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The preventive effects of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on ethylene glycol-induced hyperoxaluria

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Abstract. – OBJECTIVE: Kidney stones are a common complication of hyperoxaluria. The aim of this study is to investigate the protective and preventive effects of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on ethylene glycol-induced hyperoxaluria.

MATERIALS AND METHODS: Male Wistar rats between 110 and 145 g in weight were used in the study, *Ulva lactuca* aqueous extract and polysaccharides were prepared. The male albino rats were supplemented with 0.75 percent ethylene glycol (v/v) in their drinking water for six weeks to induce hyperoxaluria. Ulvan infusions (100 mg/kg body weight), ulvan polysaccharides (100 mg/kg body weight), and atorvastatin (two milligrams/kg body weight) to treat hyperoxaluric rats for four weeks (every other day) were used. Weight loss, serum creatinine, serum urea, serum uric acid, serum oxalate, kidney oxalate, kidney lipid peroxidation, and kidney DNA fragmentation and kidney histopathological studies were done.

RESULTS: Weight loss, rise of serum creatinine, serum urea, serum uric acid, serum oxalate, kidney oxalate, kidney lipid peroxidation, and kidney DNA fragmentation were all shown to be prevented by the addition of atorvastatin, polysaccharides, or aqueous extract, respectively. Catalase (CAT) activity, glutathione peroxidase (GPX) activity, glutathione-S-transferase (GST) activity, and histopathological perturbations were all significantly reduced by the medicines that were studied.

CONCLUSIONS: Hyperoxaluria caused by ethylene glycol may be prevented by a combination of *Ulva lactuca* aqueous extract, ulvan polysaccharides, and atorvastatin. A reduction in renal oxidative stress and an improvement of the antioxidant defense system may be responsible for these protective benefits. However, *Ulva lactuca* infusion and ulvan polysaccharides need to be studied further in humans, in order to determine their efficacy and safety.

Key Words:
Hyperoxaluria, *Ulva lactuca*, Ulvan polysaccharides, Atorvastatin, Kidney, Oxidative stress.

Introduction

One of the most common risk factors for kidney stones is a daily urine excretion of more than 40 mg/day of hyperoxaluria[1,2]. Hyperoxaluria causes an increase in the body's endogenous oxalate production, which can lead to kidney stones,

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nephrocalcinosis, and oxalosis, all of which can contribute to end-stage renal disease (ESRD)[3,4]. In males, calcium oxalate calculi are believed to affect about 12% of the population, and in females, the recurrence rate is between 70-80% and 47-60%[5]. Nephrocalcinosis and nephrolithiasis can be caused by ethylene glycol poisoning, which results in crystals deposition on the renal parenchyma and in the collector system (nephrolithiasis)[6-10]. Tubular cell damage is caused by oxalate ions and calcium oxalate crystals, and both have been determined to be toxic[11]. It has been suggested by a number of researchers that hyperoxaluria and oxalosis are linked to elevated levels of lipid peroxides and free radicals, as well as a depleted antioxidant defense system[12,13]. The pathological implications of kidney stone development are mostly mediated by an increase in free radicals[14,15].

When it comes to the treatment of various ailments, herbal and green algae medications are becoming more popular because they have less adverse effects than conventional drugs[16-20]. Many pharmacological medicines used to prevent urinary tract stones may not work for all individuals, and many have side effects that make them ineffective over time, according to Atmani et al[21]. A new field of medicine, alternative medicine, must be created as a result[22]. Seaweeds have long been a staple of the Asian diet and are now recognized as a valuable but underutilized resource[23]. Sulphated polysaccharides from marine seaweeds and algae and their sulfated polysaccharides have attracted considerable interest because of the vast range of medicinal applications and antioxidants they provide[18,24-26]. In addition, *Ulva lactuca*'s natural polysaccharides content showed a free radical quenching activity by increasing the liver's functional ability during D-Galactosamine-induced oxidative stress, according to Sathivel et al[27]. Finally, the green algae *Ulva* spp. and their separated polysaccharides have anticoagulant, antihepatotoxic, antinephrotoxic, anti-inflammatory, antitumoral, antihyperglycemic, and antihyperlipidemic activities, respectively[28-34].

Many studies[35,36] have shown that atorvastatin protects the kidneys from damage. Even in the face of high levels of plasma cholesterol, atorvastatin has a renoprotective effect in hypercholesterolemic New Zealand rabbits[35]. As Fassett et al[36] reported, atorvastatin may protect patients with chronic renal disease and cardiovascular disease from renoprotective effects.

Ulva lactuca aqueous extract and polysaccharides were compared to atorvastatin in this study to see if they could protect albino male rats against ethylene glycol-induced hyperoaluria, kidney damage, and oxidative stress.

Materials and Methods

Animals and Housing

Experimenters employed for the study, researchers used male Wistar rats between 110 and 145 g in weight. The National Research Center, Dokki, Giza, Egypt, provided the animals. Ten days before the experiment began, they were kept under surveillance to rule out any intercurrent infections. With well aerated coverings on their plastic cages, the animals were kept at room temperature (about 20 to 25°C), had access to drink, and were fed a balanced standard meal. An evaluation was made to determine whether rats gained weight as a result of this experiment. The Canadian Council on Animal Care (CCAC) provides recommendations for all animal treatments[37].

Ulva Lactuca Collection

The sea lettuce (*Ulva lactuca*) was taken from Alexandria, Egypt's Mediterranean Sea coastlines. Phycology (Algology) Professor Dr. Ibrahim Boreey from Beni-Suef University, Egypt, confirmed its authenticity. Remove any extraneous materials, such as epiphytes and contaminations, by washing the samples with seawater and tap water, and then with deionized water. The cleaned algae were then dried in an open shaded area using just natural sunlight.

Preparation of Ulva Lactuca Aqueous Extract and Polysaccharides

With an electric grinder, Teflon homogenizer (Glas-Col, Terre Haute, USA), the air-shaded dried algae were coarsely ground. When the dried powdered algae were infused in boiling water for 15 minutes, the combination was filtered to produce the aqueous extract, which was ready for use. The dried powdered algae were boiled for two hours in distilled water, precipitated by ethanol, to separate ulvan polysaccharides (sulphated and non-sulphated). As a result of this process, salts and minerals were removed from the precipitate, which was then drained and dried to remove any remaining residues of alcohol[38].

It took 15 minutes to create the infusion of aqueous extract from the powdered algae and 2

percent w/v boiling distilled water, after which it was filtered and administered orally to rats at doses of 100 mg/kg b.w. every other day for four weeks. For four weeks, the same amount of ulvan polysaccharides was given orally, dissolved in boiling distilled water. According to the dosages used in prior studies[21,32].

Preparation of Atorvastatin Dose

A dose of 2 mg/kg b.w.[39] of atorvastatin calcium (44 mg), equivalent to 40 mg atorvastatin, was administered orally to rats every other day for four weeks at a dose level of 44 mg atorvastatin calcium in 100 ml distilled water. Egyphar Pharmaceutical Company (Obour city, Cairo, Egypt) supplied the atorvastatin.

Induction of Hyperoxaluria

Adding 0.75% ethylene glycol to the drinking water of albino rats for 28 days induced hyperoxaluria[40,41]. S. D. Fine Chem Ltd. (Mumbai, Maharashtra 400013, India) provided the ethylene glycol that was used in the experiment.

Experimental Design

The animals were separated into five groups, each with six animals, and each group had six creatures. During the last four weeks of the study, the equivalent volume of vehicle (distilled water) was given orally to Group 1 (the normal control), which was given tap water (without ethylene glycol) as drinking water for six weeks. Drinking water containing 0.75% ethylene glycol was provided to the group 2 (ethylene glycol group) for six weeks and the same volume of vehicle (distilled water) was administered three times a week for the final four weeks of the study (every other day). A 0.75% ethylene glycol (v/v) solution was given to groups 3, 4 and 5 every other day (every other week) for six weeks, while *Ulva lactuca* aqueous extract was given orally at 100 mg/kg body weight, ulvan polysaccharides were given orally at 100 mg/kg body weight, and atorvastatin was given at 2 mg/kg body weight every two weeks during the last four weeks. A control group was established for groups 1, 2, 3, 4, and 5, with group 1 serving as the control group for group 2.

Blood and Tissue Sampling

Diethyl ether anesthesia was administered to the animals at the end of the trial, and they were then weighed and killed. The jugular vein was used to draw blood, while the urinary bladder was used to draw urine. After allowing the blood to coagulate at room temperature for 30 minutes, it was centrifuged at 3,000 rpm. Different biochemical markers associated with kidney function were swiftly examined using only the clear no hemolyzed supernatant serum. The serum and urine samples were stored at -30°C until they were needed. For histopathology tests, the kidneys of each rat were removed and weighed, and then one kidney was fixed in neutral buffer formalin. Homogenizers made of Teflon were used to homogenize 0.5 grams of kidney from each animal in 5 ml of 0.9% NaCl (10% w/v) (Glas-Col, Terre Haute, USA). Alkaline phosphate (ALP), aspartate aminotransferase (AST), oxalate levels, and oxidative stress markers were measured in the homogenate that was maintained in the deep freezer at -30°C for further analysis. Five minutes of centrifugation at 3,000 rpm yielded the homogenate supernatant needed to isolate the kidney sample homogenate.

Biochemical Analysis

According to Young[42], Diamond Diagnostics (Egypt) developed kits for measuring serum and urine creatinine concentrations. Kits acquired from Spectrum Diagnostics (Obour City, Cairo, Egypt) were used to measure the serum urea level, according to Shephard and Mezzachi[43]. According to Fossati et al[44], Diamond Diagnostics (Cairo, Egypt) kits were used to measure uric acid. According to Young[45], kits from Biostc were used to detect oxalate in serum and kidney samples (Italy). According to Sherwin[46], the alanine transaminase (ALT) and aspartate transaminase (AST) activities in the kidneys were measured using test kits supplied from Spectrum Diagnostics (Egypt). This study was carried out utilizing kits from Spectrum Diagnostics (Egypt) according to Marsh et al[47]. GSH levels in the kidneys, total thiol content, lipid peroxidation (LPO), catalase (CAT) activity, glutathione peroxidase (GPX) activity, glutathione-S-transferase (GST) activity, and DNA fragmentation were assessed using the methods of Beutler et al[48], Koster et al[49], Preuss et al[50], Cohen et al[51], Matkovics et al[52], Mannervik and Gutenberg[53] and Burkitt et al[54], respectively.

Histopathological Studies

According to the Banchroft et al[55], procedure, fixed kidneys were sent to Egypt's National Cancer Institute at Cairo University for wax blocking, sectioning, and staining with hematoxylin and eosin. The histological changes were then detected by examining the stained sections that had been generated.

Statistical Analysis

LSD analysis was used to compare the various groups to each other using the One-way ANOVA (PC-STAT, 1985)[56]. Expressed as the mean minus standard error, the findings were (SE). In a one-way ANOVA, the F-probability represents the interaction between groups. *p*-values lower than 0.05 were considered statistically significant.

Results

Biochemical Studies

Ethylene glycol increased serum creatinine, serum urea, and serum uric acid levels in albino rats by 59.61, 101.82, and 14.15%, respectively, after injection (Table I). On the other hand, ethylene glycol-induced hyperoxaluric rats had a markedly lower urine creatinine level and urine creatinine/serum creatinine ratio ($p<0.01$). Ethylene glycol-administered rats had a -15.38-percentage decrease in serum urea levels compared to aqueous extract-treated rats, but ulvan polysaccharides significantly ($p<0.01$) improved the deleterious effects on serum creatinine, urine creatinine and urine creatinine/serum creatinine levels, serum urea, and serum uric acid, recording percentage changes of -34.94, +166.14, +326.52, and -13.67 r. Atorvastatin, like ulvan polysaccharides, improved all of these metrics. Urine creatinine levels, urine creatinine to serum creatinine ratio, and serum urea levels all improved more quickly with ulvan polysaccharides than with atorvastatin (Table I).

Excess oxalate levels were found in both the kidney and the serum of hyperoxaluric rats, with increases of 67% and 157.14%, respectively, (Table II). Hyperoxaluric rats were treated with aqueous extract, ulvan polysaccharides, and atorvastatin, and the high levels were dramatically reduced in these animals. Atorvastatin was found to be the most successful in lowering the kidney's oxalate content (-57.14%), while ulvan polysaccharides was found to be the most effective in lowering the serum's oxalate level (-62.03%).

Hyperoxaluric rats' kidney AST and ALT activity decreased by 86.57 and 40.04 %, respectively, ($p<0.05$). When hyperoxaluric rats were treated with an aqueous extract, their ALP and AST activities were not significantly increased ($p<0.05$), but their ALT activity was significantly increased ($p<0.01$). The ulvan polysaccharides, on the other hand, reduced ALP, AST, and ALT ($p < 0.05$); -15.70%, ($p<0.01$); 113.35%, and ($p<0.05$); 46.79%, respectively) while increasing ALP, AST, and ALT. ALP, ALT, and AST activities were not significantly increased ($p>0.05$) in rats given atorvastatin, whereas AST activities were significantly increased ($p<0.05$) (Table III).

Ethylene glycol-induced hyperoxaluric rats had a 1,422.37% decrease in kidney DNA fragmentation ($p<0.05$). Excessive DNA fragmentation may have been reduced by aqueous extract, ulvan polysaccharides, and atorvastatin in rats with hyperoxaluria, recording percentage changes of -84.61%, 81.7%, and -82.7% for these treatments, respectively (Table IV).

According to oxidative stress markers and antioxidant defense system (Tables V and VI), ethylene glycol administration significantly increased LPO by 30.69%; however, GSH level and total thiol content as well as the activity of the enzymes CAT, GPX, and GST were all significantly decreased when compared to the normal control.

An aqueous extract, polysaccharides from *Ulva*, and atorvastatin reduced LPO ($p<0.01$) and increased GSH and GPX ($p<0.01$) in rats with hyperoxaluria. As a result of treatment with aqueous extract, ulvan polysaccharides, and atorvastatin, the total thiol level increased noticeably, changing by percentages of 60%, 26.4%, and 20%, respectively. A highly significant ($p<0.01$) and significant ($p<0.05$) rise in CAT and GST activities was seen after treatment with the ulvan polysaccharides, while the aqueous extract treatment resulted in an increase in CAT and GST activity. Table VI shows that atorvastatin therapy boosted CAT and GST activity by 108.53 and 18.95 percentage points, respectively.

Histological Changes

Figures 1A-B show that kidney slices from normal rats have a normal histological structure (as depicted by microscopy). The kidney's cortex and medulla are separated by a thin membrane. For example, in the cortex, you'll find the glomeruli (G), the proximal and distal convoluted tubules (PT and DT). Tubules of collecting and Henle loop segments compose the medulla.

Epithelial cell swelling in the lining of the tubules was related with endothelial cell proliferation and vacuolation in rats given ethylene glycol (P, V) (Figure 2A). Between the renal tubules, there was an infiltration of inflammatory cells (IF) (Figure 2B). As opposed to Figure 2C which depicts several tubule lumens filled with thick oxalate crystals, which shows cystic dilatation, Figure 2C depicts (CD).

Aqueous extract of *Ulva lactuca* revealed swelling and vacuolization of the endothelial cells

Table I. Effect of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on various parameters related to kidney function of normal and ethylene glycol-administered albino rats.

Parameters / Treatments	Serum creatinine (mg/dl)	Change %	Urine creatinine in (mg/dl)	Change %	Urine creatinine/serum creatinine	Change %	Serum urea (mg/dl)	Change %	Serum uric acid (mg/dl)	Change %
Dist. water (G1)	0.52±0.06^{b}		64.70±14.67^{a}		116.19±16.77^{a}		24.71±1.90^{c}		4.10±0.11^{b}	
Dist. water + Ethy. glycol (G2)	0.83±0.06^{a}	59.61	20.32±2.30^{b}	-68.59	24.36±1.79^{c}	-79.03	49.87±5.38^{a}	101.82	4.68±0.01^{a}	14.15
Aqu. ext. + Ethy. glycol (G3)	0.79±0.04^{a}	-4.82	17.57±0.49^{b}	-13.53	22.44±1.17^{c}	-7.88	44.73±2.21^{ab}	-10.31	3.96±0.11^{b}	-15.38
Ulvan poly. + Ethy. glycol (G4)	0.54±0.05^{b}	-34.94	54.08±1.04^{a}	166.14	103.90±8.48^{ab}	326.52	18.52±1.68^{c}	-62.86	4.04±0.20^{b}	-13.67
Ator. + Ethylene glycol (G5)	0.55±0.04^{b}	-33.73	46.33±7.63^{a}	128.00	83.68±9.70^{b}	243.51	36.56±5.45^{b}	-26.69	4.00±0.13^{b}	-14.53
F- Probability	$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.01$	
LSD at the 5% level	0.153		21.816		27.706		10.900		0.371	
LSD at the 1% level	0.207		29.516		37.484		14.749		0.502	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b}$ and ^{c}) are significantly different at $p<0.05$. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

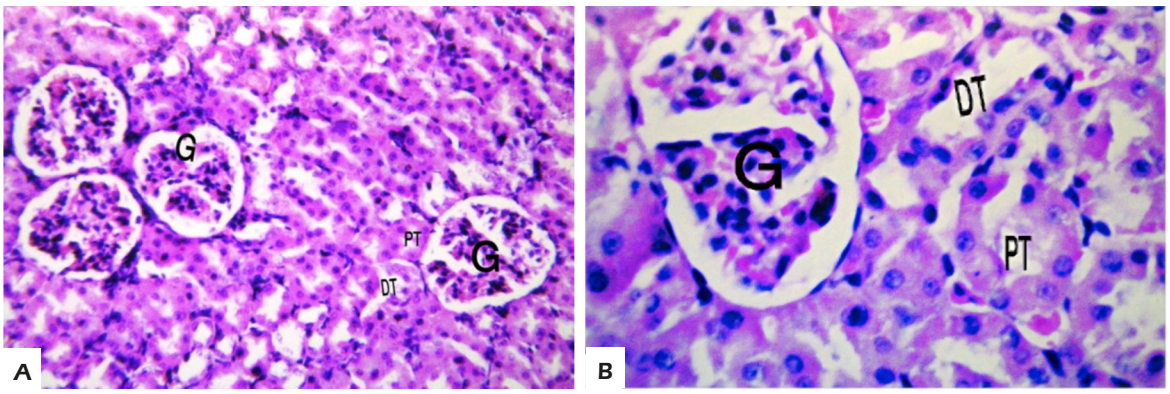


Figure 1. A photomicrograph of a sections of kidney of a normal control rat showing normal histological structure of the kidney at low magnification [(**A**); X 205] and high magnification [(**B**); X 512] depicting glomeruli (G), proximal convoluted tubules (PT) and distal convoluted tubules (DT) (H&E).

lining hyperemic tufts of glomeruli in ethylene glycol-treated rats, as well as swelling of the epithelial cells lining the tubules (S) (Figures 3A and B). Figure 3C depicted focal hemorrhages (H) between tubules and oxalate crystals in a few medulla tubules. However, despite these abnormalities in histology, rats given ethylene glycol had significantly better kidney architecture than control rats.

In rats given ethylene glycol, ulvan polysaccharides led to a considerable improvement in the kidney's structural integrity. Despite this, the hyperemic glomerular tufts of hypertrophied glomeruli (G) were associated with tubular epithelial cell swelling (S) in the cortex (Figure 4A).

Portions of the epithelial cells lining some of the cortex's tubules were necrotic after being

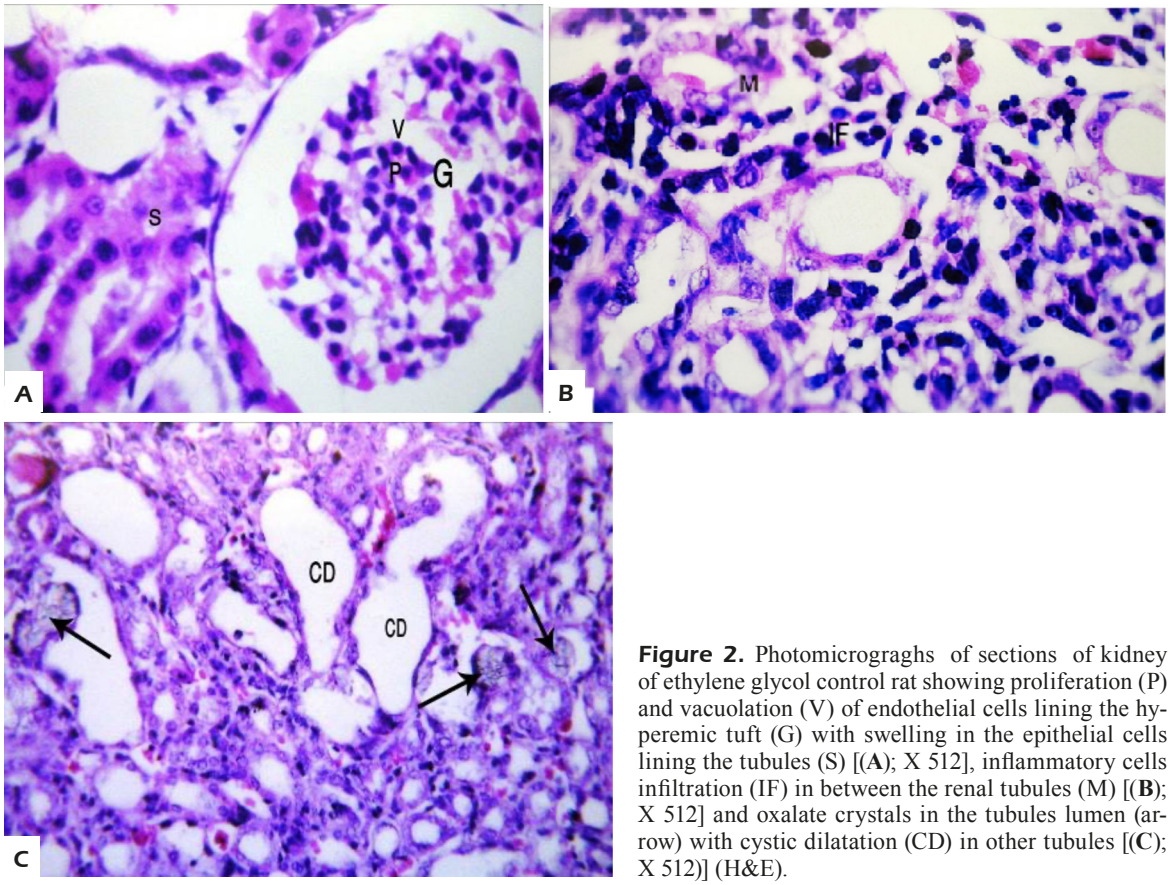


Figure 2. Photomicrograghs of sections of kidney of ethylene glycol control rat showing proliferation (P) and vacuolation (V) of endothelial cells lining the hyperemic tuft (G) with swelling in the epithelial cells lining the tubules (S) [(**A**); X 512], inflammatory cells infiltration (IF) in between the renal tubules (M) [(**B**); X 512] and oxalate crystals in the tubules lumen (arrow) with cystic dilatation (CD) in other tubules [(**C**); X 512)] (H&E).

Table II. Effect of Ulva lactuca aqueous extract, ulvan polysaccharides and atorvastatin on kidney and serum oxalate levels of normal and ethylene glycol-administered albino rats.

Parameters / Treatments	Kidney oxalate (µg/g)	Change %	Serum oxalate (mg/l)	Change %
Dist. water (G1)	46.46±7.43^{bc}		9.31±0.54^{d}	
Dist. water + Ethy. glycol (G2)	77.85±5.42^{a}	67.56	23.94±1.84^{a}	157.14
Aqu. ext. + Ethy. glycol (G3)	57.15±6.08^{b}	-26.59	19.12±0.61^{b}	-20.13
Ulvan poly. + Ethy. glycol (G4)	52.20±1.31^{b}	-32.95	9.09±0.25^{d}	-62.03
Ator. + Ethy. glycol (G5)	33.30±1.31^{c}	-57.22	13.54±0.74^{c}	-43.44
F- Probability	p<0.001		p<0.001	
LSD at the 5% level	14.57		2.79	
LSD at the 1% level	19.72		3.78	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b, c}$ and ^{d}) are significantly different at p<0.05. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

exposed to ethylene glycol in combination with atorvastatin, resulting in hyperemia in the glomeruli (G) (Figure 4B). The atorvastatin-treated rats demonstrated substantial improvement in numerous disturbed histology alterations as compared to the ethylene glycol-administered control rats.

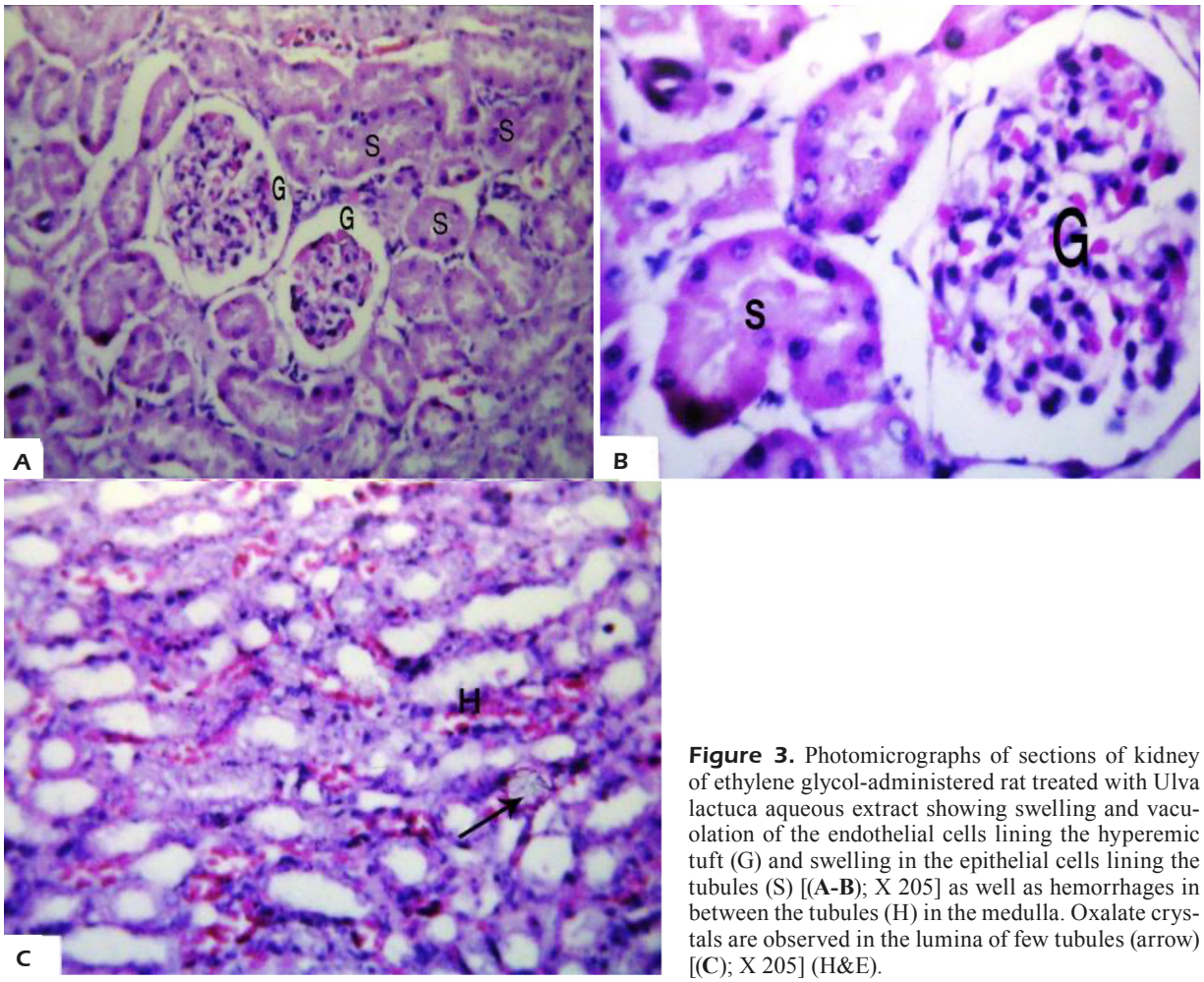


Figure 3. Photomicrographs of sections of kidney of ethylene glycol-administered rat treated with Ulva lactuca aqueous extract showing swelling and vacuolation of the endothelial cells lining the hyperemic tuft (G) and swelling in the epithelial cells lining the tubules (S) [(**A-B**); X 205] as well as hemorrhages in between the tubules (H) in the medulla. Oxalate crystals are observed in the lumina of few tubules (arrow) [(**C**); X 205] (H&E).

Table III. Effect of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on ALP, AST and ALT activities in kidney of normal and ethylene glycol-administered albino rats.

Parameters / Treatments	ALP (U/g)	Change %	AST (mU/100 mg)	Change %	ALT (mU/100 mg)	Change %
Dist. water (G1)	13.23±0.88^{a}		38.12±3.23^{a}		17.68±1.51^{a}	
Dist. water + Ethy. glycol (G2)	11.69±0.22^{ab}	-11.64	11.98±0.83^{d}	-68.57	10.60±1.02^{c}	-40.04
Aqu. ext. + Ethy. glycol (G3)	12.57±0.70^{a}	7.48	15.18±0.68^{cd}	26.71	18.80±1.97^{a}	77.36
Ulvan poly. + Ethy. glycol (G4)	9.86±0.53^{b}	-15.70	25.56±2.04^{b}	113.35	15.56±1.08^{ab}	46.79
Ator. + Ethy. glycol (G5)	13.98±1.50^{a}	19.57	18.96±0.72^{c}	58.26	12.26±1.48^{bc}	15.66
F- Probability	$p<0.05$		$p<0.001$		$p<0.01$	
LSD at the 5% level	2.56		5.252		4.267	
LSD at the 1% level	3.47		7.106		5.773	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b, c}$ and ^{d}) are significantly different at $p<0.05$. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

Table IV. Effect of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on kidney DNA Fragmentation of normal and ethylene glycol-administered albino rats.

Parameters / Treatments	DNA Fragmentation (%)	Change %
Dist. water (G1)	1.52±0.20^{c}	
Dist. water + Ethy. glycol (G2)	23.14±0.91^{a}	1,422.37
Aqu. ext. + Ethy. glycol (G3)	3.56±0.51^{b}	-84.61
Ulvan poly. + Ethy. glycol (G4)	4.3±0.53^{b}	-81.42
Ator. + Ethy. glycol (G5)	3.98±0.58^{b}	-82.79
F- Probability	$p<0.001$	
LSD at the 5% level	1.723	
LSD at the 1% level	2.331	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b}$ and ^{c}) are significantly different at $p<0.05$. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

Discussion

In the case of secondary hyperoxalouria, it is possible that the condition is brought on by a combination of hereditary and environmental factors[57]. It is routine practice to administer ethylene glycol as a means of inducing experimental hyperoxaluria and urolitiasis[58]. Many researchers are interested in testing the effects of seaweeds, algae, and their constituents on experimental animals because of their potential as therapies for a variety of ailments. As a result, this research aims to compare the preventative effects of *Ulva lactuca's* aqueous extract and polysaccharides with atorvastatin, a standard medicine, on ethylene glycol-induced hyperoxaluric rats.

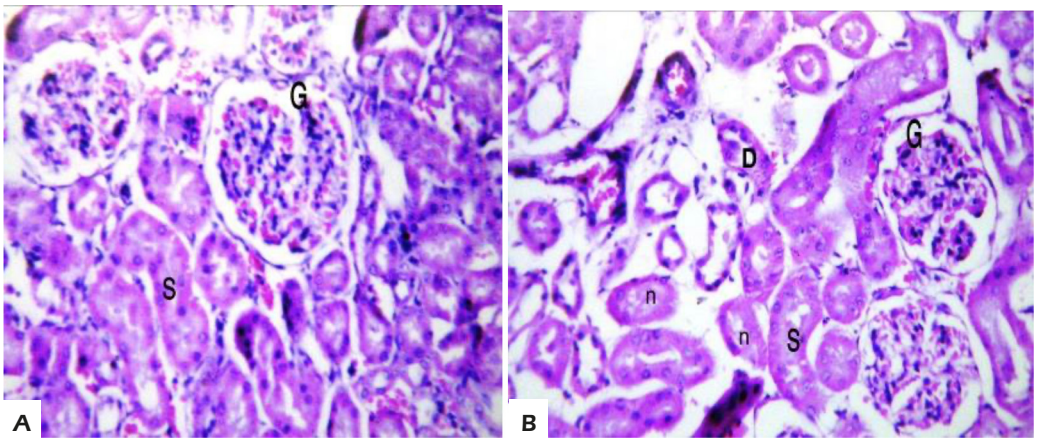
In the current study, ethylene glycol in drinking water at a 0.75% (v/v) concentration for six weeks induced hyperoxaluria, nephrolithiasis, and kidney injury, which was manifested by an increased serum and kidney oxalate level, an elevated serum creatinine, urea, and uric acid level, a decreased

Table V. Effect of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on kidney lipid peroxidation, glutathione content and total thiol content of normal and ethylene glycol-administered albino rats.

Parameters Treatments	Glutathione (nmol/100 mg)	Change %	Total thiol nmol/100 mg)	Change %	Lipid peroxidation (MDA nmol/100 mg)	Change %
Dist. water (G1)	69.12±3.76^{ab}		235.20±3.34^{a}		39.36±2.97^{b}	
Dist. water + Ethy. glycol (G2)	53.64±0.71^{d}	-22.39	95.66±12.29^{c}	-59.33	51.43±2.44^{a}	30.66
Aqu. ext. + Ethy. glycol (G3)	67.47±2.58^{bc}	25.78	153.90±13.66^{b}	60.88	26.68±1.53^{c}	-48.12
Ulvan poly. + Ethy. glycol (G4)	74.01±1.04^{a}	37.97	120.93±9.39^{bc}	26.42	35.03±3.34^{b}	-31.88
Atorvastatin + Ethy. glycol (G5)	62.64±0.79^{c}	16.78	115.51±15.12^{c}	20.75	24.47±2.12^{c}	-52.42
F- Probability	p<0.001		p<0.001		p<0.001	
LSD at the 5% level	6.253		33.611		7.465	
LSD at the 1% level	8.459		45.473		10.099	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b, c}$ and ^{d}) are significantly different at p<0.05. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

urine creatinine level, and an increased kidney DNA fragmentation. Additionally, histological evidence of hyperoxia, renal nephrolithiasis, and kidney injury was found by oxalate crystals in the lumen of numerous tubules, cystic dilatation, endothelial cell alterations, and inflammatory cell infiltration between renal tubules in patients with kidney disease. A number of researchers have come to the same conclusion[9,10,58-61]. Biochemical and histological effects on the kidney may be attributed to the fact ethylene glycol converts into two hazardous metabolites: glycolic acid, which causes acidosis; finally, oxalic acid which precipitates as calcium oxalate in several organs, particularly in the kidneys[62,63]. Hypoxia may cause kidney damage by increasing oxidative stress and decreasing the body's ability to produce antioxidants. As a result, ethylene glycol-administered rats had higher amounts of lipid peroxidation in their kidneys compared to those that did not have ethylene glycol in their bodies, according to the results of this study. It has also been shown[64,65]



Figures 4. Photomicrographs of sections of kidney of ethylene glycol-administered rat treated with ulvan polysaccharides (**A**; X 205) showing hyperemic tuft in the hypertrophied glomerulus (G) with swelling in the tubular epithelial cells (S) and kidney of ethylene glycol-administered rat treated with atorvastatin (**B**; X 205) showing hyperemia in the glomerular tuft of the glomeruli (G) with degeneration and necrosis (n) in the epithelial cells lining the tubules (D) with swelling in other (S). H & E.

Table VI. Effect of *Ulva lactuca* aqueous extract, ulvan polysaccharides and atorvastatin on kidney catalase, glutathione peroxidase and glutathione-S-transferase of normal and ethylene glycol-administered albino rats.

Parameters / Treatments	Catalase (k.10²)	Change %	Glutathione peroxidase (mU/100 mg tissue)	Change %	Glutathione-S-transferase (mU/100 mg tissue)	Change %
Dist.water (G1)	10.88±0.50^{bc}		113.13±2.66^{b}		92.59±3.26^{a}	
Dist. water + Ethy. glycol (G2)	6.92±0.70^{c}	-36.4	109.84±6.38^{b}	-2.91	35.82±2.70^{d}	-61.31
Aqu. ext. + Ethy. glycol (G3)	15.78±3.16^{ab}	128.03	161.95±7.04^{a}	47.44	46.54±2.34^{bc}	29.93
Ulvan poly. + Ethy. glycol (G4)	20.65±2.45^{a}	198.41	170.76±8.28^{a}	55.46	54.51±5.72^{b}	52.18
Atorvastatin + Ethy. glycol (G5)	14.43±1.95^{b}	108.53	163.63±6.05^{a}	48.97	42.61±1.84^{cd}	18.95
F- Probability	$p<0.01$		$p<0.001$		$p<0.001$	
LSD at the 5% level	5.91		18.55		10.056	
LSD at the 1% level	7.996		25.096		13.606	

Data are expressed as mean ± standard error. Number of animals in each group is six. Means, which do not share the same superscript symbol ($^{a, b, c}$ and ^{d}) are significantly different at $p<0.05$. Percentage changes were calculated by comparing ethylene glycol-administered control group with normal control and ethylene glycol-administered treated groups with ethylene glycol-administered control group.

that elevated levels of oxalate/calcium oxalate cause calcium-mediated cell damage and attrition of cell surface glucosaminoglycane in renal tubular epithelial cells, which eventually favors crystal adhesion and aids in stone formation. To summarize, Dal Moro et al[15] reported that Oxalate and calcium oxalate monohydrate have been shown to produce free radicals, which are the primary cause of the pathological effects that lead to kidney stone formation.

Atorvastatin and ulvan polysaccharides were found to protect against ethylene glycol-induced kidney damage, as well as lowering blood creatinine and urea, uric acid, and kidney oxalate levels. They also reduced urine creatinine and the urine creatinine/ serum creatinine ratio were also reduced. These findings are in line with those of a slew of other studies[24-26,39,66]. Sulphated polysaccharides from marine seaweeds were found to protect rats' kidneys from cyclosporine A-induced damage by Josephin et al[24-26]. Spirulina, a blue-green microalga, was found to be effective in treating kidney stones caused by the chemical ethylene glycol, according to Al-Attar[66]. So, atorvastatin could assist to prevent and treat crystal formation, according to Tsujihata et al[39] who discovered that it had an inhibitory effect on renal tubular cell injury caused by oxalate-induced oxidative stress.

AST as well as ALT activity in the kidney homogenate of the control group may indicate tubular dysfunction and tubulointerstitial injury due to ethylene glycol. These crystals are known to harm proximal tubular epithelial cells and are often associated with inflammatory reactions, shedding of brush border membranes, and enzyme leakage in urine[67,68]. Co-treatment of ethylene glycol hyperoxaluric rats with aqueous extract, ulvan polysaccharides, and atorvastatin improved the activity of kidney ALP, AST, and ALT, which may reflect the improvement of kidney integrity and function.

The delivery of ethylene glycol resulted in a rapid increase in kidney DNA fragmentation, which indicates an increase in oxidative stress and nephrotoxicity as a result of therapy with ethylene glycol. In addition to the creation of free radicals, oxalate and calcium oxalate monohydrate cause the DNA damage and proteins and lipids within cells, resulting in tissue injury, which is the primary cause of kidney stones[15,69]. The introduction of a hydroxyl group (OH) into the C-8 position of guanosine or guanine residues, generating 8-OHdG and 8-hydroxyguanine, is extensively employed as an indicator of DNA oxidation, and the formation of 8-OHdG and 8-hydroxyguanine is a more sensitive biomarker of DNA oxidation[70-72].

The results of this investigation show that treatment of ethylene glycol-administered rats with the tested medications significantly improved kidney DNA fragmentation. These findings are in line with those of a slew of other studies[73-75]. In methyl methane sulphonate-treated human lymphocytes, three extracts from *Sargassum latifolium* were found to minimize DNA damage between 24.5% and 62.5%[73]. *Fucus serratus* and *Fucus vesiculosus* extracts were shown to be the most effective in protecting Caco-2 cells from H2O2-induced DNA damage, according to O'Sullivan et al[74]. It was also found that statins reduce protein and DNA oxidation in the blood, according to Aydin et al[75]. This suggests that statins have additional beneficial effects on the body.

To better understand how the antioxidant defense system works and how oxidative stress affects it, we administered ethylene glycol, which causes hyperoxaluria, and found that it increased lipid peroxidation and decreased glutathione content (GSH), total thiol content, catalase activity (CAT), gluthathione peroxidase activity (GPX), and glutathione-S-transferase (GST). According to a number of studies, these findings are in agreement. Ethylene glycol and the accumulation of oxalate in kidneys have been linked to lipid peroxidation and oxidative stress, which can be significantly reduced by antioxidants, but if antioxidants fail, it can lead to renal tissue damage, urolithiasis, and the loss of anti-adherent glycosaminoglycan layer, which may act as a stone-forming nidus[6,13,76,77].

This treatment results in a reduction in LPO and a rise in GSH and total thiol levels as well as the activities of Cat (CAT), GST (GPX), and the glutathione peroxidase (GPX) enzymes. Many researchers have come to the same conclusion[24,30,31,78-80]. *Ulva lactuca*'s aqueous extract, polysaccharides, and ethanolic extract have been shown by Ahmed et al[31] to contain potential renoprotective properties in rats by reducing oxidative stress and increasing antioxidant defenses. Additional studies[78-80] have shown that sulfated polysaccharides can reverse the aberrant rise in LPO, which has been underlined in multiple *in vitro* experiments, by reversing the abnormal rise in LPO. Sulfated polysaccharides have also been observed to improve the activity of antioxidant enzymes, which is consistent with the findings of low molecular weight heparin supplementation on renal-

toxicity, according to Ruperez[78]. Another study by Godard et al[80] suggested that polysaccharides not only scavenge reactive oxygen species and act as an effective antioxidant, but also prevent the formation of reactive oxygen species and inhibit the formation of LPO. Cyclosporine A-induced nephrotoxicity in rats was alleviated by supplementation of sulfated polysaccharides derived from marine seaweeds[24]. *Spirulina* has been demonstrated to protect rats' livers and kidneys against oxidative stress produced by lead acetate[81]. It was found that the pretreatment with an extraction of green algae performed with hot water, *Ulva reticulata*, improved the antioxidant status in experimental animals with low levels of lipid peroxides, according to Rao et al[30]. *Dunaliella salina* algal powder extract was also reported to protect experimental animals from oxidative stress generated by carbon tetrachloride[82,83]. Although the current results are in agreement with Augusti et al[84], they noted that astaxanthin detected in algal pretreatment inhibited catalase activity.

Oxalate crystals identified in ethylene glycol-hyperxaluric rats' nephron tubular lumens are absent in hyperxaluric rats given *Ulva lactuca* aqueous extract, polysaccharides, and atorvastatin, according to the current study. Kidney structural integrity and architecture were improved as a result of oxalate crystal avoidance. Glycosaminoglycans, as well as semisynthetic and sulphated polysaccharides, have been shown in other studies[85,86] to inhibit crystal development, crystal adhesion, and renal tubular cell injury.

Conclusions

Ulva lactuca aqueous extract and ulvan polysaccharides, as well as atorvastatin, were found to have beneficial effects on kidney function indicators and histopathological changes caused by ethylene glycol in the current investigation. Oxalate toxicity prevention may be one of the reasons for the renoprotective effects of *Ulva lactuca* polysaccharides and aqueous extract, which boost the antioxidant status. *Ulva lactuca* infusion and ulvan algae need to be studied in humans further, however, in order to determine their efficacy and safety.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Ethics Approval
All animal experiments in the study were approved by the Experimental Animal Ethics Committee of Faculty of Science, Beni-Suef University, Egypt (Ethical Approval Number: BSU/ FS/ 2015/25). All efforts were done to reduce the number and suffering of animals.

Informed Consent
Not applicable.

Availability of Data and Materials
The data involved in this study are available from the corresponding author upon request.

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