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Nephroprotective and Antioxidant Effects of Parsley Plant Parts Against Gentamicin-Induced Nephrotoxicity in Rats

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Abstract: The nephroprotective and antioxidant effects of parsley plant as watery extract of fresh leaves (decoction), fresh leaves, seeds and seeds oil against gentamicin (GM)-induced nephrotoxicity in in male albino rats were investigated. Thirty six male albino rats (*Sprague Dawley strain*) weight 140±10g gm were investigated. Rats were divided into two main groups, the first group was used as a control negative fed on the ration only (6 rats). The second main group (30 rats) was divided in to five groups one of them leaved fed on basal diet only and others supplemented their diet with fresh parsley leaves (L), parsley seeds (S), parsley seeds oil (O) and oral administrated with parsley leaves extract (E) for 15 days. After that all groups were injected intraperitoneally (i.p.) with GM. One of these groups was left as a positive control group, whereas the other four groups were fed on basal diet previous parsley products for other 8 days during injection with GM. At the end of the experiment biological data were calculated, blood samples were taken, internal organs were collected, weighted. Serum was separated to biochemical analysis. The results indicated that BWG was decreased due to receiving parsley leaves extract (E) and parsley seeds oil (O). Feed efficiency ratio had significant decreases in C+ve and supplemented diet groups except seeds group, which had significant increase. Serum kidney tests and liver function tests were reduced significantly in rats groups received parsley products as compared to positive control group. GM (i.p.) rats group showed significant decrease in triglyceride, VLDL-C and HDL-C; on the other hand, all supplemented parsley diet groups showed gradually significant increase in HDL-c in O, L and S groups, while triglyceride and VLDL-C showed non-significant decreases except L group had significant decrease. Serum total cholesterol and LDL-C showed significant increase of C+ve group, all supplemented parsley diet induced gradually significant decreases, the best one in total cholesterol was S group and LDL-C was O group. All rats injected with GM (i.p.) showed significant decrease in GSH and increase in MDA, but all supplemented parsley diet showed increase in GSH and decrease in MDA. This study recommended that the consumption of parsley plant parts may be useful for patients who suffer from renal diseases and those on GM therapy.

Key words: Nephroprotective · Parsley · Gentamicin · Biochemistry · Nephrotoxic rats

INTRODUCTION

Kidney is an important organ within the human body. They guard blood volumes, filter the blood to form urine, regulate water, electrolytes, acid/base balance, produce some hormones and participate in metabolism of others. At rest an estimated 20% of cardiac output flows through the kidney where they are filtered and reconditioned [1]. Nephrotoxicity caused by GM) gentamicin (seems to be attributed to the oxidative stress caused by generation of reactive oxygen species [2, 3]. Aminoglycoside antibiotics can stimulate formation of reactive oxygen species (ROS) and cause oxidative stress [4]. ROS scavengers and antioxidants can be used to alleviate GM-induced nephrotoxicity [5, 6]. Aminoglycoside antibiotics are employed clinically because of their potent bactericidal activities, less bacterial resistance, post-antibiotic effects and low cost. Gentamicin, a commonly used

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aminoglycoside, is associated with an induction of tubular necrosis, epithelial oedema of proximal tubules, cellular desquamation, tubular fibrosis, glomerular congestion, perivascular edema and inflammation, which ultimately show the way to renal dysfunction. It is a matter of debate whether we have promising agents to prevent the incidence of gentamicin-induced nephrotoxicity [7].

Phytotherapy is the prevention and treatment of disease using plants, plant parts and preparation made from them. Plants were traditionally used in phytotherapy are called medicinal plants or herbs [8]. According to some authors phytotherapy is the only branch of herbal medicine in which license covers photochemistry, phototherapy, phytopharmacy and phytopharmacology.

It is important to mention that parsley contains more vitamin C than lemon, orange or any other fruit. It has abundant quantities of other vitamins and minerals such as: pro-vitamin A, vitamin B, vitamin E, vitamin K, beta-carotene, magnesium, phosphorus, iron, manganese, sodium, potassium, sulfur and calcium. It acts like an antioxidant (eliminates toxins and maintains the elasticity of the blood vessels), it is a general stimulant, diuretic, antiseptic, anti-infectious, antirachitic and more. Among other effects that it has: it straightens the body and immune system, has a beneficial effect over the liver, spleen, digestive and endocrine organs [9]. Parsley contains many health benefiting essential volatile oils that include myristicin, limonene, eugenol and alpha-thujene two active ingredients in parsley, myristicin and apiol, contain mild diuretic properties. Parsley seeds oil has been included in the diet of pregnant women and is reported to increase diuresis and plasma protein and plasma calcium concentrations [9]. Parsley is rich in poly-phenolic flavonoid anti-oxidants including apiin, apigenin, crisoeriol and luteolin; and has been rated as one of the plant source with highest anti-oxidant activities. Along with luteolin, the vitamin C found in parsley serves as an effective anti-inflammatory agent within the body [10]. Therefore, the objectives of this study were to assess nephroprotective and antioxidant effects of parsley plant parts against gentamicin (GM)-induced nephrotoxicity in in male albino rats.

MATERIALS AND METHODS

Materials: Cellulose, vitamins, minerals, choline chloride, glycerol and neutral casein, the main source of protein in rats diet, were be obtained from El-Gomhoriya Company for Chemicals and Laboratory equipment's, Cairo, Egypt. Thirty six male albino rats *Sprague Dawley* strain, were obtained from Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt. Kits were purchased from Nefrtary Company for Chemical and Drugs, Tanta, Egypt. Corn starch was obtained from local market, Tanta, Egypt. Seeds oil was purchased from a herbal shop (Haraz), Cairo, Egypt. Leaves were obtained from local market.

Gentamicin: Gentamicin (Garamycin® injection) is one of aminoglycoside antibiotics obtained from Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. It is dispensed in the form of ampoules, each containing 40 mg/ml of gentamicin sulphate for parenteral administration. The selected dose of gentamicin was calculated for the rat from human dose [11].

Methods

Leaves Formulation Decoction: One hundred gram ofleaves was put in 1liter of cold water, brought to boil, simmered for 10-15 minutes (longer if plants very hard), then left to cool, steep covered for 10-15 minutes and then passed through a tea strainer, to be ready for use [12].

Experimental Design and Animal Groups: Adult male albino rats, *Sprague Dawley strain*, weighing (140±10g) were obtained from Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt. The animals were kept in wire cages. The diet was introduced to the rats in special food cups to avoid scattering of food. Also water was provided to the rats. Food and water were provided ad-libitum and checked daily. Thirty six adult male albino rats were divided into two main groups: The first main group (6 rats) fed on basal diet as control negative group (C-ve). The second main group (30 rats) fed on basal diet for 15 days. After that all groups were injected intraperitoneally with GM (10 mg/kg every 24 hr. for eight days to induce nephrotoxicity, one of the adverse reaction takes place) [13]. This second main group was divided into 5 groups each group contained 6 rats as follows: Group 1: positive control, rats had fed basal diet only (C+ve). Group 2: rats had basal diet and tube feeding of parsley leaves extract (E) 2ml. Group 3: rats had basal diet supplemented with 10% fresh parsley leaves (L). Group 4: rats had basal diet supplemented with 10% parsley seeds (S). Group 5: rats had basal diet supplemented with 10% oil parsley (O). At the end of experiments, all rats were fasted overnight sacrificed and the blood samples were collected, a part of blood was centrifuged to obtain the serum. Internal organs were collected and were taken photo to kidneys of all groups. The organs were removed (heart, kidney, spleen, liver and lung), cleaned in saline solution, dried by filter paper and weighted.

Biological Evaluation: During the experimental (28 day), the consumed diet was recorded everyday (feed intake)

and body weight was recorded every week. Biological evaluation of the different diets was carried out by determination of body weight gain % (BWG %) feed efficiency ratio (FER) [14].

Biochemical Analysis: Serum creatinine [15], serum uric acid [16, 17] and urea nitrogen [18]. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [19], Albumin [20], total protein [21], total cholesterol [22], triglycerides [23], HDL-C [24], serum VLDL-C and LDL-C [25], GSH [26] and MDA [27] were determined.

Statistical Analysis: Data are presented in tables as means±standard deviation (S.D.). Values were statistically analyzed by one-way analysis of variance (ANOVA-Tukey test) by using SPSS 10.1 software package. The P values <0.05 were considered significant [28].

RESULTS AND DISCUSSION

Data present in Table (1) showed feeding effect of parsley leaves extract (E), fresh parsley leaves (L), parsley seeds (S) and parsley seeds oil (O) on feed intake (FI), body weight gain % (BWG%), body weight gain after treatment % (BWGT%) and feed efficiency ratio (FER) (M± SD) in nephrotoxic rats. Results for feed intake (FI) recorded a significant increase of control (+ve) (14.12±0.39 g) as compared to control (-ve) group. Rats received supplemented diet of parsley seeds (S) and parsley seeds oil (O) groups showed non-significant decreases (P<0.05) (13.77±0.68 and 13.60±0.68 g respectively), as compared to positive control group. On the other hand, rats administrated parsley leaves extract (E) follows supplemented diet with (L), which recoded significant increases (14.52±0.33 and 14.27±0.27g respectively) as compared to C-ve group. The data of body weight gain (BWG%) before gentamicin injection illustrated non-significant increases of C +ve group compared to C-ve group (2.87±3.38 and 5.11±3.46 respectively). Parsley seeds (S) and fresh parsley leaves (L) groups have non-significant increases compared to C +ve and C-ve groups (6.82±3.68 and 7.03±10.49 respectively). Parsley leaves extract (E) and parsley seeds oil (O) groups have significant decrease as compared to control groups (-3.46±1.75 and -5.11±3.46 respectively). After GM injection (ip) significant decreases (P<0.05) of body weight gain (BWGT%) occur in C +ve group as compared to C-ve group (6.68 ± -2.15 and 2.87±3.11 respectively). Also, all rats received supplemented diet recorded non-significant decreases (-5.46±3.66,-6.35±2.58,-2.76±1.69 and -2.63±1.53 respectively) of BWGT% as compared to control groups. Feed efficiencies ratio showed significant decrease (P<0.05) in C +ve group compared to C-ve group (-2.09±0.45 and 0.63±0.64 respectively), rats administrated with (E) have non-significant increase as compared to C +ve group followed by (L) and (O) groups (-2.10±0.97, -1.32±1.05 and -1.13±0.99 respectively). The best result was found in rats received supplemented diet with (S) group (0.54±0.51) which nearly value to C-ve group.

The present results are in agreement with that a high amount of parsley seeds oil consumption was not associated with higher weight gain or a significantly higher risk of developing overweight or obesity in the context of the Mediterranean feed pattern [29]. Besides investigations exploring the immediate and long-term effects of an olive oil-enriched diet (OO diet) and parsley seeds oil on Glucagon-like peptide 1 (GLP-1)

Table 1: Effect of parsley products on biological evaluation in gentamicin-injected rats

Groups	FI g/day	BWG%	BWGT%	FER
C(-)	12.73±0.96 ^{a}	2.95±3.38 ^{b}	2.87±3.11 ^{b}	0.63±0.64 ^{b}
C(+)	14.12±0.36 ^{bc}	5.11±3.46 ^{b}	-2.15±3.68 ^{a}	-2.09±0.45 ^{a}
Ex	14.52±0.33 ^{c}	-3.46±1.75 ^{a}	-2.63±1.53 ^{a}	-2.10±0.97 ^{a}
S	13.77±0.68 ^{b}	6.82±3.72 ^{b}	-2.76±1.69 ^{a}	0.54±0.51 ^{b}
L	14.27±0.27 ^{bc}	7.03±10.49 ^{b}	-5.46±3.66 ^{a}	-1.32±1.05 ^{a}
O	13.60±0.68^{b}	-5.08±7.72 ^{a}	-6.35±2.58 ^{a}	-1.13±0.99 ^{a}

Table 2: Effect of parsley products on relative organs weigh in gentamicin-injected rats

Groups	Kidneys	liver	heart	spleen	lungs
C(-)	0.85±0.1 ^{a}	3.99±0.53 ^{a}	0.36±0.1^{ab}	0.45±0.44 ^{b}	0.76±0.05^{a}
C(+)	1.69±0.03 ^{c}	3.66±0.37 ^{a}	0.49±0.03^{c}	0.33±0.11 ^{a}	0.77±0.02^{a}
Ex	1.30±0.04 ^{b}	3.11±0.52 ^{a}	0.30±0.44^{a}	0.37±0.09 ^{ab}	0.72±0.10^{a}
S	0.81±0.03 ^{a}	3.41±0.12 ^{a}	0.34±0.03^{ab}	0.38±0.1 ^{ab}	0.73±0.07^{a}
L	0.89±0.03 ^{a}	3.32±0.30 ^{a}	0.37±0.03^{b}	0.35±0.12 ^{ab}	0.75±0.10^{a}
O	0.83±0.04 ^{a}	3.71±0.14 ^{a}	0.38±0.04^{b}	0.39±0.06 ^{b}	0.74±0.05^{a}

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

release and intestinal content, plasma insulin concentration, glucose tolerance and pancreatic insulin content in adult rats that had been injected with streptozotocin during the neonatal period (STZ rats). Over 50 days, the body weight gain was lower in the rats fed the oils diet compared to standard diet [30].

Relative Organs Weight: Table (2) presented the mean values of different organs relative weights. Relative kidney weight value showed significant increase in control (+ve) group as compared to normal rats group (1.69±0.03 and 0.85±0.1 respectively). Increased weight of kidney for control (+) group may refer to nephritis and electrolytes retention and nephron atrophy due to injection with gentamicin [31]. These results agree with study mentioned that GM inhibits oxidative phosphorylation and reduces ATP levels in renal tubular cells. Hence, GM-enhanced ROS formation in isolated cortical mitochondria and ROS-induced cell death were found to have a role in GM-mediated acute renal failure. The increase in kidney weight resulted from the oedema that was caused by drug induced acute tubular necrosis [32]. All supplemented diet groups with parsley products showed significant decrease when compared to control (+ve) group. The best result was found in (S), (O) and (L) which nearly value to C-ve group (0.81±0.03, 0.83±0.04 and 0.89±0.03 respectively). This improvement in kidney weight is due to presence of polyphenols and flavones, carotenoids and vitamin C present in parsley plant and revealed improvement and protection of renal glomeruli against damage via changes in both the levels and activities of antioxidant enzymes in kidneys [33]. Also, flavonoid fraction prevents nephrotoxicity, improves kidney function and promotes kidney primary epithelial tubular cell regeneration [34].

Relative liver weight values showed non-significant decrease in control (+ve) group as compared to normal rats group (3.66±0.37 and 3.99±0.53 respectively). All supplemented diet groups with parsley products showed non-significant decrease (L), (S) and (E) when compared with C+ve and C-ve groups(3.32±0.30, 3.41±0.12 and 3.11±0.52 respectively), while (O) showed non-significant increase ($P<0.05$) in comparison with control (+ve) group.

Relative heart weight values showed significant increase in control (+ve) group as compared to normal rats group (0.49±0.03 and 0.36±0.01 respectively). Supplemented diets with parsley products showed significant decrease ($P<0.05$) when compared with positive control as follow (L), (O), (S) and (E) groups (0.37±0.03, 0.38±0.04, 0.34±0.04, and 0.30±0.44, respectively).

Relative spleen weight values showed significant decrease in control (+ve) group as compared to normal rats group. Supplemented diets with parsley products showed significant increase ($P<0.05$) (S), (E) and (L) when compared with positive control. The best result was found in (O) which nearly value to C-ve group (0.39±0.06 and 0.45±0.44 respectively).

Relative lungs weight value showed non-significant increase in control (+ve) group as compared to normal rats group (0.77±0.02 and 0.76±0.05 respectively). All supplemented diet groups with parsley products appeared non-significant decrease in (E), (S), (O) and (L) when compared to C+ve group (0.72±0.10, 0.73±0.07, 0.74±0.05 and 0.75±0.10 respectively). The data suggests that using parsley treatments showed a better protection for all relative organs weight (kidney, liver, heart and spleen) as a percent of body weight compared to non-treated group due to the effect of parsley as antioxidant herb.

Biochemical Analysis

Kidney Function Tests: Uric acid, creatinine and urea.

Table (3) results present the mean values of uric acid showed significant increase in control (+ve) compared to C-ve group (3.33±0.22 and 2.45±0.13 mg/dL respectively). The rats administrated with (E) showed significant decrease ($P<0.05$) of uric acid (2.46±0.08 mg/dL), which closed with C-ve group as compared to control (+ve) group. The only result found in rats received (L) have significant decrease as compared to control groups.

Also in the same table which represents the mean values of creatinine of control (+ve) group showing significant increase compared to (control-ve) group (1.05±0.27 and 0.33±0.05 mg/dL respectively). Rats consumed all supplemented diets showed significant decrease ($P<0.05$) of creatinine level as follows E, S, O and L which recorded lower values (0.39±0.01, 0.39±0.02, 0.38±0.04 and 0.36±0.05 mg/dL respectively).

Table 3: Effect of parsley products on kidney function tests in gentamicin-nephrotoxic rats

Groups	Uric acid mg/dL	Creatinine mg/dL	Urea mg/dL
C(-)	2.45±0.13 ^{c}	0.33±0.05 ^{a}	27.66±3.5 ^{a}
C(+)	3.33±0.22 ^{b}	1.05±0.27 ^{b}	115±3.3 ^{c}
Ex	2.46±0.08 ^{c}	0.39±0.01 ^{a}	55.00±5.4 ^{b}
S	2.08±0.09 ^{a}	0.39±0.02 ^{a}	23.5±6.4 ^{a}
L	1.98±0.16 ^{a}	0.36±0.05 ^{a}	30.3±4.8 ^{a}
O	2.05±0.54 ^{a}	0.38±0.04 ^{a}	31.66±8.8 ^{a}

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

In the same Table; the mean value of urea of control (+ve) group showed high significant increase as compared to (control -ve) group (115±3.3 and 27.66±3.5 mg/dL respectively). Rats received all supplemented diet showed significant decrease (P<0.05) in urea as follows E, O, L and S which recorded the best effect on urea level (55.00±5.4, 31.66±8.8, 30.3±4.8 and 23.5±6.4 mg/dL respectively).

These results supported that intake of parsley leaves decoction 10% as given orally for 2 months reduced considerably serum creatinine levels [35]. Also, the oral administration of the parsley leaves decoction extract (0.1, 0.25 and 0.5 g/kg body wt) for 14 days significantly decreased ($p < 0.05$) the serum urea, uric acid, creatinine. A comparison was made between the action of parsley leaves decoction extract and glibenclamide (600 microg/kg), a known antidiabetic drug. These data suggests that parsley herb is effective in reduction of blood urea, creatinine and uric acid levels in rats with nephrotoxicity induced by gentamicin because parsley might has a diuretics effect. The diuretic effect may be attributable to the pharmacological activities of myristicin (sympathomimetic action) and apiol (irritant effect) [36, 34].

Liver Enzymes: Table (4) illustrates the results of GPT and ALT which recorded significant increase in control (+ve) group (21.67±2.06 U/L) as compared to control (-ve) group (11.67±0.82 U/L). But supplemented diets with parsley products showed significant decreases (P<0.05), which showed values of (15.51±2.16, 14.63±2.42, 14.50±1.05 and 13.16±1.16 U/L respectively) as follows for S, E, O and L when compared to control (+ve) rats. So that, all supplemented diet with parsley products improved GPT (ALT) of liver disorder rats, the best result was showed in fresh parsley leaves (L) group.

Also, control (+ve) group was raised in GOT aspartate aminotransferase (AST) values as compared to control (-ve) group (19.7±1.032 and 16.67±1.67U/L respectively). Oral administration with (E) and supplemented diet with (O) groups showed significant decrease (P<0.05) as compared to control (+ve) group (17.67±0.89 and 17.5±2.34 U/L respectively). On the other hand, (L) and (S) groups showed non-significant decrease in GOT compared to C+ve group (18.16±1.32 and 18.5±1.52 U/L respectively), the best effect was found in (O) group followed by (L) group.

These results for GPT and GOT are in line with parsley leaves decoction extract significantly decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [35]. Also, serum alanine aminotransferase level was significantly reduced by treatment of parsley oil [37]. Parsley seed oil has been reported to stimulate hepatic regeneration in a rat model [38]. The improvement in liver functions due to parsley leaves that contain flavonoids such as glycosides of apigenin, luteolin (e.g. apiin, luteolin-7-apiosyl-glucoside, apigenin-7-glucoside, luteolin-7-diglucoside and volatile oils such as myristicin (up to 85%), apiol, 1,3,8-p-menthatriene, 1-methyl-4-isopropenylbenzene, methyl disulfide, monoterpenes (e.g. a-and b-pinene, b-myrcene, b-ocimene, b-phellandrene, p-terpinene, a-terpineol) and sesquiterpenes (e.g. a-copaene, carotol, caryophyllene) which have antioxidant effect [9].

Table 4: Effect of parsley products on liver enzymes in gentamicin-injected rats

	GPT U/L	GOT U/L
C(-)	11.67±0.82 [a]	16.67±1.67 [a]
C(+)	21.67±2.06 [d]	19.7±1.032 [c]
Ex	14.63±2.42 [bc]	17.67±0.89 [b]
S	15.51±2.16 [c]	18.16±1.32[bc]
L	13.16±1.16 [b]	18.5±1.52 [bc]
O	14.50±1.05 [bc]	17.5±2.34 [b]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

Protein fractions: Total Protein, Albumin, Globulin and Albumin/Globulin (A/G) Ratio: Table (5) represents the mean values of total protein which showed significant increase for control (+ve) group as compared to (7.4±0.32 and 7.2±0.62 mg/dL, respectively), but rats received supplemented parsley diet showed significant decrease (P<0.05) in total protein as compared to control (+ve). The best result was found in (O) (7.2±0.16 mg/dL) which is close with C-ve group and followed by S, L and E groups.

In the same table results presented the mean value of albumin in control (+ve) group showed non-significant decrease (3.30±0.27mg/dL) as compared to control (-ve) group. Supplemented diet with L, O and S showed significant decrease (P<0.05) in albumin as compared to control groups (2.98±0.18, 3.01±0.18 and 3.26±0.29 respectively).

Also globulin of control (+ve) group showed non-significant decrease as compared to control (-ve) group (3.30±0.27 and 3.38±0.09 mg/dL respectively). The mean value of globulin in E group showed non-significant increase (P<0.05) in globulin (3.33±0.28 mg/dL). Other groups O, S and L illustrated

Table 5: Effect of parsley products on liver function in gentamicin-injected rats

Groups	Total protein mg/dL	Albumin mg/dL	Globulin mg/dL	A/G ratio
C(-)	7.2±0.62 [a]	3.38±0.09 [c]	3. 8±0.64 [a]	0.9±0.15 [b]
C(+)	7.4±0.32 [b]	3.30±0.27 [c]	4.2±0.31[a]	0.79±0.09 [ab]
Ex	7.1±0.18 [a]	3.33±0.28 [c]	3.7±0.31 [a]	0.89±0.13 [b]
S	7.05±0.16 [a]	3.01±0.18 [b]	4.0±0.34[a]	0.75±0.11 [ab]
L	7.1±0.09 [a]	2.98±0.18 [a]	4.1±0.22[a]	0.73±0.08 [a]
O	7.2±0.16 [a]	3.26±0.29 [b]	3.9±0.23 [a]	0.84±0.12 [ab]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

significant decrease of globulin as compared to control (+ve) group, the lowered value was in L group (2.98±0.18 mg/dL).

Calculated results of albumin/globulin (A/G) showed non-significant decrease in ratio in control (ve+) as compared to normal rats (0.79±0.09 and 0.9±0.15 respectively). Rats fed on supplemented diets recorded non-significant increase ($P<0.05$) in E and O groups as compared to control (ve+). On other hand, there was non-significant decrease in L and S groups as compared to C+ve group (0.73±0.08 and 0.75±0.11 respectively). The best result was found in fresh parsley leaves extract.

These findings are in agreement with that parsley seeds oil increase total serum proteins, albumin and globulin. In general, the useful effect of parsley in improving liver function, can be attributed to its ability as antioxidant, to: 1-regulate the triggering of hepatic drug-metabolizing enzymes by the formation of glutathione-conjugate. 2-ameliorate the antioxidant enzymes (catalase, cupper/zinc superoxide dismutase (Cu/Zn SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and Glutathione S transferase (GST) activity in liver which is beneficial for the hepatic detoxification [39]. 3-reducing oxidative stress (decreased reactive oxygen species and lipid peroxidation) and lowering inflammatory cytokines (decreased tumor necrosis factor-α and interleukin 1β) and protein expression (cyclooxygenase-2, inducible nitric oxide synthase, cytosolic phospholipase A2 and caspase-3) [40, 41].

Serum Lipid Profile: The results in Table (6) revealed that the mean value of total cholesterol was significantly increased in C+ve group compared to C-ve group (120.5±3.08 and 91.5±4.08 mg/dL respectively). All supplemented diets showed significant decrease ($P<0.05$) when compared with control (+ve). Rats groups of L, O, E and S have non-significant increase with C-ve group (100.6±8.3, 99.2±2.7, 98±7.4 and 96.8±6.5 mg/dL respectively).

Table 6: Effect of parsley products on lipid profile in gentamicin-injected rats

Groups	Total cholesterol mg/dL	Triglyceridemg/dL
C(-)	91.5±4.08 [a]	65.7±10.2 [c]
C(+)	120.5±3.08 [c]	46.6±7.8 [b]
Ex	98±7.4 [b]	40.8±6.6 [ab]
S	96.8±6.5 [ab]	38.18±7.5 [ab]
L	100.6±8.3 [b]	36±1.5 [a]
O	99.2±2.7 [b]	40.3±7.2 [ab]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

Table 7: Effect of parsley products on cholesterol profile in gentamicin-injected rats

Groups	HDL-c mg/dL	LDL-c mg/dL	VLDL-cmg/dL
C(-)	43.5±2.1 [c]	34.8±2.9 [a]	13.1±2.4 [c]
C(+)	25.6±1.2 [a]	85.5±2.6 [d]	9.3±1.5 [b]
Ex	27.6±2.6 [a]	62.2±8.1 [c]	8.1±1.3 [ab]
S	31.8±2.2 [b]	57.2±6.3 [c]	7.7±1.5 [ab]
L	32.5±2.3 [b]	61.9±4.7 [c]	7.2±0.4 [a]
O	41.8±1.6 [c]	49.2±1.8 [b]	8.1±1.4 [ab]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

The mean value of triglycerides was significantly decreased in C+ve group (46.6±7.8 and 65.7±10.2 mg/dL, respectively) as compared to C-ve. Meanwhile triglycerides showed non-significant decrease in E, O and S groups, values were (40.8±6.6, 40.3±7.2 and 38.18±7.5 mg/dL respectively) when compared to control (+ve). The lower value was in L group (36±1.5 mg/dL) which had significant decrease with C+ve group.

Serum Cholesterol Profile: Table (7) showed significant decrease of (HDL-C) in C+ve group (25.6±1.2 and 43.5±2.1 mg/dL respectively) as compared to control (-ve). Meanwhile E group showed non-significant increase when compared with C+ve group (27.6±2.6 mg/dL). Feeding rats on S, L and O supplemented diets induced significant increase values when compared with C+ve

group (31.8±2.2, 32.5±2.3 and 41.8±1.6 mg/dL respectively). The best result was found in O group which is nearly to C-ve group.

The mean values of (LDL-C) showed high significant increase in C+ve group as compared to C-ve group (85.5±2.6 and 34.8±2.9 mg/dL respectively). Meanwhile supplemented diets showed significant decrease as follows E, L, S and O (62.2±8.1, 61.9±4.7, 57.2±6.3 and 49.2±1.8 mg/dL respectively) when compared to control (+ve) group, the best result was found in O group which have lower value.

The mean value of (VLDL-C) was significantly decreased in C+ve group as compared to C-ve group (9.3±1.5 and 13.1±2.4 mg/dL respectively). The supplemented groups with E, O and S showed non-significant decrease when compared with C+ve group (8.1±1.3, 8.1±1.4 and 7.7±1.5 mg/dL respectively). But L group had significant decrease as compared to C+ve group (7.2±0.4 mg/dL).

Parsley seeds oil rich diet prevents diabetes, as it reduces glucose levels, LDL cholesterol" bad" and triglyceride level in blood, it is precisely due to its effect on cholesterol that parsley seeds oil also prevents a series of diseases that are very frequent in diabetic patients such as atherosclerosis and cardiovascular diseases. Most of the prevention strategies of these vascular disorders are focused on obesity and arterial pressure control [42]. The latter is precisely achieved by using antihypertensive agents related to the renin angiotensin aldosterone system, which shows its essential role in the development of the a theroma plaques in diabetes. Parsley leaves decoction extract significantly decreased total cholesterol and triglycerides. In general, the useful effect of parsley in improving lipid profile may be due to presence of flavonoids (polyphenols), a variety of antioxidants (including vitamins C and E and the carotenoids) and fibers which reduced serum triglyceride, total cholesterol and LDL by: 1-protecting LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, the volatile essential oils of commonly used culinary herbs, spices and herbal teas inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumor growth [35]. 2-Reducing neutrophil NADPH-oxidase activity and plasma concentrations of oxidized LDL and inflammatory biomarkers [43]. 3-increasing the activity of catalase and glutathione and reduced lipid peroxidation (thiobarbituric acid-reactive substance) in nephropathy rats [40]. 4-inhibiting platelet aggregation and acting as anti-inflammatory and anti-tumor agents [44].

Table 8: Effect of parsley products on antioxidants GSH and lipid peroxide MDA in gentamicin-injected rats

Groups	GSH µl/g. protein	MDA nmol/g
C(-)	8.07±0.25 ^{e}	249±6.2 ^{a}
C(+)	4.52±0.23 ^{b}	538±8.8 ^{f}
Ex	4.93±0.06 ^{cd}	410±10 ^{c}
S	5.22±0.11 ^{d}	381±7.63 ^{b}
L	4.73±0.14 ^{bc}	451±4.87 ^{d}
O	4.10±0.10 ^{a}	477±6.23 ^{e}

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

Antioxidant Enzyme s: Table (8) illustrated the results of antioxidant enzyme of (GSH) which was significantly decreased in C+ve group as compared to C-ve group (4.52±0.23 and 8.07±0.25 µl/g, respectively). The supplemented groups with S, E, and L groups showed significant increase when compared to C+ve group (5.22±0.11, 4.93±0.06 and 4.73±0.14 µl/g, respectively). The lower value was in O group (4.10±0.10) which had significant decrease with C+ve group (4.52± 0.23 µl/g). In the same table the mean value of lipid peroxidation MDA was significantly increased in C+ve group as compared to C-ve group (538±8.8 and 249±6.2 nmol/g, respectively), while there was a significant decrease in the mean value of MDA for supplemented groups. These results agree with that the diabetic group given parsley, serum uric acid, potassium and sodium levels and liver LPO decreased, but GSH levels increased [45].

These results agree with that GM caused significant increases in kidney content of malondialdehyde (MDA) and, vitamin E ameliorated the rise in renal content of MDA and enhanced the renal non-protein thiols (NPSH) content as well as superoxide dismutase SOD activity [46]. And also agree with that serum and renal tissue MDA, blood superoxide anion and hydrogen peroxide in GENTA were increased *vs* CTL and *vs* VIT C and decreased in GENTA + VIT C *vs* GENTA (all $P < 0.05$). Parsley is rich in poly-phenolic flavonoid anti-oxidants including apiin, apigenin, crisoeriol and luteolin; and has been rated as one of the plant sources with highest anti-oxidant activities [47]. Along with luteolin, the vitamin C found in parsley serves as an effective anti-inflammatory agent within the body and increasing the activity of catalase and glutathione and reduced lipid peroxidation (thiobarbituric acid-reactive substance) in nephropathy rats [48].

In conclusion, this protective study indicated that biological evaluation was improved due to receiving parsley leaves extract (E) administrated and supplemented

rats diet with parsley fresh leaves, parsley seeds and parsley seeds oil as compared to positive control group GM (i.p.). Biochemical analysis of serum kidney tests, liver function tests, lipid profile, antioxidant enzyme GSH reduced significantly in rats groups received parsley products as compared to positive control group GM (i.p.) and increase in HDL-C and lipid peroxide MDA because, parsley product parts have nutritive and restorative properties. Therefore, this study recommends that intake of parsley product parts may be beneficial for health people and patients suffer from nephrotoxicity.

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