

# The Effect of Psyllium Husk, Green Coffee, Orange Peel and their Mixture on Obesity and its Related Complications Induced by High Fat Diet in Rats

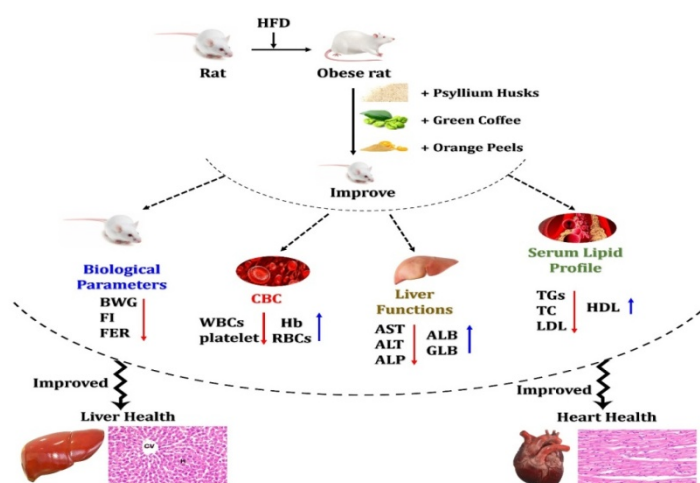
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**Abstract** The present study aims to investigate the effectiveness of psyllium husk, green coffee, orange peel and their mixture on obesity Induced by high fat diet (HFD) in rats. Rats (n= 72 rats), were divided into two main groups, the first group, normal control, (Group 1, 6 rats) still fed on basal diet (BD) and the other main group (66 rats) was used for obesity induction by feed with HFD for four weeks then classified into eleven equal sub groups as follow:. Group 2 (G2), model control, fed on BD only as a positive control (rats with obesity), groups (3-10), fed on BD containing 2.5 and 5.0% of psyllium husk, green coffee, orange peel and their mixture, and groups (11-12) fed on BD containing 1 and 2% of CHROMAX (one of the dietary supplements commonly used in obesity management) for comparative study. Rats feeding on HFD (model control) for 4 weeks leads to increase the BWG, FI and FER than the normal group by the rate of 161.7, 47.6 and 79.1%, respectively. Biochemical analysis data indicated that obesity induced a significant ( $p \leq 0.05$ ) decrease the hemoglobin (-34.4%) and RBCs (-46.7%), serum albumin (-63.9%) and globulin (-51.7%), increased liver functions enzymes activities (AST, 147%, ALT 163.2% and ALP, 107.6%), blood glucose (151.5%), serum lipid profile (TGs, 83.3%, TC, 75.9% and LDL-c , 200.1%) compared to normal controls. Intervention with selected plant parts in feeding rats protocol for 4 weeks led to significantly ( $p \leq 0.05$ ) improvement on the all biological and biochemical parameters of the obese rats by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts, followed by the groups that were fed on green coffee, orange peel and psyllium husks, respectively. Also, the feeding intervention with a mixture of plant parts was more effective in obesity treatment when compared to CHROMAX. In conclusion, the results of present study provide a basis for the use of selected plant parts for the prevention or early treatment of obesity and its related complications, but completing this important topic requires the necessity of conducting more studies and research in the future.



**Keywords:** body weight, liver functions, complete blood count, serum lipid profile, blood glucose, dietary supplement, histology

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## 1. Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems [1]. It is defined by body mass index (BMI) and further evaluated in terms of fat distribution via the waist-hip ratio and total cardiovascular risk factors. BMI is closely related to both percentage body fat and total body fat [2]. Obesity in adults and in children and adolescents has also gained epidemic proportions and has been associated with premature deaths in adults [3]. According to the International Obesity Taskforce, more than 300 million people who have a body mass index greater than 30 kg/m are categorized as obese. The number of obese-born children of developing countries is increasing, as is the number of obese adults in developed countries. One of three children born in the early current century is expected to develop obesity-related diabetes [4]. Such as mentioned in medical references, obesity is a complex disease commonly accompanied by insulin resistance, increased oxidative stress, and enhanced inflammatory marker expression [5,6]. Also, obesity is associated with the occurrence of low-level chronic inflammation, demonstrating a close link between metabolism and immunity. Fat cells known as adipocytes vigorously secrete a mixture of products that link obesity and diabetes [7,8]. Excessive body weight is associated with various diseases particularly cardiovascular diseases, diabetes mellitus type2, obstructive sleep apnea, certain types of cancer, neurological disorders, osteoarthritis and asthma [2,8,9,10,11,12]. Also, obesity, especially abdominal obesity, has an association with dyslipidemia characterized by increasing triglyceride and decreasing high-density lipoprotein cholesterol concentrations [13,14,15]. Furthermore, epidemiological studies revealed that obesity is identified as a risk factor for neurological disorders such as Alzheimer's disease [16,17]. For all of these previous reasons, researchers around the world have turned their attention to creating many pharmaceutical agents to prevent or treat obesity and its complications, but what was developed represented a small number of effective and safe products from a therapeutic standpoint [18]. In addition, most drug treatments are expensive and have many harmful effects, which lead to patient non-compliance. Hence, there was an urgent need to search for alternative treatments mainly from natural sources, because they are cost-effective and have few side effects. In this direction, many studies indicate that applying the use of plant parts to prevent or treat obesity is an effective and safe method with great economic advantages [19,20,21,22,23].

Psyllium (*Plantago ovata*) is an important medicinal plant cultivated in India, Pakistan, and Iran. It is derived from *Plantago ovata*. Psyllium is from the Latin “planta,” which means “sole of the foot,” and ovata relates to the form of the leaves [24]. The husks of the seeds are more commonly used, but the seeds themselves have also been administered. Psyllium is a mixture of polysaccharides: pentoses, hexoses, and uronic acids. Seed preparations contain approximately 47% soluble fiber by weight and

husk preparations generally consist of 67-71% soluble fiber and approximately 85% total fiber by weight [25]. Psyllium hull is a milling manufacturer good of seed and is approximately 10% to 25% of the weight of the dried seed. In the husk, more than 60% of hemicellulose and arabinoxylans that is water-soluble. The xylan backbone of arabinoxylans is related to arabinose, galacturonic and rhamnose acid units [26]. Soluble commonly (arabinoxylans), insoluble polysaccharide (cellulose, lignin and hemicellulose) as well as tannins, flavonoids and phenols are the parts of the entire psyllium crop, arabinoxylan, psyllium, and husk seeds [27]. The Psyllium husk is useful in allopathic and ayurvedic treatment. It is rich in arabinoxylans, and this component is used for treating certain gastrointestinal problems. Also, husk is used as an ingredient in foods to improve their fiber content, texture, and rheological and sensory characteristics. Soluble fibers in psyllium are related to bile acids withinside the small gut that shapes a compound that forestalls the re-absorption of bile through the small gut, thereby enhancing the manufacturing and secretion of bile acids to update the lacking acids [28]. Furthermore, psyllium husk also helps in lowering the cholesterol and increase the production of bile in this way lowers the cholesterol level in the human body which helps out in lowering the LDL-level and triglycerides [29,30]. Finally, psyllium husk has dietary fiber that aid in reducing glucose tolerance, insulin resistance and obesity as well as psyllium husk plant has specific flavonoids that prevent the formation of cancer cells [31,32].

Coffee is one of the most economically important agricultural commodities worldwide. The global coffee production is dominated by Arabica coffee (*Coffea arabica* L.) with a share of 64.5% [33]. The chemical composition of green coffee from different geographical origins is characterized by the presence of phenolic acids (chlorogenic acids and caffeic acid) and alkaloids (caffeine) [34]. Chlorogenic acids are esters of the trans-cinnamic acids whose amounts in green coffees are significantly reduced during the roasting process. Green coffee biochemical composition primarily depends on genetic variation and bean maturation. Moreover, most compounds related to the aroma of coffee are produced by partial destruction of the green bean during roasting through the process of pyrolysis, degradation and Maillard reaction. More than 950 compounds have been identified after roasting the amount and types of compounds reported being dependent on location and roasting degree [35]. Therefore, coffee shows the highest antioxidant activity (AA) and has been reported to be the major and natural source of antioxidants [36]. It has 0.15–0.30 mg/g total antioxidant content. Antioxidants are essential for a number of bioactivities in human body. With this context, phenolic compounds could antioxidant and anti-aging and able to neutralize the effects of oxidative damage to the skin. Many researchers reported that coffee extracts have revealed a set of important biological activities including antibacterial, antiviral, anti-inflammatory antioxidant and free radical scavenging [37]. Also, chlorogenic acids are effective in many biological activities within the body, where they have an anti-dyslipidemic, anti-hyperglycemic effects with anti-obesity properties [38]. It is believed that they improve fat and glucose metabolism and thus

improving insulin sensitivity through several mechanisms at the level of gene expression [39].

Citrus (*Citrus* spp., Family: *Rutaceae*) is an economically important fruit all over the world including Egypt. However, its peels generated by the juice industry are one of the major sources of agricultural wastes. Natural products present in citrus peels e.g. dietary fibers, flavonoids, carotenoids, folic acid, vitamin C, pectin and essential oils present are very useful for food industry and human health. Also, citrus peels are good source of phenolic compounds can be extracted and employed as natural antioxidants to prevent oxidation of some foods or may be utilized in designing functional foods [40]. Polymethoxyflavones (PMFs), the major components of orange peel, have been found to have health benefits, including anti-inflammatory, anti-carcinogenic, anti-viral, anti-oxidant, anti-thrombogenic and anti-atherogenic properties [41]. Also, the major phenolic compounds present in the orange peel include hydroxycinnamic acids (HCA) and flavonoids, among which flavanones are the most prevalent [42]. Furthermore, citrus flavonoids, especially hesperidin, have a wide range of therapeutic properties, including anti-inflammatory, anti-hypertensive, diuretic, analgesic, and hypolipidemic activities [43]. Previous studies have demonstrated the effects of these flavonoids on lipid and glucose metabolism in experimental animals and humans, specifically on lipid catabolism, glucose transport, the insulin-receptor function, and peroxisome proliferator-activated receptors (PPARs) activation, all of which play essential roles in weight control [44,45]. Finally, orange peel has complexes such as catechol, dimethoxy phenol, cyclohexane, coumarin, acetic acid, stigmasterol, sitosterol and vitamin E which are accountable for its antioxidant feature [46]. Thus, all the previous studies with the other reported that the peel of the orange fruit contains a higher concentration of antioxidant substances than the flesh of the fruit [43]. For the all previous reasons, the present study aims to investigate the potential effects of using different plant parts (psyllium husks, green coffee, and orange peels) as diet supplement on obesity and its complications in rats.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Plant Parts

Dried plant parts including psyllium (*Plantago ovata*) husk, Green coffee (*Coffea arabica* L.) and Orange (*Citrus* spp.) peel were purchased by Agriculture Seeds, Spices, and Medical Plant Company (Harraz), El-Darb El-Ahmar, Cairo Governorate, Egypt. Samples were validated in the Department of Agricultural Plant, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. The collected samples was transported to the laboratory and used immediately for plant parts powders preparation.

#### 2.1.2. Chemicals, Solvents, Buffers and Dietary Supplement

All chemicals, solvents and buffers (except what is

mentioned in the place) were purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Dietary supplement (CHROMAX) was purchased from local drug store, Shebin El-Kom, Egypt.

#### 2.1.3. Kits

Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA), serum lipids profile (triglycerides, TGs; total cholesterol, TC; high density lipoprotein cholesterol, HDL-c), serum glucose and insulin level were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Vitamins and salts mixtures in food grade, organic solvents and other chemicals in analytical grade were purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

#### 2.1.4. Machines

Throughout this investigation absorbance for different assays were determined using UV-160A; Shimadzu Corporation, Kyoto, Japan.

## 2.2. Methods

### 2.2.1. Preparation of Plant Parts Powder

Dried plant parts including psyllium (*Plantago ovata*) husk, green coffee (*Coffea arabica* L.) and orange (*Citrus* spp.) peel were cleaned/separated manually to remove the foreign bodies. The cleaned plant parts were dried at 60 °C for one h in hot air oven (AFOS Mini Smoker, England). This is followed by milling in high miller speed (Moulinex Egypt, Al-Araby Co., Egypt) to produce the respective powder. The material that passed through an 80 mesh sieve was retained for use.

### 2.2.2. Biological Experiments

#### 2.2.2.1. Animals

Animals used in this study, adult male albino rats (140±8 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

#### 2.2.2.2. Experimental Diets

The basic diet (BD) prepared according to the following formula as mentioned by Reeve *et al.*, [47] as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The high fat diet (HFD) [diet induced obesity (DIO)] prepared according to Research Diets, Inc. NJ, as follow: casein, 80 mesh (23.3%), L-cystine (0.35%), corn starch (8.48%), maltodextrin (11.65%), sucrose (20.14%), soybean oil (2.91%), lard fat (substituted by lamb fat) (20.69%), mineral mixture (1.17%), dicalcium phosphate (1.52%), calcium carbonate (0.64%), potassium citrate.1 H<sub>2</sub>O (1.92%), vitamin mixture (1.17%), choline bitartrate (0.23%). The used vitamin and minerals

mixtures component were formulated according to Reeves *et al.*, [47]

#### 2.2.2.3. Experimental Design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council [48]. Rats (n= 72 rats), were housed individually in wire cages in a room maintained at  $25 \pm 4$  °C and kept under normal healthy conditions. All rats were fed on BD for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group, normal control, (Group 1, 6 rats) still fed on BD and the other main group (66 rats) was used for obesity induction by feed with diet-induced obesity (DIO) for four weeks then classified into eleven equal sub groups as follow: Group 2 (G2), model control, fed on BD only as a positive control (rats with obesity), groups 3 and 4 (G3 and G4) fed BD containing 2.5 and 5% of psyllium husk powder, respectively, groups 5 and 6 (G5 and G6) fed containing 2.5 and 5% of orange peel powder, respectively, groups 7 and 8 (G7 and 8) fed BD containing 2.5 and 5% of green coffee powder, respectively, , groups 9 and 10 (G9 and G10) fed BD containing 2.5 and 5% of Mix powder (mixture of psyllium husk, orange peel and green coffee powders by equal parts), respectively, and groups 11 and 12 (G11 and G12) fed BD containing 2.5 and 5% of selected dietary supplement (CHROMAX) powder, respectively.

#### 2.2.2.4. Biological Evaluation

The diet consumed was recorded every day and body weight was recorded every week during the experimental period (4 weeks days). The body weight gain (BWG, %), food intake (FI), and food efficiency ratio (FER) were determined according to Chapman *et al.*, [49] using the following equations: BWG (%) = (Final weight – Initial weight)/ Initial weight $\times$ 100 and FER = Grams gain in body weight (g/28 days)/ Grams feed intake (g/28 days).

#### 2.2.2.5. Blood Sampling

At the end of experiment period, 4 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, [50]. Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20 °C until analysis. Also, internal organs were taken immediately after sacrificing rats, weighted and liver and heart immersed in 10% neutral buffered formalin for histopathological examination.

#### 2.2.2.6. Hematological analysis

##### Liver functions

Liver functions were determination using specific methods as follow: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities according to Yound, [51], Tietz, [52] and Yound, [51], respectively.

#### Blood lipids profile

Triglycerides (TG), Total cholesterol (TC) and HDL-Cholesterol were determined in serum using specific kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were assayed according to the equations of Fmiedewald *et al.*, [53] as follow: Very low density lipoprotein (VLDL cholesterol) = TG/5 and LDL cholesterol = Total cholesterol – HDL cholesterol – V LDL cholesterol

#### Serum glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to Yound, [51]

#### Blood proteins

Blood proteins (albumin and globulin) were determined by a colorimetric method described by Vanessa *et al.*, [54].

#### Complete blood count (CBC)

CBC including [hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and platelets] were performed by automatic measurement using an Avantor Performance Materials Inc. Business, Center Valley, USA (H32 VET 3-Part differential analyzer of hematology).

#### Histopathological Examination

Specimens of the internal organ (liver and heart tissues) were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned (4-6  $\mu$ m thickness), stained with hematoxylin and eosin and examined microscopically [55].

#### 2.2.3. Statistical Analysis

All data were statistically analyzed using a computerized Costat program by one way ANOVA. Results were given as means  $\pm$  standard deviation (SD). Differences between treatments at  $P \leq 0.05$  were considered significant [56].

## 3. Results and Discussion

### 3.1. Effect of a Dietary Intervention with Psyllium Husks, Green Coffee, Orange Peels Powder and Their Mixture on Body Weight(BW), Feed Intake (FI) and Feed Efficiency Ratio (FER) of Obese Rats

Data in Table 1 and Figure 1 were shown the effect of a dietary intervention with psyllium husks, green coffee, orange peels powder and their mixture on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of obese rats (as a percent of change). Such data indicated that feeding of rats on HFD (model control) for 4 weeks leads to increase the BWG, FI and FER than the normal group by the rate of 161.7, 47.6 and 79.1%, respectively. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to



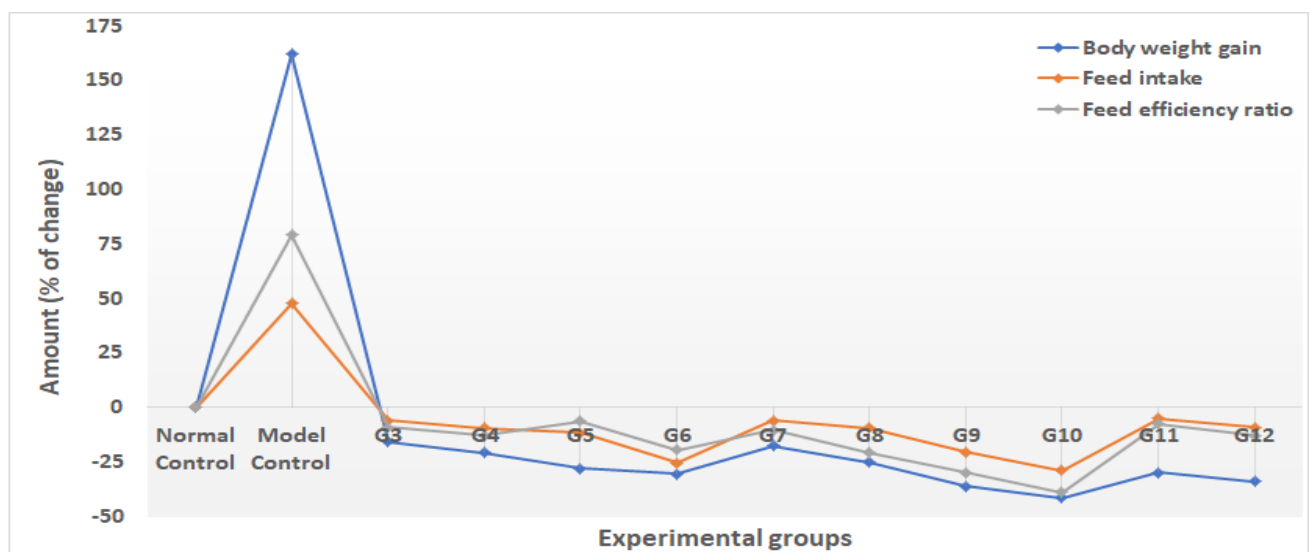
significantly ( $p \leq 0.05$ ) decrease on the BWG, FI and FER of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts, followed by the groups that were fed on green coffee, orange peel and psyllium husks, respectively. Also, the feeding intervention with a mixture of plant parts was more effective in reducing BWG, FI, and ER when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in BWG, FI and FER of the obese rats as the result of plant parts intervention were exhibited a dose-dependent manner. The current data agree well with several studies conducted on the same plant parts tested in this study, but separately for each e.g. psyllium husks [57,58,59,60,61], green coffee [62,63,64,65,66] and orange peel [67,68,69,70]. Such study reported that the tested plant parts helps in boosting the immune system and thus redirecting the energy back on the weight loss process. They also help are full of antioxidants that are

essential for weight loss and overall well-being. The natural antioxidants/bioactive compounds found in the tested plant parts include polyphenols, phenolic acids, alkaloids, flavonoids, terpenoids, anthocyanins, carotenoids, polysaccharides, quercetin, terpenes, pectin, dietary fiber and essential oils [71,72,73,74,75,76]. Several previous studies indicated that all of these bioactive compounds exhibited various biological activities including antioxidant and scavenging activities, inhibition the lipid oxidation, and anti-inflammatory and anti-obesity effects [8,12,14,77]. All of the previous studies with the others indicated that the possible mode of action of weight-lowering activity (anti-obesity) effects of the tested plant parts could be explained by one or more of the following mechanisms such as antioxidant, anti-inflammatory, reduced fat accumulation, increased lipolysis, decreased leptin and resistin levels, increased adiponectin, inhibited differentiation of adipocytes, suppressed the preadipocyte proliferation and reduced adipogenesis [66] [78-85].

**Table 1. Effect of psyllium husks, green coffee, orange peels powder and their mixture on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of obese rats**

| Groups                         | BWG (%)                  | FI (g/day)              | FER (%)                  |
|--------------------------------|--------------------------|-------------------------|--------------------------|
| G1: Control (-ve) (Normal)     | 21.10±0.21 <sup>g</sup>  | 17.45±1.22 <sup>d</sup> | 0.043±0.002 <sup>g</sup> |
| G2: Control (+ve)              | 55.21±0.12 <sup>a</sup>  | 25.75±1.20 <sup>a</sup> | 0.077±0.004 <sup>a</sup> |
| G3: Psyllium husks (2.5%)      | 46.40±0.10 <sup>b</sup>  | 24.18±1.15 <sup>a</sup> | 0.070±0.001 <sup>b</sup> |
| G4: Psyllium husks (5.0%)      | 43.65±0.40 <sup>c</sup>  | 23.27±1.35 <sup>b</sup> | 0.067±0.002 <sup>c</sup> |
| G5: Green coffee (2.5%)        | 39.62±0.23 <sup>d</sup>  | 22.70±1.14 <sup>b</sup> | 0.072±0.005 <sup>b</sup> |
| G6: Green coffee (5.0%)        | 38.35±0.30 <sup>d</sup>  | 19.15±1.30 <sup>c</sup> | 0.062±0.003 <sup>d</sup> |
| G7: Orange peels (2.5%)        | 45.37±0.10 <sup>b</sup>  | 24.15±1.20 <sup>a</sup> | 0.069±0.003 <sup>c</sup> |
| G8: Orange peels (5.0%)        | 41.25±0.42 <sup>cd</sup> | 23.28±1.23 <sup>b</sup> | 0.061±0.004 <sup>d</sup> |
| G9: Mixture powder (2.5%)      | 35.17±0.10 <sup>e</sup>  | 20.50±1.14 <sup>c</sup> | 0.054±0.004 <sup>e</sup> |
| G10: Mixture powder (5.0%)     | 32.20±0.42 <sup>f</sup>  | 18.27±1.30 <sup>d</sup> | 0.047±0.005 <sup>f</sup> |
| G11: Dietary supplement (1.0%) | 38.60±0.23 <sup>d</sup>  | 24.42±1.20 <sup>a</sup> | 0.071±0.006 <sup>b</sup> |
| G12: Dietary supplement (2.0%) | 36.33±0.30 <sup>e</sup>  | 23.35±1.23 <sup>b</sup> | 0.067±0.005 <sup>c</sup> |
| LSD ( $P \leq 0.05$ )          | 2.460                    | 1.752                   | 0.0024                   |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ )



**Figure 1.** Effect of a dietary intervention with psyllium husks, green coffee, orange peels powder and their mixture on body weight gain, feed intake and feed efficiency ratio of obese rats (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

### 3.2. Effect of Psyllium Husks, Green Coffee, Orange Peels Powder and Their Mixture on Internal Organs Weight of Obese Rats

Data in Table 2 and Figure 2 were shown the effect of a dietary intervention with psyllium husks, green coffee, orange peels powder and their mixture on internal organs weight of obese rats (as a percent of change). Such data indicated that feeding of rats on HFD (model control) for 8 weeks leads to increase the weight of liver, kidney and heart than the normal group by the rate of 44, 97.5 and 33.3%, respectively. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) decrease the weight of liver, kidney and heart of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts and green coffee, followed by the

groups that were fed on orange peel and psyllium husks, respectively. Also, the nutritional intervention with a mixture of plant parts and green coffee were more effective in reducing the organ weight when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in organ weight of the obese rats as the result of plant parts intervention was exhibited a dose- dependent manner. The same data was observed by Boraey, [8] and Elhassanneen *et al.*, [12] who found that a dietary intervention with Ashwagandha root powder which have some of bioactive compound found in the tested plant parts decreased the organs (liver, kidney, heart, brain, spleen and lungs) weight of obese rats. Thus, such behavior could be attributed to the biological activities of their bioactive compound content. Also, in population studies, Mandal *et al.*, [86] demonstrated that in both men and women, there were statistically significant increases in organ weights with body mass index (BMI).

Table 2. Effect of Psyllium husks, green coffee, orange peels powder and their mixture on internal organs weight of obese rats

| Groups                         | Liver (g)              | Kidney (g)             | Heart (g)              |
|--------------------------------|------------------------|------------------------|------------------------|
| G1: Control (-ve) (Normal)     | 6.53±1.22 <sup>c</sup> | 1.21±0.25 <sup>c</sup> | 0.72±0.05 <sup>b</sup> |
| G2: Control (+ve)              | 9.43±1.37 <sup>a</sup> | 2.39±0.38 <sup>a</sup> | 0.96±0.16 <sup>a</sup> |
| G3: Psyllium husks (2.5%)      | 8.98±1.35 <sup>a</sup> | 1.89±0.32 <sup>a</sup> | 0.88±0.13 <sup>a</sup> |
| G4: Psyllium husks (5.0%)      | 8.28±1.34 <sup>b</sup> | 1.56±0.30 <sup>b</sup> | 0.84±0.10 <sup>a</sup> |
| G5: Green coffee (2.5%)        | 7.54±1.30 <sup>b</sup> | 1.80±0.36 <sup>b</sup> | 0.87±0.11 <sup>a</sup> |
| G6: Green coffee (5.0%)        | 6.35±0.76 <sup>c</sup> | 1.35±0.20 <sup>b</sup> | 0.80±0.10 <sup>a</sup> |
| G7: Orange peels (2.5%)        | 8.70±1.32 <sup>a</sup> | 1.90±0.37 <sup>a</sup> | 0.89±0.13 <sup>a</sup> |
| G8: Orange peels (5.0%)        | 8.30±1.34 <sup>b</sup> | 1.50±0.36 <sup>b</sup> | 0.85±0.10 <sup>a</sup> |
| G9: Mixture powder (2.5%)      | 7.10±0.87 <sup>c</sup> | 1.60±0.33 <sup>b</sup> | 0.79±0.05 <sup>a</sup> |
| G10: Mixture powder (5.0%)     | 6.89±0.59 <sup>c</sup> | 1.30±0.24 <sup>c</sup> | 0.75±0.04 <sup>b</sup> |
| G11: Dietary supplement (1.0%) | 8.97±1.35 <sup>a</sup> | 1.93±0.42 <sup>a</sup> | 0.90±0.11 <sup>a</sup> |
| G12: Dietary supplement (2.0%) | 8.14±1.30 <sup>b</sup> | 1.84±0.38 <sup>b</sup> | 0.85±0.09 <sup>a</sup> |
| LSD ( $P \leq 0.05$ )          | 0.973                  | 0.537                  | 0.203                  |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ )

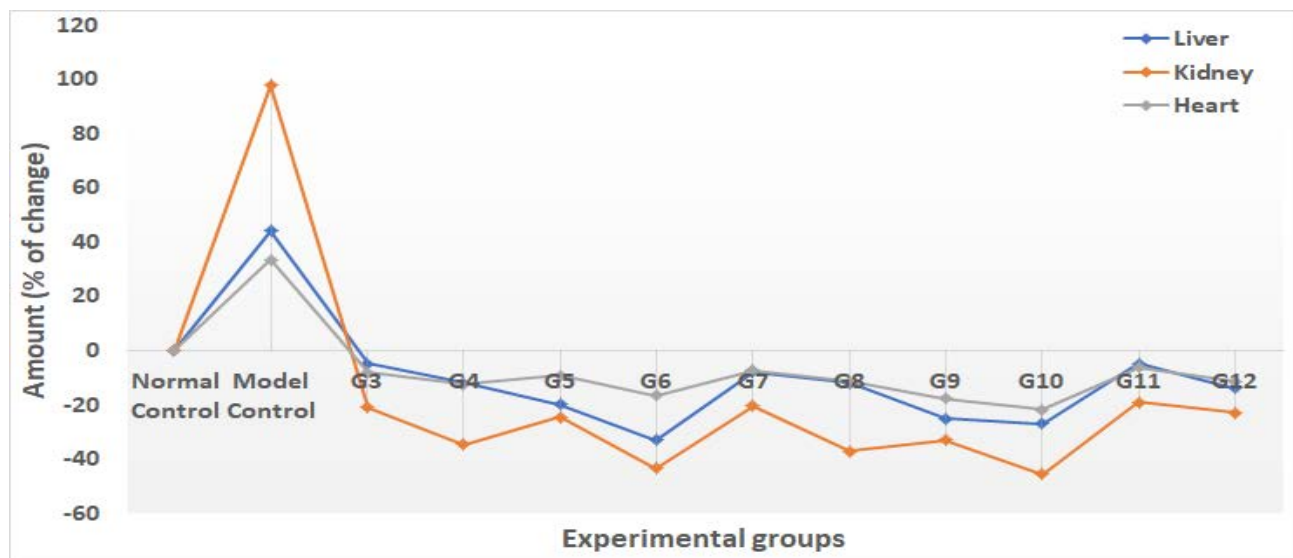


Figure 2. Effect of Psyllium husks, green coffee, orange peels powder and their mixture on internal organs weight of obese rats (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

### 3.3. Effect of Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Blood Picture of Obese

Effect of psyllium husks, green coffee, orange peels powder and their mixture on hematological data of obese rats was shown in Table 3 and Figure 3. Such data indicated that feeding of rats on HFD (model control) for 8 weeks leads to decrease the hemoglobin and RBCs than the normal group by the rate of -34.4 and -46.7%, respectively. The opposite direction was observed for platelet and WBCs. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) increase the hemoglobin and RBCs and decrease the platelet and WBCs of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts and green coffee followed by the groups that were fed on orange peel and psyllium husks, respectively. Also, the nutritional intervention with a mixture of plant parts and green coffee were more effective in improving the CBC when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in RBCs and Hb and increasing in WBCs and platelets of the obese rats as the result of plant parts intervention were exhibited a dose- dependent manner.

The present research data confirmed that obesity has adverse effects on all tested hematological parameters, with characteristic decrease in RBCs and hemoglobin level. With this line, Kelsey *et al.*, [87] found that blood donor obesity is associated with changes in RBCs metabolism and susceptibility to hemolysis in cold storage and in response to osmotic and oxidative stress. Obesity may alter RBC metabolism and susceptibility to hemolysis via diverse pathways. For example, RBCs from obese subjects with metabolic syndrome exhibited 25% reduction in deformability and increased membrane rigidity as compared with RBCs from nonobese subjects. Also, according to Ballinger [88], depletion in erythrocytes count and Hb level leads to iron-deficiency anemia which is characterized by a microcytic hypochromic blood picture, also hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation.

On another side, obesity leads to increase in both WBCs and platelets. With this direction, Yeging *et al.*, [89] reported that obese people with elevated WBC count, a marker of subclinical inflammation, are at increased risk of developing type 2 diabetes. Also, Herishanu *et al.*, [90] found that fat tissue produces and releases inflammatory cytokines, and that obesity may be regarded as a state of

low-grade inflammation. In this regard, an association between obesity and persistent leukocytosis was established. For platelet count, Vauclard *et al.*, [91] reported that platelet activation is enhanced during obesity increasing the thrombosis risk. Thus, it is well known that obesity is associated with increased thrombosis risk due to platelet activation. Data of the present study showed that the selected plant parts caused an improvement in the all tested hematological parameters to a nearly normalized value. Such protective effect may be explained through several mechanisms since the tested plant parts are complex mixture of several nutrients (vitamins and minerals) and phytochemicals (polyphenols, phenolic acids, alkaloids, flavonoids, terpenoids, anthocyanins, carotenoids, polysaccharides, quercetin, terpenes, pectin, dietary fiber and essential oils) [71,72,73,74,75,76]. For example, vitamins C and E are strong antioxidants which play an important role in detoxification process through removing the free radicals, protecting DNA from oxidative damage and reducing micronucleus frequencies in polychromatic erythrocytes of bone marrow [92,93]. Phytochemicals detected in the tested plant parts exhibited several biological effects including antioxidant and scavenging activities and inhibiting the lipid peroxidation's [8,15,21,94,95,96].

### 3.4. Effect of Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Liver Functions of Obese Rat

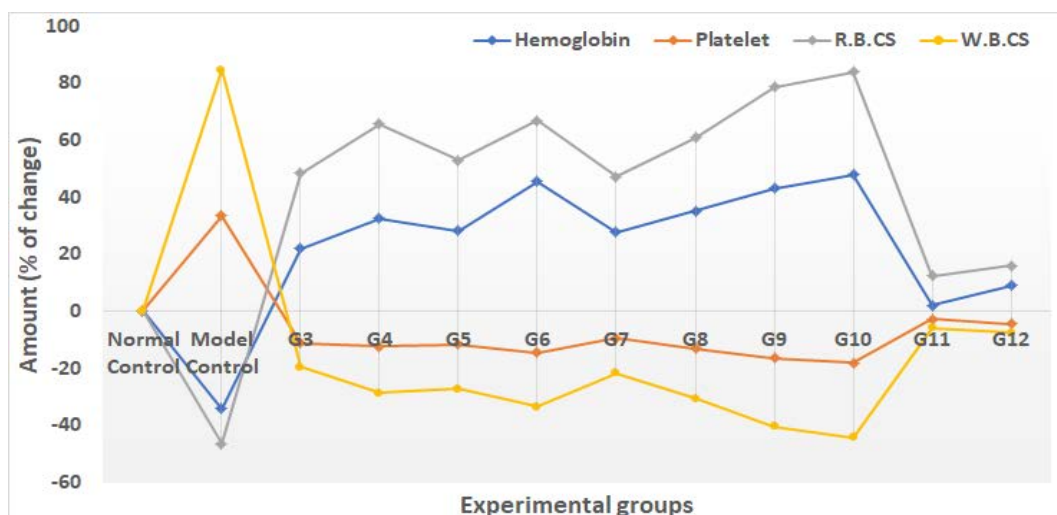
Data in Table 4 and Figure 4 showed the effect of Psyllium husks, green coffee, orange peels powder and their mixture on liver functions of obese rat. From such data it could be noticed that obesity induced a significant increased ( $p \leq 0.05$ ) in AST (147%), ALT (163.2%) and ALP (107.6%) compared to normal group. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) decrease on the AST, ALT and ALP of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts, followed by the groups that were fed on green coffee, psyllium husks and orange peel, respectively. Also, the feeding intervention with the all tested plant parts and their mixture was more effective in reducing AST, ALT and ALP when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in AST, ALT and ALP of the obese rats as the result of plant parts intervention were exhibited a dose- dependent manner.

**Table 3. Effect of psyllium husks, green coffee, orange peels powder and their mixture on Blood picture of obese rats**

| Groups                     | Hemoglobin (g/dl)               | Platelet ( $10^6/\text{mm}^3$ ) | R.B.CS ( $10^3/\text{mm}^3$ ) | W.B.CS ( $10^3/\text{mm}^3$ ) |
|----------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|
| G1: Control (-ve) (Normal) | 16.54 $\pm$ 1.62 <sup>a</sup>   | 180.0 $\pm$ 4.10 <sup>f</sup>   | 4.95 $\pm$ 0.37 <sup>a</sup>  | 5.56 $\pm$ 0.30 <sup>d</sup>  |
| G2: Control (+ve)          | 10.85 $\pm$ 1.25 <sup>c</sup>   | 240.0 $\pm$ 6.45 <sup>a</sup>   | 2.64 $\pm$ 0.28 <sup>c</sup>  | 10.25 $\pm$ 0.75 <sup>a</sup> |
| G3: Psyllium husks (2.5%)  | 13.20 $\pm$ 1.35 <sup>b,c</sup> | 213.0 $\pm$ 4.80 <sup>c</sup>   | 3.91 $\pm$ 0.36 <sup>b</sup>  | 8.23 $\pm$ 0.60 <sup>b</sup>  |
| G4: Psyllium husks (5.0%)  | 14.37 $\pm$ 1.45 <sup>b</sup>   | 210.0 $\pm$ 4.20 <sup>d</sup>   | 4.37 $\pm$ 0.41 <sup>a</sup>  | 7.30 $\pm$ 0.47 <sup>b</sup>  |
| G5: Green coffee (2.5%)    | 13.90 $\pm$ 1.37 <sup>b</sup>   | 212.0 $\pm$ 4.21 <sup>c</sup>   | 4.03 $\pm$ 0.44 <sup>a</sup>  | 7.45 $\pm$ 0.42 <sup>b</sup>  |
| G 6: Green coffee (5.0%)   | 15.75 $\pm$ 1.50 <sup>a</sup>   | 205.0 $\pm$ 5.22 <sup>d</sup>   | 4.40 $\pm$ 0.35 <sup>a</sup>  | 6.80 $\pm$ 0.35 <sup>c</sup>  |

| Groups                         | Hemoglobin<br>(g/dl)          | Platelet<br>( $10^9/\text{mm}^3$ ) | R.B.CS<br>( $10^3/\text{mm}^3$ ) | W.B.CS<br>( $10^3/\text{mm}^3$ ) |
|--------------------------------|-------------------------------|------------------------------------|----------------------------------|----------------------------------|
| G7: Orange peels (2.5%)        | 13.85 $\pm$ 1.53 <sup>b</sup> | 217.0 $\pm$ 5.23 <sup>c</sup>      | 3.88 $\pm$ 0.32 <sup>b</sup>     | 8.01 $\pm$ 0.64 <sup>b</sup>     |
| G8: Orange peels (5.0%)        | 14.65 $\pm$ 1.57 <sup>b</sup> | 208.0 $\pm$ 5.10 <sup>d</sup>      | 4.24 $\pm$ 0.30 <sup>a</sup>     | 7.10 $\pm$ 0.51 <sup>c</sup>     |
| G9: Mixture powder (2.5%)      | 15.50 $\pm$ 1.55 <sup>a</sup> | 200.0 $\pm$ 4.52 <sup>e</sup>      | 4.71 $\pm$ 0.40 <sup>a</sup>     | 6.10 $\pm$ 0.45 <sup>cd</sup>    |
| G10: Mixture powder (5.0%)     | 16.01 $\pm$ 1.61 <sup>a</sup> | 196.0 $\pm$ 4.25 <sup>e</sup>      | 4.85 $\pm$ 0.42 <sup>a</sup>     | 5.70 $\pm$ 0.53 <sup>d</sup>     |
| G11: Dietary supplement (1.0%) | 11.04 $\pm$ 1.40 <sup>c</sup> | 233.0 $\pm$ 5.40 <sup>b</sup>      | 2.96 $\pm$ 0.27 <sup>b</sup>     | 9.64 $\pm$ 0.65 <sup>a</sup>     |
| G12: Dietary supplement (2.0%) | 11.80 $\pm$ 1.43 <sup>c</sup> | 229.0 $\pm$ 5.30 <sup>b</sup>      | 3.06 $\pm$ 0.28 <sup>b</sup>     | 9.45 $\pm$ 0.60 <sup>ab</sup>    |
| LSD ( $P \leq 0.05$ )          | 1.205                         | 5.170                              | 1.031                            | 1.055                            |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ )



**Figure 3.** Effect of psyllium husks, green coffee, orange peels powder and their mixture on CBC of obese rats (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

In general, aminotransferases, ALS and ALT, are normally intracellular enzymes and elevated levels in plasma indicates damage to cells rich in these enzymes. These enzymes are elevated in nearly all liver diseases, but are particularly high in conditions that the causes extensive cell necrosis, such as severe viral hepatitis and prolonged circulatory collapse i.e. liver damage [97-102]. Additionally, aminotransferases may be elevated in non-hepatic disease, such as myocardial infraction, muscle disorders [103]. On the other side, Alkaline phosphatase (ALP) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline pH to give pi and the corresponding alcohol, phenol or sugar. It is found in different tissues and the highest values are found in liver, biliary tract, epithelium, bone, intestinal mucosa and placenta [97,98,104]. Elevated serum and leukocytic ALP leaves in patients with Hodgkin's and non-Hodgkin's lymphoma, hairy cell leukemia [105,106]. Data of the present study with the other reported that aminotransferases (AST and ALT) and ALP may be elevated significantly ( $p \leq 0.05$ ) in additionally non-hepatic disease such as obesity in both human and experimental animals [107,108,104, 20]. Several previous studies found that the selected plant parts, and their mixture are a rich source of different classes of phytochemicals such polyphenols, phenolic acids, alkaloids, flavonoids, terpenoids, anthocyanins, carotenoids, polysaccharides, quercetin, terpenes, pectin, dietary fiber and essential oils [71,72,73,74,75,76]. Several studies reported that the effect of the plant parts on decreasing the serum liver function enzymes activity and improve the liver disorders

could be attributed to their high level content of these bioactive compounds and their biological activities. For example, psyllium husks phytochemicals was reported to protect liver cell and improve the fatty liver [60,109,110]. Also, green coffee bioactive compounds were effective to avoid cirrhosis of the liver [111,112,113]. Furthermore, bioactive constituents of orange peel disrupts the formation of liver cancer, hepatocarcinogenesis [114]. Taking into account all these biological effects, the higher improvement in liver function indicators recorded in rats fed with the mixture of the tested plant parts can be attributed to the simultaneous accumulation effects resulting from their content of different phytochemical classes. In this context, several studies have confirmed that the presence of biologically active compounds, such as those found in the plant parts that were tested, have effective roles in protecting the liver from various damages that may affect it. For example, Ho and Chang, [115] recorded that phenolic compounds and anthocyanins and their antioxidative capacities contributes greatly to protecting the liver from harmful effects. Also, Ragaa *et al.*, [116] reported that several natural compounds that have the antioxidant potent such as phenolic compounds and pigments that might lower oxidative stress and hepatic protective. Furthermore, alkaloids, carotenoids, terpenoids, flavonoids and polysaccharides have recorded high degrees of protection against the harmful effects caused by various xenobiotics in the liver [12] [100-120]. Data of the present study with the others indicated that the possible mode of action of liver serum enzymes-lowering activity of the tested plant parts could be explained by one or more



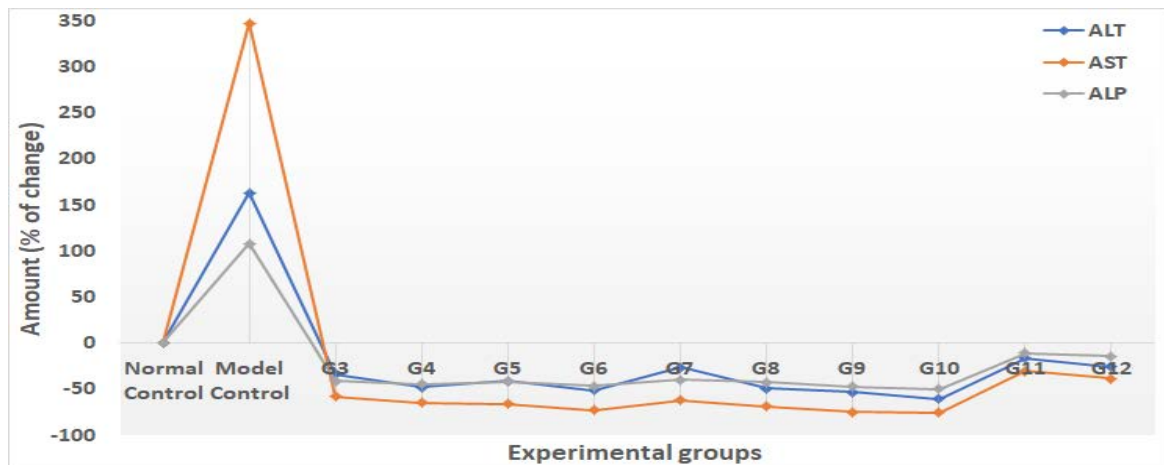
of the following mechanisms including antioxidant and scavenging activities against the ROS [121,122,123,124], Boost the immune system [125], inhibition the lipid oxidation [96,126,127], anti-inflammatory [15],

improvement of antioxidant defense system in red blood cells [5] and block the hepatocellular uptake of bile acids, diminished the bilirubin concentration [97,128,129,130]

**Table 4. Effect of psyllium husks, green coffee, orange peels powder and their mixture on liver functions of obese rat**

| Groups                         | ALT (U/L)                | AST (U/L)                | ALP (U/L)                 |
|--------------------------------|--------------------------|--------------------------|---------------------------|
| G1: Control (-ve) (Normal)     | 9.80±1.28 <sup>f</sup>   | 15.06±1.13 <sup>gh</sup> | 90.70±2.00 <sup>h</sup>   |
| G2: Control (+ve)              | 25.79±1.75 <sup>a</sup>  | 67.26±2.46 <sup>a</sup>  | 188.30±3.80 <sup>a</sup>  |
| G3: Psyllium husks (2.5%)      | 17.03±1.43 <sup>c</sup>  | 28.02±1.95 <sup>d</sup>  | 110.50±2.90 <sup>d</sup>  |
| G4: Psyllium husks (5.0%)      | 13.40±1.39 <sup>e</sup>  | 23.80±1.74 <sup>e</sup>  | 103.60±2.72 <sup>e</sup>  |
| G5: Green coffee (2.5%)        | 15.06±1.40 <sup>d</sup>  | 23.20±1.70 <sup>ef</sup> | 108.70±3.0 <sup>de</sup>  |
| G 6: Green coffee (5.0%)       | 12.55±1.33 <sup>e</sup>  | 18.08±1.82 <sup>g</sup>  | 100.80±2.88 <sup>e</sup>  |
| G7: Orange peels (2.5%)        | 18.90±1.41 <sup>c</sup>  | 25.40±1.93 <sup>e</sup>  | 113.07±3.10 <sup>d</sup>  |
| G8: Orange peels (5.0%)        | 13.00±1.38 <sup>e</sup>  | 20.70±1.42 <sup>f</sup>  | 107.90±2.65 <sup>de</sup> |
| G9: Mixture powder (2.5%)      | 11.98±1.29 <sup>e</sup>  | 17.01±1.64 <sup>g</sup>  | 99.06±2.31 <sup>f</sup>   |
| G10: Mixture powder (5.0%)     | 10.04±1.24 <sup>ef</sup> | 15.90±1.48 <sup>g</sup>  | 94.21±2.10 <sup>g</sup>   |
| G11: Dietary supplement (1.0%) | 21.45±1.65 <sup>b</sup>  | 46.09±2.85 <sup>b</sup>  | 167.80±3.63 <sup>b</sup>  |
| G12: Dietary supplement (2.0%) | 19.09±1.51 <sup>c</sup>  | 41.29±2.69 <sup>c</sup>  | 160.60±3.41 <sup>c</sup>  |
| LSD (P≤0.05)                   | 1.571                    | 2.100                    | 3.282                     |

Each value represents mean ± SD (n = 5). Mean under the same column with different superscript letters are different significantly (P≤ 0.05)



**Figure 4.** Effect of psyllium husks, green coffee, orange peels powder and their mixture on liver functions of obese rat (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2).

### 3.5. Effect of Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Glucose Levels of Obese Rat

Effect of psyllium husks, green coffee, orange peels powder and their mixture on glucose levels of obese rat was shown in Table 5 and Figure 5. From such data it could be noticed that obesity induced a significant increase ( $p \leq 0.05$ ) in serum glucose (151.5%) compared to normal controls. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) decrease on the glucose level of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts, followed by the groups that were fed on green coffee, psyllium husks and orange peel, respectively. Also, the feeding intervention with the all tested plant parts and their mixture was more effective in reducing blood

glucose when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in blood glucose of the obese rats as the result of plant parts intervention were exhibited a dose- dependent manner.

Data of the present study with the other reported that serum glucose may be elevated significantly ( $p \leq 0.05$ ) in additionally induced diabetic disease such as obesity in both human and experimental animals [8,20,104,107,108]. Several previous studies found that the selected plant parts, and their mixture are a rich source of different categories of phytochemicals including polyphenols, phenolic acids, alkaloids, flavonoids, terpenoids, anthocyanins, carotenoids, polysaccharides, quercetin, terpenes, pectin, dietary fiber and essential oils [71,72,73,74,75,76]. Several studies reported that the effect of the plant parts on decreasing the blood glucose and improve the obesity complications (hyperglycemia) could be attributed to their high level content of these bioactive compounds and their biologically activities. For example, psyllium husks phytochemicals was reported to control the blood glucose

(Antidiabetic) [58,74,110]. Also, green coffee bioactive compounds were effective in modulation of the postprandial glucose metabolism [111,113]. Furthermore, bioactive constituents of orange peel ameliorate the hyperglycemia and decrease glycosylation [69,70,131]. Taking into account all these biological effects, the higher improvement in blood glucose recorded in rats fed with the mixture of the tested plant parts can be attributed to the simultaneous accumulation effects resulting from their content of different phytochemical classes.

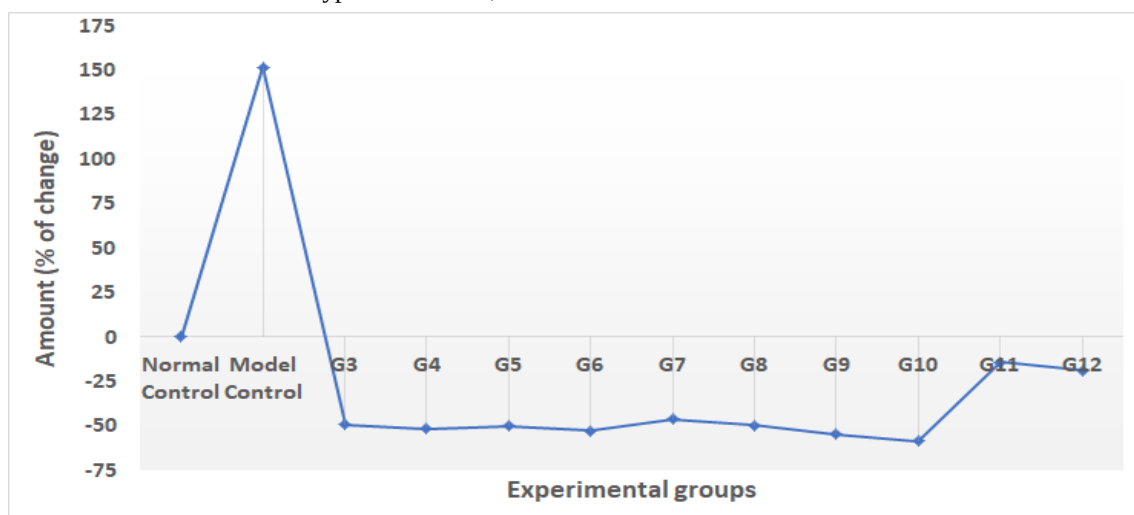
Data of the present study with the others indicated that the possible mode of action of blood glucose-lowering levels of the tested plant parts could be explained by one or more of the following mechanisms including enhance the antioxidant defense mechanism, and reduce the accumulation of free radicals, inhibiting lipid oxidation, affect hormonal-induced glucose hemostasis, promote glucose uptake and energy metabolism, reduce glucose absorption in the intestine, the prevention of erythrocyte impairments associated with a high risk of vascular complications in diabetes, improve glucose response and insulin resistance associated with type 2 diabetes, and

improved pancreatic islet morphology and increasing the amount of pancreatic  $\beta$ -cells [104,108] [132-137].

**Table 5. Effect of psyllium husks, green coffee, orange peels powder and their mixture on glucose levels of obese rat**

| Groups                         | Glucose (mg/dl)                |
|--------------------------------|--------------------------------|
| G1: Control (-ve) (Normal)     | 94.90 $\pm$ 3.40 <sup>b</sup>  |
| G2: Control (+ve)              | 238.70 $\pm$ 4.62 <sup>a</sup> |
| G3: Psyllium husks (2.5%)      | 121.20 $\pm$ 3.00 <sup>e</sup> |
| G4: Psyllium husks (5.0%)      | 115.03 $\pm$ 2.84 <sup>f</sup> |
| G5: Green coffee (2.5%)        | 119.20 $\pm$ 3.35 <sup>e</sup> |
| G6: Green coffee (5.0%)        | 112.60 $\pm$ 2.75 <sup>f</sup> |
| G7: Orange peels (2.5%)        | 128.70 $\pm$ 3.43 <sup>d</sup> |
| G8: Orange peels (5.0%)        | 120.03 $\pm$ 3.15 <sup>e</sup> |
| G9: Mixture powder (2.5%)      | 108.01 $\pm$ 2.84 <sup>g</sup> |
| G10: Mixture powder (5.0%)     | 98.70 $\pm$ 2.50 <sup>h</sup>  |
| G11: Dietary supplement (1.0%) | 204.63 $\pm$ 4.46 <sup>b</sup> |
| G12: Dietary supplement (2.0%) | 193.75 $\pm$ 4.03 <sup>c</sup> |
| LSD ( $P \leq 0.05$ )          | 4.170                          |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ )



**Figure 5.** Effect of psyllium husks, green coffee, orange peels powder and their mixture on glucose levels of obese rat (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

### 3.6. Effect of Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Major Blood Proteins of Obese Rat

Effect of psyllium husks, green coffee, orange peels powder and their mixture on major blood proteins of obese rats was shown in Table 6 and Figure 6. From such data it could be noticed that obesity induced a significant decreased ( $p \leq 0.05$ ) in serum albumin (-63.9%) and globulin (-51.7%) compared to normal controls. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) increase on the albumin and globulin levels of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts, followed by the groups that were fed on green coffee, orange peel and psyllium husks, respectively. Also, the

feeding intervention with the all tested plant parts and their mixture was more effective in increasing the blood proteins when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of increasing in blood proteins of the obese rats as the result of plant parts intervention were exhibited a dose-dependent manner. In general, Globulins, albumins, and fibrinogen are the major blood proteins. Albumin is an important metal binding protein. It is a sacrificial antioxidant that can bind copper tightly and iron weakly to its surface serving as a target for their related free radical reactions. Thus it inhibits copper ion dependent lipid peroxidation [138]. Globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins can be synthesized in the liver but immunoglobulins are synthesized exclusively in immune system, lymphoid tissue [139]. Plasma globulins are made up of a range of proteins including carrier proteins and immunoglobulins or antibodies. Globulins

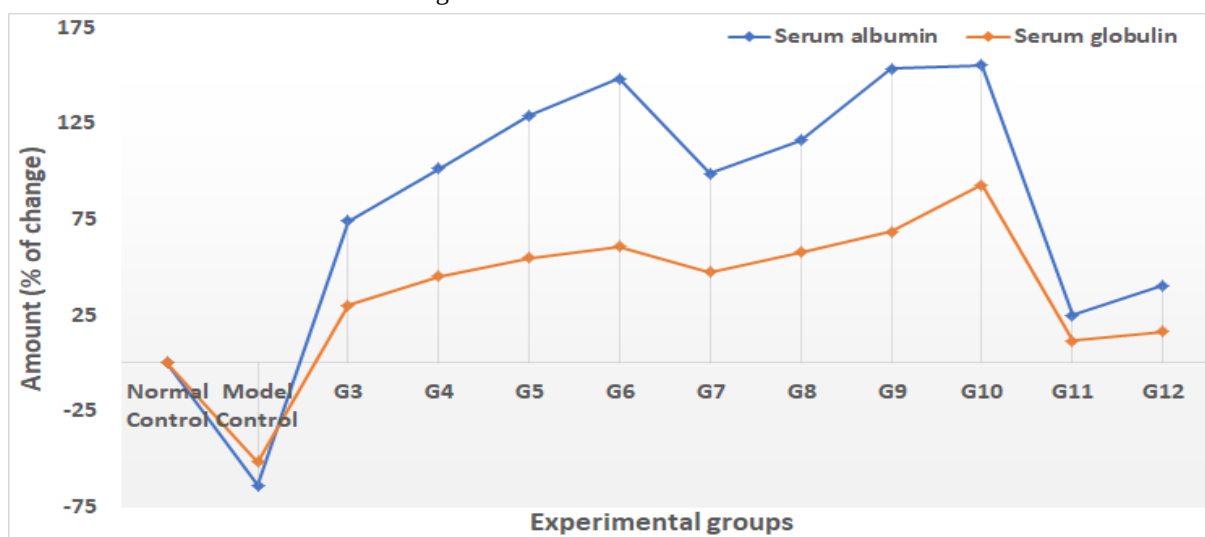
play an important role in liver function, blood clotting, and fighting infection. There are different types of globulins called alpha, beta, and gamma globulins. Reduction of serum proteins has been observed in nephritis, liver injuries, malnutrition, injury reactions, and oedema [136,140,141,142,143,144]. Also, Madhuvanthi and Lathadevi [145] underweight individuals showed a decrease in serum protein levels whereas there were no significant changes in the serum protein levels in overweight and obese individuals. So, it is worthy to report that feeding with some food items produced significant improvement in serum albumin as the result of suffering diseases. In the present study, intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture led to significantly ( $p \leq 0.05$ ) increase on the albumin and globulin levels of the obese rats. Perhaps some of the reasons that explain this matter are the noticeable improvement that these selected plant parts and their mixtures have caused in the functions of the liver, which is the main organ in the synthesis of blood proteins. Also, several previous studies reported that such plant parts rich in bioactive compounds which have the ability to protect liver cell and improve the fatty liver, effective to avoid cirrhosis of the liver, and disrupts the formation of liver cancer [60,110,111,113,114]. Taking into account all of these previous studies it could be proposed that the higher improvement in blood protein recorded in rats fed with the mixture of the tested plant parts can be attributed to the simultaneous accumulation effects resulting from their

content of different phytochemical classes and various biological activities.

**Table 6. Effect of psyllium husks, green coffee, orange peels powder and their mixture on serum albumin & Serum globulin of obese rat**

| Groups                         | Serum albumin (g/dl)   | Serum globulin (g/dl)   |
|--------------------------------|------------------------|-------------------------|
| G1: Control (-ve) (Normal)     | 6.53±0.56 <sup>a</sup> | 5.32±0.41 <sup>a</sup>  |
| G2: Control (+ve)              | 2.36±0.20 <sup>b</sup> | 2.57±0.28 <sup>b</sup>  |
| G3: Psyllium husks (2.5%)      | 4.10±0.23 <sup>e</sup> | 3.34±0.35 <sup>e</sup>  |
| G4: Psyllium husks (5.0%)      | 4.75±0.33 <sup>d</sup> | 3.73±0.37 <sup>d</sup>  |
| G5: Green coffee (2.5%)        | 5.40±0.23 <sup>c</sup> | 3.97±0.39 <sup>cd</sup> |
| G6: Green coffee (5.0%)        | 5.85±0.60 <sup>b</sup> | 4.13±0.34 <sup>c</sup>  |
| G7: Orange peels (2.5%)        | 4.69±0.35 <sup>d</sup> | 3.78±0.30 <sup>d</sup>  |
| G8: Orange peels (5.0%)        | 5.10±0.20 <sup>c</sup> | 4.05±0.35 <sup>c</sup>  |
| G9: Mixture powder (2.5%)      | 5.98±0.45 <sup>a</sup> | 4.32±0.36 <sup>c</sup>  |
| G10: Mixture powder (5.0%)     | 6.02±0.50 <sup>a</sup> | 4.95±0.38 <sup>b</sup>  |
| G11: Dietary supplement (1.0%) | 2.94±0.28 <sup>f</sup> | 2.86±0.24 <sup>f</sup>  |
| G12: Dietary supplement (2.0%) | 3.31±0.37 <sup>f</sup> | 2.99±0.29 <sup>f</sup>  |
| LSD ( $P \leq 0.05$ )          | 0.430                  | 0.357                   |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ )



**Figure 6.** Effect of Psyllium husks, green coffee, orange peels powder and their mixture on major blood proteins of obese rats (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

### 3.7. Effect of Psyllium Husks, Green Coffee, Orange Peels and their Mixture As Powder on Serum Triglycerides, and Serum Total Cholesterol of Obese Rats

Effect of psyllium husks, green coffee, orange peels and their mixture as powder on serum triglycerides, total cholesterol, HDL-c and LDL-c of obese rats was shown in Table 7 and Figure 7. Such data indicated that feeding of rats on HFD (model control) for 8 weeks leads to increase the triglycerides, total cholesterol and LDL-c than the

normal group by the rate of 83.3, 75.9 and 200.1%, respectively. The opposite direction was observed for HDL-c. Intervention with selected plant parts such psyllium husks, green coffee, orange peels and their mixture (2.5 and 5%) in feeding rats for 8 weeks led to significantly ( $p \leq 0.05$ ) decrease the triglycerides, total cholesterol and LDL-c and increase the HDL-c of the obese rats (model control) by different rates. The best results for all previous measures were recorded in the groups of rats that were fed on a mixture of selected plant parts followed by the groups that were fed on green coffee, orange peel and psyllium husks, respectively. Also, the

feeding intervention with all of plant parts and their mixture were more effective in improving the serum lipid profile when compared to one of the dietary supplements commonly used in this regard (CHROMAX). Furthermore, the rate of decreasing in triglycerides, total cholesterol and LDL-c and increase the HDL-c of the obese rats as the result of plant parts intervention were exhibited a dose-dependent manner.

Data of the present study with the other reported that serum lipid profile parameters disturbance significantly ( $p \leq 0.05$ ) as the result of obesity in both human and experimental animals [6,8,14,20,21,77,146]. Several previous studies found that the selected plant parts, and their mixture are a rich source of different categories of phytochemicals including polyphenols, phenolic acids, alkaloids, flavonoids, terpenoids, anthocyanins, carotenoids, polysaccharides, quercetin, terpenes, pectin, dietary fiber and essential oils [71,72,73,74,75,76]. Several authors reported that the effect of the plant parts on improving the serum lipid profile (obesity complications) could be attributed to their high level content of these bioactive compounds and their biological activities. Psyllium husks, green coffee and orange peel phytochemicals were reported to decrease the triglycerides and improve the cholesterol fractions [59,61,110,147,148,149,150]. Taking into account all these biological effects, the higher improvement in serum lipid profile recorded in rats fed with the mixture of the tested plant parts can be attributed to the simultaneous accumulation effects resulting from their content of different phytochemical classes.

In general, coronary heart disease (CHD) is a major health problem in both industrial and developing countries including Egypt. Many studies have now shown that blood

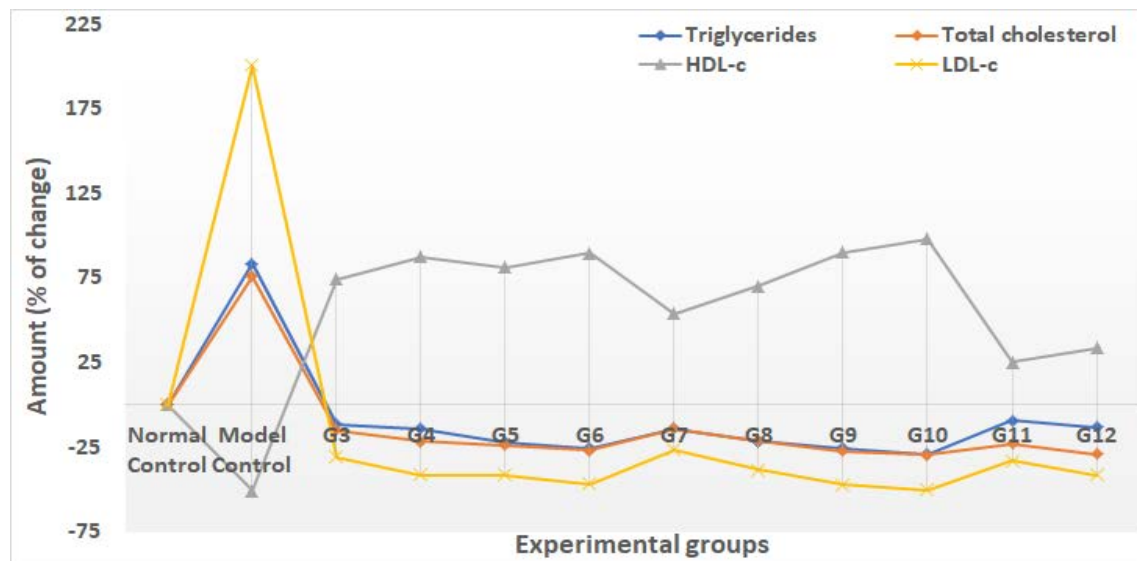
elevated concentrations of total cholesterol and LDL-c are powerful risk factors for CHD, whereas high concentrations of HDL-c or a low LDL-c represent the safety factors [5,151]. The composition of the human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. Reduction in saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering the risk for cardiovascular disease. In recent years, however, the possible hypocholesterolemic effects of several dietary (phytochemicals) such as found in our selected plant parts have attracted much interest. Several authors reported that diets rich in plant foods (vegetables and fruits) were also associated with a reduced risk of heart disease and many chronic diseases of aging [66,151,152,153]. These foods contain phytochemicals that have anti-cancer and anti-inflammatory properties, which could confer many health benefits. Also, Jayanta *et al.*, [154] found that anthocyanin-rich foods significantly prevented elevation in serum and tissue lipids, circulating levels of cardiac and hepatic damage markers, and resulted in excretion of lipids through feces. Data of the present study with the others indicated that the possible mode of action of blood lipid profile improving of the tested plant parts could be explained by one or more of the following mechanisms including enhance the antioxidant and anti-inflammatory activities [20,125,155,156,157], reduce the LDL oxidation and endothelial cell damage which is believed to be involved in the early development of atherosclerosis [151,158,159], inhibit consumption of antioxidant vitamins such as  $\alpha$ -tocopherol [158,160], protect human serum paroxonase (PON 1) activities [159,161], bind to albumin and never incorporated into the LDL particle [162].

**Table 7. Effect of psyllium husks, green coffee, orange peels and their mixture as powder on serum lipid profile of obese rats**

| Groups                         | Triglycerides (mg/dl)     | Total cholesterol (mg/dl) | HDL-c (mg/dl)           | LDL-c (mg/dl)            |
|--------------------------------|---------------------------|---------------------------|-------------------------|--------------------------|
| G1: Control (-ve) (Normal)     | 107.31±3.13 <sup>h</sup>  | 125.00±3.75 <sup>g</sup>  | 51.87±2.40 <sup>a</sup> | 51.67±2.60 <sup>i</sup>  |
| G2: Control (+ve)              | 196.65±5.17 <sup>a</sup>  | 219.85±5.65 <sup>a</sup>  | 25.45±1.36 <sup>f</sup> | 155.07±3.75 <sup>a</sup> |
| G3: Psyllium husks (2.5%)      | 173.59±4.10 <sup>c</sup>  | 186.20±5.45 <sup>b</sup>  | 44.33±2.21 <sup>c</sup> | 107.15±3.12 <sup>c</sup> |
| G4: Psyllium husks (5.0%)      | 168.71±4.60 <sup>cd</sup> | 171.63±5.27 <sup>c</sup>  | 47.65±2.35 <sup>b</sup> | 90.24±3.00 <sup>f</sup>  |
| G5: Green coffee (2.5%)        | 152.61±3.70 <sup>e</sup>  | 167.05±4.65 <sup>cd</sup> | 46.08±2.30 <sup>b</sup> | 90.45±3.10 <sup>f</sup>  |
| G6: Green coffee (5.0%)        | 146.10±3.85 <sup>f</sup>  | 160.08±4.55 <sup>e</sup>  | 48.29±2.47 <sup>b</sup> | 82.57±2.85 <sup>g</sup>  |
| G7: Orange peels (2.5%)        | 168.45±4.75 <sup>cd</sup> | 188.74±5.40 <sup>b</sup>  | 39.17±2.72 <sup>d</sup> | 95.29±3.11 <sup>e</sup>  |
| G8: Orange peels (5.0%)        | 154.02±4.20 <sup>e</sup>  | 172.67±5.35 <sup>c</sup>  | 43.23±2.39 <sup>c</sup> | 90.79±2.95 <sup>f</sup>  |
| G9: Mixture powder (2.5%)      | 145.88±4.11 <sup>f</sup>  | 159.10±4.65 <sup>e</sup>  | 48.38±2.50 <sup>b</sup> | 81.54±2.61 <sup>g</sup>  |
| G10: Mixture powder (5.0%)     | 139.06±3.50 <sup>g</sup>  | 155.00±4.13 <sup>f</sup>  | 50.32±2.58 <sup>a</sup> | 76.87±2.30 <sup>h</sup>  |
| G11: Dietary supplement (1.0%) | 178.05±5.10 <sup>b</sup>  | 169.05±5.30 <sup>c</sup>  | 31.79±2.23 <sup>e</sup> | 113.96±3.34 <sup>b</sup> |
| G12: Dietary supplement (2.0%) | 170.73±4.80 <sup>c</sup>  | 155.60±4.40 <sup>f</sup>  | 34.01±2.47 <sup>e</sup> | 103.57±3.34 <sup>d</sup> |
| LSD ( $P \leq 0.05$ )          | 2.740                     | 3.670                     | 2.640                   | 2.814                    |

Each value represents mean  $\pm$  SD (n = 5). Mean under the same column with different superscript letters are different significantly ( $P \leq 0.05$ ). HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol





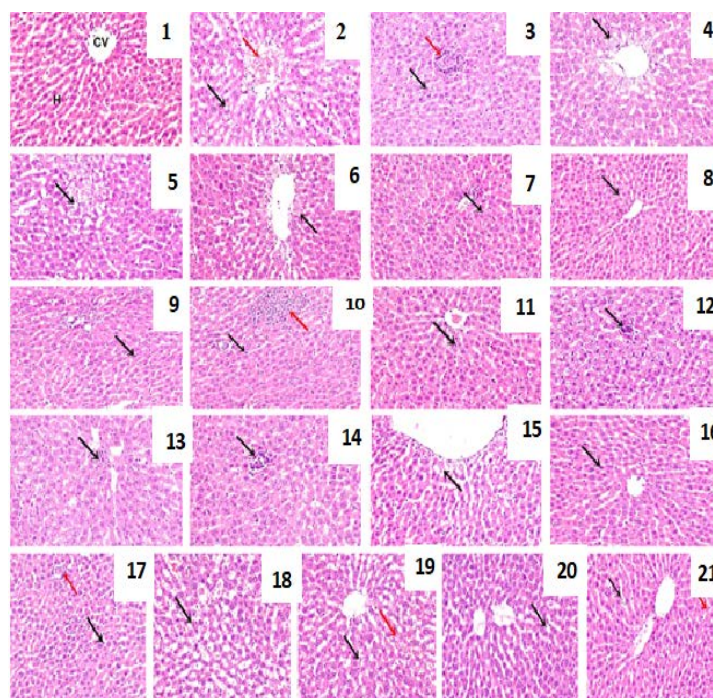
**Figure 7.** Effect of psyllium husks, green coffee, orange peels and their mixture as powder on serum triglycerides, and serum total cholesterol of obese rats (as a percent of change). The positive control group (C2) is calculated as a percentage of the negative control group (G1), and the remaining groups (groups treated with plant parts) are calculated as a percentage of the positive control group (G2)

### 3.8. Histopathological studies

#### 3.8.1. Effect of Feeding Intervention with Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Liver Tissue Histopathological Examination of Obese Rats

Effect of feeding intervention with psyllium husks, green coffee, orange peels powder and their mixture on liver tissue histopathological examination of obese rats was shown in [Figure 8](#). Microscopically, liver of rats from group 1 (normal control) revealed the normal histological structure of hepatic lobule, from central vein and hepatocytes (Photo 1). On contrary, liver of rats from group 2 showed hepatocellular steatosis, congestion of central vein (Photo 2), Kupffer cells activation, focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 3). Meanwhile, liver of rats from group 3 described steatosis of focal hepatocytes (Photos 4 and 5). Furthermore, liver of rats from group 4 exhibited vacuolar degeneration of centrilobular hepatocytes (Photo 6) and Kupffer cells activation (Photo 7). Moreover, sections from group 5 revealed Kupffer cells activation (Photo 8). However, liver of rats from group 6 showed Kupffer cells activation (Photo 9), and focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 10). Likewise, liver of rats from group 7 showed Kupffer cells activation (Photo 11), and small focal hepatocellular necrosis (Photo 12). On the other hand, liver of rats from group 8 manifested Kupffer cells activation (Photo 13) and focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 14). Liver of rats from group 9 showed vacuolar degeneration of centrilobular hepatocytes (Photo 15), necrosis of sporadic

hepatocytes (Photo 16). Meanwhile, liver of rats from group 10 showed small focal hepatocellular necrosis (Photo 17). Examined sections from group 11 described dilatation and congestion of hepatic sinusoids (Photos 18 and 19), vacuolar degeneration of hepatocytes (Photo 19). Meanwhile, liver of rats from group 12 exhibited Kupffer cells activation (Photo 20 and 21) and necrosis of sporadic hepatocytes (Photo 21). In similar studies, Qaiis *et al.*, [163] found that treatment of animals with ochratoxin A alone induced histological changes in liver tissue such as dilatation of the veins and necrosis, degeneration hampering the normal function of this tissue. Yemeni coffee powder represents a good functional food having a potential of boosting the antioxidant capacity in liver of rats in induced with toxicity using Ochratoxin A and thus protecting them from oxidative stress and tissue damage. Also, Hu *et al.*, [164] reported that the hepatocytes of HFD group are scattered and perform balloon like changes, the edges of the hepatic lobules are not clear, and the fatty deposits in the liver form more white fatty vacuoles. The results of histological observations showed that citrus peel powder (CPP) was effective in reducing the accumulation of liver fat, which in turn had an anti-obesity effect. The enlargement of fat cells leads to the growth of adipose tissue. In the HFD rats, addition of CPP significantly reduced adipose tissues size and intracellular fats. Furthermore, Doaa *et al.*, [165] reported that histological examination of the liver in HFD/diabetic rats showed lipid accumulation in the vesicles of hepatocytes and eventually fatty degeneration of hepatocytes. Treatment with green coffee bean extract largely attenuated the vacuolar degeneration, as shown by the tremendous decrease in the formation of fat vacuoles in the liver sections.



**Figure 8.** Effect of feeding intervention with psyllium husks, green coffee, orange peels powder and their mixture on liver tissue histopathological examination of obese rats

**Photo 1,** Photomicrograph of liver of group 1 (normal control) rat showing the normal histological architecture with normal central vein (CV) and normal hepatocytes (H); **Photo 2,** Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow) and congestion of central vein (red arrow); **Photo 3,** Photomicrograph of liver of rat from group 2 showing Kupffer cells activation (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow); **Photo 4,** Photomicrograph of liver of rat from group 3 showing steatosis of focal hepatocytes (arrow); **Photo 5,** Photomicrograph of liver of rat from group 3 showing steatosis of focal hepatocytes (arrow); **Photo 6,** Photomicrograph of liver of rat from group 4 showing vacuolar degeneration of centrilobular hepatocytes (arrow); **Photo 7,** Photomicrograph of liver of rat from group 4 showing Kupffer cells activation (arrow); **Photo 8,** Photomicrograph of liver of rat from group 5 showing Kupffer cells activation (arrow); **Photo 9,** Photomicrograph of liver of rat from group 6 showing Kupffer cells activation (arrow); **Photo 10,** Photomicrograph of liver of rat from group 6 showing Kupffer cells activation (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow); **Photo 11,** Photomicrograph of liver of rat from group 7 showing Kupffer cells activation (arrow); **Photo 12,** Photomicrograph of liver of rat from group 7 showing small focal hepatocellular necrosis (arrow); **Photo 13,** Photomicrograph of liver of rat from group 8 showing Kupffer cells activation (arrow); **Photo 14,** Photomicrograph of liver of rat from group 8 showing focal hepatocellular necrosis associated with inflammatory cells infiltration (arrow); **Photo 15,** Photomicrograph of liver of rat from group 9 showing vacuolar degeneration of centrilobular hepatocytes (arrow); **Photo 16,** Photomicrograph of liver of rat from group 9 showing necrosis of sporadic hepatocytes (arrow); **Photo 17,**

Photomicrograph of liver of rat from group 10 showing Kupffer cells activation (black arrow) and small focal hepatocellular necrosis (red arrow); **Photo 18,** Photomicrograph of liver of rat from group 11 showing dilatation and congestion of hepatic sinusoids (arrow); **Photo 19,** Photomicrograph of liver of rat from group 11 showing dilatation and congestion of hepatic sinusoids (red arrow) and vacuolar degeneration of hepatocytes (black arrow); **Photo 20,** Photomicrograph of liver of rat from group 12 showing Kupffer cells activation (arrow); **Photo 21,** Photomicrograph of liver of rat from group 12 showing Kupffer cells activation (black arrow) and necrosis of sporadic hepatocytes (red arrow). (H & E, X 400).

### 3.8.2. Effect of Feeding Intervention with Psyllium Husks, Green Coffee, Orange Peels Powder and their Mixture on Heart Tissue Histopathological Examination of Obese Rats

Effect of feeding intervention with psyllium husks, green coffee, orange peels powder and their mixture on heart tissue histopathological examination of obese rats was shown in [Figure 9](#). Microscopically, heart of rat from group 1 revealed the normal histological structure of cardiac myocytes (Photo. 1). In contrast, heart of rats from group 2 showed vacuolation of the sarcoplasm of cardiac myocytes, congestion of myocardial blood vessel (Photo. 2,3), and necrosis of sporadic cardiac myocytes and vacuolation in the wall of myocardial blood vessel (Photo 4). Meanwhile, heart of rats from group 3 exhibited congestion of myocardial blood vessel (Photo. 5) and few mononuclear cells infiltration (Photo 6). Otherwise, sections from group 4 manifested no histopathological alterations (Photo. 7, 8). Moreover, heart of rats from group 5 showed no histopathological alterations (Photo. 9) except congestion of myocardial blood vessels in some sections (Photo 10). Some examined sections from groups 6 & 7 revealed no histopathological lesions (Photo. 11 &



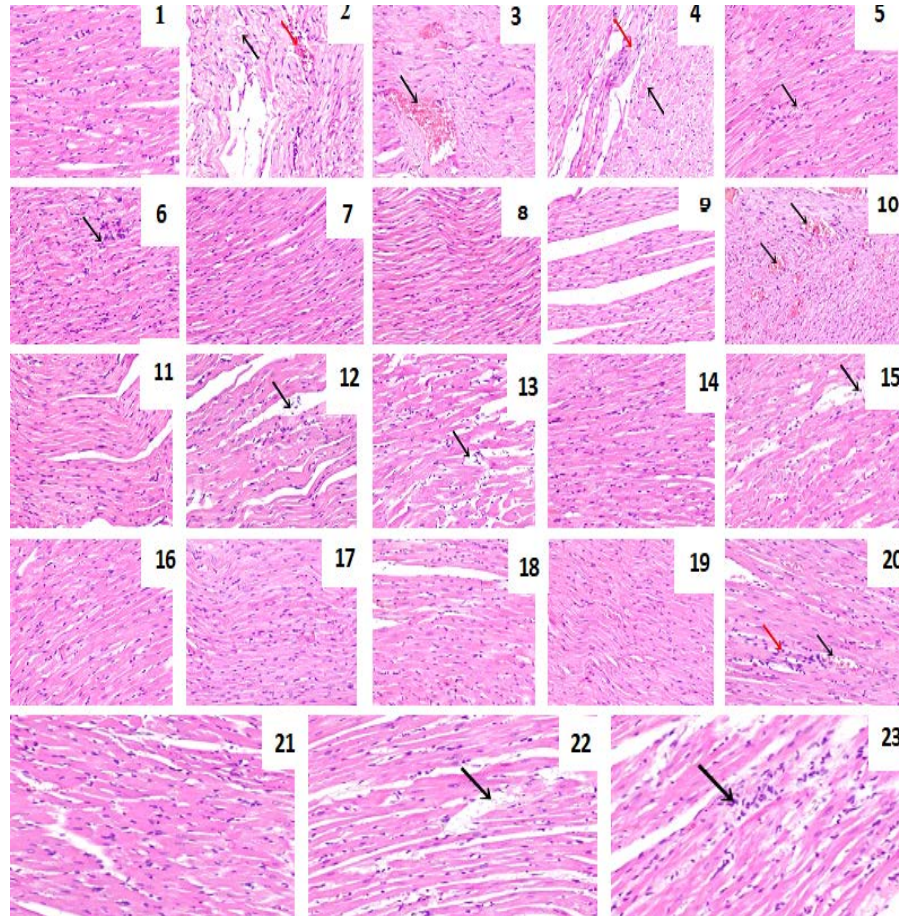
12), whereas, other sections from this group described slight intermyocardial edema (Photo. 13 & 14). Meanwhile, some examined sections from group 8 showed slight intermuscular edema (Photo 15) whereas, other sections exhibited no histopathological changes (Photo 16). Moreover, some sections from group 9 revealed no histopathological lesions (Photo. 17 & 18). Furthermore, heart of rats from group 10 exhibited no histopathological changes except slight intermyocardial edema in some sections (Photo. 19). Otherwise, heart of rats from group 11 showed slight congestion of myocardial blood vessel and few mononuclear cells infiltration (Photo 20). Few sections from this group revealed apparent normal myocytes (Photo 21). Moreover, some heart sections from group 12 manifested slight intermyocardial edema (Photo 22) and few mononuclear cells infiltration (Photo 23). In similar study, Fransiska *et al.*, [166] found that administration of green coffee exxtract in all doses (200, 400, and 800 mg/kg odyweight) significantly reduced the atherogenic index of plasma and improved cardiac histopathology in the hyperlipidemic rats. Also, Fransiska *et al.*, [167] reported that orange peel extract nanoparticles in granular form improve cardiac and aortic histopathology in rats, with a dose of 100 mg/kg BB being the best dose for improving cardiac and aortic histopathology. This study and others have attributed these effects to orange peel has pharmacological effects that include hypoglycaemic and hypolipidaemic benefits, antioxidant properties, and improved myocardial energy metabolism and oxidative stress [168,169].

## 4. Conclusion

The present study has demonstrated the potency of the selected plant parts including psyllium husks, green coffee, orange peels and their mixture for the prevention or early treatment of obesity and its related complications which include decrease on body weight and blood glucose level, enhance the serum lipid profile and CBC parameters, minimize the liver functions and positively manipulate the obesity-related histopathological changes in liver and heart tissues. Also, nutritional intervention with a mixture of selected plant parts was more effective in reducing the effects of obesity and its complications compared to some nutritional supplements spread on the market and commonly used in this regard. These results support the benefits of modifying the diet while interfering with plant parts, in alleviating obesity and its associated complications. However, completing this important topic requires the necessity of conducting more studies and research in the future.

## Ethical Considerations

The ethical issues of this study was reviewed and approved by the Scientific Research Ethics Committee (SREC, Approval # 33-SREC-09-2021), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.



**Figure 9.** Effect of feeding intervention with psyllium husks, green coffee, orange peels powder and their mixture on heart tissue histopathological examination of obese rats

**Photo 1**, Photomicrograph of heart of rat from group 1 showing the normal histological structure of cardiac myocytes; **Photo 2**, Photomicrograph of heart of rat from group 2 showing vacuolation of the sarcoplasm of cardiac myocytes (black arrow) and congestion of myocardial blood vessel (red arrow); **Photo 3**, Photomicrograph of heart of rat from group 2 showing congestion of myocardial blood vessel (arrow); **Photo 4**, Photomicrograph of heart of rat from group 2 showing necrosis of sporadic cardiac myocytes (black arrow) and vacuolation in the wall of myocardial blood vessel (red arrow); **Photo 5**, Photomicrograph of heart of rat from group 3 showing congestion of myocardial blood vessel (arrow); **Photo 6**, Photomicrograph of heart of rat from group 3 showing few mononuclear cells infiltration (arrow); **Photo 7**, Photomicrograph of heart of rat from group 4 showing no histopathological alterations; **Photo 8**, Photomicrograph of heart of rat from group 4 showing no histopathological alterations; **Photo 9**, Photomicrograph of heart of rat from group 5 showing no histopathological alterations; **Photo 10**, Photomicrograph of heart of rat from group 5 showing congestion of myocardial blood vessels; **Photo 11**, Photomicrograph of heart of rat from group 6 showing no histopathological alterations; **Photo 12**, Photomicrograph of heart of rat from group 7 showing no histopathological lesions; **Photo 13**, Photomicrograph of heart of rat from group 6 showing slight intermyocardial edema; **Photo 14**, Photomicrograph of heart of rat from group 7 showing slight intermyocardial edema; **Photo 15**, Photomicrograph of heart of rat from group 8 showing slight intermyocardial edema; **Photo 16**, Photomicrograph of heart of rat from group 8 showing no histopathological changes; **Photo 17**, Photomicrograph of heart of rat from group 9 showing no histopathological changes; **Photo 18**, Photomicrograph of heart of rat from group 9 showing no histopathological changes; **Photo 19**, Photomicrograph of heart of rat from group 10 showing no histopathological changes; **Photo 20**, dial blood vessel (black arrow) and few mononuclear cells infiltration (red arrow); **Photo 21**, Photomicrograph of heart of rat from group 11 showing apparent normal cardiac myocytes; **Photo 22**, Photomicrograph of heart of rat from group 12 showing slight intermyocardial edema; **Photo 23**, Photomicrograph of heart of rat from group 12 showing few mononuclear cells infiltration (arrow) (H & E, X 400).

## Conflicts of Interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENT

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## Authors' contribution

Yousif Elhassaneen participated in developing the study protocol, retrieving conceptual information, validating the results and statistical analysis, preparing a draft of the manuscript, performing a critical review to intellectually organize the content, and granting approval to publish the final version of the manuscript. Hend Kashaf conducted the experimental trials, and collected, tabulated, analyzed and interpreted the results. She was also involved in conceptual information retrieval and preparation of the draft of the manuscript. Emad El-Kholie participated in retrieving conceptual information, validating the study results, and preparing a draft of the manuscript. Olfat Khater made contributions to the concept and design of the work.

## Abbreviations

ALT, Alanine aminotransferase, ALP, alkaline phosphatase, AST, aspartate aminotransferase, BD, basal diet; BWG, body weight gain; FER, feed efficiency ratio; FI, feed intake; HB, hemoglobin; HDL-c, high density lipoprotein; HFD, high fat diet; RBCs, red blood cells; TC, total cholesterol, TG's, triglycerides; WBCs, white blood cells.

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