Effect of Dry Leaves of Grape and Black Mulberry and their Mixture on Gentamicin-Induced Nephrotoxicity in Rats

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Abstract

Natural plants were reported to have healing functions in many diseases; leaves of grape *Vitis vinifera* and Mulberry *Morus nigra* have been used in Egyptian culinary as a cooked food due to its pleasant flavor; and showed significant antioxidant properties which offer positive health effects. This study was undertaken in order to examine the impact of dried leaves of grape (GL), mulberry (ML), and their mixture on some parameters of renal functions and antioxidants status in rats with nephrotoxicity induced by gentamicin. Adult male Albino rats (total 48 rats) *Sprague-Dawley* Strain were assigned to this study; the rats were divided into ٨ groups; groups of rats were fed on varied levels of grape or mulberry leaves and their mixtures throughout the experiment (28 days). Results of this study indicated that there were significant increments in renal function parameters, and decrements of liver enzymes and MDA and increases in antioxidants of rat groups fed on mixture of grape and mulberry leaves. The study results indicated the potential effectiveness of grape and mulberry leaves in limiting the effects of oxidative stress and nephrotoxicity.

Key words: Grape leaves, mulberry leaves, kidney functions, liver and oxidative stress.

تأثير أوراق العنب والتوت الاسود المجففة وخليطهما على السمية الكلوية الناتجة عن الجنتاميسين في الجرذان

المستخلص

العديد من الابحاث اشارت الى الخصائص العلاجية للعديد من النباتات الطبيعية في امراض متعددة، ومن اهم النباتات التي تستخدم بالمطبخ المصري نظرا لنكهتها المفضلة ولما لها من خصائص لاحتوائها على مضادات الاكسدة والتي تحقق فؤائد صحية للانسان كلا من اوراق العنب واوراق التوت، لذا هدفت الدراسة الى تقييم التاثير الوقائي لكلا من اوراق العنب واوراق التوت المجففة على وظائف الكلى والاجهاد التاكسدي في ذكور الجرذان المصابة بالسمية الكلوية الناتجة من الجنتاميسين.

استخدمت الدراسة ٤٨ جرذا مقسمة الى ٨ مجموعات وتناولت بعض المجموعات اوراق العنب او التوت بنسب مختلفة، منفردة او مخلوطة، لمدة ٢٨ يوما. اظهرت نتائج الدراسة تحسنا واضحا في مؤشرات وظائف الكلى، انخفاضا في انزيمات الكبد والمالون داي الدهيد، وارتفاعا في مضادات الاكسدة بالدم في المجموعات التي تناولت خليط اوراق العنب والتوت. وتؤكد الدراسة الفعالية المحتملة لاوراق العنب والتوت في الحد من الاضرارالكلوية والاجهاد التاكسدي.

الكلمات المفتاحية: اوراق العنب– اوراق التوت– وظائف الكلى– الكبد– الاجهاد التاكسدي.

Introduction:

Chronic kidney disease (CKD) considered as a major public health problem, and it was regarded as a health problem in Egypt, affecting about 13% of Egyptian population **(Nagib, et al., 2023)**. In addition, **Davis et al., (2016)** stated that one of the important reasons to develop acute kidney injury is the use of nephrotoxic medication which initiates chronic kidney diseases (CKD). Gentamicin (GM) along other antibiotics has been reported as the most nephrotoxic **(Campbell et al., 2023)**. Gentamicin has been reported to provoke nephrotoxicity by inhabiting the synthesis of protein in kidney cells and hence initiating cell necrosis of renal proximal tubule, moreover gentamicin increases the production of free radicals in renal cortex and thus resulting in renal damage **(Balakumar et al., 2010)**.

Incorporating medicinal plants in human diet gained much interest recently, since the advantages of using natural remedy compared with synthetic chemicals include safety and cost effectively **(Xue et al., 2023)**; furthermore, these natural plants play essential part in treatment of many diseases in folk medicine **(Almalki, et al., 2019)**. Leaves of Grape *Vitis v.* used as a cooked food in Egyptian culinary due to its pleasant flavor. Varieties of white Grape showed higher antioxidant activity, which is associated with its polyphenolic compounds **(Lima, et al., 2016)**. The leaves of *Vitis v*. have been used to treat inflammatory turmoil **(Walker, 2002).** Phenolic compounds of grape leave have many positive health effects including antioxidants, and anti-inflammation properties **(Cheah et al., 2014)**. Grape leaves offer benefits for many long-term health benefits in addition to their antioxidant properties **(Keklik et al., 2024)**.

Mulberry *Morus nigra* fruits and leaves put this plant in the group of functional foods that are helpful to individual's health **(Kadam, 2019)**; the leaf extract has been reported to efficiently scavenged NO, $O2^-$, and DPPH radicals and showed significant reducing power **(Andallu et al., 2014)**. Furthermore, **Ren et al., (2015)** demonstrated that, mulberry leaf polysaccharides considerably raise antioxidant enzymes and diminish the MDA levels in diabetic rats, where **Diab et al., (2020)** illustrated that, *M. nigra* extract suppressed DNA damage in hepatic and renal tissues; and appear to be effective in management of oxidative stress-induced organ toxicity. In addition, extract of *M. nigra* leaves considerably enhanced kidney functions in streptozotocin-induced diabetic rats **(Lim and Choi, 2019).**

In accord to the increasing interest in bioactive compounds of grapes and mulberry leaves, this study aims to examine the impact of dried grape (GL), mulberry (ML) leaves and their mixture on some parameters of renal and liver functions and on oxidative stress in a gentamicin-induced nephrotoxicity rats.

Materials and Methods:

Fresh leaves of grape and black mulberry were obtained from the Agricultural Research Center (Cairo, Egypt). Grape leaves (GL) and mulberry leaves (ML) were washed in running water and after drying in an oven at 40 c° for 12 hours, the leaves grinded and by using a sieve a dried powder were obtained. Gentamicin (Gentamicin- Alex 80mg/ 2ml) was purchased from Memphis Co., Egypt.

All used chemicals were of analytical grade and purchased from Al Gomhoria Co., Kits for biochemical blood analysis were obtained from Sigma-Aldrich.

The method of **Singleton et al., (1999)** was followed to determine the total phenolic content; total flavonoids content of leaves of grape and mulberry were determined in accord to **Matejic et al., (2012).**

The grape and mulberry leaf powders were mixed with a normal basal diet at predetermined concentration and fed to treated groups. The method of **Reeves, et al., (1993)** was used to prepare the standard diet. Forty eight (48) adult male albino rats *Sprague-Dawley* Strain weighing (180± 15 g) were assigned to this study, the rats were divided into two main groups as follow: first group (1) negative control group (6 rats) fed on a standard diet, the second main group (42 rats) were injected subcutaneously with gentamicin (100 mg/kg body weight for 7 days) to induce nephrotoxicity **(Udupa and Prakash, 2019)**; then divided into the following 7 groups (each 6 rats): group (2) positive control group fed normal standard diet, group (3) fed on 5% GL, group (4) fed on 10% GL, group (5) fed on 5% ML, group (6) fed on 10% ML, group (7) fed on mixture of 2.5% GL+2.5% ML, and group (8) fed on mixture of 5% GL+ 5 % ML. By the end of experiment (28 days) the rats were anesthetized using diethyl ether, and blood was withdrawn for hepatic portal vein and then centrifuged at 4000 rpm for 10 minutes; serum was separated in a disposable Cuvette and frozen till further analysis.

Blood biochemical analysis: following the methods of **Searcy et al (1967), Caraway (1955),** and **Bohmer, (1971)** kidney function parameters including serum urea, uric acid and creatinine, respectively were estimated. Aspartate Amino Transferase (AST) and Alanine Amino Transfers (ALT) were estimated in accord with **Reitman and Franke (1957)**, and Alkaline Phosphatase (ALP) was estimated according to **Roy (1970)**.

Malondialdehyde (MDA) as an oxidative stress biomarker was determined following the method mentioned by **Draper et al., (1993)**. Total Antioxidant Capacity (TAC) and glutathione reductase (GSH) were determined as mentioned by **Miller et al., (1993)** and **Beutler et al., (1963)**, respectively. Methods of **Kakkar et al., (1984)** and **Aebi (1984)** were applied in order to estimate activities of Super Oxide Dismutase (SOD); and Catalase (CAT) respectively.

Statistical Analysis: the data were statistically analyzed using the one-way analysis of variance (ANOVA) followed by Duncan test, by using Statistical Package for Scientific Search (V. 16), Chicago, USA; the level of significant was considered at ($P \leq 0.05$).

Results and Discussion:

Total phenolic (TP) and flavonoids (TF) content of grape and black mulberry leaves in figure (1); illustrated that, both leaves are rich sources of total phenolic and flavonoids, although mulberry leaves had higher concentration of total phenolic (41.37$\pm$ 9.73 mg GAE/g DW) and flavonoids (6.55$\pm$ 1.34 mg QUE/g DW) than that in grape leaves (10.43$\pm$ 3.51 mg GAE/g DW) and (5.65$\pm$ 0.81 mg QUE/g DW) respectively.

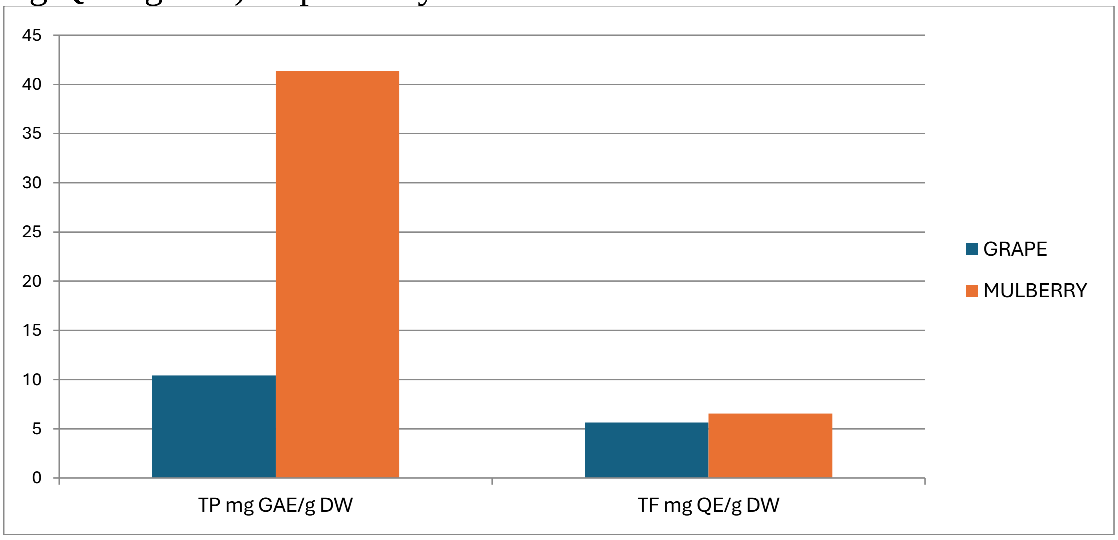

Figure (1): Mean total phenolic (TP) and flavonoids (TF) content of dried grape and black mulberry leaves.

Yu et al. (2018) found that, the total phenol (TP) content in varied varieties of mulberry leaves were 30.4 mg GAE /g DW to 44.7 mg GAE /g DW. Leaves of

Mulberry were considered as a major resource of phenolic **(Gryn-Rynko et al., 2016)**. Total Phenolic was estimated between 9.72 and 14.25 mg GAE/ g; where flavonoids were between 5.08 and 6.22 mg/ g fresh grape leaves **(Güler and Candemir, 2014)**. Variation in the literature data concerning total phenols and flavonoids in grape and mulberry leaves could be due to the fact that, varieties and ecological factors affect synthesis of phytochemical and polyphenols.

Table (1): Mean serum Urea, Uric acid and Creatinine (mg/dl) of rat groups fed on varied level of dried grape and mulberry leaves and their mixtures (Mean + S.E).

Groups	Urea mg/dl	Uric Acid mg/dl	Creatinine mg/dl
(-ve) Control	47.99±1.13 d	2.15±0.12 d	0.57± 0.12 h
(+ve) Control	181.28±2.35 a	8.08±1.47 a	5.32± 0.4 a
5%GL	138.47±1.73 b	4-14±1.14 b	1.67±0.14 b
10% GL	135.86±2.63 b	4.09±1.08 b	1.42±0.08 d
5% ML	126.83±3.00 c	2.14±0.17 d	1.18±0.17 e
10% ML	122.71±2.58 c	2.13±0.22 d	0.98±0.21 f
2.5% GL + 2.5% ML	137.26±2.20 b	3.61±1.05 c	1.52±0.05 c
5% GL + 5% ML	121.76±1.08 c	2.14±0.10 d	0.86±0.10 g

Means within same column with dissimilar letter are significantly different at $P \leq 0.05$.

As shown in Table (1) the positive control group showed significantly higher levels of serum urea, uric acid and creatinine compared to that of negative control group, while rats groups injected with gentamicin and fed on varied levels of grape, mulberry leaves and their mixture showed significant reduction of serum urea, uric acid and creatinine compared to positive group. The best result was noticed of GL 5%+ ML 5% group. The use of nephrotoxic drugs is responsible for about 20% of renal insufficiencies among hospitalized patients **(Schortgen, 2005)**. Recent study by **Bencheikh et al., (2022)** showed that, when gentamicin was administered to rats, significant increases were noticed in the serum urea, uric acid, and creatinine. Renal functions impairment might be noticed by decreases in glomerular filtration rate (GFR), and subsequent increase in level of urea **(Li et al., 2020)**.

The grape leaves are sources for wide variety of polyphenols **(Fan et al., 2004)**, The bioactive compounds present in grape leaves were quercetin, resveratrol, gallic acid, caffeic acid, and kaempferol **(Singh et al., 2023)**; whereas **Cao et al., (2021)** reported that, mulberry leaf extract contained 1-deoxynojirimycin (5.47%), chlorogenic acid (1.21%), resveratrol (0.39%), scopoletin (0.09%), and astragaloside (0.04%). On the other hand, in mulberry leaf bioactive compounds were phenolic acids, flavonoids, stilbenes, phytosterols, phytoalexins, terpenoids, benzofurans, polysaccharides, alkaloids, and fibers that work separately or synergistically to improve human health **(Zou et al., 2024)**.

Negm et al., (2020) reported that there were a noteworthy decrease in serum uric acid, urea nitrogen and creatinine levels in rats' groups treated with varied levels of grape leaves ($P< 0.05$) when compared to Carbon Tetrachloride induced liver toxicity rats. Polyphenols in mulberry leaf resulted in considerable decrements in levels of serum urea and creatinine in diabetic rats **(Varghese and Thomas, 2019)**.

Treatment with quercetin at a dose of 5& 10 mg/kg in rats' model of adenine-induced chronic kidney disease resulted in decreasing of blood urea and creatinine levels; it also decreased oxidative stress, kidney inflammation and improved renal functions **(Yang et al., 2018)**. Quercetin alleviates hyperuricemia in hyperuricemic mice, through inhibiting Xanthine oxidase (XOD) activity, decreasing absorption of the uric acid and lowering the inflammation of the kidney **(Li et al., 2024)**. It is well known that XOD is an enzyme that contributes to the generation of reactive oxygen species, significantly influencing cellular oxidative stress and facilitates the conversion of hypoxanthine to xanthine and finally catalyzes the conversion of xanthine into uric acid **(Rajendran et al., 2014)**.

Nabavi et al., (2013) found that gallic acid (GA) decreased oxidative stress in kidneys of sodium fluoride-induced oxidative stress rat. Also, **Baradaran et al., (2014)** stated that, in gentamicin-induced nephrotoxicity rats, gallic acid prevented oxidative stress due to its antioxidant properties. In addition, **Asci et al., (2017)** illustrated that gallic acid role in decreasing oxidative stress was due to its strong chelating properties in cells and tissues which give gallic acid its characteristic as antioxidant and anti-inflammatory; furthermore, the liver and kidneys regenerative and reparative capacity was enhanced by gallic acid.

Chander and Chopra (2006) stated that, Resveratrol (3,5,4′-hydroxystilbene), all plant leaves contain polyphenols compounds, which acted as anti-inflammatory and antioxidative effect in glycerol-induced renal injury. **Holthoff et al. (2012)** demonstrated that, resveratrol could through two actions, one is to restore the renal microcirculation and the second is to scavenge reactive nitrogen species, thus protecting the tubular epithelium. Resveratrol was reported to have protection against renal diseases **(Morales et al., 2006)**; and these beneficial effects are thought to be due to its antioxidative properties, resveratrol is considered as a strong scavenger of free radicals. In another study, Resveratrol was reported to have numerous effects of protection against age-related disorders, including renal diseases **(Kitada and Koya, 2013)**.

Table (2): Mean serum AST, ALT and ALP (U/L) of rat groups fed on varied level of dried grape and mulberry leaves and their mixtures (Mean + S.E).

Groups	AST U/L	ALT U/L	ALP U/L
(-ve) Control	34.97±0.88 [e]	60.96± 1.60 [e]	63.69± 0.61 [e]
(+ve) Control	72.27±1.18 [a]	108.82± 2.40 [a]	128.92±0.99 [a]
5%GL	53.96± 1.16 [b]	92.93± 1.81 [b]	105.47±2.48 [b]
10% GL	41.29± 1.29 [c]	77.93± 2.64 [c]	96.79± 2.64 [c]
5% ML	44.09± 2.75 [c]	76.44±1.60 [c]	92.85± 1.84 [c]
10% ML	38.37±1.87 [d]	67.44±1.07 [d]	81.47±1.52 [d]
2.5% GL + 2.5% ML	41.53± 0.77 [c]	80.71± 1.95 [c]	94.72±1.39 [c]
5% GL + 5% ML	33.19± 2.64 [e]	65.33± 1.37 [d]	82.35±1.38 [d]

Means in a same column with dissimilar letter are significantly different at $P \leq 0.05$.

Data in table (2) indicated that liver enzymes (ALT, ALT and ALP) were significantly higher in gentamicin-induced nephrotoxicity rats (+ve control group) as compared to their levels in normal rat (-ve control group). **Al-Badrany et al., (2012)** illustrated that, ALT and AST both enzymes are used as accurate indicators of hepatocellular injuries and necrosis. Gentamicin induces hepatotoxicity secondary to nephrotoxicity, it initiates hepatic injury via increasing free radicals' production which attack and damaged hepatocytes. **Gardner et al., (2016)** indicated that nephrotoxicity caused by gentamicin can result in damage to the liver. Gentamicin provokes oxidative stress in liver, and results in hepatotoxicity **(Alkahtani et al., 2009)**.

In group of gentamicin induced nephrotoxicity rats fed on grape leaves and mulberry leaves and their mixture showed significantly lower level of AST, ALT and ALP comparing to that of positive control values. The group fed on leaves mixture of 5% grape+5% mulberry showed the lowest levels followed by group fed on 10% mulberry leaf.

Mulberry *M. nigra* leaves extract significantly reduce the level of AST, ALT and ALP in rats treated with anti-rheumatic drug (Methotrexate) which mitigated liver injury **(Tag, 2015)**. Various positive effects of mulberry could be due to its phenolic compounds including the phenolic acids, flavonols and anthocyanins **(Ghorbani and Hooshmand, 2021)**. In addition, **Pari and Suresh (2008)** indicated that grape leaf extract had hepatoprotective effects, which could be attributed to the fact that, procyanidins present in grape might reduce the free radicals damaging actions in cells **(Li and Zhong, 2004)**.

Ahmadvand et al., (2019) affirmed that, antioxidant compounds such as gallic acid which is one of the phenolic acids abundant in grape and mulberry leaves; has hepato-protective effects against gentamicin injuries of liver tissues. Moreover, gallic acid treatment resulted in noticeable decrements of liver enzymes ALT, AST and ALP in nephrotoxic rats; and this could be linked to its anti-oxidative and anti-inflammatory properties in the kidney and its effects on hepatic tissues (**Ahmadvand et al., 2020)**. Polysaccharides, phenolic acids, flavonoids and stilbenes that were found in grape and mulberry leaf could act independently or synergistically to protect the liver cell against ROS and further hepatotoxicity.

Table (1): Mean serum MDA, TAC, GSH, CAT and SOD of rat groups fed on varied level of dried grape and mulberry leaves and their mixtures (Mean + S.E).

Groups	MDA nmol/ ml	TAC µmol/ L	GSH µmol/ L	CAT U/ ml	SOD U/ ml
(-ve) Control	1.91±0.08 ^{f}	1.98±0.05 ^{a}	111.06±2.63 ^{a}	48.05±1.53 ^{a}	21.42±1.93 ^{a}
(+ve) Control	24.36±1.61 ^{a}	0.40±0.02 ^{e}	30.09±1.79 ^{e}	39.02±1.37 ^{c}	14.29±0.74 ^{d}
5%GL	7.82±0.29 ^{b}	1.62±0.07 ^{d}	78.03±2.66 ^{d}	44.80±1.96 ^{b}	16.90±1.15 ^{c}
10% GL	6.87±0.13 ^{c}	1.81±0.04 ^{b}	93.43±2.13 ^{c}	48.75±1.46 ^{a}	18.99±0.31 ^{b}
5% ML	4.38±0.38 ^{d}	1.91±0.02 ^{a}	79.42±2.73 ^{d}	45.15±2.96 ^{b}	19.61±1.38 ^{b}
10% ML	3.56±0.44 ^{e}	1.90±0.01 ^{a}	100.28±2.99 ^{b}	49.50±2.22 ^{a}	19.95±1.29 ^{b}
2.5% GL + 2.5% ML	4.95±0.16 ^{d}	1.76±0.04 ^{c}	91.61±2.43 ^{c}	48.45±1.14 ^{a}	19.33±1.80 ^{b}
5% GL + 5% ML	3.91±0.08 ^{e}	1.97±0.01 ^{a}	112.04±3.19 ^{a}	49.25±1.28 ^{a}	19.98±1.37 ^{b}

Means within same column with dissimilar letter are significantly different at $P \leq 0.05$.

Table (3) showed that, gentamicin induces oxidative stress as noticed from MDA significant increment; and a significant decrements of TAC, GSH, CAT and SOD when compared with negative control group values. This could be explained on the basis that, gentamicin affects mitochondria through activating the intrinsic pathway of apoptosis and generating oxidative stress by increasing superoxide anion and hydroxy radicals **(Gamaan et al., 2023)**, ROS scavenging enzymes including CAT and SOD considered as an important defense against ROS; and when the indigenous antioxidant system inhabited hydrogen peroxide accumulated **(Halliwell, 1994).**

On the other hand, groups of rats fed on mixture of 5% grape leaf + 5% mulberry leaf and groups fed on mulberry 10% followed by group fed on mixture of 2.5% grape leaf + 2.5% mulberry leaf showed significant decrements in malondialdehyde MDA when compared with positive control. Whereas, all groups of rats fed on grape leaf, mulberry leaf and their mixture showed significant increments in Total Antioxidant Capacity, Glutathione reductase (GSH), and Catalase (CAT) and Super Oxide Dismutase (SOD) compared to positive control values.

Mulberry leaf extract has been reported to efficiently scavenge NO, $O2^-$, and DPPH radicals and showed significant reducing power **(Andallu, et al., 2014)**. Furthermore, **Ren et al., (2015)** demonstrated that, polysaccharides found in mulberry leaf enhanced activities of antioxidant enzymes and decreased MDA concentration in diabetic rats. Where flavonoids in grape leaves showed inhibitory effects against lipid peroxidation, which could be due to their scavenging action against free radicals **(Ruf et al., 1995, Kravchenko et al., 2003)**. In a study by **Dani et al., (2010)** it was found that, the treatment with grape leaves prevent the changes in antioxidant enzymes which could be happened due to the accumulation of H_2O_2; and the fact that there were significant positive correlation between content of polyphenols and activities of antioxidant enzymes **(Halliwell, 2008)**. Furthermore, the extract of grape leaves decreased the lipid peroxidation and enhanced the liver and kidneys' enzymatic and non-enzymatic antioxidants levels in alcohol induced oxidative stress in rats.

In conclusion, results indicated that grape leaves and mulberry synergistically protect kidney against oxidative stress, the mixture of both leaves enhanced kidney and liver functions.

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