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BIOMEDICAL SCIENCES

Does *Sciaena umbra* (Linnaeus 1758) otolith protect tissues against nephropathy, oxidative stress and inflammation induced by ethylene glycol?

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Abstract: *Sciaena umbra* is a species of fish with large otoliths. These otoliths are used for treatment of kidney stone disease with high morbidity among the public. In present study, the first group was determined as a control. Group 2 was applied to rats by adding 1% ethylene glycol to drinking water. Group 3 rats were given 50 mg/kg otolith by gavage daily. Group 4 rats were administered by adding ethylene glycol and otolith was given. Group 5 rats were added ethylene glycol for the first 30 days. Then next 15 days, the rats were given only otolith. the Serum CREA and BUN levels and urine calcium, phosphate and pH levels were determined to be damaged by ethylene glycol. Free radicals and oxidative damage caused by ethylene glycol were determined from oxidative/antioxidative parameters. Ethylene glycol has also been shown to be inflammatory. There is no positive effect on oxidative stress. From the levels of TNF-α and IL-1β in renal tissue, SUO has shown the triggering effect of inflammation. All data indicate that otolith is not an agent that can be used in nephropathy in the kidney. It is thought that caution should be exercised regarding its use.

Key words: Ethylene glycol, inflammation, otolith, oxidation, *Sciaena Umbra* (Linnaeus 1758).

INTRODUCTION

Sciaena umbra (Linnaeus 1758), is a sea fish, belongs to the Sciaenidae family. The three genera of the family Sciaenidae are Argyrosomus, Sciaena and Umbrina and five different species respectively. *Sciaena umbra* (Linnaeus 1758) generally lives on the eastern Atlantic coast, the Mediterranean Sea, the Black Sea and the Azov Sea. It is a fish species that lives near the shore, on rocky places and at the sea bottom (depth between 5 m-100 m). Despite its ecological and economic importance, there is not enough information about the ecology and biology of *S. umbra*. There are several studies on development, sexual development and diet (Engin & Seyhan 2009). They are generally found in herds of 4-5 to 30 (Mesa et al. 2008). Its back is humpback and dark brown to navy blue. The abdomen is yellow-white in color. Their average length is 50-70 cm. They live about 20 years (Artuz 2006, Marini et al. 2006).

Otoliths are calcium constituents in the ear, whose main functions are the recognition of sounds, hearing and balance. Otoliths that are not attached to the skull are located under the brain, inside the soft transparent inner ear canal. They are also called cephalitis. The main component of otolith is $CaCO_3$. Otoliths are found in all fish except stingray, jawless fish and sharks. Otolith formation and growth are associated with somatic growth. The formation and development

of otolith depends on environmental factors. Otoliths used in the growth and age calculation of fish explain biological history of fish. The studies in fish are usually based on age determination from age rings, and consequently, height-age relationships, seasonal growths and reproductive periods. In addition, it has been reported that otolith weight and fish age can be determined in some fish species (Samsun & Samsun 2006). *Sciaena umbra* (Linnaeus 1758) is a species of fish with very large. These otoliths have traditional usage aganist to urolithiasis in Turkey (Ergin et al. 2017, Béarez et al. 2005, Marini et al. 2006, Cruz & Lombarte 2004).

Factors such as prolonged metabolic diseases, ischemia, trauma, radiation, aging process, xenobiotics, oxidation of catecholamines increased by stress also cause the formation of reactive oxygen species (ROS). Ethylene glycol is the colorless, odorless compound of choice for metabolism of CaOx kidney stones by inducing acute or chronic hyperoxaluria. ROS, oxidative stress and subsequent inflammation occur in renal cells exposed to CaOx crystals formed with ethylene glycol. It is important to prevent the breakdown of biochemical reactions with ROS in one or more steps, the attack of the mediator-activated inflammatory cells instead of the lesion and the excessive accumulation there. Cytokines are a number of hormone-like polypeptide molecules by some cells during inflammation. they direct the activities of immune system cells (Aksoy & Sözbilir 2012).

In this study, ethylene glycol induced nephropathy was tried to be established. The effects of *Sciaena umbra* otolith against oxidative stress and inflammation, were investigated. For this purpose, malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), total antioxidant status (TAS), total oxidant status (TOS), catalase (CAT), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin 18 (IL-18) parameters and creatinine (CREA), blood urea nitrogen (BUN), calcium, phospate concentration and pH in blood, tissue and urine samples were determined.

MATERIALS AND METHODS

Material

Approval of animal ethics was obtained from the Afyon Kocatepe University ethics committee (AKUHADYEK-86-12 dated 29.02.2012). Thirty five male Wistar Albino rats weighing 300-380 g used in the study were obtained from Afyon Kocatepe University Experimental Animals Research and Application Center. The rats were cared for at the Kocatepe University Experimental Animals Research and Application Center, in 12 hours ideal light environment, at suitable temperature, in polypropylene 17x30x42 cm special cages designed for rat care. The rats were fed with tap water and standard pellet feed. After the adaptation of the rats to the environment, the experimental stage was started.

Treatment

The experimental animals were kept under observation for one week in Afyon Kocatepe University Experimental Animals Research and Application Center with standard laboratory food. Five groups of 7 rats were formed by random sampling method.

Group 1 is organized as a Control group. Substance injection or administration was not performed during the experiments. Group 2 was arranged as Ethylene Glycol (EG) group. In this group, 1% ethylene glycol was added to the drinking water of the rats during the study period. Group 3 was assigned as *Sciaena umbra* otolith (SUO) group. All rats in this group were given 50 mg / kg *Sciaena umbra* otolith (SUO) daily by gavage. Group 4 was assigned as *Sciaena umbra* otolith + Ethylene Glycol (SUO+EG) group. 1%

ethylene glycol was added to the drinking water of rats in this group where the protective effects of *Sciaena umbra* otoliths were investigated. In addition, *Sciaena umbra* otolith was given to these rats by gavage at a rate of 50 mg / kg each day. Group 5 was assigned as Ethylene Glycol + *Sciaena umbra* otolith (EG+SUO) group. Ethylene glycol was added to the drinking water for the first 30 days during the study. For the next 15 days, rats were given *Sciaena umbra* otolith at a rate of 50 mg/kg by gavage and ethylene glycol was not added to the drinking water. At the end of the study, all rats were sacrificed and blood and tissue samples were taken. Rats in anesthetized using ketamine (Alpha, Egevet, Istanbul, Turkey) and xylazine (Ramp from Bayer AG, Germany) was administered.

Bıochemıcal analysıs

Measurement of MDA (Malondialdehyde) levels in erythrocyte samples is described by Jain et al. (1989), tissue samples Ohkawa et al. (1979) according to the method. Both methods are based on TBA (thiobarbituric acid) reaction. The reaction of MDA with TBA produces a pink complex. The degree of lipid peroxidation is determined by measuring the absorbance of this complex. GSH (Glutathione) concentration in erythrocyte specimens and tissue homogenates is described by Beutler et al. (1963); 5,5'-Dithiobis-2-Nitrobenzoic Acid (DTNB) was determined by GSH method based on the reduction principle.

Catalase (CAT) is the enzyme that breaks down H_2O_2 into water and oxygen. The activity of this enzyme is measured using H_2O_2 as a substrate. Aebi (1974) method is used to monitor the decrease in H_2O_2 concentration at 240 nm. NO (Nitric Oxide) determination was performed in plasma samples and tissue homogenates by Miranda et al. (2001) reported by the method. Measurement was performed using VCl_3 and Griess reagents. Total antioxidant levels measured in plasma samples and tissue homogenates were measured using a commercial kit (Rel Assay, Gaziantep, Turkey). The method used was reported by Erel (2004). Total oxidant levels measured in plasma samples and tissue homogenates were measured at 540 nm using a commercial kit (Rel Assay, Gaziantep, Turkey). The method used was reported by Erel (2005)

Measurements of inflammation markers (TNF-α, IL-1β, IL-18) were performed in renal homogenates and serum samples with TNF-α (eBioscience, San Diego, CA, USA) IL-1β (eBioscience, San Diego, CA, USA), IL-18 (Novex) commercial kits. All biochemical analyzes were determined on ELISA (BIOTEK ELx800).

Urine analysis (calcium, phosphate and pH) were performed in the laboratory of Medical Biochemistry Department of Afyon Medical Sciences University Hospital.

Statıstıcal analysıs

All numerical results are expressed as mean±standard deviation. Statistical significance was calculated by the ANOVA test with the Duncan posttest and results were considered significant at $p < 0.05$. Data were analyzed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS AND DISCUSSION

Ethylene glycol is a colorless, odorless, water soluble chemical and used as a solvent in the plastics, cosmetics and paint industries. However, it is a chemical commonly used as antifreeze. Accidental use of ethylene glycol in humans and animals results in serious permanent damage and death (Zimina et al. 1977, Khan et al. 2006). Ethylene glycol, which is rapidly absorbed from the gastrointestinal tract, is metabolised in the liver. Ethylene

glycol is first oxidized to glycolaldehyde by the alcohol dehydrogenase (ADH) enzyme. The glycolaldehyde is then converted to glycolate (glycolic acid). This mechanism is mediated by aldehyde dehydrogenase, a mitochondrial enzyme, and aldehyde oxidase, a cytosolic enzyme. Glycolatin alcohol group is oxidized to glyoxylate (glyoxcylic acid). CO_2 is separated from glyoxylate and its metabolites formic acid, CO_2, glycine, serine and oxalate are formed. Oxalic acid forms Ca-oxalate crystals with calcium. These crystals are often deposited in the renal tubules and produce acute tubular necrosis after 24-72 hours. Some of the calcium oxalate crystals also accumulate around the small arteries in the brain and cerebellum. Thus, oxalates are shown as important pathological factors in EG poisoning (Kaiser et al. 1997, Khan et al. 2006). Sodium oxalate, ammonium oxalate, hydroxy-L-proline, ethylene glycol and CaClx by inducing acute or chronic hyperoxaluria. Hyperoxaluric agents are often administered with a diet containing vitamin D or magnesium deficiency, and sometimes with a pH reduction protocol. Lithogenic substances are usually injected orally or by gavage intraperitoneally in food or water. There are many studies (Zimina et al. 1977, Khan & Hackett 1987). Nephropathy was created with ethylene glycol in this study.

Renal cellular exposure to oxalate and CaOx crystals leads to the production of reactive oxygen species (ROS), development of oxidative stress, and subsequent injury and inflammation (Khan 1997). Hyperoxaluria and subsequent formation of calcium oxalate crystal can damage the renal tubular epithelial cells. In the case of oxalate formation, increased ROS damage the renal cells; however, it causes obstruction of the urinary tract and increases the intrarenal tube pressure. ROS affects tissues by disrupting different pathways related to DNA damage and protein modifications. Cell damage, lipid peroxidation, ROS and oxidative stress formation during hyperoxaluria should be evaluated together (Aksoy & Sözbilir 2012, Koul et al. 1999, Davalos et al. 2010). In this study, MDA, a marker of lipid peroxidation, was found to be statistically significant ($p < 0.05$) in the erythrocytes and tissues in the groups where ethylene glycol was applied compared to the control. In many studies, it has been reported that the application of ethylene glycol increases the MDA level (Ashok et al. 2010). Nitric oxide (NO) is a radical with a high concentration of harmful effects. In the present study, a statistically significant decrease in plasma and tissue NO levels was observed ($p<0.05$). Plasma and kidney tissue in the therapeutic group and liver tissue in the prophylactic group appear low NO concentration such as the EG group. NO concentrations was shown in Table I. Narter et al. (2018) examined the effects of propolis against kidney stones formed by applying ethylene glycol in early phase (7 days) and late phase (28 days). They stated that EG decreased NO level in both cases and propolis increased NO level especially in late phase.

When hyperoxaluria occurs, oxidative stress and cell damage from ROS has been confirmed by tissue culture studies by exposure to Ox/CaOx crystals of renal epithelial cells and by animal model studies (Thamilselvan et al. 2000, 2003). Determination of total oxidant status (TOS) and total antioxidant status (TAS) is very important to include the most general and comprehensive information used to determine the oxidative damage caused by ethylene glycol whether SUO has antioxidative effect or not. In the Table I, it was seen that the TOS values in the plasma and tissues in the ethylene glycol treated group were higher than the other groups ($p<0.05$). This can be considered as a marker of oxidative damage. When the plasma and renal TOS values were examined, it was seen that TOS

Table I. Erythrocyte, plasma, kidney and liver tissue oxidative stress marker (MDA, NO, TOS) levels.

Group	Control	EG	SUO	SUO+EG	EG+SUO
Erytrocyte MDA (nmol/gHb)	1.80±0.11^{b}	2.61±0.18^{a}	2.35±0.12^{c}	2.48±0.06^{ac}	2.30±0.11^{c}
Renal MDA nmol/g protein	7.87±0.10^{b}	10.03±0.37^{c}	9.04±0.13^{a}	10.04±0.28^{c}	8.17 ±0.33^{b}
Liver MDA (nmol/g protein)	4.17±0.07^{d}	7.74±0.13^{a}	4.53±0.08^{b}	7.23±0.12^{c}	6.00±0.24^{e}
Plasma NO (µmol (NO)x /L)	62.48±0.79^{d}	47.08±0.23^{a}	60.52±0.99^{b}	52.02±0.83^{c}	48.45±1.07^{a}
Renal NO (µmol(NO)x/mg protein)	23.71±0.96^{b}	17.25±1.15^{a}	21.03±1.27^{b}	20.44±1.19^{b}	19.09±0.32^{ab}
Liver NO (µmol (NO)x/mg protein)	20.31±0.68^{b}	9.82±0.56^{a}	13.76±0.98^{c}	11.01±1.35^{a}	14.39±0.83^{c}
Plasma TOS (µmol H_2O_2 Equiv./L)	5.39±0.28^{a}	10.49±0.34^{c}	7.72±0.77^{b}	10.32±0.46^{c}	9.48±0.23^{c}
Renal TOS mmol H_2O_2 Equiv/mg protein	8.87±0.35^{a}	15.54 ±0.37^{d}	12.21±0.53^{b}	14.87±0.42^{c}	143.08±0.26^{c}
Liver TOS mmol H_2O_2 Equiv/mg protein	11.49±1.15^{a}	22.90±0,48^{d}	17.83±0.92^{c}	16.52±0.61^{bc}	16.80±1.25^{b}

Data were presented as mean±standard deviation (n=7). Differences in the statistics (p<0.05) between the experimental groups are expressed in the letters in superscripts (a-e). EG; ethylene glycol treated group, SUO; *Sciaena umbra* otolith treated group, SUO + EG; ethylene glycol and *Sciaena umbra* otolith treated group; EG + SUO; ethylene glycol treated first 30 days, after 15 days *Sciaena umbra* otolith treated group.

was significantly higher (p<0.05) in all groups where EG was administered alone and together with SUO. It was seen SUO is not effective on oxidative stress. When plasma TAS values were analyzed in Table II, it was seen that TAS was lower (p<0.05) in all groups where EG was applied alone and together with SUO. In tissues, TAS values in prophylactic group were found to be the same as in EG group. This shows that SUO has no antioxidant effect.

The accumulation of CaOx in the kidneys significantly reduces their efficacy with treatments with antioxidants and free radical scavengers (Khan 1997). Many studies have demonstrated the production of reactive oxygen species and changes in renal cellular endogenous antioxidant defenses that cause oxidative stress caused by hyperoxaluria. Cellular GSH is very important in defense against oxidative damage. When the study was examined, it was seen in Table II that EG group GSH concentrations in erythrocytes and tissues were significantly lower (p <0.05) than those of all groups. This shows that damage caused by EG application and GSH are effective for the elimination of this damage. Accumulation of CaOx crystals in the kidney has been associated with a decrease in total renal cellular glutathione and an increase

in lipid peroxides (Muthukumar & Selvam 1998). Although the GSH concentrations of SUO groups were increased, this increase was not statistically significant. It is seen that SUO is not an antioxidant system-supporting agent. Mohanasundari et al. (2005) reported that renal epithelial cell damage caused by ethylene glycol due to hyperoxaluria is associated with free radical formation and lipid peroxidation. The damage starts with hyperoxaluria and continues with crystal accumulation of renal tubules. The metabolic end product of ethylene glycol is calcium oxalate nephrotoxic. They thought that GSH decreased in EG treated groups because of free radical formation. As a result of this, they reported that renal epithelial cell damage caused by ethylene glycol due to hyperoxaluria is associated with free radical formation and lipid peroxidation. The damage starts with hyperoxaluria and continues with crystal accumulation of renal tubules. The metabolic end product of ethylene glycol is calcium oxalate nephrotoxic. They thought that GSH decreased in EG treated groups because of

Table II. Erythrocyte, plasma, kidney and liver tissue antioxidative marker (TAS, GSH, CAT) levels.

Group	Control	EG	SUO	SUO+EG	EG+SUO
Plasma TAS (µmol Trolox Equiv./L)	1.64±0.02^{c}	0.42±0.06^{a}	1.03±0.072^{d}	0.54±0.08^{a}	0.62 ±0.03^{b}
Renal TAS (mmol Trolox Equiv./mg protein)	0.96±0.06^{a}	0.37±0.03^{b}	0.51±0.05^{c}	0.38±0.03^{b}	0.48±0.02^{c}
Liver TAS (mmol Trolox Equiv./mg protein)	0.70±0.03^{a}	0.37±0.02^{b}	0.50±0.06^{c}	0.39±0.03^{b}	0.51 ±0.02^{c}
Erytrocyte GSH (nmol/g Hb)	84.41 ± 1.89^{d}	61.06 ± 1.01^{a}	70.75 ± 2.01^{c}	66.04 ± 1.01^{b}	72.57 ± 0.99^{c}
Renal GSH (µmol/g protein)	76.47 ± 0.94^{b}	54.89 ± 1.34^{a}	65.51 ± 1.09^{c}	59.42 ± 1.29^{d}	61.9 ± 0.93^{e}
Liver GSH (µmol/g protein)	76.73 ± 1.47^{c}	49.77 ± 1.09^{b}	60.19 ± 0.89^{a}	54.50± 0.77^{d}	58.15 ± 0.62^{e}
Erytrocyte CAT (k/g Hb)	4.49±0.08^{d}	2.41±0.05^{a}	3.13±0.06^{b}	2.81±0.04^{c}	2.78±0.05^{c}
Renal CAT (k/g protein)	2.37±0.05^{c}	1.59±0.05a^{b}	2.28±0.04^{c}	1.99±0.06^{b}	1.98±0.04^{b}
Liver CAT (k/g protein)	20.87±0.66^{c}	12.98±0.73^{a}	19.56±1.16^{c}	14.26±0.43^{a}	17.07±0.87^{b}

Data were presented as mean±standard deviation (n=7). Differences in the statistics ($p<0.05$) between the experimental groups are expressed in the letters in superscripts (a-e). EG; ethylene glycol treated group, SUO; *Sciaena umbra* otolith treated group, SUO + EG; ethylene glycol and *Sciaena umbra* otolith treated group; EG + SUO; ethylene glycol treated first 30 days, after 15 days *Sciaena umbra* otolith treated group.

free radical formation. Reduction or complete removal of crystal deposition relates to the rearrangement of defenses by increasing the activity of antioxidative enzymes such as catalase, glutathione peroxidase (GPx), reduced glutathione (GSH).

Catalase (CAT) is an enzyme involved in the destruction of peroxides and the antioxidant defense of the organism. In this study, it was seen that catalase enzyme activity in the erythrocytes EG group was significantly ($p<0.05$) lower than all other groups. On the other hand, it was observed that CAT enzyme activities in the prophylactic group and EG group were lower than the other groups. Oxidative stress is a condition that leads to a reduction in endogenous enzyme levels that allow inhibition of many free radical species. It is seen that SUO is not very effective antioxidant against oxidative stress caused by EG. In their study, Abhirama & Sundaram (2018) examined the effects of ethanolophilic extract of *Biophytum sensitivum* on nephrotoxicity caused by the application of ethylene glycol for 28 days. In line with our study, they indicated that EG administration decreased antioxidant enzyme levels. They argued that because of the bioactive contents contained in the *Biophytum sensitivum*, it could also be nephroprotective and antiurolytic.

The accumulation of nitrogenous substances such as urea, creatinine and uric acid in the blood increases due to the formation of stones in the urinary system or a blockage due to renal parenchymal damage. This indicates significant damage to the kidney. Serum Creatinine (CREA) and Blood Urea Nitrogen (BUN) concentration was shown in Table III. In this study, serum creatinine, blood urea nitrogen and urine calcium and phosphate levels were significantly ($p<0.05$) increased in the EG-treated group compared to the control group. Therapeutic or prophylactic administration of SUO appears to have no effect on urolithiasis. Urinary calcium concentration in EG groups increased in accordance with previous reports (Divakar et al. 2010).

Increased urinary calcium promotes nucleation and precipitation of calcium oxalate/phosphate from urine, followed by crystal growth. The increased urinary inorganic phosphate excretion observed on ethylene glycol application creates calcium phosphate crystals that induce calcium oxalate accumulation, providing a suitable environment for stone formation. Nucleation in urine and aggregation of crystals depend on pH. Uric acid stones occur

Table III. Serum Creatinine (CREA), Blood Urea Nitrogen (BUN) Concentration.

Group	CREA (mg/mL)	BUN (mg/mL)
Control	0.38 ± 0.03^{a}	30.35 ± 1.57^{a}
EG	0.69 ± 0.02^{d}	45.82 ± 1.13^{d}
SUO	0.50 ± 0.02^{b}	32.80 ± 1.06^{b}
SUO+EG	0.61 ± 0.02^{c}	43.31 ± 0.56^{c}
EG+SUO	0.63 ± 0.01^{c}	33.57 ± 1.05^{b}

Data were presented as mean±standard deviation (n=7). Differences in the statistics ($p<0.05$) between the experimental groups are expressed in the letters in superscripts (a-d). EG; ethylene glycol treated group, SUO; *Sciaena umbra* otolith treated group, SUO + EG; ethylene glycol and *Sciaena umbra* otolith treated group; EG + SUO; ethylene glycol treated first 30 days, after 15 days *Sciaena umbra* otolith treated group.

in acidic urine whereas calcium oxalate and calcium phosphate stones occur in alkaline urine (Kohri et al. 1988). Urine analysis was shown in Fig. 1. It is seen that urine pH of EG treated group is 7.84±0.29 in this study. Consistent with the study, Abhirama & Sundaram (2018) found that serum CREA and BUN levels and urine pH, calcium and phosphate concentrations of EG were high.

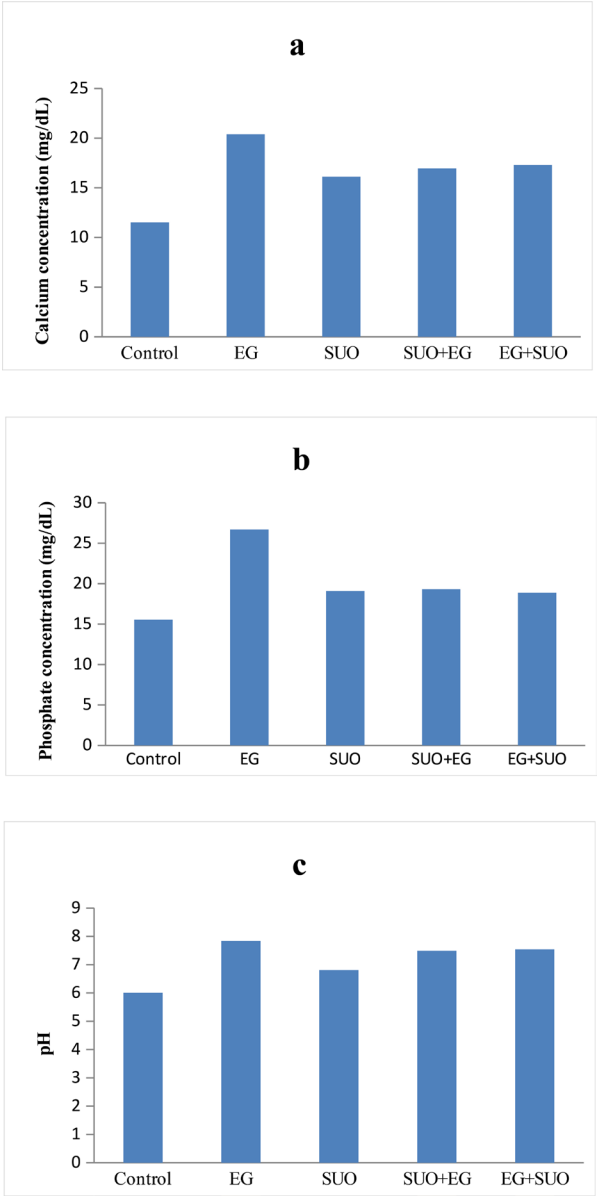

Figure 1. Urine Analysis; a: Urine calcium concentration; b: Urine phosphate concentration; c: Urine pH.

Exposure of renal cells to oxalate and CaOx crystals lead to generation of ROS, development of oxidative stress, and subsequent injury and inflammation. Renal injury and inflammation appear to play an important role in stone formation. Research has shown that renal epithelial cells' response to Ox and CaOx crystals are biphasic and concentration dependent. The oxide is mitogenic at low concentrations and is toxic at high concentrations as well as with CaOx crystals. Injury of renal epithelial cells causes cellular disruption and the production of membranous vesicles. The crystals are either passed as crystalluria particles or endocytosed by epithelial cells to be processed by the lysosomal system or transported to the interstitium. The CaOx crystal accumulation in the kidney up-regulates the expression and synthesis of macromolecules that increase inflammation and can lead to fibrosis (Hackett et al. 1994, Koul et al. 1994, 1996).

Tumor necrosis factor (TNF) is a powerful proinflammatory cytokine and an important means of inflammatory tissue damage. It also has important immunomodulatory functions. Studies support the role of TNF in the pathogenesis of acute and chronic renal disease. However, TNF mediates immunosuppressive effects in both proinflammatory and especially chronic renal disease and systemic autoimmunity (Vielhauer & Mayadas 2007). In Table IV, serum and kidney tissue inflammation marker (TNF-α, IL-1β ve IL-18) levels were shown. Serum TNF-α levels were found to be statistically different in all groups ($p < 0.05$) and the highest TNF-α levels belonged to the EG group. The renal TNF-α levels of the EG group and SUO + EG group were also higher than the other groups ($p < 0.05$). The TNF-α levels of SUO group are higher than control, so SUO is effective both in serum and kidney, exclusively. Studies on the model of acute oxalosis show that inflammation signals are limited to intrarenal

Table IV. Serum and Kidney Tissue Inflammatory Cytokine (TNF-α, IL-1β ve IL-18) levels.

	Control	EG	SUO	SUO+EG	EG+SUO
Serum TNF-α (pg/mL)	33.38 ± 0.7^{b}	74.19 ± 1.33^{a}	43.24 ± 1.86^{d}	67.23 ± 0.96^{c}	49.74 ± 0.81^{e}
Renal TNF-α (pg/mg protein)	358.44 ± 2.35^{a}	408.88 ± 2.21^{d}	387.98 ± 0.83^{b}	412.13 ± 2.35^{d}	393.42 ± 2.17^{c}
Serum IL-1β (pg/mL)	101.09 ± 0.74^{a}	197.13 ± 4.46^{c}	118.61 ± 2.91^{b}	119.16 ± 1.18^{b}	119.00± 1.19^{b}
Renal IL-1β (pg/mg protein)	1418.76 ± 19.52^{a}	2000.26 ± 18.15^{c}	1654.35 ± 41.74^{b}	1981.78 ± 23.37^{c}	1994.12 ± 12.28^{c}
Serum IL-18 (pg/mL)	1018.83 ± 37.34^{c}	1406.18 ± 57.49^{d}	1135.85 ± 36.16^{a}	1385.19 ± 26.73^{d}	1229.13 ± 30.86^{b}
Renal IL-18 (pg/mg protein)	2343.93± 49.00^{b}	3780.63 ± 59.11^{a}	2811.66 ± 40.50^{b}	3185.03 ± 108.92^{b}	56.09^{b}

Data were presented as mean±standard deviation (n=7). Differences in the statistics (p<0.05) between the experimental groups are expressed in the letters in superscripts (a-e). EG; ethylene glycol treated group, SUO; *Sciaena umbra* otolith treated group, SUO + EG; ethylene glycol and *Sciaena umbra* otolith treated group; EG + SUO; ethylene glycol treated first 30 days, after 15 days *Sciaena umbra* otolith treated group.

dendritic cells. Numerous studies have shown that IL-1α may not be mediator of intrarenal inflammation and tubular necrosis in models of postischemic or toxic acute kidney injury (AKI). In many cases, IL-1α release is probably more important in the kidney, whereas IL-1β causes systemic inflammation (Melnikov et al. 2002). In the present study, it was observed that the application of SUO alone or with EG was significantly higher in serum IL-1β concentrations compared to control (p<0.05). Therefore, it cannot be said that SUO has a protective and therapeutic effect. At the same time it was determined that IL-1β levels in the 4th and 5th groups were the same as the EG group in the kidney and showed an inflammatory effect similar to ethylene glycol. Many cytokines are released into the kidney injured by leukocytes and renal tubular cells and are important components of the initiation and spread of inflammation in AKI. IL-18 is a proinflammatory cytokine produced by proximal tubules, lymphocytes, neutrophils and macrophages in ischemic AKI. IL-18 mediated ischemic AKI has mechanisms independent of neutrophils and macrophages. Cisplatin-induced AKI is associated with increases in IL-6 and IL-18 in cytokines and neutrophil infiltration in the kidney (Melnikov et al. 2002, He et al. 2009, Faubel et al. 2007). In the present study EG group IL-18 concentration was higher (p<0.05) than other groups. However, when the serum IL-18 levels were examined, it was found that the

prophylactic group had a similar effect to the EG group. Numerous tissue culture and animal model studies have shown that treatments with antioxidants and free radical scavengers reduce injuries caused by the Ox/CaOx crystal. However, CaOx crystal accumulation in the kidneys, antioxidants and free radical scavengers were significantly reduced the effectiveness of treatment (Khan 1997).

CONCLUSION

Sciaena umbra otolith has been widely and traditionally used in the treatment of kidney stones, but there are no studies on its effects and mechanism of action. In this study, the effects of *Sciaena umbra* otolith on ethylene glycol induced nephropathy were investigated. Serum CREA and BUN levels and urine calcium, phosphate and pH levels were determined to be damaged by ethylene glycol. When oxidative/antioxidative parameters were examined in erythrocytes/plasma and tissue, free radicals generated by ethylene glycol and oxidative damage were determined. SUO does not exhibit antioxidative character, but acts as ethylene glycol. There is also an inflammatory effect caused by EG from tissue and serum cytokine levels. From TNF-α and IL-1β levels in renal tissue, it is seen that SUO has enhancing effects on the inflammation. Therefore, it cannot be said that *Sciaena umbra* otolith is nephroprotective on kidney and may have antioxidative and anti-inflammatory effect. It was concluded that *Sciaena umbra* otolith has no protective effect on stone formation/nephropathy.

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Laçine Aksoy and Mesturiye Yaylalı contributed to the study by conducting the study, experimental design and project design. Laçine Aksoy, Mesturiye Yaylalı and Mukhriddin Suyundikov have contributed to experimental animal practices, laboratory studies and data analysis.