Archives of Razi Institute, Vol. 77, No. 5 (2022) 1865-1871

DOI: 10.22092/ARI.2022.358160.2164

Original Article

Investigation of the Effect of *Rhubarb* stalks extracts on Mice Exposed to Oxidative Stress

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Received 21 March 2022; Accepted 23 May 2022
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Abstract

Normal blood lipid levels have a crucial role in lowering cardiovascular mortality. This study was designed to investigate the effect of aqueous rhubarb extract on serum glucose, cholesterol, total lipids, peroxynitrite, malondialdehyde, glutathione, and ceruloplasmin levels, as well as glutathione and malondialdehyde levels in the liver, kidney, and heart tissue in mice exposed to oxidative stress. 40 Balb/c mice were randomly allocated into 8 groups (n=5). Group 1: The control group was left eating feed and water without treatment for (15) days. Group 2: A group exposed to oxidative stress by giving hydrogen peroxide at a rate of (0.5%) with drinking water for 15 days. Group 3: A group exposed to oxidative stress induced by hydrogen peroxide at a rate of (0.5%) for 15 days with injecting on the seventh day, daily for a week, with insulin subcutaneously (15) units/kg. Group (4-8): the Groups were exposed to the oxidative stress induced by hydrogen peroxide (0.5%) for 15 days with injecting on the seventh day into the peritoneal cavity with both the cold aqueous and nonprotein extract, the extract of flavonoids at a dose of 400, 400, 0.4, 8.8, 1.96 mg/kg body weight, respectively. All animals were anesthetized on the last day of the experiment, blood samples were obtained for biochemical testing, and tissue samples from the livers were collected for research. The results revealed that the cold crude aqueous, non-proteinous extracts, flavonoids, proteinous precipitate, and proteinous compound caused a significant decrease (P<0.05) in serum glucose, cholesterol, total lipids, peroxynitrite, malondialdehyde levels in kidney, liver, and heart. The recorded data showed a significant increase (P<0.05) in serum glutathione and ceruloplasmin in serum and glutathione levels in liver, kidney, and heart tissues in male mice exposed to oxidative stress. The results showed that all *Rhubarb* extracts have an antioxidant effect in mice exposed to oxidative stress.

Keywords: Oxidative stress, Antioxidant, *Rhubarb*

1. Introduction

Herbs, spices, and plants have been demonstrated to have a broad spectrum of pharmacological and therapeutic properties, and most recent studies have focused on isolating and identifying the molecules found in these plants for medical and biomedical purposes. The Polygonaceae family includes *Rhubarb*. Rheum comes in a variety of forms. Some, like Rheum *Officinale B.* and *Rheum palmatum L.*, are known as medicinal Rhubarb, while others, like *Rheum rhabarbarum L.*, are known as vegetable *rhubarb*. The vegetable *rhubarb*, *Rheum rhabarbarum L.*, is a central Asian wild plant (1). *Rhubarb* (*Rheum rhabarbarum L.*) has been used as a traditional remedy in far eastern nations. For over a thousand years, medicinal *rhubarb* (peeled dried root and rhizome of *Rhubarb*) has been used as a laxative (2). For decades, medicinal *Rhubarb* has been used to treat chronic renal failure in China and Japan (3).

R. rhaponticum (in beer or mead) was used to treat gastrointestinal discomfort, gastritis, liver and spleen

problems, heartbreak and pericardium pain, pulmonary system dysfunctions, and reproductive system diseases, such as uterine and breast pains. It was thought that drinking *R. rhaponticum*-based concoctions or eating its root would help with dyspepsia. Externally, a vinegar macerate from this plant was used to treat skin conditions, including itching and scratching, while a water macerate was indicated for ulcer treatment. *R. rhabarbarum* was traditionally used as a purgative (4). The antioxidant action of methanolic extracts of *Rheum palmatum* and *Rheumribes* leaves and stalks was investigated with the discovery of emodin. Berköz, YILDIRIM (1) reported that the leaves of *Rheum rhabarbarum L.* had antioxidant activity.

The antioxidant activities of *R. rhaponticum*-derived extracts have been examined in early radical-scavenging experiments (based on the DPPH and ABTS+ radicals), mainly at concentrations well above physiologically attainable levels of natural compounds and their metabolites (5). Therefore, the current research aimed to investigate the effect of aqueous extract from *Rheum rhabarbarum L.* on lipid oxidation and oxidative stress.

2. Materials and Methods

2.1. Plant Collection

Rhubarb stalks were collected in their flowering season from several locations in Mosul, Iraq. It was accorded a category based on medicinal plant classification reference. At the University of Mosul's College of Education's herbariums, a voucher specimen of the plant was also identified and recorded. Plant specimens' roots were removed from their shoots, rinsed in tap water, and dried in the shade for seven days.

2.2. *Rhubarb* Extraction

Flavonoids, protein, and nonprotein extracts from the *Rhubarb* Stalks plant were prepared according to Abed and Saadon (6) technique.

2.3. Animals Experimentation

White male mice weighing 185 to 235 grams were obtained from the College of Veterinary Medicine at the University of Mosul. The animals were housed in standard cages with free access to water and food (a typical laboratory pellet diet). With a 12-hour light/dark cycle, the temperature in the animal habitat was adjusted between 24 and 29 ° C.

2.4. Design Experimentation

40 Balb/c mice were randomly allocated into 8 groups (n=5). Group 1: The control group was left eating feed and water without treatment for (15) days. Group 2: A group exposed to oxidative stress by giving hydrogen peroxide at a rate of (0.5%) with drinking water for 15 days. Group 3: A group exposed to oxidative stress induced by hydrogen peroxide at a rate of (0.5%) for 15 days with injecting on the seventh day, daily for a week, with insulin subcutaneously (15) units/kg. Group (4-8): the Groups were exposed to the oxidative stress induced by hydrogen peroxide (0.5%) for 15 days with injecting on the seventh day into the peritoneal cavity with both the cold aqueous and nonprotein extract, the extract of flavonoids at a dose of 400, 400, 0. 4,8. 8, 1.96 mg/kg body weight, respectively.

2.5. Determination of the Effective Dose of *Rhubarb* Stalks

Healthy mice weighing 22-26 g were used to determine the effective dose. Then they were divided into 6 groups of (5) mice each and were treated as follows:

1. The first group was injected into the peritoneal cavity with (0.1) ml of local physiological solution (Normal Saline) and returned as a control group.

2. Groups of (2-6) were injected into the peritoneal cavity with doses of (100, 200, 300, 400, and 500) mg/kg of body weight, respectively, with the cold crude aqueous extract. Two hours after the injection into the peritoneal cavity, the glucose level in the blood was estimated. The most effective dose for lowering the blood glucose level was chosen.

2.6. Analysis of Biochemical Parameters

They estimated glucose, total cholesterol, and total lipids using the Standard Kits from Biolabo Company. The level of peroxy nitrate in the serum was estimated using the modified method for researchers (7). The

ceruloplasmin was estimated by the method for the researcher (8). Using Ellman's reagent (DTNB) [5,5-dithiobis(2-Nitrobenzoic acid)], the amount of GSH in the liver, kidney, and heart tissues was evaluated using a modified technique described by (9), while MDA levels were estimated using the method described by Guidet and Shah (10).

2.7. Statistical Analysis

To analyze the data, a one-way ANOVA was used. Duncan's novel multiple range tests were used to solve the variation between treatment means. For all statistical studies, the statistical tool SPSS 28.0 was utilized (SPSS Ltd., Surrey, UK).

3. Results

3.1. Separation of Protein Sediments from Cold Aqueous Extract of *rhubarb* Stalks

Protein sediments were separated by gel filtration technique using a dimensional column (90 × 2 cm) and containing the gel of Sephadex G- 100, as shown in the figure 1, where figure 1 shows the separation of the concentrated solution of the protein precipitate isolated from the cold aqueous extract of *rhubarb* stalks. The separation result was detectable by the presence of one peak at the elution volume of 120.4 mL.

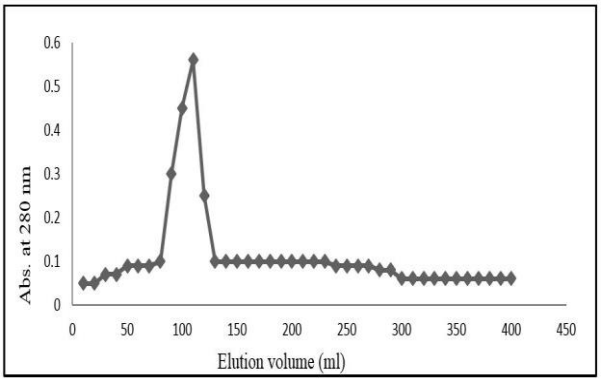

Figure 1. Elution volume for a cold proteinous extract from *Rhubarb* stalks using column (90 x 2) cm containing Sephadex G-100

To determine the approximate molecular weights of protein compounds separated by the gel filtration technique. Several known molecular weight materials were passed through the separating column with dimensions of 90 × 2 cm^2. The recorded data showed that the molecular weight at 204,000,000) was Dalton. Then to determine the size of the Rogan, as shown in table 1, the plotting of the size of the substance was used for each substance against the logarithm of the molecular weight, and the standard curve for estimating the molecular weight was obtained as shown in figure 2 through which the approximate molecular weight of the separated protein compound was determined.

Table 1. Elution volumes of materials with known molecular weights on Sephadex G-100

Material	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	2000000	66
BSA	67000	101
Egg albumin	45000	121
Trypsin	23000	134
Insulin	5734	285
Tryptophan	204	436

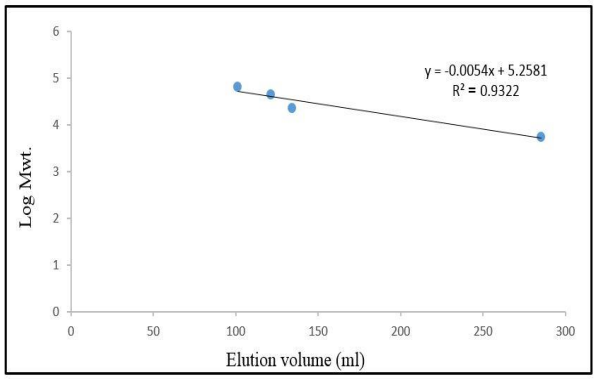

Figure 2. The plot of the logarithmic molecular weight of known proteins versus elution volume using column (90 x 2) cm containing Sephadex G-100

By projecting the volumes of the protein compounds separated by the gel filtration technique onto the standard curve in figure 2, the approximate molecular weight of the protein compound separated from *rhubarb* stalks was determined as follows: Molecular weight of 51118.9 Dalton. Table 2 shows the determination of the most effective dose in reducing glucose levels in healthy male mice of the cold crude aqueous extract. The practical dose value was 400 mg/kg body weight.

The treatment with insulin at a dose of (10) IU/kg body weight under the skin in healthy male mice led to a significant decrease (P<0.05) in the levels of glucose, cholesterol, total lipids, malonaldehyde and peroxynitrite, and a significant increase (P<0.05) in the levels of glutantalion and ceruloplasmin in the blood serum compared with the group of mice exposed to oxidative stress induced with hydrogen peroxide with drinking water only, as shown in table 3. Through the treatment of mice exposed to oxidative stress mediated by hydrogen peroxide with crude and nonprotein aqueous extract, flavonoid extract, protein precipitate, and protein compound, which was separated by gel filtration technique by intraperitoneal injection at a dose of (400, 400, 0.4, 8.8, 1.96) mg/ It was found that treatment with the above extracts led to a significant decrease (P<0.05) in the levels of glucose, total lipids, cholesterol, peroxynitrite, and malondialdehyde in the blood serum as shown in table 3 and the levels of malonaldehyde in liver, kidney and heart tissues as shown in table 4 It also caused a significant increase (P<0.05) in the levels of ceruloplasmin and glutathione in the blood serum as shown in table 3 and tissue glutathione levels as shown in table 4.

Table 2. Determination of the effective dose in reducing glucose level in healthy male mice of the cold crude aqueous extract

Glucose levels Mm/L	Dose of the cold crude aqueous extract					
	Control	100	200	300	400	500
	5.3± 0.22	4.89±0.31	4.01±0.41	3.63±0.28	0.26±3.11*	4.02 ±0.18

Table 3. Effect of *rhubarb* extracts on levels of some biochemical parameters

Parameters / Study groups	Glucose mM/L	Cholesterol mM/L	Total lipids Mg/100cm3	Peroxynitrite µmol /L	Glutathione µmol /L	Malondialdehyde µmol /L	Ceruloplasmin µmol /L
Control	6.43±0.04^{d}	3.11±0.22^{ab}	374.8±11.4^{c}	175.42±22.1^{c}	13.03±0. 22^{d}	0.66±0.04^{de}	273.6±5.2^{g}
Mice treated with hydrogen peroxide	7.75±0. 28^{c}	3.86±0.54^{cd}	535.14±13.3^{f}	246.4±12.1^{i}	9.64±0.11^{a}	0.94±0.03^{g}	130.4±4.2^{a}
Insulin (10U/Kg)	4.32±0.22^{a}	3.23±0.09^{ab}	372.61±20.11^{c}	185.8±17.1^{e}	11.82±0.31^{c}	0.71±0.05^{of}	250.8±5.6^{f}
Code cold extract 400mg/Kg	5.83±0.22^{b}	3.11±0.05^{ab}	261.41±30.39^{b}	203.6±14.1^{g}	10.62±0.42^{b}	0.78±0.07^{f}	196.46±4.1^{c}
Nonprotienous cold extract 400mg / Kg	5.21±0.26^{b}	3.31±0.33^{ab}	251.25±8.21^{b}	178±11.1^{d}	12.82±0.31^{d}	0.54±0.06^{cde}	212.8±4.5^{d}
Flavonoid extract 0.4mg/Kg	5.08±0.12^{b}	2.98±0.06^{a}	186.26±7.33^{b}	152.6±12.1^{a}	14.04±0.36^{e}	0.42±0.07^{a}	292.4±6.2^{h}
Cold Potienous Precipitate 8.8 mg / Kg	6.51±0.3^{d}	3.42±0.32^{ab}	196.42±23.1^{a}	192.6±26.2^{f}	11.62±0.52^{c}	0.56±0.05^{bc}	185.9±4.2^{b}
Proteins compound 1.96 mg/Kg	6.62±0.32^{d}	4.1±0.22^{d}	399.2±26.1^{d}	160±13.1^{b}	13.33±0.75^{d}	0.61±0.05^{cd}	231.9±10.7^{e}

*Different letters indicate significant differences at the $P \leq 0.05$

* Similar letters indicate no significant differences at the $P \leq 0.05$

Table 4. Effect of different extracts from *Rhubarb* stalks on levels of malonaldehyde and glutathione in liver, kidney, and heart tissues

Parameters / Study groups	Glutathione (Nano mole/g)			Malonaldehyde (Nano mole/g)		
	Liver	**Kidney**	**Heart**	**Liver**	**Kidney**	**Heart**
Control	6945.6±20.2^{h}	2460.2±20.3^{f}	2280.8±30.2^{d}	349.3±10.6^{b}	286.4^{b}	245.3±21.4^{a}
Mice treated H^2O^2 0.5% with water	4482.3±19.3^{a}	1740.6±23.5^{a}	2110.2±24.1^{a}	534.6±14.6^{f}	499.5±19.2^{d}	694.3±23.3^{f}
Insulin 10 units/kg	6252.3±17.5^{f}	2420.9±22.6^{f}	2260.6±20.3^{d}	320.5±13.5^{ab}	250.5±17.4^{a}	326.4±20.1^{b}
Cold extract 400 mg/kg	5325.4±19.3^{d}	2240.4±20.2^{d}	2215.9±20.4^{c}	412.6±16.6^{cd}	406.4±20.2^{c}	511.8±13.2^{e}
Non proteinous extract 400 mg/kg	5722.5±24.6^{e}	2210.7±19.9^{d}	2243.2±19.3^{cd}	382.8±18.2^{c}	396.2±14.2^{b}	515.6±19.2^{e}
Flavonoids 0.4 mg/kg	7024.2±21.5^{i}	2450.4±18.4^{f}	2265.7±17.5^{d}	316.87±22.58^{a}	243.4±18.2^{a}	220.7±15.8^{a}
Proteins participate 8.8 mg/kg	4915.61±20.4^{b}	2334.6±20.2^{e}	2164.8±13.3^{b}	436.8±15.5^{d}	398.5±17.5^{c}	476.3±14.4^{d}
Proteins compound 1.96 mg/kg	5102.6±17.4^{c}	1990.6±15.5^{b}	2215.8±20.5^{c}	406.2±16.7^{cd}	264.8±16.2^{ab}	318.6±24.6^{b}

*Different letters indicate significant differences at the $P \leq 0.05$

* Similar letters indicate no significant differences at the $P \leq 0.05$

4. Discussion

Drug manufacturing depends solely on medicinal plants. Because of the increased focus on side effects associated with using synthetic substances to treat diseases, pharmaceutics is increasingly focused on finding new, effective, and less hazardous medications. Natural chemicals derived from medicinal plants have significantly contributed to the development of medications for treating various ailments (11). A 15-day treatment with 0.5% hydrogen peroxide in drinking water resulted in a considerable rise in glucose levels in male mice. Suggesting this increase could be that peroxide Hydrogen causes an increase in oxygen pressure, hyperoxia, and thus an increase in active types of oxygen that attack and destroy beta cells in the pancreatic islets of Langerhans, nullifying insulin synthesis and stimulating glucose formation and glycogenolysis (12).

Total cholesterol levels were dramatically raised after treatment with hydrogen peroxide. This rise might be due to the oxidation of (100-apo B) in total low-density lipoprotein (12). Oral injection of hydrogen peroxide increased total lipid levels significantly, possibly owing to suppression of the enzyme lipoprotein lyase (4). The impact of *rhubarb* stalk fiber on hypercholesterolemia in humans was investigated in a study. In this trial, using *rhubarb* stalk powder twice daily for four weeks reduced total and LDL cholesterol considerably (13). Another research found that rhubarb hydro alcoholic extract lowered cholesterol in hypercholesterolemic rabbits compared to nicotinic acid. In addition, the mechanism of action of Rhubarb hydroalcoholic extract on blood cholesterol reduction in experimental animals remains unknown. In another study, Rhubarb increased bile production while decreasing blood cholesterol and cholesterol esters in the liver in mice (13). Another study found that *rhubarb* extracts lowered cholesterol and triglyceride levels caused by hypothyroidism, suggesting that they may be able to prevent hypercholesterolemia, hypertriglyceridemia, and other cardiovascular diseases in hypothyroid individuals. However, the minimal dose did not significantly lower blood cholesterol in the preventive 1 group, which might be attributed to reduced amounts of beneficial components in the extract (13). The level of serum MDA was significantly different between the groups compared to the negative control group, which agrees with several studies suggesting that *rhubarb* stalk can remove free radicals and reduce malondialdehyde using existing flavonoids (14). Another study found that rabbits on cholesterol-rich diets had higher plasma MDA levels. Similar findings were also obtained in their research.

MDA is a lipid peroxidation product that, by generating internal and intermolecular bridges, can inactivate membrane carriers. Cholesterol-rich diets significantly alter antioxidant defense systems.

Rhubarb can help with constipation, hyperlipidemia, hepatic damage, and diabetes, according to modern pharmacology (15). According to a chemical study, Anthraquinones, tannins, polyose, phenylbutazones, and stilbenes are found in *Rhubarb* (16). The findings also showed that insulin administration reduced total fat levels in mice subjected to oxidative stress generated by hydrogen peroxide compared to animals treated with hydrogen peroxide and drinking water, which is in line with earlier research (6). The results, on the other hand, indicated a rise in the levels of glutathione and ceruloplasmin in the blood of mice, as well as the level of glutathione in the tissues, and these findings were consistent with the researcher's findings (17). In summary, this case-control analysis demonstrated that *Rhubarb* positively affects albino mice through reduced levels of blood glucose, lipid profile, Peroxynitrite, Glutathione, Malondialdehyde, and Ceruloplasmin.

Authors' Contribution

Study concept and design: L. F. B., L. A. A. B. and S. Z. J. A.
Acquisition of data: L. F. B., L.
Analysis and interpretation of data: L. A. A. B.
Drafting of the manuscript: L. A. A. B.
Critical revision of the manuscript for important intellectual content: L. F. B., L. A. A. B. and S. Z. J. A.
Statistical analysis: L. F. B.
Administrative, technical, and material support: L. F. B., L. A. A. B. and S. Z. J. A.

Ethics

The Study Protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to express their appreciation to the biology departments of Mosul University's College of Education, where the research was conducted in their laboratory.

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