

The Protective Effect of White and Red Radish as Hypoglycemic and Hypocholesterolemic Agents

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Abstract: Radish, *Raphanus Sativus L.* (family: Brassicaceae) has been recognized since ancient times, roots and leaves are used for cooking and treatment of a wide variety ailments. White radish leaves (WRL), white radish roots (WRR) and red radish roots (RRR) were used in this study. Four major active compounds were identified by HPLC in the methanolic extracts of radish, those included catechin, ferulic, sinapic and coumaric acid. The edible parts of radish were used as hypoglycemic and hypocholesterolemic experiments by using albino rats. Water extracts of WRL, WRR and RRR were administered orally at doses 100, 200 and 400 mg/kg b.w/day/rat. Results show the effect of the extracts on glucose level of diabetic rats. The highest effect of tested radish extracts was with G12 (rats administered 400 mg RRR/kg b.w/day). Also, there was a significant decrease in TG, TC, LDL-c and increase in the HDL-c parameter in G12. Furthermore, results of the influence of WRL, WRR and RRR as hypocholesterolemic action was in a positive way especially G12 of RRR on the reduction of the levels of TC, LDL-c and TG and increased the level of glutathione peroxidase. Moreover, results of G11 show the reduction of malodialdehyde (MDA) in serum of rats. G11(HFD(high fat diet) + 1%cholesterol +400 mg RRR/kg b.w/day)recorded the best results as hypocholesterolemic effect which could be attributed to the content of main phenolic acids in red radish roots in high level. Our results indicates the hypoglycemic and hypocholesterolemic effect of radish extracts.

Keywords: Radish, leaves, roots, hyperglycemic, hypercholesterolemia, rats

I. Introduction

Raphanus sativus Linn is a root vegetable of cruciferaceae family and widely grown in India for its culinary and medicinal purposes. Medicinal uses of *Raphanus sativus* have been documented in India since the tenth century [1, 2]. The ancient inhabitants of Greek prized radishes above all root crops. The root crop was a common food in Egypt long before the pyramids were built, and was popular in ancient Rome as well. The word "radish" is a derivation of the Latin word "radix," or root. Columbus and the early settlers brought radishes to America. Radish can sprout from seed to small plant in as little as 3 days [3]. The main season for sowing the radish in Egypt is mainly from September to March. This crop is harvested within 30-50 days of sowing and is pulled out from the soil when it reaches edible size. Radish is of considerable economic importance for India, Japan and China. In Egypt, the annual production of radish is estimated as (2295 tons) as reported by the agricultural statistics (2015) [4].

Robust health-promoting properties of *Raphanus sativus* have also been attributed to polyphenolic compounds. Polyphenolics are a large family of natural compounds widely distributed in plant food. Polyphenolic have become the focus of current nutritional and therapeutic interest largely due to their disease-preventing and health-promoting effects. Polyphenolics appear to play a significant role as antioxidants in the protective effect-derived foods [5]. The antioxidant activity of the dietary phenolics is considered to be superior to that of the essential vitamins and is ascribed to its high redox potential which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups [6].

Raphanus sativus L., or the radish, is reported to be closely related to reduction in cancer development, belongs to the cruciferous vegetable family [7], and contains a range of digestive enzyme [8]. Radishes consist of a root and green leafy parts. The radish leaves contain more vitamin A and C and calcium than the root [9].

Diabetes mellitus is one of the metabolic diseases that results in carbohydrate, lipid, and protein metabolic dysfunction. Due to insulin deficiency, lipolysis decreases which, in turn, results in hyperlipidemia and an increase in blood cholesterol and triglyceride, eventually leading to atherosclerosis and increase in the risk of heart attack [10]. Therefore, although controlling the blood glucose in these patients is very important, prevention of long term side effects should be taken into account very seriously. Any treatment which could control triglyceride and cholesterol in diabetic patients is very useful. In some cases, phytotherapy is used for treatment of hyperlipidemia [11].

Since synthetic drugs have undesirable side effects or contraindications, the World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes [12]. One of these plants is *Raphanus sativus* which increases high level of cholesterol in the blood is a major risk for coronary heart disease, hypertension and stroke [13]. Too much circulatory blood cholesterol slowly builds up in the inner walls of the arteries that feed the heart and brain. Together with other substances, cholesterol then progressively form plaque and narrows the arteries, leading to atherosclerosis [14]. Radish (*Raphanus sativus*) increases the serum HDL in normal rats [15]. In rats with a high-fat diet, the use of extract of the *Raphanus Sativus* causes epithelium repair and an increase in enteroocytes [16].

The objective of this work was to extract the phenolic compound from white radish (leaves and roots) and red radish roots. Fractionation and identification of these phenolic compounds were also have been done. The availability of radish phenolic compounds as hypoglycemic and hypocholesterolemic action were also evaluated.

II. Materials And Methods

2.1. Materials

Two varieties of fresh Radish (*Raphanus Sativus* L.) "white and red" were obtained from the Horticultural Research Institutes, Agriculture Research Centre, Giza, Egypt in January 2013 at their full maturity. The samples were authenticated by Assistant Prof. Mostafa Kamal, Vegetable Crop Department, Horticultural Research Institutes, Agriculture Research Centre, Giza, Egypt . After harvesting, the radish roots and leaves were transported to the laboratory, then washed under running water to remove dirt. DPPH (1,1-diphenyl 2-picrylhydrazyl) , Standards of phenolic acids: Sinapic , Coumaric, Ferulic, Vanillic, Caffeic acid, Catechin, Gallic, *p*-hydroxy-benzoic acid , Syringic acid were purchased from Sigma Aldrich Company. Methyl alcohol (99.9%) was purchased from El Nasr Pharmaceutical Chemical Company. Alloxan monohydrate was purchased from Kemet Medical Company, Cairo, Egypt. Male albino rats weighing 180–200 g were purchased from the Research Institute of Ophthalmology, Giza, Egypt. Diagnostic kits were purchased from Bio-Diagnostics Company, Giza, Egypt.

2.2 Preparation of Dried Radish

Leaves of white radish were cut into small pieces and dried in an oven at 50°C for 18 hours. The dried samples were packed into plastic bags and stored at room temperature (25°C) until analysis. Roots of red and white radish were cut into thick slices (5 mm) by using Fruits and vegetable slicer , then the slices were dipped in water containing 10 mmol /litre of glutathione (reduced form) and 100 mmol /litre of citric acid for 5 min. The slices were dried in an oven at 60°C for drying. The dehydrated slices were grounded into Broun mixer (AG Frankfurt/M- type 4250).

2.3 Determination of proximate Composition of dried Samples

Moisture, ash, crude protein, crude fiber, ether extract and total carbohydrate were determined in all the dried samples (leaves and roots) of white and red radish according to the method described by the [17].

2.4 Extraction of polyphenols

The powdered samples of leaves and roots were extracted with methanol at room temperature for 48 h with a mass to volume ratio of 1:5 (g/ml) as described by [18] and [19]. The extracts were evaporated in a rotary evaporator (Heidolph- Rotacool, Germany) at 40°C. , then they were lyophilized. Dried residues were subsequently redissolved in methanol to yield a concentration (w/v) of 10 mg/mL

2.5 Determination of total phenolic content

Total phenolic content was determined colorimetrically by the Folin Ciocalteu method according to the method described by [20] with some modifications as described by [21] Plant extracts were dissolved in methanol to yield a concentration (w/v) of 10 mg/mL. 50 µL aliquots were mixed with 1.25 mL of Folin–Ciocalteu reagent (diluted 1:10 fold) and 1 mL of 7.5% sodium carbonate solution. After 30min, absorbance was measured by Janway model 6705 spectrophotometer (England) at a $\lambda=765$ nm, at room temperature. The results were expressed gram as Catechin equivalents per 100 g dried plant (g Catechin /100 g).

2.6 Fractionation and identification of phenolic compounds

The polyphenolic compounds of radish extracts were fractionated and identified for phenolic compounds by HPLC, according to the method described by [22]. Identification of individual phenolic compounds was performed on Hewlett- Packard HPLC (Model 1100), using a hypersil C18 reversed- phase column (25 × 4.6 mm) with 5µm particle size. Injection was done by means of a Rheodyne injection valve (Model 7125) with 50µl fixed loop. The mobile phase was composed of solvent A (4.5% formic acid) and

solvent B (80% of acetonitrile and 20% of solvent A). The program began with isocratic elution with 95% A (0-1min); then a linear gradient was used until 16 min, lowering A to 20%; from 17 min to 24 min, and A decreased to 0%. The flow rate was 1 ml min⁻¹, and the runs were integrated at 280 and 320, 360 nm for hydroxycinnamic acid and flavonoid derivatives, respectively. Scanning was performed from 200 to 600 nm. Phenolic compounds were identified by comparing retention times and UV-VIS spectra with those of pure standards and the range of calibration curves. The repeatability of the quantitative analysis was $\pm 4\%$. The analysis were replicated (n=3), and the contents given as means values, plus or minus the standard deviation. The results were expressed as grams of each compound per total phenolic compounds.

2.7 Determination of antioxidant activity

The diphenyl picrylhydrazyl (DPPH) radical scavenging activity was performed as described by [23]. 250 μg /mL of methanolic solution of extract was prepared . An aliquot (10 μL) of methanolic extract was mixed with 90 μL of distilled water and 3.9 ml methanolic DPPH solution (0.025 g/L), then incubated for 30 min in darkness. The absorbance was measured at 515nm against methanol as blank, using a Janway model 6705 spectrophotometer (England). Negative control was prepared with 10 μL methanol, 90 μL distilled water and 3.9 mL DPPH solution. The antioxidant activity was calculated using the following equation.

$$AA\% = \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}}$$

where:

AA is the antioxidant activity.

Abs_{DPPH} is the absorbance of DPPH free radical solution in methanol; Abs_{sample} is the absorbance of DPPH free radical solution mixed with sample.

2.8 Animal studies

One hundred and thirty eight male Albino rats weighing 180–200 g were obtained from the Research Institute of Ophthalmology, Giza, Egypt. The animals were housed individually in well aerated cages with screen bottom and fed on basal diet as described in A.O.A.C. [24] for 12 days as an adaptation period. Salt mixture and vitamin mixture were prepared as described in A.O.A.C. [24, 25] respectively. Temperature and humidity were maintained at 25°C and 60% respectively, food and water were given ad libitum.

2.8.1 Preparation of different radish extracts

This experiment was carried out according to the method described by [26] and [19]. One hundred grams of each dried and powdered of white radish leaves (WRL) or white radish roots (WRR) as well as red radish roots (RRR) were extracted with distilled water (w:v ratio of 1:5) at room temperature (25°C) for 48 hours. The extracts were then filtered and evaporated by rotary evaporation . The semi-solids extracts were lyophilized with final weights 8.3 g (10.5%) , 9.6 g (11.7%) and 10.2 g (12.1%) respectively.

2.8.2 Experimental design:

The rats were divided into two main groups as follows:

(I) Diabetic group

(II) hypercholesterolemic group

The group of diabetic experiment was carried out for 4 weeks. This main group contained 72 rats, divided into 12 sub-groups of rats, each having 6 rats as recommended by [26] and [27]. All the diabetic rats were administrated orally (by stomach tube) with WRL, WRR and RRR extracts. Table (1) summarized the groups of experimental rats.

Diabetes was induced in overnight fasted animals by a single intraperitoneal injection of alloxan monohydrate, dissolved in 5% w/v normal saline at a dose of 150 mg/kg BW. The dose of alloxan was injected periodically for 3 days. Five days later. The rats with blood glucose level ≥ 300 mg/dL were considered to be diabetic as recommended by [28]. Blood samples were collected from the eye plexuser by a fin capillary glass tube. The samples were centrifuged for 10 min at 3000 rpm and the serum was collected, blood glucose level was measured.

The groups were as follows: (G1) Control basal diet, (G2) diabetic control, (G3) diabetic injected with 20 units insulin, (G4) diabetic + 100 mg WRL/kg/day (20 mg /200 g rat/ day) , (G5) diabetic + 200 mg WRL/kg/day (40 mg /200 g rat/ day), (G6) diabetic + 400 mg WRL/kg/day (80 mg /200 g rat/ day), (G7) diabetic + 100 mg WRR/kg/day (20 mg /200 g rat/ day), (G8) diabetic + 200 mg WRR/kg/day (40 mg /200 g rat/ day) ,(G9) diabetic + 400 mg WRR/kg/day (80 mg /200 g rat/ day), (G10) diabetic + 100 mg RRR/kg/day (20 mg /200 g rat/ day) , (G11) diabetic + 200 mg RRR/kg/day (40 mg /200 g rat/ day) and (G12) diabetic + 400 mg RRR/kg/day (80 mg /200 g rat/ day) .

The second main group of rats "hypercholesterolemia group" was carried out to 6 weeks. This second main group contained 66 rats, divided into 11 sub-groups, each having 6 rats as recommended by [29]. All the hypercholesterolemic rats were administered orally using stomach tube. The groups of experimental rats are shown in Table (2).

The groups of rats were as follows: (G1) Control basal diet, (G2) Control high fat diet (HFD)+1%cholesterol, (G3) HFD + 1% cholesterol + 100 mg WRL/kg/day (20 mg /200 g rat/ day) , (G4) HFD + 1% cholesterol + 200 mg WRL/kg/day (40 mg /200 g rat/ day) , (G5) HFD + 1% cholesterol + 400 mg WRL/kg/day (80 mg /200 g rat/ day) , (G6) HFD + 1% cholesterol + 100 mg WRR/kg/day (20 mg /200 g rat/ day),(G7) HFD + 1% cholesterol + 200 mg WRR/kg/day (40 mg /200 g rat/ day) ,(G8) HFD + 1% cholesterol + 400 mg WRR/kg/day (80 mg /200 g rat/ day), (G9) HFD + 1% cholesterol + 100 mg RRR/kg/day (20 mg /200 g rat/ day), (G10) HFD + 1% cholesterol + 200 mg RRR/kg/day (40 mg /200 g rat/ day) and (G11) HFD + 1% cholesterol + 400 mg RRR/kg/day (80 mg /200 g rat/ day) .

At the end of the experiment rats were sacrificed . The blood was collected in tubes and centrifuged at 3000 rpm to obtain serum. Serum glucose, total cholesterol, HDL- cholesterol, LDL - cholesterol, triglycerides (TG), and were measured by using the diagnostic kits. Glutathione peroxidase (GSH- PX) and malondialdehyde (MDA) levels in organs (liver and heart) and blood were determined according to the method of [30] and [31], respectively. Results were calculated by using extinction coefficient ($6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) .Protein level was determined according to the method of [32].

2.8.3 Organs preparation before glutathione peroxidase analysis

Organs were preparation according to the method of [14] as follows : 1- Homogenize the sample in 4-8 volumes (per weight tissue) of cold buffer (50 mM phosphate buffer, pH 7.0, containing 5 mM EDTA and 1 mM 2-mercaptoethanol) 2- Centrifuge at 4000 rpm for 10-20 minutes at 2-8°C. 3- Remove the supernatant fluid containing the enzyme.

2.8.4 Organs preparation before malondialdehyde analysis

Organs were preparation according to the method of [31] as follows 1- Prior to dissection, perfuse tissue with a phosphate buffered saline solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots 2- Homogenize the tissue in 5-10 ml cold buffer (50mM potassium phosphate, pH 7.5) per gram tissue 3- Centrifuge at 4000 rpm for 15 minutes 4- Remove the supernatant for assay.

2.9 Statistical analysis

Results were statistically analyzed by the least significant differences (L.S.D) at the level of probability procedure, according to [33].

III. Result and Discussion

3.1 Chemical composition of white and red radish

The chemical composition of white and red radish was calculated on dry wt. basis as shown in Table (3). Results show that, the red radish roots (RRR) recorded the highest amount of crude protein compared with the white radish either white radish leaves (WRL) or white radish roots (WRR). However WRR had higher amounts of crude protein than WRL. On contrary leaves of white radish (WRL) had the highest amounts of crude fat, crude fiber and the ash content comparing with WRR and RRR. Meanwhile, the total carbohydrate of red radish roots was significantly the highest percentage as comparing with white radish either leaves or roots. The moisture content of fresh red radish roots (RRR) and fresh white radish roots (WRR) was statistically non significant (on fresh wt. basis), however their were in significant difference with white radish leaves (WRL), that recorded the lowest value of moisture content. Our results are in accordance with the results of [34] and [35] who reported that one of the nutritional benefits of radish roots is its high concentration of carbohydrates and dietary fiber. They also added that, the leaves of radish had higher percent of ash, fat and crude fiber than those of the roots.

3.2 Total phenolic content and antioxidant activity

Total phenolic content and antioxidant activity of white radish leaves and roots as well as red radish roots are shown in Table (4). Results of total phenolic content were calculated as g catechin/100g dried sample. Statistical analysis showed significant variations through the tested samples. White radish leaves had the highest amount of total phenols (1.612 g/100g) followed by red radish roots, meanwhile white radish roots recorded the lowest amount of total phenols (0.831 g as catechine/ 100g dried sample).

Antioxidant activity of the tested samples was determined by DPPH assay as shown in Table (4). Significance could be shown through the samples under investigation. White radish leaves recorded the highest

percentage of antioxidant activity, on contrary, the roots of white radish had the lowest value. So, as the total phenolic increased, the antioxidant activity also increased directly proportional.

Our results are in accordance with the results of [36] and [37] who reported that the environmental factors, such as solar radiation, altered antioxidant properties together with total phenolic content. [38] revealed that the highest difference between day and night temperature showed the greatest production of phenolics and antioxidant activities. So high land areas can be good places for the production of radish containing higher functional compounds. Although the dietary intake of phenolics varies considerably among geographic regions, it is estimated that daily intake range from about 20 mg to 1 mg, which is higher than that for vitamin E [39]. Phenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory action [40]. Dietary plant phenolic compounds have been described to exert a variety of biological actions such as free radical scavenging, metal chelation, modulation of enzymatic activity and more recently to affect signal transduction, activation of transcription factors and gene expression [41].

3.3 Identification of phenolic compounds of white and red radish extracts

Several phenolic compounds were identified by HPLC in the leaves and roots of white radish as well as in the red radish roots. There included ferulic, sinapic, catechin, coumaric, vanillic, caffeic, gallic, syringic, rutin and protocatechuic acid as shown in Table (5). Results also show that, catechin was the most abundant phenolic acid in the methanolic extract that recorded 0.500 and 0.852 g/100g wt of extract for white radish leaves (WRL) and white radish roots (WRR), respectively. Meanwhile, coumaric acid was the most abundant phenolic acid for red radish roots (RRR) that recorded 0.520 g/100g. The amount of vanillic acid for WRL and RRR was almost the same (0.423 and 0.410 g/100g respectively), however, it was only 0.140 g/100 g for WRR. On the other hand, the phenolic acid ferulic was high amount for RRR (0.333 g/100 g dry wt extract) as compared with WRL and WRR those had only 0.130 and 0.180 g/100g dry wt extract respectively. Sinapic acid was in high amounts for all the tested samples for radish either leaves or roots. Results also show that, both of rutin and protocatechuic acid were found in high concentration for white radish leaves, meanwhile they were in low concentration for white radish roots and red radish roots. Our results are in accordance with the results of [42], [5], [35] and [43] who reported that catechin, ferulic, sinapic and coumaric acid were identified as major phenolic compounds in the methanolic extract. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid), this phenolic compound possesses three distinctive structural motifs that can possibly contribute to the free radical scavenging capability of this compound [44]. Ferulic acid exhibits a wide range of therapeutic effects against various diseases, like cancer, diabetes, cardiovascular, neuro-degenerative. Ferulic acid, a phenolic compound is a strong membrane antioxidant and known as positively affect human health [45]. Moreover, sinapic acid is widespread in fruits, vegetables, cereal grains, oilseed crops, and some spices and medicinal plants and as such is common in the human diet. Sinapic acid shows antioxidant, antimicrobial, anti-inflammatory, anticancer and anti-anxiety activity [46]. On the other hand, *p*-Coumaric acid (4-hydroxycinnamic acid) is a phenolic acid that serves as a precursor of other phenolic compounds, and exists either in free or conjugated form in plants. Its biological activities, including antioxidant, anti-cancer, antimicrobial, antiviral, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities [47].

3.4 Effect of radish leaves and roots extracts on the serum levels of glucose, total cholesterol, LDL, HDL, and triglycerides in diabetic rats:

Results of the influence of white radish leaves or roots as well as red radish roots extracts on the glucose level, total cholesterol (TC), LDL, HDL and triglycerides (TG) are shown in Table (6). Glucose levels (mg/dl) for all the tested groups of rats were in significant difference. The highest level was with G2 "Diabetic group of rats" that recorded 340.3 mg/dl. The lowest level of glucose was found to be with G1 "control basal diet" followed by G3 "Diabetic injected with insulin. Groups 4 to 12 show the effect of radish leaves or roots extracts on the level of serum glucose. The lowest level of glucose through these groups was with G12 that supplemented with 400 mg red radish roots/kg b.w/day which recorded 130.12 mg/dl/serum glucose level. G9 "diabetic group that had 400 mg white radish roots recorded 140.63 mg/dl serum glucose level. Meanwhile G6 "diabetic group had 400 mg white radish leaves recorded only 156.63 mg/dl serum glucose level. These results appear the importance of red radish roots on lowering the glucose level. Our results are in agreement with the results of [27] and [48] who reported that aqueous extracts of radish had shown significant protection and maximum reduction in blood glucose in alloxan induced diabetic rats. They also reported that, alloxan (beta cyto toxin) induced diabetes in a wide variety of animals by damaging the insulin secreting beta cell resulting in a decrease in endogenous insulin release, which decreased the utilization of glucose by the tissues. They attributed the significant antidiabetic activity of *Raphanus sativus* L may be due to inhibition of subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect.

So the highest effect of G 12 for glucose level reduction could be attributed to the highest level of ferulic acid, sinapic acid and catechin in radish red roots (RRR) as shown in Table (5). [49] explained the hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK- Ay mice, which is a model of non-insulin dependent diabetes mellitus. Ferulic acid also restored blood glucose to near normal levels and protected against apoptosis of pancreatic β -cell in STZ-diabetic rats. Thus ferulic acid can possibly be at least one of the components of methanolic radish root extract responsible for the observed antihyperglycemic effect. On the other hand, [50] and [51] reported that sinapic acid and catechin have a potential antihyperglycemic effect in streptozotocin-induced diabetic rats. Moreover, *p*-coumaric acid has mitigatory effects against diabetes [47].

Results in Table (6) also show the influence of radish roots or leaves on the total cholesterol (TC), LDL, HDL and triglycerides (TG) determined as mg/dl of rat serum. All the results were statistically significant difference, the lowest levels of TC, LDL and TG among the groups of rats supplemented with white radish root or leaves and red radish roots was belongs to the G12 followed by G9 and G6 who had 400 mg dried radish extract who could lowered the level of TC from 124.87 mg/dl for G2 to 99.12 for G12. Also reduced the LDL started for 78.99 for G2 to 41.82 only for G12. In addition, the reduction of TG was from 88.26 for G2 to 59.73 for G12. The HDL level was increased up to 45.28 mg/dl for G12 and 40.43 mg/dl for G9 and 39.16 mg/dl for G6 who supplemented 400 mg dried radish extract red radish roots, white radish roots or white radish leaves respectively. Moreover, the other concentrations of different dried radish extracts 100 mg, or 200 mg/kg b.w/day also affected on the levels of these parameters but in different ways, that the reduction in TC, LDL and TG was less than the highest concentration (400 mg dried radish extract), all were in significant difference. Our results are in accordance with the results of [27].

On the other hand, [52] reported that metabolic dyslipidemia is the most common complication of insulin resistance and type 2 diabetes. Moreover, this is characterized by distinct changes from a normal plasma lipid which induced elevated triglyceride, cholesterol and LDL [52, 53]. These parameters are major factors for cardiovascular diseases. Some reports show that *Raphanus sativus* can decrease the plasma cholesterol, triglyceride and phospholipids in normal rats [54,16]. It seems that *Raphanus sativus* increases the lipid metabolism and lowers the lipid plasma by increasing the activity of lipoprotein lipase.

3.5 Influence of radish leaves and roots extracts on cholesterol, LDL, HDL, glutathione peroxidase and malondialdehyde in livers, blood and serum hypercholesterolemic rats

Changes in total cholesterol, LDL, HDL, glutathione peroxidase (GSH- PX) and malondialdehyde (MDA) for different groups of hypercholesterolemic rats those had radish leaves or roots extracts compared with other groups of rats represented as basal diet and high fat diet (HFD), are shown in Table (7). Results show that, the total cholesterol of different group of rats was in significant difference. The highest value (140.88 mg/dl serum) was recorded for the G2 group of rats, that had HDF+ 1% cholesterol. This value reduced up to 100.03 mg/dl serum for G11 those had 400 mg RRR/kg b.w/day with the HDF+ 1% cholesterol, followed by G8 and G10 those had 400 mg WRR and 200 mg RRR/kg b.w/day respectively. However, G5 (group of rats had 400 mg WRL/ kg b.w/ day with HDF + 1% cholesterol) was in significant difference with G11 and G8 that was had higher total cholesterol than them.

Results in Table (7) also show the effect of radish on LDL, HDL and TG of different tested group of rats. G2, group of rats had the highest value of LDL (88.22 mg/dl serum). G11 had the lowest value of LDL (37.34 mg/dl serum), those had 400 mg RRR/kg b.w/day followed by G10 that had 200 mg RRR/kg b.w/day, they were in significant difference. G8 and G7 were in non significant difference, although they had different amount of WRR either 400 mg or 200 mg WRR/kg b.w/day respectively.

The results of HDL values as (mg/dl serum) are shown in Table (7), G1 (control group of rats) was statistically in nonsignificant difference with G11 (group of rats had 400 mg RRR/kg b.w/ day) those recorded 48.57 and 46.89 mg/dl respectively. Moreover, the groups of rats G3 and G6 were nonsignificant difference they recorded the lowest value of HDL related to these had radish extracts.

Results of triglycerides (TG) for different groups of rats show the effect of different radish extracts on TG. G2 had the highest value of TG (group of rats didn't had any radish extracts that recorded 91.41 mg/dl. Significant difference could be shown among all the tested groups of rats. However, G11 (had 400 mg RRR) recorded the lowest value of TG. G10 and G8 were in nonsignificant difference of TG, although they had different amounts of radish, 200 mg RRR and 400 mg WRR/kg b.w/day. Our results are in accordance with the results of [29] and [55] who reported that the levels of serum TC, TG and LDL cholesterol were significantly increased in the hypercholesterolemic (HC) rats, as compared to those of the control rats. They also reported that, feeding by stomach tube of the *Raphanus sativus* extract (RSE), however, significantly decreased the levels of these atherogenic lipids in the HC + RSE rats. They also added that the levels of HDL increased in the HC + RSE rats, as compared to that of the control rats. On the other hand, [56] showed that ferulic acid has the ability to reduce the level of low density lipoproteins in rats. They also suggested that synthesis of cholesterol was decreased by competitive inhibition of hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase) by

ferulic acid. This enzyme is the most important regulatory step in the biosynthesis of cholesterol. On the other hand, [57] reported that Caffeic acid, ferulic acid and coumaric acid supplements decrease plasma cholesterol concentration, they attributed this to the increased fecal sterol, which in turn led to a decreased absorption of dietary cholesterol.

Results in Table (7) also show the effect of white and red radish leaves or roots on glutathione peroxidase (GSH-PX) in blood and liver for all different groups of rats. Results show that all the results were statistically significant difference. Control group of rats (G1) had the highest level of GSH-PX that recorded 44.3 nmole/ml blood. On contrary G2 of rats those had high fat diet recorded the lowest value (22.39 nmole/ml of blood). The other groups of rats rated from G3 to G11 showed improvement in the level of GSH-PX resulting from administrating with radish. Groups of rats included G11, G8 and G5 those had 400 mg red or white radish were superior to the rest of corresponded groups of rats that they had high values of GSH-PX.

On the other hand, results in Table (7) show the different values of GSH-PX in the liver tissue of different groups of rats. Results show that the concentration values of GSH-PX increased with the increasing of radish extracts. So, the groups of rats those had 400 g RRR/kg/day (G11) and those had 200 mg RRR/kg/day had the highest values of GSH-PX compared with the other corresponded groups of rats. However, G8 and G5 (groups had 400 mg WRR/kg/day and 400 mg WRL/kg/day) were not as the same with G11 (400 mg RRR/kg/day), this could be attributed to the strength of phenolic compounds in RRR than WRR or WRL. Our results are in accordance with the results of [58] who reported that the activity of glutathione peroxidase in erythrocytes of the black radish juice-supplemented hyperlipidaemic rats was higher than that in non-supplemented hyperlipidaemic animal. Moreover, caffeic acid, ferulic acid and coumaric acid might lessen cholesterol induced oxidative injury and may be capable of lowering or slowing down oxidative stress [59, 60].

It could be shown from the results in Table (7) the effect of different radish extracts on the levels of MDA (nmole/ml serum) and MDA (nmole/g tissue). Results show the decrement for all the different groups of rats that were in significant difference those affected by the administration of radish extracts. Control sample (G1) recorded the lowest values of MDA either in serum or in live tissue of rats. The lowest values of MDA (nmole/ml serum) or (nmole/g of liver tissue) could be shown for G11 followed by G10, this decrement could be attributed to red radish root extracts in the concentrations of 400 mg or 200 mg/RRR/kg/day. Our results are in agreement with the results of [61] and [62] who reported that cholesterol, triglyceride, malondialdehyde and conjugated diene concentrations were significantly higher in the sera of hyperlipidaemic rats compared to the control. Lipid peroxidation characteristics and cholesterol content in the steatotic liver were also significantly higher. Also they added that supplementation of the lipid rich diet with black radish juice resulted in a significant decrease in the parameters measured.

IV. Conclusion

It could be concluded that white radish leaves or roots and red radish roots extracts have phenolic compounds like ferulic, sinapic, catechin and coumaric, they have antioxidant activity and therefore they have an important role as hypoglycemic and hypocholesterolemic agents. From the results it could be concluded that the best effect was attributed to the red radish extract.

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Table 1. Composition of basal and different diabetic diets (g/100g diet)

Ingredient	(G1) Control basal diet	(G2) diabetic control	(G3) diabetic treated with insulin	(G4) diabetic +100 mg WRL /kg/day	(G5) diabetic +200 mg WRL /kg/day	(G6) diabetic + 400 mg WRL /kg/day	(G7) diabetic +100 mg WRR /kg/day	(G8) diabetic + 200 mg WRR /kg/day	(G9) diabetic + 400 mg WRR /kg/day	(G10) diabetic + 100 mg RRR /kg/day	(G11) diabetic + 200 mg RRR /kg/day	(G12) diabetic + 400 mg RRR /kg/day
Starch	63.3	63.3	63.3	63.2	63.1	62.9	63.2	63.1	62.9	63.2	63.1	62.9
*casein	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
Com oil	10	10	10	10	10	10	10	10	10	10	10	10
celulose	5	5	5	5	5	5	5	5	5	5	5	5
Vit-mix	1	1	1	1	1	1	1	1	1	1	1	1
Salt-mix	4	4	4	4	4	4	4	4	4	4	4	4
*WRL	-	-	-	100	200	400	-	-	-	-	-	-
*WRR	-	-	-	-	-	-	100	200	400	-	-	-
*RRR	-	-	-	-	-	-	-	-	-	100	200	400

*Casein contained 90% protein, *WRL :White Radish Leaves,*WRR :White Radish Roots,*RRR : Red Radish Roots

Table 2. Composition of basal and high fat diets (HF diets) (g/100g diet)

Ingredient	(G1) Control basal diet	(G2) HFD + 1% cholesterol	(G3) HFD + 1% cholesterol+ 100 mg WRL /kg/day	(G4) HFD + 1% cholesterol +200 mg WRL /kg/day	(G5) HFD + 1% cholesterol +400 mg WRL /kg/day	(G6) HFD + 1% cholesterol +100 mg WRR /kg/day	(G7) HFD + 1% cholesterol +200 mg WRR /kg/day	(G8) HFD + 1% cholesterol +400 mg WRR /kg/day	(G9) HFD + 1% cholesterol +100 mg RRR /kg/day	(G10) HFD + 1% cholesterol + 200 mg RRR /kg/day	(G11) HFD + 1% cholesterol +400 mg RRR /kg/day
Starch	63.3	50.62	50.52	50.42	50.22	50.52	50.42	50.22	50.52	50.42	50.22
*casein	16.7	15	15	15	15	15	15	15	15	15	15
Com oil	10	8	8	8	8	8	8	8	8	8	8
Cellulose	5	5	5	5	5	5	5	5	5	5	5
Vit-mix	1	1	1	1	1	1	1	1	1	1	1
Salt-mix	4	4	4	4	4	4	4	4	4	4	4
Beef tallow	-	15	15	15	15	15	15	15	15	15	15
Cholesterol	-	1	1	1	1	1	1	1	1	1	1
Colin bitartate	-	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cholic acid	-	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
*WRL	-	-	100	200	400	-	-	-	-	-	-
*WRR	-	-	-	-	-	100	200	400	-	-	-
*RRR	-	-	-	-	-	-	-	-	100	200	400

*Casein contained 90% protein,*WRL :White Radish Leaves,*WRR :White Radish Roots,*RRR : Red Radish Roots

Table 3. Chemical composition of the white radish leaves^{*}, white radish roots^{**} and red radish roots^{***} (on dry weight basis).

Component	WRL	WRR	RRR	LSD (P≥0.05)
Crude protein	13.2b ± 0.22	14.9 ^a ± 0.25	12.4 ^c ± 0.30	0.517
Crude fat	3.00 ^a ± 0.11	2.11 ^b ± 0.21	1.54 ^c ± 0.24	0.389
Crude fiber	23.2 ^a ± 0.41	12.9 ^b ± 0.35	12.63 ^b ± 0.36	0.748
Ash	15.2a ± 0.55	13.2 ^b ± 0.25	12.9 ^b ± 0.35	0.805
Total carbohydrates	45.40 ^c ± 1.69	56.89 ^b ± 1.55	60.53 ^a ± 1.24	3.007
Moisture	90.06 ^b ± 1.12	95.37 ^a ± 1.14	97.46 ^a ± 1.53	3.260

* WRL, ** WRR, *** RRR

Table 4. Total phenolic content and antioxidant activity of the white radish leaves^{*}, white radish roots^{**} and red radish roots^{***} (on dry weight basis).

Plant part	White radish		Red radish	LSD (P≥0.05)
	Leaves (WRL)	Roots (WRR)	Roots (RRR)	
Total phenolic content (g catechin/ 100g dried sample)	1.612 ^a ±0.03	0.831 ^c ±0.03	1.240 ^b ±0.10	0.125
Antioxidant activity %	75.4 ^a ±0.90	35.6 ^c ±0.83	66.4 ^b ±0.65	1.60

* WRL, ** WRR, *** RRR

Table 5. Phenolic compounds (g/100g) of white radish leaves^{*}, white radish roots^{**} and red radish roots^{***} extracts.

Phenolic acids g/100g ^{****}	White radish		Red radish roots
	Leaves	Roots	
Ferulic	0.130	0.180	0.333
Sinapic	0.344	0.480	0.340
Catechin	0.500	0.852	0.365
Coumaric	0.213	0.121	0.520
Vanillic	0.423	0.140	0.410
Caffeic	0.170	0.200	0.301
Gallic	0.020	0.018	0.054
Syringic	0.053	0.149	0.120
Rutin Protocatechuic acid	0.350	0.050	0.085
	0.330	0.038	0.040
Total	2.533	2.228	2.568

* WRL, **WRR, ***RRR ****g/100g dry wt extract

Table (6) : Effect of white radish leaves^{*}, white radish roots^{**} and red radish roots^{***} extracts on the level of serum glucose, total cholesterol, LDL, HDL cholesterol and triglycerides in diabetic rats.

Groups	Glucose (mg/dl serum)	Total cholesterol (mg/dl serum)	LDL (mg/dl serum)	HDL (mg/dl serum)	Triglycerides (mg/dl serum)
G1	99.4 ± 88	86.89 ± 0.88	27.22 ± 0.73	48.57 ± 0.74	52.46 ± 1.67
G2	340.3 ^a ± 2.03	124.87 ^a ± 1.34	78.99 ^a ± 1.27	32.04 ^a ± 1.42	88.26 ^b ± 1.61
G3	119.04 ^b ± 1.82	93.43 ^b ± 1.23	25.53 ^b ± 0.64	46.35 ^b ± 0.86	53.53 ^b ± 0.92
G4	178.32 ^b ± 1.98	118.23 ^b ± 1.2	59.33 ^b ± 0.98	34.15 ^b ± 1.28	82.05 ^b ± 1.11
G5	165.41 ^c ± 1.25	112.77 ^c ± 0.86	55.4 ^c ± 1.28	36.49 ^b ± 0.87	76.24 ^b ± 1.85
G6	156.63 ^c ± 1.64	108.97 ^{de} ± 1.42	52.28 ^d ± 1.56	39.16 ^{de} ± 1.33	73.8 ^d ± 2.11
G7	169.34 ^c ± 0.99	113.74 ^c ± 1.67	53.92 ^{cd} ± 1.35	35.73 ^{gh} ± 1.82	79.22 ^c ± 2.51
G8	153.12 ^f ± 0.98	107.36 ^e ± 0.88	49.98 ^e ± 0.87	38.14 ^{ef} ± 1.53	74.35 ^d ± 0.92
G9	140.63 ^g ± 1.73	103.63 ^g ± 1.55	46.61 ^{fg} ± 1.99	40.43 ^{cd} ± 0.75	68.82 ^e ± 0.77
G10	155.91 ^e ± 0.73	110.32 ^d ± 2.1	48.12 ^{ef} ± 0.66	38.97 ^{de} ± 0.95	75.89 ^d ± 0.86
G11	142.42 ^g ± 1.88	105.68 ^{fg} ± 1.97	45.05 ^g ± 0.84	41.93 ^c ± 1.09	63.24 ^f ± 1.58
G12	130.12 ^h ± 1.05	99.12 ^h ± 0.85	41.82 ^h ± 1.52	45.28 ^b ± 0.81	59.73 ^g ± 1.54
LSD (P>0.05)	2.50	2.37	2.04	1.97	2.61

* WRL, ** WRR, *** RRR

G1: Control (basal diet), G2: Diabetic, G3: Diabetic injected with 20 units insulin /kg b.w/day, G4: Diabetic+ 100 mg WRL/kg b.w/day, G5: Diabetic+ 200 mg WRL/kg b.w/day, G6: Diabetic+ 400 mg WRL/kg b.w/day, G7: Diabetic+ 100 mg WRR/kg b.w/day, G8: Diabetic+ 200 mg WRR/kg b.w/day, G9: Diabetic+ 400 mg WRR/kg b.w/day, G10: Diabetic+ 100 mg RRR/kg b.w/day, G11: Diabetic+ 200 mg RRR/kg b.w/day, G12: Diabetic+ 400 mg RRR/kg b.w/day.

Different letters within each column indicate significant difference. The data are expressed in mean ± S.D.

Table (7) : Effect of white radish leaves* and white radish roots** and red radish roots***extracts on the level of total cholesterol, LDL, HDL, triglycerides, glutathione peroxidase (GSH-PX) and malondialdehyde (MDA) in liver, blood and serum hypercholesterolemic rats.

Groups	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	GSH-PX		MDA	
					Liver (nmol/mg)	Blood (nmol/ml)	Liver (nmol/g T)	Serum (nmol/ml)
G1:	86.89 [±] 1.22	27.22 [±] 1.42	48.57 ^{a±} 0.99	43.46 ^{h±} 1.60	44.3 ^{a±} 1.84	844.88 ^{a±} 2.45	4.77 ^{g±} 1.22	210.16 [±] 2.04
G2:	140.88 ^{a±} 2.01	88.22 ^{a±} 1.52	30.31 ^{g±} 0.84	91.41 ^{a±} 2.10	22.39 ^{g±} 2.01	643.32 [±] 1.98	17.8 ^{a±} 1.19	246.33 ^{a±} 2.11
G3:	119.12 ^{b±} 1.2	50.84 ^{b±} 0.68	36.57 ^{f±} 2.05	60.21 ^{b±} 1.01	29.93 ^{f±} 1.42	788.12 [±] 2.19	12.46 ^{b±} 1.33	223.72 ^{b±} 1.86
G4:	113.21 ^{d±} 1.8	47.91 ^{c±} 1.60	39.68 ^{de±} 1.89	55.82 ^{cd±} 0.74	34.35 ^{de±} 1.89	792.36 ^{h±} 2.09	10.96 ^{bcd±} 1.57	219.10 ^{c±} 1.88
G5:	108.30 ^{f±} 1.2	44.01 ^{de±} 0.59	41.05 ^{d±} 1.33	51.38 ^{f±} 1.43	37.14 ^{cd±} 2.11	803.49 ^{f±} 1.77	9.16 ^{cde±} 0.99	214.32 ^{de±} 1.79
G6:	115.91 ^{c±} 0.98	46.05 ^{c±} 1.73	37.28 ^{ef±} 2.07	57.59 ^{c±} 1.71	33.98 ^{e±} 1.55	795.81 ^{g±} 1.17	11.33 ^{bc±} 1.25	218.73 ^{c±} 2.09
G7:	109.69 ^{ef±} 1.07	42.15 ^{ef±} 0.85	40.45 ^{d±} 1.45	52.26 ^{ef±} 1.56	36.43 ^{cde±} 1.08	801.13 ^{f±} 2.09	9.06 ^{de±} 1.12	215.07 ^{d±} 1.42
G8:	104.31 ^{g±} 1.44	40.23 ^{fg±} 0.91	44.13 ^{b±} 0.88	48.02 ^{g±} 0.67	38.61 ^{bc±} 1.88	812.85 ^{d±} 1.11	7.89 ^{ef±} 1.82	211.63 ^{ef±} 1.56
G9:	110.85 ^{e±} 0.92	43.16 ^{e±} 1.66	41.38 ^{cd±} 1.67	54.66 ^{de±} 1.31	35.87 ^{cde±} 1.14	807.03 ^{e±} 1.62	10.15 ^{cd±} 1.26	213.43 ^{def±} 1.79
G10:	105.05 ^{g±} 1.56	39.21 ^{g±} 0.66	43.69 ^{bc±} 0.66	48.53 ^{g±} 0.77	38.13 ^{bc±} 1.77	818.63 ^{e±} 1.58	7.12 ^{ef±} 1.27	210.43 ^{fg±} 1.88
G11:	100.03 ^{h±} 0.85	37.34 ^{h±} 0.82	46.89 ^{a±} 1.70	45.55 ^{h±} 1.93	40.94 ^{b±} 1.91	830.07 ^{b±} 1.62	5.89 ^{fg±} 0.89	205.16 ^{h±} 1.94
LSD (P≥0.05)	2.28	2.05	2.53	2.42	2.92	3.06	2.18	3.15

*WRL, **WRR, ***RRR

G1: Control (basal diet) , G2: HFD+1%cholesterol , G3: HFD+1%cholesterol + 100 mg WRL/kg b.w/day, G4: HFD+1%cholesterol + 200 mgWRL /kg b.w/day, G5: HFD+1%cholesterol + 400 mg WRL/kg b.w/day, G6: HFD+1%cholesterol + 100 mg WRR/kg b.w/day, G7: HFD+1%cholesterol + 200 mg WRR/kg b.w/day, G8: HFD+1%cholesterol + 400 mg WRR/kg b.w/day, G9: HFD+1%cholesterol + 100 mg RRR/kg b.w/day,G10: HFD+1%cholesterol + 200 mg RRR/kg b.w/day, G11: HFD+1%cholesterol + 400 mg RRR/kg b.w/day

Different letters within each column indicate significant difference.

The data are expressed in mean ± S.D.