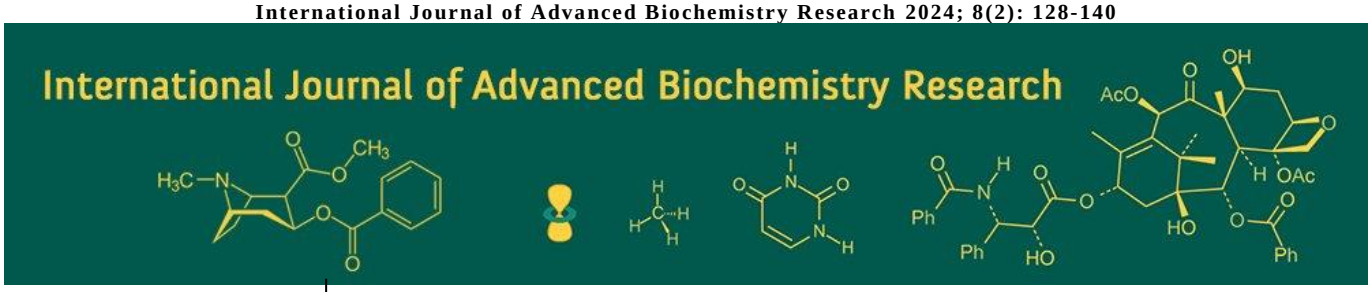


ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(2): 128-140
www.biochemjournal.com
Received: 21-12-2023
Accepted: 27-01-2024

Hanan A Abd Elmonem
Department of Biological Application, Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Doaa S Mansour
Department of Biological Application, Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Reham M Morsi
Department of Biological Application, Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Wafa M Wafa
Department of Radiation Protection and Civil Defence, Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Corresponding Author:
Doaa S Mansour
Department of Biological Application, Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Comparative study between some fixed and essential oils on toxicity induced by gentamicin in male albino rats

Hanan A Abd Elmonem, Doaa S Mansour, Reham M Morsi and Wafa M Shahin

DOI: https://doi.org/10.33545/26174693.2024.v8.i2b.634

Abstract
The present study was conducted to assess the effect of fixed mix oils (radish and parley) and essential mix oils (clove and peppermint) administration on gentamicin -induced inflammation, oxidative stress toxicity and kidney injury in male rats. In the present work seventy male albino rats were arranged into seven equal groups. Control (G1); carrier group (G2); gentamicin group (G3); fixed mix oils group (G4); essential mix oils group (G5); co-treated group included rats that received fixed mix oils and injected at the same time by gentamicin (G6); co-treated group included rats that received essential mix oils and injected at the same time by gentamicin (G7). The administration of gentamicin revealed a significant increase in levels of serum kidney function (urea, creatinine and uric acid), liver function (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase), lipid profile (cholesterol and triglyceride), tumor necrosis factor-alpha, malondialdehyde, parathyroid hormone and white blood cells count as compared to control group. In contrast; a significant decrease in albumin, superoxide dismutase, catalase, calcium and phosphorus ions levels, Osteocalcin, triiodothyronine, red blood cells count and hemoglobin were observed in gentamicine group as compared to control. The oral supplementation of fixed mix oils and essential mix oils has significantly attenuated the severity of gentamicin-induced oxidative stress. Both two mixtures of oils have potential antioxidant synergistic effect to ameliorate nephrotoxicity and changes in hematological, bone homeostasis and liver function induced by gentamicin. It could be concluded that fixed mix oils (radish and parsley) as well as essential mix oils (clove and peppermint) considered as a natural substance and has a promising protective effect against toxicity induced by gentamicin. Both of these mixtures of oils have nearly the same antioxidant activities. So it is easier to use the fixed oil mixture of low coast than essential oil of high coasted.

Keywords: Gentamicin, kidney, radish extract, parsley extract, clove oil, peppermint oil

Introduction
The most dangerous adverse effect of gentamicin (GM), an aminoglycoside antibiotic that is clinically efficient against infections caused by Gram-negative bacteria (Ali, 1995) [4], is nephrotoxicity, which makes it less useful in clinical settings. Within one week of beginning GM therapy, thirty percent of patients begin to exhibit symptoms of nephrotoxicity (Paterson *et al*., 1998) [49]. It was recently demonstrated that GM can induce acute kidney injury at a single dosage. Although it's unclear exactly how genetically modified organisms cause nephrotoxicity. However, it has been linked to GM buildup in the proximal and distal collecting duct tubular epithelial cells (Fujiwara, *et al*., 2012) [24]. Reactive oxygen species (ROS) are produced in greater quantities in mitochondria as a result of genetic modification (GM); these free radicals damage biomolecules such proteins, lipids, and nucleic acids. (Lopez-Novoa *et al*., 2011) [37]. Moreover, the main effects of GM-induced ROS overproduction are the induction of inflammation and the suppression of the natural antioxidant system (Cao, *et al*., 2019) [11].
Therefore, the deposition of GM in tubular epithelial cells causes a host of harmful events in tubular cells, including inflammation, necrosis, apoptosis, phospholipidosis, mitochondrial dysfunction, and endoplasmic reticulum stress. These events can lead to glomerular filtration rate decline, tubular dysfunction, and cell death (Lopez-Novoa *et al*., 2011) [37].

According to Babaeenezhad *et al*. (2021) [9], gentamicine caused hepatotoxicity in an animal model.

Reactive oxygen species (ROS) are produced in greater quantities in mitochondria as a result of genetic modification (GM); these free radicals damage biomolecules such proteins, lipids, and nucleic acids. (Lopez-Novoa *et al*., 2011) [37]. Moreover, the main effects of GM-induced ROS overproduction are the induction of inflammation and the suppression of the natural antioxidant system (Cao, *et al*., 2019) [11]. Therefore, the deposition of GM in tubular epithelial cells causes a host of harmful events in tubular cells, including inflammation, necrosis, apoptosis, phospholipidosis, mitochondrial dysfunction, and endoplasmic reticulum stress. These events can lead to glomerular filtration rate decline, tubular dysfunction, and cell death (Lopez-Novoa *et al*., 2011 [37] Peppermint oil (*Mentha piperita* L.) is one of the essential oils that is commonly utilized in alternative medicine and traditional therapies because of its antioxidant properties (Riachi & De Maria, 2015) [55]. The main constituents of peppermint essential oil include flavonoids, pulegone, piperitone, menthofurane, and menthol (50-30%), menthone (10-30%), menthyl esters (Up to 10%), and various monoterpene derivatives (Dawidowicz *et al*., 2014) [13]. The main constituents of peppermint essential oil include flavonoids, pulegone, piperitone, menthofurane, and menthol (50-30%), menthone (10-30%), menthyl esters (up to 10%), and various monoterpene derivatives (Dawidowicz *et al*., 2014) [13]. Peppermint may have been utilized as early as 1000 BC, according to the finding of dried peppermint leaves in the Egyptian pyramids (Spirling and Daniels, 2001) [62] According to Keifer *et al*. (2008) [33], it possesses anticancer, antibacterial, and antiallergenic properties in addition to reducing cramps, digestive issues, anorexia, nausea, and diarrhea.

A cruciferous vegetable, radish (*Raphanus sativus* Linn) has been used in folk medicine to combat a variety of toxicants (Salah-Abbe`s *et al*., 2008) [56]. Alkaloids, glycosides, saponins, tannins, carbohydrates, phenolic chemicals, flavonoids, amino acids, and volatile oil have all been identified in *Raphanus sativus* Linn extract (Manivannan *et al*., 2019) [38]. According to Jan and Badar (2012) [32], radishes are high in potassium, magnesium, copper, calcium, ascorbic acid, folic acid, vitamin B6, and riboflavin. Furthermore, radishes have been shown in many studies to have antioxidant, antibacterial, and anticancer properties (Rakhmawati *et al*., 2009; Pocasap *et al*., 2013; Noman *et al*., 2021) [52, 81, 43]. Radish oil has significant antioxidant and anti-carcinogenic properties against toxicity brought on by a variety of chemical agents, according to Chung *et al*. (2012) [12].

Due to its high water content (78-82%, w/w), parsley (*Petroselinum crispum*) is an extremely rich source of vitamins C and E, carotene, thiamin, and organic minerals. It comes from the Mediterranean region and is used in cooking and medicine. Additionally, oleic *Petroselinum*, palmatic linoleic, and other fatty acids are present in parsley fixed oil (Farah *et al*., 2015) [22]. According to Papay *et al*. (2012) [48], apigenin and its glucosidal flavonoids, which are present in parsley leaves, have anti-inflammatory, antioxidant, and anticancer properties.

Because of their antioxidant action, natural products like plant extracts and essential oils are widely utilized worldwide to protect people from a variety of ailments brought on by oxidative stress. The synergistic interactions between various antioxidant components found in blends of natural and synthetic antioxidants, as well as blends of various plant essential oils or herbal extracts, can increase antioxidant activity. Moreover, non-volatile fixed oils have a lower carbon footprint than essential oils. Accordingly, the primary goal of this study is to assess any potential synergistic effects on nephrotoxicity and alterations in hematological, bone homeostasis, and liver function brought on by gentamicin between two mixed essential (Volatile) oils extracted from clove buds and peppermint leaves and two mixed fixed (Non-volatile) oils extracted from radish and parsley seeds.

Materials and Methods

Materials

Chemical

Gentamicin (GM) was purchased from Devo Pharmaceuticals Company as pharmaceutical ampoules (80 mg).

Preparation of plant and seeds

We bought dried clove buds (*Syzygium aromaticum*) from a local Cairo, Egypt market. The glass jar contained the clove blossoms. We bought the parsley seeds (*Petroselinum Cspum*), peppermint leaves (*Mentha piperta* L.), and radish seeds (*Raphanus sativus* L.) from El-Maghrabi farm of fragrant plants in El-Nobareya, Egypt. Glass jars contained peppermint leaves, parsley seeds, and radish seeds.

Extraction of oils

The essential oils of clove buds and peppermint leafs were extracted by steam distillation method for 6hrs, as described by Guenther, (1961) [25]. The obtained essential oils were dried over anhydrous sodium sulphate and stored in dark bottles at 5 °C until used.

Extraction of radish and parsley seeds

Both of radish and parsley seeds were grinded by a clean grinder for 15s. The powder of radish and parsley seeds (500 g) were subsequently soaked in 2500 ml of n-hexane for h at 25 °C, filtered and concentrated by rotary evaporator to remove n-hexane according to Zhao, *et al*., (2017) [75]. The obtained oils stored at 4 °C until used. The extract oils were dissolved in Tween 80 before administration.

Animals

The study used 70 male albino rats, weighing between 130 and 150 g and aged between 9 and 10 weeks. Before beginning the experiment, the rats were housed in an animal home for one week, fed a normal rodent food, and given unlimited access to water. After acclimatization for one week, the rats were split evenly into seven groups. The Institutional Animal Care and Use Committee (IACUC-SCI-TU-0019) authorized the biology department's and the nuclear research center's animal care guidelines, which were followed in the upkeep and treatment of the animals.

Experimental Groups

Rats were equally divided into seven groups

- **1st group:** Control group included rats injected intraperitoneally (I.P.) with normal saline.

- **2nd group:** Carrier group included rats that received orally by stomach tube with 1ml of 1% of tween 80 for two weeks.
- **3rd group:** gentamicine group included rats that received gentamicin (80 mg/kg /day) i.p for two weeks daily I/P as described by Ohtani *et al.*, (1995) [45] to induce experimentally acute renal failure.
- **4th group:** Fixed mix oils group included rats received radish oil by oral gavages at a dose of (2ml/kg/day) according to (Omran and Soliman, 2005) [46] and parsley oil at a dose of (250 mg/kg/day) according to (Elkomy *et al*., 2020) [20] for two weeks.
- **5th group:** Essential mix oils group included rats received clove oil at a dose of (200 mg/kg /day) according to (El-Hadary and Hassanien, 2016) [18] and peppermint oil at a dose of (100 mg/kg /day) by oral gavages for two weeks.
- **6th group:** Co-treated group included rats that received fixed mix oils of radish oil at a dose of (2 ml/kg /day) and parsley oil at a dose of (250 mg/kg /day) for two weeks and injected at the same time by gentamicin (80 mg/kg/day) for two weeks.
- **7th group:** Co-treated group included rats that received essential mix oils clove oil at a dose of (200 mg/kg /day) and peppermint oil at a dose of (100 mg/kg/day) for two weeks and injected at the same time by gentamicin (80 mg/kg/day) for two weeks

Samples

At the end of the experimental period, rats were fasted overnight; euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy. Blood sample was collected in two tubes, one with EDTA for determination of blood picture including RBCs, Hb, and WBCs were estimated using automatic blood cell counter (Abacus 380 CBC counter). The other tube without anticoagulant then centrifuged at 3000rpm for 15 minutes to obtain serum for evaluate biochemical parameters.

Tissue samples were homogenized in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) through glass homogenizer. Then, homogenates were centrifuged at 5,000 r.p.m for 15 minutes at 4 °C then the supernatant was used to evaluate biochemical parameters.

Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total cholesterol (TC), triacylglycerols (TG), creatinine, urea, uric acid, calcium (Ca) and Phosphorus (P) were estimated by using colorimetric assay kits from Biodiagnostic Co, Egypt. Serum levels of tumor necrosis factor-alpha (TNF-α) was performed by Ray Bio mouse ELISA kit. Osteocalcin and Parathyroid hormone (PTH) levels were determined by ELISA technique. Determination of serum triiodothyronine hormone (T_3) level by Radioimmunoassay (RIA) using kits purchased from DIA source Immuno Assay S.A.-Rue du Bosquet, 2-B 1348 Louvain- La- Neuve- Belgium. In kidney homogenate activities of superoxide dismutase (SOD) and catalase (CAT)as well as malondialdehyde (MDA) were measured according to the method of Sun *et al*., (1988) [63], Aebi (1984) [2], and Ohkawa *et al*., (1979) [44] respectively, by using assay kits from Biodiagnostic Co, Egypt.

Statistical Analysis

The obtained data were presented as means ± SD. One-way analysis of variance (ANOVA) was carried out. The statistical comparisons among the groups were performed with Duncan's test, using a statistical package program (COSTAT). Differences among the groups were considered significant at $p<0.05$.

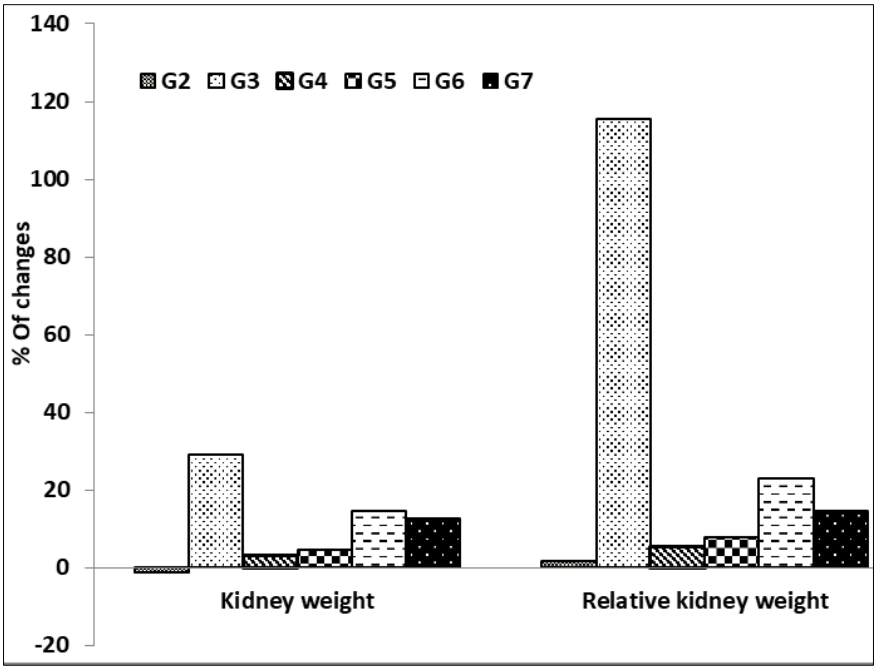
Results

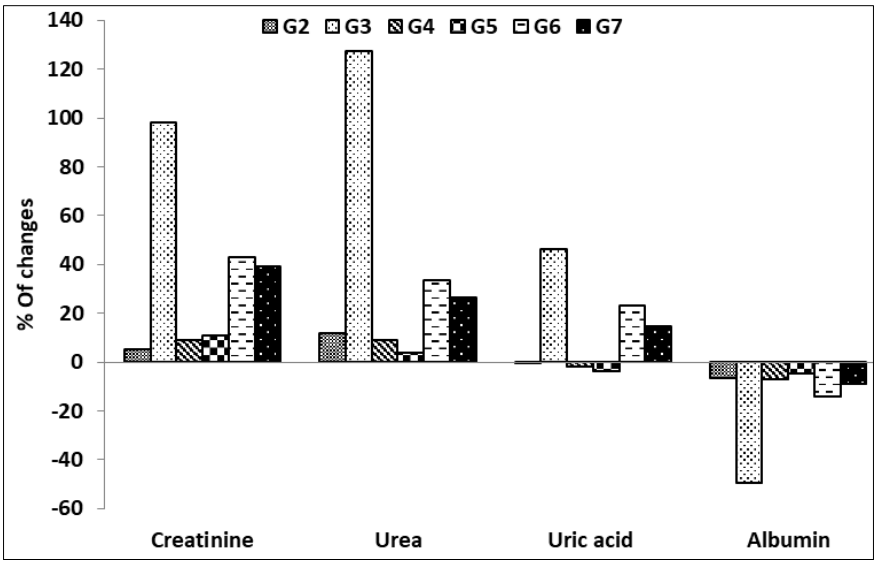
Animals of gentamicin (GM) treatment shows significant increase ($p\leq 0.05$) in kidney weight and relative kidney weight as compare to normal control. Also, there were significant increases in serum creatinine, urea, uric acid and TNF-α with a marked decrease ($p\leq 0.05$) in albumin and serum triiodothyronine levels in the animals treated with GM relative to the control group. Whereas those treated with fixed mix or essential mix oils showed a marked improvement in the mentioned measurements compare to those of GM treated group (Table 1 & Figure1).

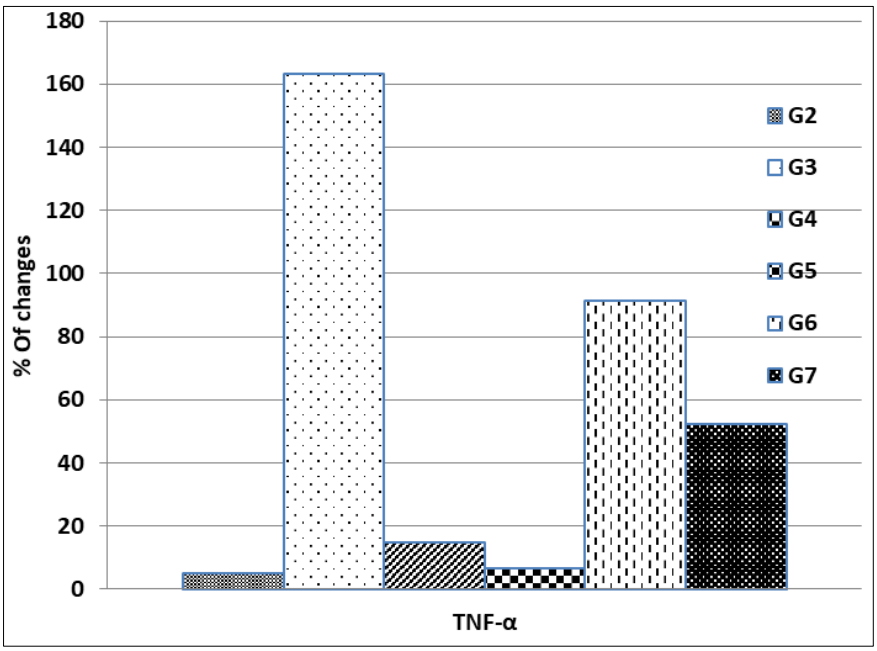
Table 1: Kidney weight and function tests, tumor necrosis factor-alpha (TNF-α) and triiodothyronine (T_3) in all experiment groups.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7
Kidney weight	0.956±0.04^{c}	0.944±0.02^{c}	1.234±0.07^{a}	0.986±0.03^{c}	1.0±0.06^{c}	1.094±0.05^{b}	1.078±0.09^{b}
Relative kidney weight	0.542±0.01^{d}	0.551±0.02^{d}	1.168±0.10^{a}	0.572±0.01^{cd}	0.584±0.02^{cd}	0.667±0.03^{b}	0.621±0.04^{bc}
Creatinine (mg/dL)	0.56 ±0.06^{c}	0.59±0.07^{c}	1.11±0.05^{a}	0. 61±0.02^{c}	0. 62±0.04^{c}	0.80±0.03^{b}	0.78±0.01^{b}
Urea (mg/dL)	37.96±10.46^{c}	42.41±7.62^{bc}	86.33±9.24^{a}	41.41±5.12^{bc}	39.49±8.52^{bc}	50.76±4.35^{b}	48.01±6.78^{bc}
Uric acid (mg/dL)	4.65±0.12^{d}	4.62±0.33^{d}	6.79±0.23^{a}	4.56±0.29^{d}	4.48±0.36^{d}	5.73±0.15^{b}	5.33±0.25^{c}
Albumin (g/dL)	4.53±0.16 ^{a}	4.23±0.26 ^{ab}	2.28±0.32 ^{c}	4.22±0.31 ^{ab}	4.31±0.46 ^{ab}	3.89±0.43 ^{b}	4.12±0.18 ^{ab}
TNF-α (pg/ml)	79.08±8.04^{d}	83.13±11.22^{d}	208.2±16.67^{a}	90.63±10.31.45^{d}	84.36±10.61^{d}	151.22±16.98^{b}	120.54±17.11^{c}
T_3 (nmol/L)	2.82 ±0.03^{b}	2.79 ±.035^{b}	1.69 ±.036^{a}	2.78±.092^{b}	2.74±0.031^{b}	2.63±0.019^{b}	2.15±0.019^{b}

Values are presented as Mean ± SD (n=10).
Different letters indicate significant difference ($p<0.05$)


140
120
100
80
60
40
20
0
-20
% Of changes
G2 G3 G4 G5 G6 G7
Kidney weight
Relative kidney weight


140
120
100
80
60
40
20
0
-20
-40
-60
% Of changes
G2 G3 G4 G5 G6 G7
Creatinine
Urea
Uric acid
Albumin


180
160
140
120
100
80
60
40
20
0
% Of changes
G2
G3
G4
G5
G6
G7
TNF-α

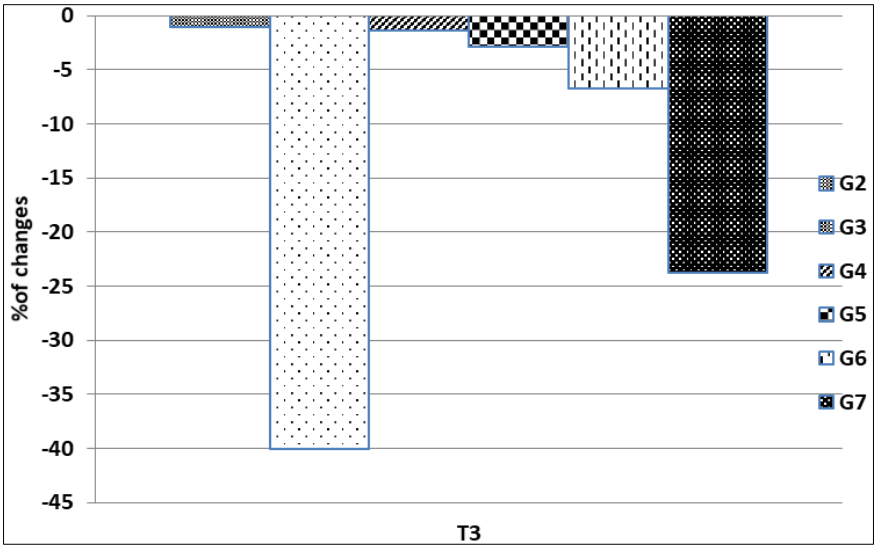


Fig 1: Percent of change in the kidney weight, relative kidney weight, levels of serum creatinine, serum urea, serum uric acid, serum albumin, TNF-α and T_3 on male albino rats in different experimental groups.

Administration of gentamicin caused oxidative stress demonstrated by a significant increase (p>0.05) of MDA level associated with a significant decrease (p>0.05) in SOD and catalase activities compared to control rats (Table 2 and fig. 2). The oral supplementation of fixed mix oils (Radish and parsley) as in group 6 and essential mix oils (Clove and peppermint) as in group 7 has significantly attenuated the severity of gentamicin-induced oxidative stress.

Table 2: Oxidative stress biomarkers in kidney tissues of all experimental groups.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7
MDA (nmol/mg)	21.48±1.16^{d}	22.48±0.41^{d}	36.3±4.23^{a}	21.08±1.64^{d}	22.28±1.76^{d}	28.90±0.96^{b}	25.86±3.31^{c}
SOD (U/mg)	21.0±2.45^{a}	22.2±2.77^{a}	11.2±1.26^{c}	23.8±2.15^{a}	20.8±1.31^{a}	13.8±2.70^{b}	15.22±2.41^{b}
CAT (U/min/mg)	15.8±2.57^{a}	16.01±1.25^{a}	7.06±1.58^{c}	15.89±3.05^{a}	15.22±2.41a	11.00±1.58^{b}	12.22±0.83^{b}

Values are presented as Mean ± SD (n=10).
Different letters indicate significant difference (p<0.05)

The results in Table (3) and fig. (3) Shows mean values of serum calcium, phosphorus, osteocalcin and parathyroid hormone for all tested groups. The results of the present study revealed significant decrease (p< 0.05) of calcium, phosphorus and osteocalcin as well as significant increase level of PTH in gentamicin administered animals (G3), compared to the other groups. These alternations were almost returned to normalcy in rats supplemented with fixed mix oils (Radish and parsley) in group 6 and essential mix oils (Clove and peppermint) in group 7.
Data presented in table (4) and fig. (4) illustrate the changes of the hematological parameters in response to gentamicin, fixed mix oil and essential mix in rats. These data demonstrated the negative impact of gentamicin on RBCs and WBCs count, Hb concentration in treated animals. This was evident from the significant (p>0.05) decrease in RBCs count, Hb concentration and significant increase (p>0.05) in WBCs induced by gentamicin. Data also showed that treatment by fixed mix oils (radish and parsley) in group 6 and essential mix oils (clove and peppermint) in group7 restored the negative effect of gentamicin treatment on these parameters to almost the control level.

Table 3: Bone markers in all experimental groups

Groups Parameters	G1	G2	G3	G4	G5	G6	G7
Ca (mg/dL)	8.02±0.13^{a}	7.7±0.56^{a}	5.1±0.16^{b}	7.54±0.82^{a}	7.68±0.72^{a}	7.32±0.41^{a}	7.92±0.24^{a}
Ph (mg/dL)	4.61±0.48^{a}	4.36±0.42^{a}	3.37±0.41^{b}	4.52 ±0.58^{a}	4.56 ±0.50^{a}	4.08 ±0.53^{a}	4.24 ±0.46^{a}
PTH (pg/ml)	44.6±6.14^{bc}	41.6±5.8^{c}	82.6±5.13^{a}	46.0±6.52^{bc}	43.0±5.70^{c}	53.8±4.43^{b}	50.2±10.96^{bc}
Osteocalcin (ng/ml)	21.62±2.07^{a}	22.36±1.8^{a}	15.96±1.54^{c}	21.42±1.96^{ab}	21.56±2.2^{ab}	19.58±1.31^{a}	20.98±1.60^{a}

Values are presented as Mean ± SD (n=10).
Different letters indicate significant difference (p<0.05)

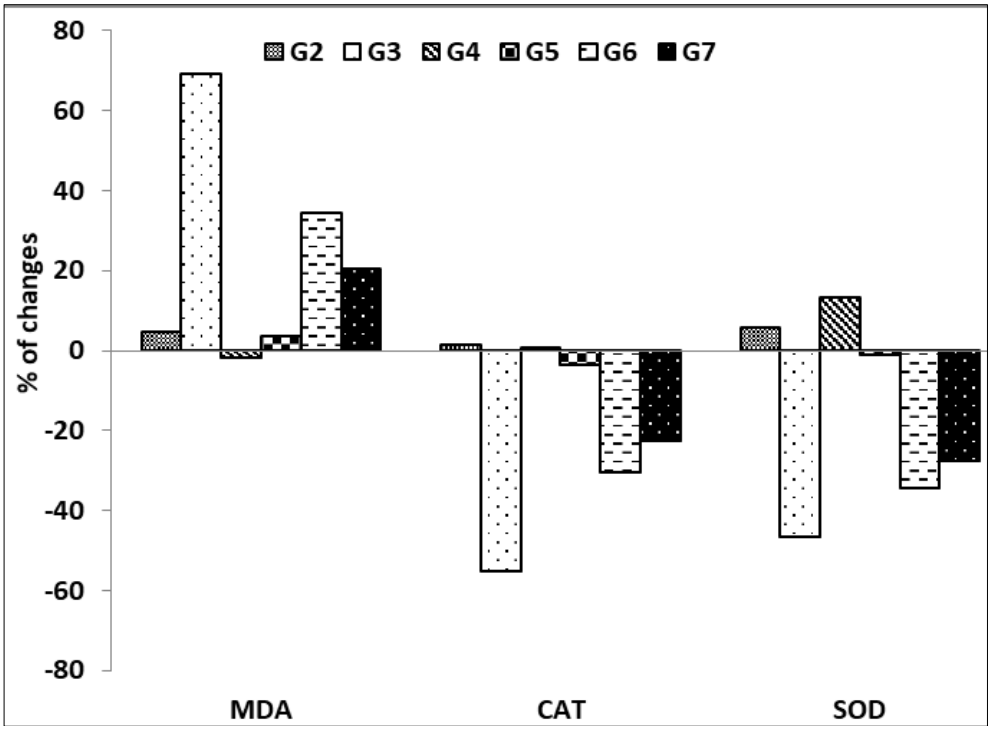


Fig 2: Percent of change in the levels of MDA (nmol/mg), SOD (U/mg), CAT (ng/mg) in kidney homogenate on male albino rats in different experimental groups

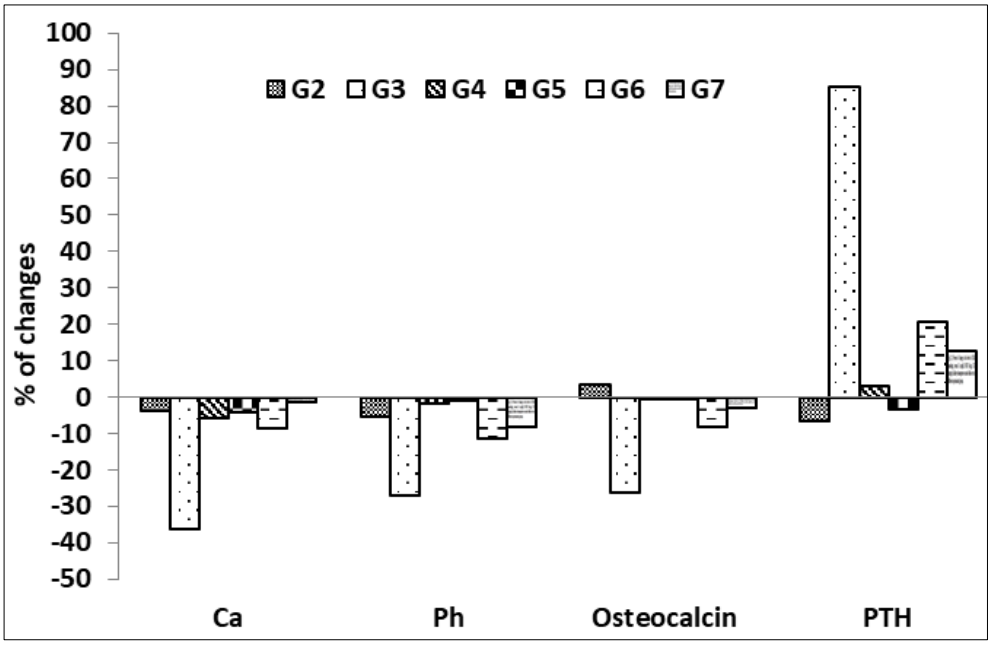


Fig 3: Percent of change in the levels of serum Ca (mg/dl), Ph (mg/dl), OST (ng/ml) and serum PTH (pg/ml) on male albino rats in different experimental groups

Table 4: Hematological parameters in all experimental groups

Groups Parameters	G1	G2	G3	G4	G5	G6	G7
Hemoglobin (g/dl)	14.41±0.51^{a}	14.53±0.53^{a}	11.97±0.80^{c}	13.81±0.74^{ab}	14.42±0.63^{a}	13.56±0.69^{b}	13.91±0.12^{ab}
RBCs (x10^6/cmm)	5.38±0.51^{a}	5.48±0.31^{a}	4.18±0.16^{b}	5.46±0.36^{a}	5.42±0.53^{a}	5.12±0.40^{a}	5.31±0.32^{a}
WBCs (x10^6/cmm)	9.44±0.58^{cd}	9.39±0.75^{cd}	13.38±1.5^{a}	9.02±1.5^{d}	9.71±1.4^{cd}	10.51±1.3^{b}	10.18±0.45^{bc}

Values are presented as Mean ± SD (n=10).
Different letters indicate significant difference (p<0.05)

Table 5 and fig. (5) shows significant increase in, AST, ALT, ALP, TC and TG in G3 (gentamicin group), compared to the control group. Treatment with gentamicin either by fixed mix oil in group 6 or essential mix oils in group 7 significantly decreased the increased levels of these parameters to the control levels compared to gentamicin group.

Table 5: Some biochemical parameters in all experimental groups

Groups Parameters	G1	G2	G3	G4	G5	G6	G7
ALT (U/L)	55.53±6.03 ^{bc}	56.50±6.20^{bc}	102.62±21.64^{a}	50.72±5.17^{c}	49.1±2.98 ^{c}	63.8±4.48^{b}	62.56±7.91 ^{bc}
AST(U/L)	81.90±7.35 ^{d}	80.38±7.12 ^{d}	189.84±5.32 ^{a}	82.64±11.53 ^{d}	78.92±9.41 ^{d}	138.64±10.76 ^{b}	97.7±15.41 ^{c}
ALP(U/L)	130.14±12.29^{c}	142.24±5.39^{bc}	256.96±30.6 ^{a}	132.12±13.93 ^{c}	128.72±0.01 ^{c}	153.22±4.741^{b}	128.62±13.47 ^{c}
TC (mg/dL)	86.17±4.67^{bc}	88.01±6.01^{bc}	170.96±8.34^{a}	81.46±6.99^{c}	88.66±7.20^{bc}	95.82±7.17^{b}	92.26±6.87^{b}
TG(mg/dL)	93.41±6.97^{b}	89.14±7.44^{b}	117.14±5.78^{a}	88.24±7.69^{b}	90.56±10.30^{b}	95.42±7.11^{b}	93.84±7.18^{b}

Values are presented as Mean ± SD (n=10).
Different letters indicate significant difference (p<0.05)

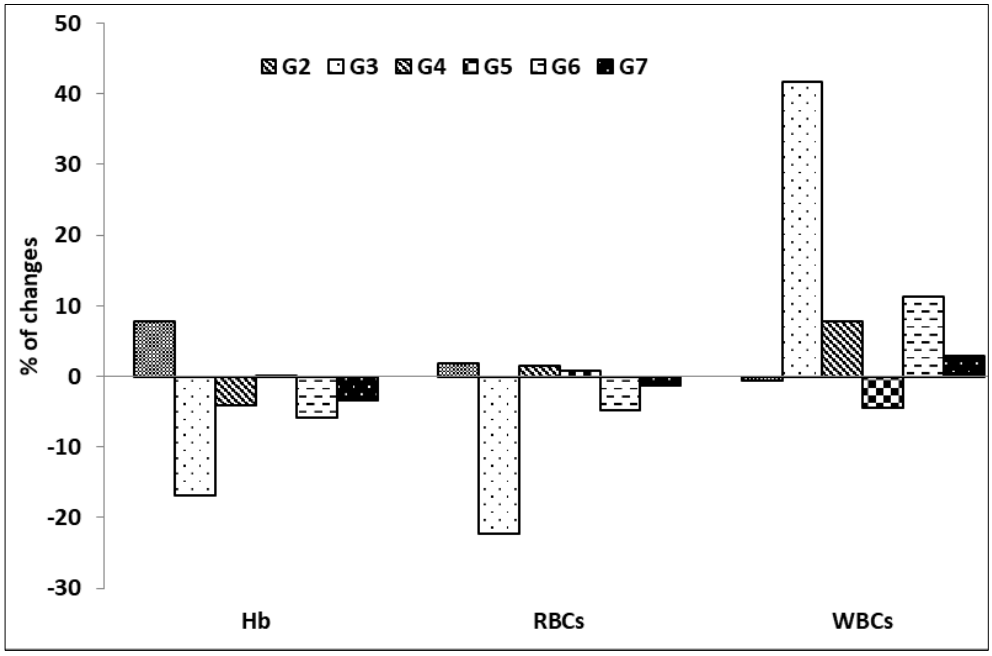


Fig 4: Percent of change in the levels of hematological parameters on male albino rats in different experimental groups

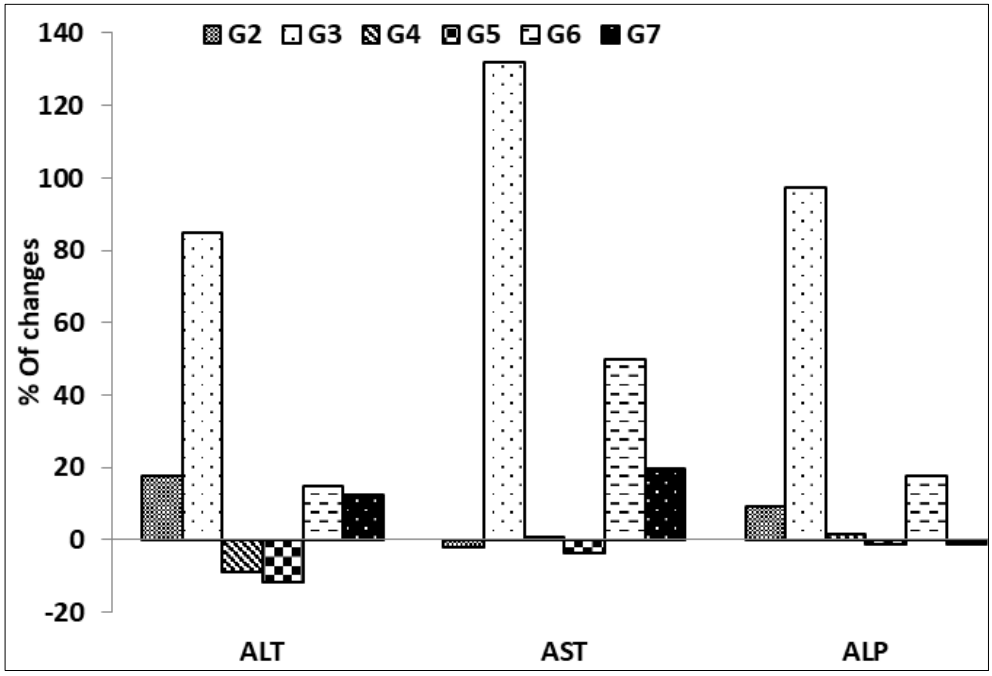


Fig 5: Percent of change in the levels of serum ALT (U/I), serum AST (U/L) and serum ALP (IU/L) parameters on male albino rats in different experimental groups

The data presented in table (6) demonstrate that there were significant strong positive correlations were found between MDA and kidney weight, creatinine, urea, uric acid, parathyroid hormone, WBC, ALT, AST, ALP, TC, TG and TNF-α. Statistical analysis showed significant strong negative correlations between MDA and each of the following; albumin, calcium, phosphorus, osteocalcin, RBC, Hb and T_3.

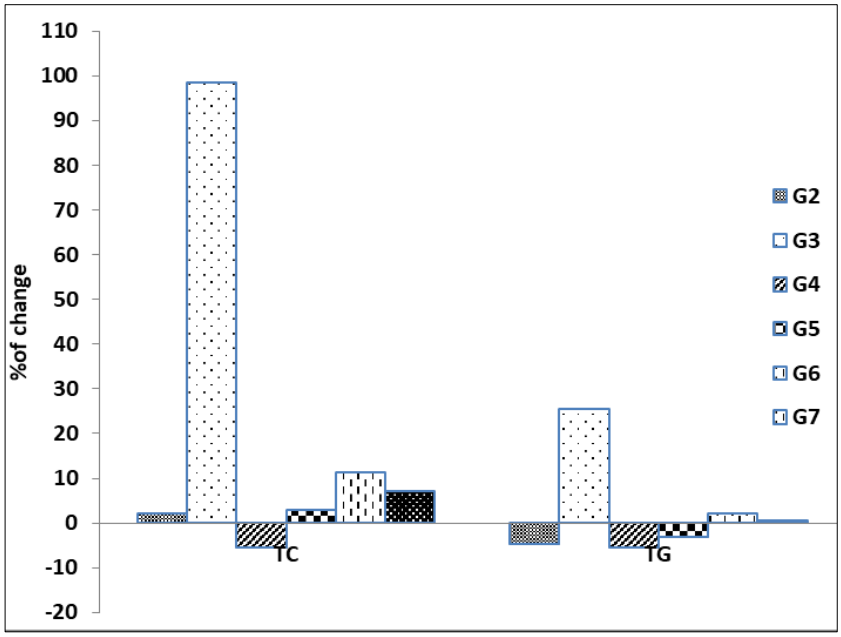


Fig 6: Percent of change in the levels of serum cholesterol (mg/dl), serum triglyceride (mg/dl) and serum triiodothyronine on male albino rats in different experimental groups.

Fig. (6). Percent of change in the levels of serum cholesterol (mg/dl), serum triglyceride (mg/dl) and serum triiodothyronine on male albino rats in different experimental groups.

Table 6: Correlation coefficient between lipid peroxidation product (MDA) and biochemical parameters

Parameters					
Parameters	Kidney weight	Creatinine	Urea	Uric acid	Albumin TNF-α T_3
MDA	0.788646	0.901287	0.806406	0.877652	-0.82859 0.890116 -0.8754
			Parameters		
	SOD	CAT	PTH	Ca	P Osteocalcin
	-0.73179	-0.768675	0.833128	-0.8078	-0.63434 -0.63621
Parameters					
Parameters	RBC	WBC	Hb	ALT	AST ALP
MDA	-0.68679	0.787006	-0.72744	0.781321	0.899423 0.798416
Parameters	TC	TG			
MDA	0.583938	0.757967			

All parameters significant correlation with MDA at $p<0.01$

Discussion

Research has demonstrated that (GM) negatively impact the kidneys by producing reactive oxygen species (ROS) and weakening the antioxidant defense system. This, in turn, causes a build-up of free radicals that can cause severe tissue damage (Lopez-Novoa *et al*., 2011) [37]. Therefore, using antioxidants could provide protection against GM-induced oxidative damage. Fixed (Non-volatile) oils and essential (Volatile) oils have antioxidants and reactive oxygen species scavenger properties. To the best of our knowledge, no previous studies have designed to compare a possible antioxidant effect between two mixed essential oil extracted from clove buds& peppermint leafes, and two mixed fixed oil extracted from seeds of radish & Parsley on nephrotoxicity and changes in hematological, bone homostasis and liver function induced by gentamicine.

According to the current results, GM caused renal damage, which was shown by a discernible rise in the serum levels of urea, uric acid, and creatinine as well as a decrease in albumin when compared to the control group. These findings are consistent with the findings of Mishra *et al*. (2021) [39]; Althunibat *et al*. (2022) [6] about the impairment of renal function induced by GM. This may be due to GM accumulate in the renal proximal convoluted tubes, producing free radicals which elevated lipid peroxidation in renal tissues, this leads to destruction of organelle membrane and reduction of glomerular filtration rate (GFR) (Hussain *et al*., 2012) [30].

Additionally, GM resulted in a marked decrease in serum albumin levels; this might be attributed to problems with albumin production in the liver as well as impairment of albumin reabsorption through proximal tubules (Dickson *et al*., 2014) [15]. GM is strongly linked to both inflammation and tissue remodeling, which ultimately results in renal fibrosis. It also causes kidney edema and inflammation, as seen by an increase in kidney weight and pro-inflammatory cytokines (TNF-α).

Moreover, this study showed that total T_3 significantly decline in GM group, this result is in contract with Wiederkehr *et al*., (2004) [69] who found the impaired of thyroid function in chronic kidney disease. Decrease T_3 level may be attributed to variety of reasons including chronic protein deficiency, lowering peripheral T_4 to T_3 conversion and protein binding (Zoccali *et al*., 2005) [74].

In this study, the GM group showed a significant elevation in MDA and a decrease in CAT and SOD in the

renal tissues when compared to the control group. This might be attributed to GM deposited in renal cortex where it enhanced oxidative stress by released iron ion from mitochondria and forms an Iron-GM complex (Yanagida *et al*., 2004) [71]. This generates reactive oxygen species (ROS) as (OH, O^{-2}, H_2O_2) and reactive nitrogen species as (NO, $ONOO^{--}$) thus increasing free radicals which suppress antioxidant defense system in renal tissues. Tomsa *et al*., (2021) [82] reported that MDA (Marker of lipid peroxidation) is a very sensitive marker for kidney damage by GM. Lipid peroxidation produces MDA inside the tissues and lowers the amount of polyunsaturated fatty acids, which act as a substrate for free radicals, especially superoxide and hydrogen peroxide. The development of GM toxicity begins with this interaction between aminoglycosides and phospholipids (Ozbek, 2012) [47]. There was a positive correlation found between the raised levels of serum creatinine, uric acid, and urea and the increased MDA levels seen in the current study. This implies a connection between renal failure, oxidative stress, and nephrotoxicity and lipid peroxidation (Ungur *et al*., 2022) [65]. The reduction in activities of CAT (Decomposer of H2 O2) and SOD (A free radical scavengers) in the renal tissues of GM rats group, may be due to increase ROS induced by GM thus occur diminished antioxidant defenses.

In the current study both the mixture of essential and fixed oils could considerably ameliorate renal function testes, antioxidant profile of kidney and thyroid function. This may be attributed to the phenolic compound in these oils and the synergistic effects of its bio compounds which improve renal filtration barrier function and GFR, through elevation antioxidant defense system. Also, decrease in kidney weight and TNF-α indicating that these oils have anti-inflammatory effects. Furthermore, as compared to the GM group, these two oil combinations significantly lower the activity of the SOD, CAT, and MDA enzymes in the kidney tissues. This may be explained by the oils' capacity to protect kidney cells' structural integrity from the damaging impacts of GM since they contain bioactive components, particularly phenolic compounds, which scavenging free radicals, metal ion chelation, suppression of cell membrane lipid peroxidation, and antioxidant enzyme control (Yosr *et al*., 2013) [73].

Fiqardina *et al*. (2022) [23] showed that administering to rats 10 mg/kg of clove oil decreased the rise in serum urea, creatinine, and kidney MDA levels following a 28-day levofloxacin treatment. Eugenol, a powerful antioxidant molecule, eugenol acetate, and thymol are examples of phenolic chemicals that may contribute to clove oil's antioxidant action (Nassar *et al*., 2007) [42]. By scavenging free radicals, chelating transient metal ions, blocking oxidant enzymes, or regenerating α-tocopherol from the α-mecofol radical, clove oil can prevent cell damage (Pulikottil *et al*., 2015) [50]. Furthermore, flavonoids have the ability to suppress LPO activity and scavenge OH, O^{-2}, and peroxyl radicals (Van Acker *et al*., 1996) [66]. As a result, they can raise SOD, GPx, and GSH while lowering MDA.

According to Aryanti *et al*. (2018) [7], peppermint oil's high phenolic component content, which includes mono and dicaffeoylquinic acids, monoterpenes, and flavonoids (Luteolin), might enhance endogenous antioxidant enzyme activity. It has been demonstrated that these compounds have strong antioxidant activity (Riachi LG & De Maria, 2015) [55]. According to Khalil *et al*. (2015) [34], giving rats peppermint leaves oil improves their antioxidant activity when they are administered CCl4.

Strong antioxidants like radish can effectively stop tissue damage from getting worse by scavenging free radicals and acting as an antioxidant. According to Shehzadi *et al*. (2021) [58], rats' nephrotoxicity caused by carbon tetrachloride (CCl4) was reversed by co-administration of *Raphanus sativus* seed extract. This may be attributing to its sulforaphene content which have antioxidant and free radical scavenging properties that augments the antioxidant defense mechanism, decrease lipid peroxidation and reduce oxidative stress (Sita *et al*., 2018) [60]. According to research by Thangapandiyan *et al*. (2018) [64], sulforaphane is a potential antioxidant medication that effectively lowers oxidative stress and repairs tissue/cell damage in both *in vitro* and *in vivo* experimental animals.

Mohammed *et al*. (2020) [40] found that by increasing SOD, CAT, and GSH and decreasing lipid peroxides, the extract from *Raphanus sativus* seeds increased the oxidative stress of CCl4. The presence of alkaloids such as coumarins, saponins, flavonoids, and anthocyanins in the extract from *Raphanus sativus* seeds may explain its capacity to directly scavenge oxygen free radicals (El-Sayed, 2001) [21].

Serum urea, uric acid, and creatinine were significantly reduced when parsley and radish seed extract was administered in addition to gentamicin, compared to the group that received gentamicin treatment. These results were consistent with those obtained by Elkhamisy *et al*. (2015) [19] using rats that were induced to become nephrotoxic due to gentamicin. Parsley extract's method of action seems to be mediated by inhibition of the Na+/K+ pump, which would decrease the reabsorption of K+ and Na+ and, as a result, the lumen's osmotic water flow and dieresis. Parsley has diuretic effects because of two ingredients: Meristic and apiol (Eidi *et al*., 2009) [17]. Thus, the decrease in creatinine, urea, and uric acid in the data might be attributed to parsley's diuretic action. According to Al-Seeni *et al*. (2018) [76], parsley methanolic extract improved kidney function, reduced lipid peroxidation, and raised antioxidant levels. Furthermore, by corrected renal and hepatic function tests, Salama *et al*. (2020) [57] revealed that parsley extracts had defensive benefits against gentamicin toxicity. Parsley's antioxidant properties, which include flavonoids, carotenoids, and other phenolic components, may be the cause of its effectiveness (Liberal *et al*., 2020) [35]. Furthermore, parsley's phytochemicals have been shown by Haidari *et al*. (2011) [26] to increase overall antioxidant capacity, inhibit harmful oxygen free radicals, and protect against oxidative stress damage.

Moreover, these two mixtures of oils were attenuated the increases in level of TNF-α in gentamicin group, this indicated its have anti-inflammatory effect. TNF-α expressions are modulated by the parsley leaf extract, according to Malik *et al*. (2017) [80].

Furthermore, Elkomy *et al*. (2020) [20] discovered that parsley's ability to alleviate thyroid gland dysfunction can be attributed either directly to its antioxidant properties or indirectly to its hepatoprotective properties, which reduce thyroid hormone metabolic abnormalities connected to liver damage.

Gentamicin causes electrolyte imbalances by inhibiting a number of cell membrane transporters in the brush border and basolateral membranes, regardless of cell damage. According to Raghavan and Weisz (2016) [51], transport

inhibition impairs cell viability in addition to tubular reabsorption, which eventually leads to necrosis or apoptosis. According to Rhee *et al*. (2015) [54], the notable decrease in calcium and phosphorus concentrations after GM treatment may be caused by antagonistic calcium ions that inhibit acetylcholine release from motor neuron terminals and generate are-like effects. This may lessen rats' forced motor activity. Also, GM dramatically lowers serum phosphorus and total calcium levels while dramatically raising PTH (Mineral-regulating hormone) levels. In line with these findings, Abdel-Azeem *et al*. (2017) [78].

The scientists linked these disruptions to the increased PTH level that resulted from gentamicin treatment. It's possible that in order to preserve calcium homeostasis. Because of the hyperfiltration of calcium generated by GM, significant levels of serum PTH are always present when calcium is liberated from the bone. Moreover, in damaged skeletal muscle, elevated glucocorticoid levels promote calcium deposition as calcium phosphate and carbonate (Heibashy & Abdel Moneim, 1999) [28]. Additionally, gentamicin intoxication may increase the amount of calcium excreted in urine, restrict calcium absorption into mitochondria, and induce the release of ionized calcium from mitochondria (Heibashy *et al*., 2009) [29].

Osteoblasts produce osteocalcin, a non-collagenous protein found in bone that is essential for calcium ion homeostasis, metabolic control, and bone mineralization (Delmas, 1993) [14]. It could be measured as an indicator of osteoblast activity and bone formation. Our findings demonstrated that, in comparison to the control, GM adversely impacted or slowed down the formation of bones by lowering the serum osteocalcin level. The most probable cause of low osteocalcin levels is GM's increase in free radicals, which may be causing a drop in osteoblastic activity.

The treatment by mixture of essential (Volatile) or mixture of fixed (Nonvolatile) oils restored PTH hormone, total calcium, phosphorus and osteocalcine to normal range compared to GM group. This may be related to rich phenolic component in these two mixtures of oils could be having ameliorative effect on renal function and stimulates osteoblastic activity and bone formation. Due to their antioxidant effects, a number of studies have discovered associations between consumption of polyphenols and bone health (Rao *et al*., 2012; Welch and Hardcastle, 2014) [53, 68]. Callaway and Jiang (2015) [10] discovered that oxidative stress promotes an increase in bone resorption connected to direct and indirect effects on osteoclast activity and differentiation, which is a significant role in the pathophysiology of osteoporosis. Polyphenols have a protective effect on bone metabolism via modifying calcium signaling. This is primarily accomplished by suppressing bone resorption, with osteoclastogenic genes serving as the principal targets (Dudarić *et al*., 2015) [16].

Hematological parameter measurements are thought to be the first sign of the harmful impact caused by the nephrotoxic medication and its metabolite (Lim *et al*., 2022) [36]. According to Ashour *et al*. (2007) [8], exposure to hazardous compounds can alter the human body's morphology, biochemistry, and physiology, leading to haematological diseases and reduced kidney function, among other effects on many organ systems. Certain drugs primarily target the hematopoietic system, negatively impacting heme synthesis enzymes, normal erythropoietin production rates, and the normal range, shape, and distribution of different blood cell types (Sharma *et al*., 2013) [59]. According to Vaziri *et al*. (1999) [67], toxic medications cause harm to erythrocytes and impair their ability to carry oxygen, which raises the risk of hypertension and cardiac arrest. The pathophysiology of anemia is caused by reduced red blood cell survival, uraemic toxin-induced marrow suppression, and iron or folate deficiencies. According to Naeshiro *et al*. (1997) [41], gentamicin primarily harms the kidney because the kidney's erythropoietin-producing cells do not produce enough of it, and the reduced plasma erythropoietin level inhibits the bone marrow from manufacturing a normal number of new erythrocytes. GM induced elevation in the number of WBC, this may attributed to immunological response against the adverse effect induced by GM. The antioxidant activities of components present in each mixture of volatile or nonvolatile oils reversed these hematological changes to normal levels. Waheeba *et al*., (2020) [79] reported that parsley can ameliorate change in hematological parameter induced by potassium bromate due to phenolic compound and vitamin C presence in parsley.

Hepatotoxicity was indicated by a significant increase in the level of ALT, AST, and ALP following gentamicin injection. Transaminases and ALP are considered sensitive markers of liver function because they are cytoplasmic compounds that are released into the circulation by modifications in the permeability of the hepatocyte membrane. As a result, their levels in the serum are enhanced. The results of this study are consistent with earlier research (Salama *et al*., 2020) [57], which discovered that rats receiving an intraperitoneal injection of 100 mg of gentamicin for eight days had increased levels of serum AST, ALT, and ALP activities.

According to Heidaria *et al*. (2011) [26], oxidative stress generated by gentamicin may have caused a change in lipid profile, which may have contributed to the notable increase in serum cholesterol and triglycerides seen in the rats in the gentamicin group. Additionally, particularly in cases when GM-induced liver injury results in increased synthesis of cholesterol or decreased hepatic catabolism. As a result, the accumulating of filtered LDL fractions in the glomeruli, which decrease the glomerular filtration surface area, may be linked to the elevated lipid levels observed in rats administered GM treatment. This is in agreement with Rashid & Khan, (2017) [83] and Hijazi, & Mouminah, (2017) [31], who reported that injecting rats with gentamicin (80 mg/kg) increased the levels of total cholesterol and TGs as compared with control animals.

When compared to GM groups, administration of a combination of volatile (Essential) or non-volatile (Fixed) blend oils significantly enhances liver enzymes, TC, and TG. This may be because these oils include polyphenols, which prevent oxidative stress, lower hazardous oxygen free radicals, and improve total antioxidant capacity. As a result, damage to the liver tissue's membranes, proteins, and DNA was prevented. The toxicity of liver indicators was therefore substantially adjusted. The mixture of volatile oils produced the greatest results, followed by the combination of non-volatile oils. This might be because the mixture of volatile oils included a higher concentration of phenolic components than the mixture of nonvolatile oils. Yıldız & Öztürk (2020) [72] found that Eugenol have high antioxidant properties on oxidative stress in liver rats induced by ethanol. Ali *et al*.,

(2014) [4] reported that eugenol, an antioxidants, found in dried flower bud of clove can protect liver rats against injury by thioacetamide. According to Al-Okbi *et al*. (2014) [5], clove oil and eugenol microemulsions are beneficial for fatty liver and dyslipidemia. Furthermore, giving gentamicin-treated rats volatile clove oil reduces the increase in ALP activity to levels close to normal, suggesting that clove oil prevented liver damage. According to Ullah *et al*. (2014) [77], gentamicin's harmful effects might be eliminated from rats by giving them peppermint leaf oils. Based on the findings of Abed *et al*. (2015) [2], radish oil used orally may effectively repair liver tissue damage by reducing serum levels of AST, ALT, and ALP. This suggests that radish oil is useful in protecting against hepatotoxicity. Its antioxidant concentration, which can guard against membrane fragility and reduce the amount of marker enzyme leaking into the bloodstream, may be responsible for this. Parsley possesses anti-inflammatory and antioxidant properties that protect against liver damage caused by gentamicine, according to Salama *et al*. (2020) [57].

Conclusion

Both two mixtures of oils have potential antioxidant synergistic effect to ameliorate nephrotoxicity and changes in hematological, bone homeostasis and liver function induced by gentamicin. And both of them have nearly the same antioxidant activities, so it is easier to use the fixed (Non-volatile) oils mixture of low coast other than the high coasted essential (volatile oils).

References

1. Abed SA, El-Shazely MO, Ahmed KA, Abdelmawla EM, Ibrahim AK. Pathological, Immunohistochemical and Biochemical Studies on The Therapeutic Effect of *Raphanus sativus* Oil on Streptozotocin Induced Diabetic Rats. Egypt. J Comp. Path & Clinic Path. 2015;28(1):1-7.
2. Aebi H. Catalase *in vitro*. Enzymol. 1984;105:121-126.
3. Ali S, Prasad R, Mahmood A, Routray I, Shinkafi TS, Sahin K, *et al*. Eugenol-rich fraction of *Syzygium aromaticum* (clove) reverses biochemical and histopathological changes in liver cirrhosis and inhibits hepatic cell proliferation. Journal of cancer prevention. 2014;19(4):288.
4. Ali BH. Gentamicin Nephrotoxicity in Humans and Animals: Some Recent Research. Gen. Pharma. 1995;26(7):1477-1487.
5. Al-Okbi SY, Mohamed DA, Hamed TE, Edris AE. Protective effect of clove oil and eugenol microemulsions on fatty liver and dyslipidemia as components of metabolic syndrome. J Med Food. 2014;17:764-71. DOI: 10.1089/jmf.2013.0033.
6. Althunibat OY, Abukhalil MH, Aladaileh SH, Qaralleh H, Al-Amarat W, Alfwuaires MA, *et al*. Formononetin Ameliorates Renal Dysfunction, Oxidative Stress, Inflammation, and Apoptosis and Upregulates Nrf2/HO-1 Signaling in a Rat Model of Gentamicin-Induced Nephrotoxicity. Frontiers in Pharmacology; c2022. p. 13.
7. Aryanti D, Agustiningsih D, Wahyuningsih MSH. Peppermint oil prevented oxidative stress in experimental animal-induced acute single bout of eccentric exercise (ASBEE): Study on blood catalase and hydrogen peroxide (H_2O_2) and glucose transporter-4 (GLUT-4) expression on the muscle cells. Journal of the Medical Sciences (Berkala Ilmu Kedokteran), 2018, 50(3).
8. Ashour AE, Yassin MM, Aasi NM, Ali RM. Blood, serum glucose and renal parameters in lead-loaded albino rats and treatment with some chelating agents and natural oils. Turkish journal of Biology. 2007;31(1):25-34.
9. Babaeenezhad E, Nouryazdan N, Nasri M, Ahmad VH, Moradi SM. Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities. Heliyon. 2021;7(7):e07465.
   DOI: 10.1016/j.heliyon.2021.e07465.
   PMID: 34278037; PMCID: PMC8264605.
10. Callaway DA, Jiang JX. Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. Journal of Bone and Mineral Metabolism. 2015;33(4):359-370.
11. Cao L, Zhi D, Han J, Kumar SS, Xie Y. Combinational effect of curcumin and metformin against gentamicin-induced nephrotoxicity: Involvement of antioxidative, anti-inflammatory and antiapoptotic pathway. J Food Biochem. 2019;43(7):e12836.
12. Chung DH, Kim SH, Myung N, Cho KJ, Chang MJ. The antihypertensive effect of ethyl acetate extract of radish leaves in spontaneously hypertensive rats. Nutr. Res. Pract. 2012;6(4):308-314.
13. Dawidowicz AL, Olszowy M. Does antioxidant properties of the main component of essential oil reflect its antioxidant properties? The comparison of antioxidant properties of essential oils and their main components. Nat Prod Res. 2014;28(22):1952-1963.
14. Delmas PD. Biochemical markers of bone turnover. J Bone Miner Res. 1993;8(S2):S549-555.
15. Dickson LE, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really. J Am Soc. Nephrol. 2014;25(3):443-453.
16. Đudarić L, Fužinac-Smojver A, Muhvić D, Giacometti J. The role of polyphenols on bone metabolism in osteoporosis. Food Res Int. 2015;77:290-298.
17. Eidi A, Eidi M, Badiei L. Antinociceptive effects of ethanolic extract of parsley (*Petroselinum crispum* L.) leaves in mice. Med Sci. J Islamic Azad University-Tehran Med Branch. 2009;19(3):181-186.
18. El-Hadary AE, Hassanien MFR. Hepatoprotective effect of cold-pressed *Syzygium aromaticum* oil against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. Pharm Biol. 2016;54:58.
19. Elkhamisy AE. Protective effect of parsley leaves and turmeric roots extracts against gentamicin induced nephrotoxicity in male rats. World J Dairy Food Sci. 2015;10(1):1-8.
20. Elkomy A, Aboubakr M, Elsayed F, Medhat Y. Protective effect of cinnamon and/or parsley oils against carbon tetrachloride (CCl4) induced hepatotoxicity in rats. J Pharmacol. Clin. Res. 2020;8:555734.
21. El-Sayed ST. Purification and characterization of raphanin, A neutral protease, from *Raphanus sativus* leaves. Pak J Biol Sci. 2001;4:564-568.
22. Farah H, Elbadrawy E, Al-Atoom AA. Evaluation of antioxidant and antimicrobial activities of ethanolic extracts of Parsley (*Petroselinum crispum*) and Coriander (*Coriandrum sativum*) plants grown in Saudi Arabia. Int. J. 2015;3:1244-1255.

23. Fiqardina A, Yusrini DY, Santoso A, Nurul SS, Ismail I. The Nephroprotective Effect of Clove Oil (*Oleum Caryophylli*) Against Levofloxacin Toxicity in Rats. Iran J Toxicol. 2022;16(1):27-34.
24. Fujiwara K, Yoshizaki Y, Shin M, Miyazaki T, Saita T, Nagata S, *et al*. Immunocytochemistry for vancomycin using a monoclonal antibody that reveals accumulation of the drug in rat kidney and liver. Antimicrob. Agents Chemother. 2012;56(11):5883-5891.
25. Guenther E. The essential oils. Vol. I, III, IV. 4th ed. Princeton, NY, USA: D. Van Nostrand Company, Inc; c1961.
26. Haidari F, Keshavarz SA, Shahi MM, Mahboob SA, Rashidi MR. Effects of parsley (*Petroselinum crispum*) and its flavonol constituents, kaempferol and quercetin, on serum uric acid levels, biomarkers of oxidative stress and liver xanthine oxidoreductase activity in oxonate-induced hyperuricemic rats. Iran J Pharm Res. 2011;10(4):811.
27. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. Clove Essential Oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules. 2021;26(21):6387.
28. Heibashy MIA, Abdel Moneim AE. Kidney and liver function tests after late Dimethyl sulfoxide (DMSO) administration in rats with gentamicin induced acute renal failure. J Egypt Ger Soc Zool. 1999;30(A):35-48.
29. Heibashy MIA, El-Nahla AM, Ibrahim AI, Saleh SYA. Comparative study between dimethyl sulfoxide (DMSO), allopurinol and urate oxidase administration in nephrotoxic rats induced with gentamicin. 43rd Annu Vet Med Symp, Coll Vet Med Nurs. Allied Health, Tuskegee Univ., AL, USA; c2009.
30. Hussain T, Gupta RK, Sweety K, Eswaran B, Vijayakumar M, Rao CV, *et al*. Nephroprotective activity of *Solanum xanthocarpum* fruit extract against gentamicin-induced nephrotoxicity and renal dysfunction in experimental rodents. Asian Pac J Trop Med. 2012;5(9):686-691.
31. Hijazi MA, Mouminah HH. Studies on effects of celery leaves on lipids profile and nephrotoxicity in rats induced by gentamicin. Curr. Sci. Int. 2017;6(4):711-722.
32. Jan M, Badar A. Effect of crude extract of *Raphanus sativus* roots on isolated trachea of albino rat. Pakistan Journal of Physiology. 2012;8(1):23-26.
33. Keifer D, Ulbricht C, Abrams TR, Basch E, Giese N, Giles M, *et al*. Peppermint (*Mentha X piperita*): An evidence-based systematic review by the natural standard research collaboration. Journal of herbal pharmacotherapy. 2008;7(2):91-143.
34. Khalil AF, Elkatry HO, El Mehairy HF. Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats. Annals of Agricultural Sciences. 2015;60(2):353-359.
35. Liberal Â, Fernandes Â, Polyzos N, Petropoulos SA, Dias MI, Pinela J, *et al*. Bioactive properties and phenolic compound profiles of turnip-rooted, plain-leafed and curly-leafed parsley cultivars. Molecules. 2020;25(23):5606.
36. Lim JY, Jung WW, Kim W. Nephrotoxicity evaluation and proteomic analysis in kidneys of rats exposed to thioacetamide. Sci. Rep. 2022;12:6837. https://doi.org/10.1038/s41598-022-11011-3.
37. Lopez-Novoa JM, Yaremi Q, Laura V, Ana IM, Francisco J. New insights into the mechanism of aminoglycoside nephrotoxicity: An integrative point of view. Kidney international. 2011;79(1):33-45.
38. Manivannan A, Kim JH, Kim DS, Lee ES, Lee HE. Deciphering the nutraceutical potential of *Raphanus sativus* - A comprehensive overview. Nutrients. 2019;11(2):402.
39. Mishra P, Mandlik D, Arulmozhi S, Mahadik K. Nephroprotective role of diosgenin in gentamicin-induced renal toxicity: Biochemical, antioxidant, immunological and histopathological approach. Future Journal of Pharmaceutical Sciences. 2021;7(1):1-3.
40. Mohammed AT, Mohammed ES, Mohammed EG. Effect of *Raphanus sativus* seed oil on chronic renal failure-induced infertility in male rats. Journal of Environmental Sciences, Mansoura University. 2020;49(3):78-87.
41. Naeshiro I, Ishimura Y, Chatani F, Sato S. Possible mechanism for the anaemia induced by gentamicin in rats. Comparative Haematology International. 1997;7(4):220-225.
42. Nassar MI, Gaara AH, El-Ghorab AH, Farrag A, Shen H, Huq E, *et al*. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. Revista Latino-Americana de Química. 2007;35(3):47.
43. Noman OM, Nasr FA, Alqahtani AS, Al-zharani M, Cordero MA, Alotaibi AA, *et al*. Comparative study of antioxidant and anticancer activities and HPTLC quantification of rutin in white radish (*Raphanus sativus* L.) leaves and root extracts grown in Saudi Arabia. Open Chemistry. 2021;19(1):408-416.
44. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Biochem. 1979;95:351-358.
45. Ohtani H, Wakui H, Komatsuda A, Satoh K, Miura AB, Itoh H, *et al*. Induction and intracellular localization of 90-kilodalton heat-shock protein in rat kidneys with acute gentamicin nephropathy. Laboratory investigation: A journal of technical methods and pathology. 1995;72(2):161-165.
46. Omran MF, Soliman NKI. Protective role of radish oil (*Raphson sativus*) against gamma radiation on lipids and carbohydrate in male rats. Isotope and Radiation Research. 2005;37(7):1805-1814.
47. Ozbek E. Induction of oxidative stress in kidney. International journal of nephrology; c2012.
48. Pápay ZE, Kósa A, Boldizsár I, Ruszkai A, Balogh E, Klebovich I, *et al*. Pharmaceutical and formulation aspects of *Petroselinum crispum* extract. Acta Pharmaceutica. Hungarica. 2012;82(1):3-14.
49. Paterson DL, Robson J, Wagener MM. Risk factors for toxicity in elderly patients given aminoglycosides once daily. Journal of general internal medicine. 1998;13(11):735-739.
50. Pulikottil SJ, Nath S. Potential of clove of *Syzygium aromaticum* in development of a therapeutic agent for periodontal disease: A review. South African Dental Journal. 2015;70(3):108-115.
51. Raghavan V, Weisz OA. Discerning the role of mechanosensors in regulating proximal tubule function. Am J Physiol. Renal Physiol. 2016 Jan 1;310(1):F1-5.
52. Rakhmawati RI, Anggarwulan EN, Retnaningtyas ES. Potency of Lobak leaves (*Raphanus sativus* L. var.

Hortensis Back) as anticancer and antimicrobial candidates. Biodiversitas J Biol. Divers, 2009, 10(3).
53. Rao LG, Kang N, Rao AV. Polyphenol antioxidants and bone health: a review. In: Phytochemicals - A global perspective of their role in nutrition and health; c2012.
54. Rhee WJ, Lee SY, Lee JH, Choi SR, Lee SC, Lee JH, *et al*. The effect of high concentration of magnesium with ropivacaine, gentamicin, rocuronium, and their combination on neuromuscular blockade. Korean J Anesthesiol. 2015 Feb 1;68(1):50-61.
55. Riachi LG, De Maria CA. Peppermint antioxidants revisited. Food Chem. 2015 Jan 1;176:72-81.
56. Salah-Abbès JB, Abbès S, Ouanes Z, Houas Z, Abdel-Wahhab MA, Bacha H, *et al*. Tunisian radish extract (*Raphanus sativus*) enhances the antioxidant status and protects against oxidative stress induced by zearalenone in Balb/c mice. J Appl. Toxicol. 2008 Jan;28(1):6-14. DOI: 10.1002/jat.1240. PMID: 17385802.
57. Salama AA, Abd El-Wahed AS, Mostafa AE. Protective effect of some plants against the toxicity of kidneys caused by gentamicin. J Med Sci. Res. 2020 Jan 3;3(1):5.
58. Shahzadi AK, Bano H, Ogbaga CC, Ayyaz A, Parveen R, Zafar ZU, *et al*. Coordinated impact of ion exclusion, antioxidants and photosynthetic potential on salt tolerance of ridge gourd [*Luffa acutangula* (L.) Roxb.]. Plant Physiol. Biochem. 2021 Feb 1;167:517-528.
59. Sharma R, Panwar K, Mogra S. Alterations in developing RBCs after prenatal and postnatal exposure to lead acetate and Vitamins. Int. J Pharm Sci. Res. 2013;4:3214-3224.
60. Sita G, Hrelia P, Graziosi A, Morroni F. Sulforaphane from cruciferous vegetables: Recent advances to improve glioblastoma treatment. Nutrients. 2018 Nov 14;10(11):1755.
61. Sohilait HJ, Kainama H. Free radical scavenging activity of essential oil of Eugenia caryophyllatum from Amboina Island and derivatives of eugenol. Open Chem. 2019 Jan 1;17(1):422-428.
62. Spirling LI, Daniels IR. Botanical perspectives on health peppermint: More than just an after-dinner mint. J R Soc. Promot. Health. 2001 Jan;121(1):62-73.
63. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin. Chem. 1988 Apr;34:497-500.
64. Thangapandiyan S, Ramesh M, Miltonprabu S, Hema T, Nandhini V, Bavithrajothi G, *et al*. Protective Role of Sulforaphane against Multiorgan Toxicity in Rats: An *In vivo* and *In vitro* Review Study. Res Rev: J Toxicol. 2018;8(1):1-8. ISSN: 2231-3834, ISSN: 2349-1264.
65. Ungur RA, Borda IM, Codea RA, Ciortea VM, Năsui BA, Muste S, *et al*. Flavonoid-Rich Extract of *Sambucus nigra* L. Reduced Lipid Peroxidation in a Rat Experimental Model of Gentamicin Nephrotoxicity. Materials. 2022 Jan 20;15(3):772.
66. Van Acker SA, Tromp MN, Griffioen DH, Van Bennekom WP, Van Der Vijgh WJ, Bast A, *et al*. Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med. 1996 Feb 1;20(3):331-342.
67. Vaziri ND, Liang K, Ding Y. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int. 1999 Nov;56:1492-1498.
68. Welch AA, Hardcastle AC. The effects of flavonoids on bone. Curr. Osteoporos. Rep. 2014 Apr;12(2):205-210.
69. Wiederkehr MR, Kalogiros J, Krapf R. Correction of metabolic acidosis improves thyroid and growth hormone axes in haemodialysis patients. Nephrol Dial Transplant. 2004 May 1;19(5):1190-1197.
70. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, *et al*. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. Int. J Mol. Sci. 2017 Jan 5;18(1):96.
71. Yanagida C, Ito K, Komiya I, Horie T. Protective effect of fosfomycin on gentamicin-induced lipid peroxidation of rat renal tissue. Chem. Biol. Interact. 2004 Nov 2004;8(3):139-147.
72. Yildiz H, Özturk E. Histopathological and Biochemical Effects of Eugenol on Alcohol-Treated Rat Liver. Adıyaman Univ. J Sci. 2020 Jun 25;10(1):83-99.
73. Yosr Z, Hnia C, Rim T, Mohamed B. Changes in essential oil composition and phenolic fraction in *Rosmarinus officinalis* L. var. typicus Batt. Organs during growth and incidence on the antioxidant activity. Ind. Crop Prod. 2013 Jan 1;43:412-419.
74. Zoccali C, Tripepi G, Cutrupi S, Pizzini P, Mallamaci F. Low triiodothyronine: A new facet of inflammation in end-stage renal disease. J Am Soc. Nephrol. 2005 Sep 1;16(9):2789-2795.
75. Zhao G, Ren Y, Ma H. Extraction and characterization of radish seed oils using different methods. Trop J Pharm Res. 2017;16(1):165-169.
76. Al-Seeni A, Madeha N, *et al*. Assessment of the antioxidant activity of parsley and carob in hypercholesterolemic male rats. Biomeo. Res. 2018;29:3370-3377.
77. Ullah N, Khan MA, Khan T, Asif AH, Ahmad W. *Mentha piperita* in nephrotoxicity - a possible intervention to ameliorate renal derangements associated with gentamicin. Indian J Pharmacol. 2014;46(2):166-170. DOI: 10.4103/0253-7613.129309. PMID: 24741187; PMCID: PMC3987184.
78. Abdel-Azeem AS, Hegazy AM, Zeidan HM, Ibrahim KS, El-Sayed EM. Potential renoprotective effects of rosemary and thyme against gentamicin toxicity in rats. J Dietary Suppl. 2017;14(4):380-394.
79. Waheeba E, Ahmed A, El-Sayed A, Ihssan MO. Antioxidant activities of parsley (*Petroselinum crispum*) on the induced biochemical and histopathological changes of potassium bromate-fed rats. Pak J Nutr. 2020;19:80-85.
80. Malik A, Kanneganti TD. Inflammasome activation and assembly at a glance. Journal of cell science. 2017 Dec 1;130(23):3955-3963.
81. Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of Thai rat-tailed radish (*Raphanus sativus* L. var. caudatus Alef). Journal of Functional Foods. 2013 Jul 1;5(3):1372-1381.
82. Tomsa R, Gutu S, Cojocaru D, Gutiérrez-Bermejo B, Flores N, Jenaro C, *et al*. Prevalence of sexual abuse in adults with intellectual disability: Systematic review and meta-analysis. International journal of environmental research and public health. 2021 Feb;18(4):1980.
83. Saleem SS, Moosa K, Imam A, Khan RA. Service quality and student satisfaction: The moderating role of university culture, reputation and price in education sector of Pakistan. Iranian Journal of Management Studies (IJMS) http://ijms. Ut. Ac. Ir. 2017 Jan 1;10(1):237-258.