@	206.60±3.91@

Statistical analysis was conducted using one-way ANOVA followed by Tukey-Kramer multiple comparisons test for the respective time period. (* vs. control group and @vs. obese group) at $P < 0.05$

and copper levels correlated with a substantial reduction in the Zn level in adipose tissue. Oral administration of a low or high GB dose significantly decreased Fe and Cu while concurrently increasing Zn.

3.7. Effect of GB Supplementation on Hepcidin Expression in the Adipose Tissues of Obese Rats

Figure 4 illustrates the means fold change of HAMP expression in the adipose tissue samples of the studied groups that show significant upregulation ($P < 0.05$) in obese adipose tissue's samples compared to control and significant downregulation ($P < 0.05$) in the adipose tissue samples with a low and high dose of GB when compared with the obese group.

3.8. Effect of GB Supplementation on Oscillator Strength of Hemoglobin (Hb) in Obese Rats

There was a dramatic increment in the oscillator strength per molar heme of obese rats in the normal control group. The opposite was clear as a highly significant decrement in rats subjected to either a low or high GB dose compared to obese rats [Table 6].

4. DISCUSSION

Obesity has emerged as a significant worldwide health issue, as it is associated with a slew of adverse health outcomes, including increased rates of cardiovascular morbidity and death [29]. Overfeeding causes

obesity and obesity-related systemic inflammation, which can lead to dysmetabolic Fe overload syndrome (DIOS) and anemic inflammation (AI) [3]. Treatment of Fe overload induced by obesity with GB extract has not been previously documented. Therefore, this study targets to treat the Fe overload status caused by obesity and thus get rid of the most severe problems associated with obesity.

Anthropometric measures are a non-invasive and low-cost way of measuring the nutritional condition of rats and are widely recommended for the detection of obesity symptoms. In infancy and adulthood, waist circumference is a predictor of abdominal or visceral fat distribution, as well as insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular disease as hypertension [30]. However, it must be emphasized that this high-calorie diet resulted in fast weight gain in experimental animals. As a result, anthropometric measures of these animals have changed over time, including b.w. gain, NAL, waist circumference, and BMI. These findings were consistent with recent reports of comparable alterations [23,31,32].

Obese rats had considerably higher cholesterol, TGs, and LDL levels, whereas HDL levels were significantly lower. Our findings were compatible with prior studies [33,34]. Consumption of GB has been shown to have hypocholesterolemia-lowering effects in rats on a HFD. GB supplementation also appears to protect the liver from oxidative stress while reducing the amount of fatty liver that develops due to the HFD. It has been proposed that people with coronary atherosclerosis should take golden berries to prevent the condition from progressing. Minor components, such as flavonoids, effectively reduce LDL oxidation and platelet aggregation, and carotenoids, which are thought to function mainly as antioxidants, may potentially have beneficial benefits [35]. Supplementation of GB extract (fruit with husk) increased HDL cholesterol levels. Since HDL is involved in the transport of cholesterol from peripheral cells to the liver, high blood HDL cholesterol levels from dietary GB extract are believed to be beneficial in reducing the risk of cardiovascular disease. Our study of the fruit and husk as a single extract reveals the full extent of their anti-obesity potential.

Table 4: Iron status parameters of the Obese, Obese+low dose of GB extract, and Obese+high dose of GB extract groups compared to the control group.

Groups parameters	Control	Obese	Obese+low dose of GB Extract	Obese+high dose of GB Extract
Fe (µg/dL)	115.7±5.29	38.71±2.9*	71.14±2.86@	97.7±3.6@
Ferritin (ng/ml)	638.6±40.79	6142.9±383.5*	2064.28±142.49@	837.14±26.3@
sTfR (µg/dl)	13.16±0.96	61.74±3.73*	37.9±1.57@	18.95±1.32@
TIBC (µg/dl)	346±4.27	395±6.07*	362.8±3.4@	332.14±5.37@
Transferrin Saturation%	33.83±1.62	9.8±0.7*	19.6±0.72@	29.41±1.06@
Hepcidin (ng/ml)	64.8±3.5	131.8±4.5*	94±4.3@	77.8±2.6@

All data are expressed as mean ± SE, sTfR, soluble serum transferrin receptor; TIBC, total iron binding capacity; (*vs control group and @ vs obese group) at P<0.05.

Table 5: Changes in trace elements in the adipose tissue of Obese, Obese+low dose of GB Extract, and Obese+high dose of GB Extract groups compared to the control group.

Groups Parameters	Zn (µg/g)	Fe (µg/g)	Cu (µg/g)
Control	4.2±0.31	9.4±0.422	3±0.17
Obese	0.85±0.18*	14.3±0.28*	10.05±0.53*
Obese+low dose of GB Extract	2.65±0.21@	11.25±0.39@	6.05±0.45@
Obese+high dose of GB Extract	3.85±0.18@	10.7±0.21@	2.7±0.25@

All data are expressed as mean ± SE, (* vs control group and @ vs obese group) at P<0.05

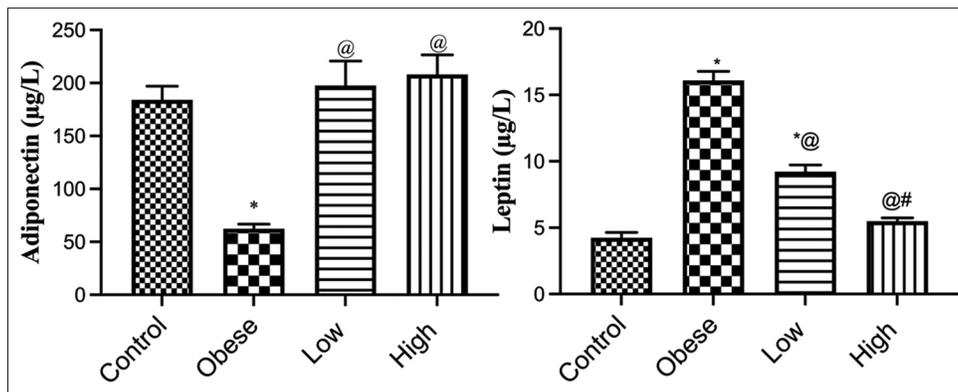


Figure 3: Adiponectin and leptin for obese, obese + low dose of GB extract, and obese + high dose of GB extract groups in comparison with the control. Each bar represents the mean±SE. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test for the respective time period. (*vs. control group, @vs. obese group, and #vs. low dose GB) at P < 0.05.

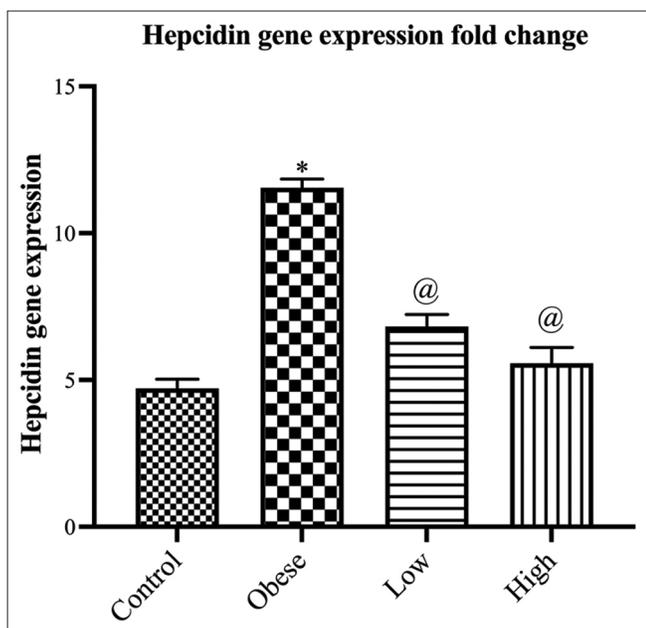


Figure 4: Fold change of hepcidin gene expression for obese, obese + low dose of GB extract, and obese+ high dose of GB extract groups compared to the control group. Each bar represents the mean±SE. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test for the respective time period. (*vs. control group and @vs. obese group) at $P < 0.05$.

Table 6: Oscillator strength for Obese, Obese + low dose of GB Extract, and Obese + high dose of GB Extract groups compared to control group.

Group	Oscillator strength
Control	1.86 ± 0.63
Obese	3.042 ± 0.85*
Obese + low dose of GB Extract	2.68 ± 0.78@
Obese + high dose of GB Extract	2.01 ± 0.69@

All data are expressed as mean ± SE, (* vs control group and @ vs obese group) at $P < 0.05$

It has also been observed that the fruit from *Physalis* species is rich in Vitamins A, B, and C, making them very nutritious. A-carotene, β-carotene, and β-cryptoxanthin are the major active components of Vitamin A in fruits. Phytosterols are extremely important because of their antioxidant properties and their impact on total and LDL cholesterol [36].

Compared to the normal control group animals, rats fed a high fructose diet had a significant rise in blood leptin and a reduction in adiponectin levels. Administration of either a low or high dose of GB extracts dramatically reduced the increase in leptin levels to near-normal levels, as well as improved adiponectin levels. Our results agreed with previous research [37,38]. Because of the aberrant buildup and malfunction of adipose tissue, obesity is characterized by a wide range of circulating adipokine levels. Obesity problems, as well as an increased risk of obesity-related comorbidities, including type 2 diabetes, are linked to altered levels of leptin and adiponectin. In rat models, leptin and adiponectin are also indicators of metabolic syndrome. Leptin is a neuroendocrine and energy homeostasis regulator, reflecting energy reserves, fat mass, and energy deprivation.

The present results showed that the studied obese rats had highly significantly decreased serum Fe and transferrin saturation levels

and significantly increased ferritin, TIBC, and sTfR levels compared with the normal control group. Fe deficiency is considered one of the most prevalent nutritional disorders worldwide by the WHO. Fe deficiency may occur if Fe supplement is reduced to meet our average nutritional needs. Some medical studies have concluded that there is a negative relationship between Fe levels and BMI. However, the serum Fe concentration in the obese group was lower than that of the normal control group, which agreed with those in several other studies [39-41]. Lower serum Fe concentrations can be clarified by the fact that plasma lipid in obesity can increase the expression of the hepatic bactericidal protein, which can inhibit intestinal Fe uptake and macrophages in the release of hemoglobin Fe [42-45]. Since serum ferritin is an acute-phase reactant in addition to an inflammatory condition, it may prevent Fe mobilization from reticuloendothelial storage, showing Fe excess and transferrin saturation below 20%, indicating Fe insufficiency [46]. Surprisingly, fruit with husk GB extract, which has not been documented before, contains high levels of TPC and flavonoid content as kaempferol, a potent reactive scavenger, and provides promising results chelating effects for Fe overload. Meanwhile, they also interacted with Fe to enhance Fe excretion rates.

Despite appropriate dietary Fe consumption, obesity is associated with hypoferrremia, decreased Fe absorption, and reduced Fe storage in adults and children [47]. Hepcidin is a hormone that regulates body Fe homeostasis and is produced by the liver. Elevation of serum hepcidin inhibits ferroportin-mediated Fe export from macrophages and enterocytes in the duodenum, leading to hypoferrremia and reduced dietary Fe absorption. Total body fat predicts increased serum hepcidin in OW and obese subjects (OW/OB), which may disrupt Fe homeostasis and increase the risk of Fe deficiency [48].

As a response to increased Fe reserves in the body, hepcidin is produced to prevent the absorption of additional Fe. Hepcidin production is substantially boosted by inflammation or Fe excess [49]. It was discovered that with extreme obesity, HAMP expression increases in adipose tissue. This rise is attributed to the chronic and continuous inflammatory stimulation in the obese state [50-52]. The quantitative expression levels of hepcidin mRNA in adipose tissue and plasma hepcidin concentration were substantially improved ($P \leq 0.05$) in the groups treated with GB extract compared to the obese group. As a result, more Fe is transported to the plasma by the Fe transporter ferroportin, improving anemia, and preventing Fe overload in fatty tissues. This investigation was in line with several other studies [12,53,54]. The organism modulates intestinal Fe absorption, uptake, and mobilization from storage to meet the body's Fe needs by regulating hepcidin production [55,56].

Another significant conclusion of our research is that Hb oscillator strength is a unique biophysical parameter that represents the entire light absorption of an Hb electronic transition. It offers quantitative information on the heme group's electrical states. This parameter's substantial rise in obese rats verifies alterations in the heme electronic structure and emphasizes hemoglobin's macromolecular instability. Then, the instability could be related to the percentage of oxidized Hb and the degree of oxidation rather than oxygenation. This result is attributed to a disturbance in the levels of Fe^{2+} , Cu^{2+} , and Zn^{2+} observed in this study [57]. Furthermore, ferric compounds resulting from obesity are expected to be paramagnetic [58], and the presence of one or more of them tends to form higher values of oxyhemoglobin oscillation strength since the ferric atom in any form of chemical binding has an odd number of electrons. It seems to be related to methemoglobin percentage or sulfhemoglobin, that is, degree of

oxidation rather than oxygenation. Not surprisingly, the observed significant decrease in the oscillator strength values in rats undergoing GB extract at different doses (200 and 400 mg/kg b.w.) was attributed to the role of GB extract, which has the potential to improve oxidative homeostasis of some trace elements induced by obesity in adipose tissue. Moreover, GB contains a large amount of Vitamin C, which acts as a powerful antioxidant. GB also possesses a ferric reducing antioxidant power which is based on electron transfer reactions [59]. Thus, it converts methemoglobin to oxyhemoglobin, confirming the stability of hemoglobin as a folding process.

5. CONCLUSION

Ultimately, the Egyptian GB (*P. peruviana L.*) boasts a high quality and quantity of nutrients and probiotics, with a lot of potential as a functional food. GB feeding was an effective intervention in ameliorating Fe overload in adipose tissues, redox balance of trace elements in obese rats, and suppressing fat accumulation. Furthermore, there is an upward assessment of the role of GB as a natural Fe chelator. Moreover, it counteracts oxidative stress, improving blood hemoglobin spin state, which reflects on its function and recovers the conformational structure of hemoglobin and its function as a macromolecule.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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