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Tamoxifen and sodium thiosulfate reduces hepatic and renal damage induced by *Xanthium strumarium* L. through controlling mitochondrial permeability

ZELİHA KESKİN ALKAÇ[1], FATİH AHMET KORKAK[2], GÜRDAL DAĞOĞLU[2], YESARİ ERÖKSÜZ[3], SADETTİN TANYILDIZI[2]

[1]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Firat University, TR-23200 Elazig, Türkiye
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Firat University, TR-23200 Elazig, Türkiye
[3]Department of Pathology, Faculty of Veterinary Medicine, Firat University, TR-23200 Elazig, Türkiye

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Keskin Alkaç Z., Korkak F. A., Dağoğlu G., Eröksüz Y., Tanyıldızı S.
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Summary

***Xanthium strumarium* is toxic and causes cell death as a result of oxidative stress and mitochondrial dysfunction. Tamoxifen (TAM) is an anticancer agent that prevents the opening of mitochondrial permeability transition pores (mPTP) and the formation of reactive oxygen species (ROS). Sodium thiosulfate (STS) is a hepatoprotective agent with antioxidant effect. In this study, the therapeutic effects of TAM and STS on liver and kidney toxicity caused by *X. strumarium* were investigated. For this purpose, 35 female Sprague-Dawley rats were used. Rats were administered TAM and STS 6 and 24 hours after *X. strumarium* seeds extract administration. The rats were sacrificed 2 hours after the last administration. Increased serum biochemistry parameters and decreased glucose levels following *X. strumarium* toxicity approached the control group with TAM and STS treatment. Oxidative stress, which was more pronounced in liver tissue, decreased with TAM and STS treatment. mPTP opening was blocked by TAM-containing groups, whereas the increased ATP-synthase activity was decreased by TAM and STS alone and in combination. However, both compounds were effective in reducing histopathologic damage limited to liver tissue. The reduction of liver and kidney damage by TAM and STS in *X. strumarium* toxicity suggests the potential use of these compounds for treatment in case of poisoning.**

Keywords: *Xanthium strumarium*, tamoxifen, sodium thiosulphate, histopathology, immunohistochemistry

Xanthium strumarium L. plant, which grows in many parts of the world, is a member of the Asteraceae family and among the people traditionally used to treat diseases, such as rhinitis, headaches, stomach diseases, bacterial and fungal infections, arthritis, epilepsy, and diabetes(8). *X. strumarium* L. contains more than 170 bioactive compounds, mainly sesquiterpenes and phenylpropanoids (8). Among these compounds, atractyloside (Atr) and carboxyatractyloside (Catr), which are in glycoside structure, are highly toxic to animals and humans (8, 23). Poisoning can occur through the use of these plants in alternative medicine among the public, consumption by children as a curiosity, and animals consuming seeds mixed with silage (23, 27).

The adenine nucleotide translocase (ANT), which is found in the inner membrane of the mitochondria, has 2 important roles in the cell. Its first function is to transport adenosine diphosphate (ADP) for oxidative phosphorylation from the cytoplasm to the mitochondrial matrix, where ADP is catalyzed by ATP synthase and converted to ATP. The generated ATP energy is used to maintain all functions of the cell (36). Secondly, it is part of the mitochondrial permeability transition pores (mPTP) located in the inner and outer mitochondrial membrane (13). mPTP in the inner mitochondrial membrane allows the passage of substances that dissolve up to 1.5 kDa (13). However, high Ca^{2+} levels, reactive oxygen species and some xenobiotics in the mitochondrial matrix lead to the opening of mPTP

causing various damages resulting in cell death (12). Therefore, ANT is required for mitochondrial ATP production and cell integrity (13). In *X. strumarium* L. poisoning, Atr and Catr formed via biotransformation, inhibit competitively ANT with high affinity. As ADP cannot be transported to the phosphorylation site in mitochondria due to this inhibition, ATP cannot be synthesized and an energy crisis occurs in the cell. Further, the binding of Atr and Catr to ANT leads to the opening of mPTP, resulting in necrotic or apoptotic cell death (14).

Tamoxifen (TAM) is a synthetic, nonsteroidal anti-estrogenic agent widely prescribed in the treatment of estrogen-dependent neoplasias, such as breast cancer (39). It has been suggested that TAM might be an effective inhibitor of the mPTP, which is implicated in the mechanisms of chemical-induced tissue injury and apoptosis (39). TAM has been shown to inhibit the induction of the mPTP in various studies (14, 39). For example, TAM was found to suppress the opening of the mPTP induced by Ca^{2+} overload through enhancing phosphate uptake into the mitochondria (39). In a study using Catr-induced mitochondrial permeability transition, TAM, at a concentration of 10 μM, completely inhibited nonspecific membrane permeability induced by 1 μM Catr in freshly prepared mitochondria (14). It also acts as an antioxidant by detoxifying intracellular peroxide radicals and prevents apoptosis by suppressing mPTP opening (13, 31, 40). However, the therapeutic role of TAM *in vivo* in Atr- and Catr-containing *X. strumarium* L. poisoning is unknown.

Sodium thiosulfate (STS) is used as an antidote for cyanide poisoning (16). Furthermore, due to its wide safety range (LD50: 4500-5000 mg/kg), it is clinically used in the treatment of calciphylaxis in patients with renal impairment (2). In studies, STS has been found to have antioxidant, antiapoptotic, and mitochondria-repairing effects on heart, liver, kidney and brain tissues (33). Due to these pharmacological effects it has been shown to have potential therapeutic effects in various health conditions, including chronic kidney disease, liver, heart and brain damage (5, 32, 33).

X. strumarium L. toxications occur worldwide causing widespread fatal poisoning cases in animals and humans. The toxic compounds it contains damage cells at the mitochondrial level, causing an intracellular energy crisis. It also causes necrotic or apoptotic cell death. For this reason, the clinical picture is severe (14, 23, 27). However, no specific treatment is available against *X. strumarium* L. poisoning. In this study, we hypothesized that TAM and STS may be effective therapeutic candidates in *X. strumarium* L. toxicity due to their antioxidant and mitochondria-repairing effects. Therefore, in the current study, we investigated the therapeutic effects of TAM and STS in rats with experimental *X. strumarium* L. toxicity.

Material and methods

Extraction of plants and dosage calculation. In October, *X. strumarium* L. plants were harvested in the Elazig region. The seeds were separated from cothylodones and crushed to make it powder. After adding distilled water to the *X. strumarium* seeds (XSS), it was kept at 100°C for about 30 minutes. The resulting mixture was filtered through filter papers (S&H Labware-125mm). Atr levels in the extract were determined by Gas Chromatographic-Mass Spectrophotometer (GC-MS-QP2010, Shimadzu) according to the method proposed by Laurens et al (19). According to this method, 1 mL of the extract was mixed with 2 mL of hydrochloric acid (HCL) (2 mol/L) and incubated at 25°C for 12 hours. The hydrolysate was then centrifuged at 3500 rpm for 5 minutes and the supernatant was transferred to another glass tube. 2 mL of ethyl acetate was added to the supernatant, vortexed for 2 minutes and centrifuged at 3500 rpm for 5 minutes. The resulting supernatant was transferred to a separate tube and the process was repeated 4 more times for the lower part by adding ethyl acetate. Finally, the organic extract sample was collected in a separate tube and dried under nitrogen gas at 45°C. The residue was derivatized with 100 μL pyridine and 100 μL (trimethylsilyl) imidazole (TMSI) at 100°C for 2 hours. At the end of two hours of derivatization, 2 μL of sample was taken and injected into the GC-MS. As a result of the analyzes, it was determined that the Atr level in XSS was 4 mg/g. The toxicity model in rats was carried out considering the quantity of Atr determined in the extract of XSS. Rats were given 75 g/kg XSS (containing 300 mg/kg Atr) extract orally.

Animals. A total of 35 female Sprague-Dawley rats, aged 6-7 weeks and weighing 200-250 g, were supplied from Laboratory Animal Resources Unit of Firat University. All rats were kept in designated cages under regular circumstances (22°C, 12 : 12 h of light/dark cycle). The rats were given unlimited access to fresh water and food. The Animal Experiments Ethics Committee of Firat University (2019/204) approved all experimentations.

Experimental model and samples handling. The Sprague-Dawley rats were split up into a total of seven groups (n = 5 per group). Groups: (1) control, (2) XSS, (3) TAM, (4) STS, (5) XSS + TAM, (6) XSS + STS, (7) XSS + TAM + STS. XSS extract was dissolved in aqueous solution, TAM and STS in normal saline and administered to rats by oral gavage. The control group (Group 1) received normal saline by the same route to provide the same conditions as the other six groups. Rats in the XSS exposed groups (Groups 2, 5, 6, 7) were administered 75 g/kg XSS extract at 0 hour. The dose of XSS was determined by reference to a previous experimental toxicity study in rats (17). 6 and 24 hours after XSS toxicity, rats in the TAM exposed groups (Groups 3, 5, 7) were administered 20 mg/kg TAM: rats in the STS exposed groups (Groups 4, 6, 7) were administered 50 mg/kg STS. The study duration was determined as 2 days because XSS toxicity is an acute toxicity and clinical symptoms of XSS toxicity appear in a short time in experimental studies (4, 17).

Two hours after the last drug administration, rats were killed by cervical dislocation and blood, liver, and kidney tissues were taken. Blood samples were centrifuged at 4°C 3500 × g for 5 min and stored at –20°C until the serum was used. Part of the liver and kidney were fixed in 10% formalin for histopathological and immunohistochemical analyses. The rest of the tissues were stored at –20°C until biochemical analyses were performed.

Assessment of serum biochemical. An automatic chemical analyzer was used to determine the levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CK), creatinine, blood urea nitrogen (BUN) and serum glucose (Olympus AU-660, Tokyo, Japan).

Assessment of tissue biochemical. Homogenization of liver and kidney tissues was performed in a Teflon-glass homogenizer and malondialdehyde (MDA) was measured in the homogenate. MDA was measured using thiobarbituric acid procedure (29). The homogenate was centrifuged at 4°C 12000 × g for 45 min. Protein, reduced glutathione (GSH) and superoxide dismutase (SOD) analyses were performed in the supernatant. GSH activity was estimated based on the ability of 5,5-dithio-bis[2-nitrobenzoic acid] (DTNB) to be reduced by sulfhydryl compounds. The optical density of the disulfide compound formed was measured at a wavelength of 412 nm (7). The colored complex released by the reduction of nitrobluetetrazolium (NBT) by superoxide radicals produced by xanthine/xanthine oxidase system was measured spectrophotometrically at 560 nm and SOD activity was determined (37). The method of Lowry et al. (22) was used to determine the protein content of the tissues.

Mitochondria isolation. The procedure for mitochondrial isolation was carried out in accordance with what Li et al. (21) had previously outlined. Liver and kidney tissues were homogenized (at 1000 × g for 10 min at 4°C) with buffer A (220 mM mannitol, 70 mM sucrose, 2.0 mM ethylenediaminetetraacetic acid, 5.0 mM 4-mor-pholinepropanesulphonic acid and 0.5% bovine serum albumin (BSA), pH 7.4) and the homogenate was centrifuged at 1000 × g for 10 min at 4°C. The obtained supernatant was centrifuged a second time under the same conditions. The final supernatant was centrifuged (at 8000 × g for 20 min at 4°C) with BSA-free and 7.5-fold diluted buffer A. The released mitochondrial pellet was suspended with BSA-free buffer A. The approach outlined by Lowry et al. (22) was used to quantify the amount of protein in the mitochondria.

Detection of Ca^{2+} amounts in mitochondria. The method outlined by Wang et al. (41) was used to detect the levels of mitochondrial Ca^{2+}. This procedure involved adding 1 mL of mitochondrial sample (1.0 mg protein), 2 mL of nitric acid, and 1 mL of ultra-pure water. Next, 1 mL of lanthanum oxide solution was added, and the mixture was agitated at room temperature. An atomic absorption spectrophotometer was used to detect the amount of Ca^{2+} in the mixture (AAS, Perkin-Elmer, Analyst 800, Norwalk, CT, USA). By creating a six-point calibration curve from the $CaCO_3$ stock solution made with ultra-pure water and nitric acid, the results were assessed.

Detectionof mPTP opening in the mitochondria. When mPTP is opened, the mitochondrial inner membrane exhibits significant permeability for sucrose and mannitol, as seen by a reduction in absorbance at 540 nm. The method outlined by Lowry et al. (22) was used to measure the concentration of mitochondrial protein after the mitochondrial pellet had been suspended in 3 mL of cold test media. The protein concentration was then altered using the cold test medium to 0.3 mg/mL. This suspension was mixed with 3 mL of cold test medium, and the absorbance at 540 nm was assessed using UV spectrophotometry (VWR-3100PC UV Spectrophotometer) (15).

Determination of Atr levels

Determination of Atr levels in serum. 0.5 mL serum sample was taken into a glass tube and mixed with 1 mL acetone. After centrifugation at 3500 × g for 10 minutes, the supernatant obtained was dried at 45°C under nitrogen gas. The residue was redissolved by adding 1 mL of distilled water. After adding 2 mL of hydrochloric acid, the procedures used for the determination of the ATR level in the plant were applied (19).

Determination of Atr levels in liver and kidney tissues. Liver and kidney tissues were homogenized with 1 : 4 (g/v) phosphate buffered saline solution (PBS, pH 4.5) and centrifuged at 3500 × g for 5 min. The supernatant was dried under nitrogen gas at 45°C. The residue was redissolved by adding 1 mL of distilled water. After adding 2 mL of hydrochloric acid, the procedures used for the determination of the Atr level in the plant were applied (19).

Analytical procedure. Shimadzu Gas Chromatography-Mass Spectroscopy (GC-MS, QP2010) was used for the determination of Atr levels in serum and tissues. DB-1 capillary column (diameter 30 m × 250 μm, film thickness 0.1 μm) and SGE 10 μL injector were used for the analyses. Injector temperature was set at 250°C and detector temperature at 200°C. The column temperature started at 215°C and was increased by 2.30°C/min up to 310°C. The helium gas flow rate was set to 1.9 mL/min. Samples were read in splitless scanning mode in a volume of 2 μL (19).

Histopathological method. Liver and kidney tissue samples were fixed in 10% neutral buffered formalin. The hematoxylin-eosin (H-E) method was used to prepare the paraffin blocks using the normal techniques, cutting them to a thickness of 5 μm, and staining them.

Immunohistochemistry. Using the avidin-biotin-peroxidase method, immunohistochemical staining was carried out to detect the expression of ATP synthase (Abcam, AB181243) (17). The chromogen was 3-amino-9-ethylcarbozol (AEC), obtained from Thermo Fischer Scientific in Massachusetts, USA, using their Ultra Vision AEC Substrate System. The positive and negative controls are performed and checked. For negative control, PBS was used instead of primary antibody.

Statistical analyses. Statistical analyses were performed using the 'IBM SPSS Statistics 21' package program. One-way ANOVA and Tukey were performed for group comparisons. Values for all parameters were given as mean ± standard deviation (mean ± SD). p-Values less than 0.05 were considered statistically significant.

Results and discussion

The body distribution of Atr. Table 1 presents the effects of administering STS and TAM (both as single treatments and in combination) on the plasma, liver and kidney tissue concentrations of Atr in rats. Administration of STS and TAM alone or in combination did not change Atr levels in tissues ($p > 0.05$).

Serum biochemistry. As shown in Table 2 and Table 3, XSS administration significantly increased serum biochemical parameters such as AST, LDH, CK, and BUN in rats (AST, LDH, CK $p < 0.001$, BUN $p < 0.05$). ALT, ALP, and creatinine increased slightly. However, this increase was not significant ($p > 0.05$). Glucose levels were also significantly reduced in the XSS group compared with the control group ($p < 0.01$). STS treatment significantly reduced the increased AST level due to XSS toxicity ($p < 0.05$). There was a non-significant decrease in TAM and TAM + STS groups. The decrease in LDH levels was significant in STS and TAM + STS groups ($p < 0.001$, $p < 0.01$), while the decrease in CK levels was significant in all three treatment groups (STS $p < 0.01$; TAM $p < 0.001$, TAM + STS $p < 0.01$, respectively). However, the decrease in BUN levels was not significant ($p > 0.05$). Although glucose increased in all treatment groups, this increase was not significant ($p > 0.05$).

Tissue biochemistry. Figure 1 shows that XSS administration significantly increased liver and kidney MDA levels in rats (liver $p < 0.001$, kidney $p < 0.01$). STS-containing groups were more effective than TAM treatment alone in reducing elevated MDA levels ($p < 0.001$). GSH levels in the liver decreased significantly in the XSS group ($p < 0.001$), whereas the decrease in kidney tissue was not significant ($p > 0.05$).

Tab. 1. Atr levels in serum, liver and kidney of rats after XSS exposure

Group	Atr levels (µg/mL)		
	Serum	Liver	Kidney
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
XSS	3.24 ± 0.17[b]	4.66 ± 0.58[b]	4.29 ± 0.28[b]
XSS + STS	3.31 ± 0.15[b]	4.40 ± 0.24[b]	4.42 ± 0.18[b]
XSS + TAM	3.34 ± 0.22[b]	4.41 ± 0.42[b]	4.37 ± 0.18[b]
XSS + STS + TAM	3.20 ± 0.11[b]	4.48 ± 0.34[b]	4.37 ± 0.23[b]

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values reported are mean ± SD; a, b – differences between groups in the same column are statistically significant ($p < 0.05$). Atr – atractyloside; XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen

Tab. 2. Effects of STS and TAM administration on some serum biochemistry parameters of rats after XSS exposure

Group	Parameters			
	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)
Control	228. 20 ± 8.71[a]	59.80 ± 2.16[a]	222.40 ± 4.82[a]	1897.0 ± 145.60[a]
XSS	312.20 ± 57.92[b]	66.60 ± 4.61[a]	245.00 ± 15.55[a]	2520.0 ± 119.30[b]
STS	229.20 ± 13.23[a]	60.40 ± 6.30[a]	240.40 ± 7.66[a]	1965.6 ± 177.95[a]
TAM	295.20 ± 22.70[b]	65.20 ± 4.65[a]	234.60 ± 7.36[a]	2149.6 ± 347.70[ab]
XSS + STS	253.80 ± 53.69[ac]	58.00 ± 2.81[a]	215.00 ± 28.06[a]	1780.8 ± 190.03[ac]
XSS + TAM	265.40 ± 78.06[abc]	61.60 ± 5.00[a]	224.20 ± 11.12[a]	2157.6 ± 246.90[abc]
XSS + STS + TAM	257.60 ± 37.19[abc]	59.60 ± 3.89[a]	211.40 ± 14.29[a]	2068.8 ± 181.78[ac]

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values reported are mean ± SD; a, b, c – differences between groups in the same column are statistically significant ($p < 0.05$). XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen; AST – aspartate aminotransferase; ALP – alkaline phosphatase; ALT – alanine aminotransferase; LDH – lactate dehydrogenase

Tab. 3. Effects of STS and TAM on glucose and some serum biochemistry parameters in rats with XSS toxicity

Group	Parameters			
	CK (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)
Control	6733.60 ± 438.82[a]	41.00 ± 2.55[a]	0.33 ± 0.08[a]	115.00 ± 12.10[a]
XSS	12939.60 ± 675.66[b]	48.00 ± 1.00[b]	0.37 ± 0.03[a]	98.40 ± 7.43[b]
STS	6981.60 ± 617.73[a]	43.60 ± 2.70[ab]	0.33 ± 0.01[a]	112.00 ± 4.67[a]
TAM	9586.00 ± 779.50[c]	46.00 ± 3.60[ab]	0.33 ± 0.03[a]	110.60 ± 4.72[ab]
XSS + STS	9421.20 ± 862.95[ac]	44.40 ± 3.36[ab]	0.31 ± 0.01[a]	104.00 ± 2.44[ab]
XSS + TAM	8793.00 ± 646.93[ac]	47.60 ± 2.54[b]	0.33 ± 0.03[a]	101.60 ± 7.63[ab]
XSS + STS + TAM	9461.80 ± 750.98[c]	44.20 ± 3.27[ab]	0.32 ± 0.02[a]	107.40 ± 4.67[ab]

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values reported are mean ± SD; a, b, c – differences between groups in the same column are statistically significant ($p < 0.05$). XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen; CK – creatine phosphokinase; BUN – blood urea nitrogen

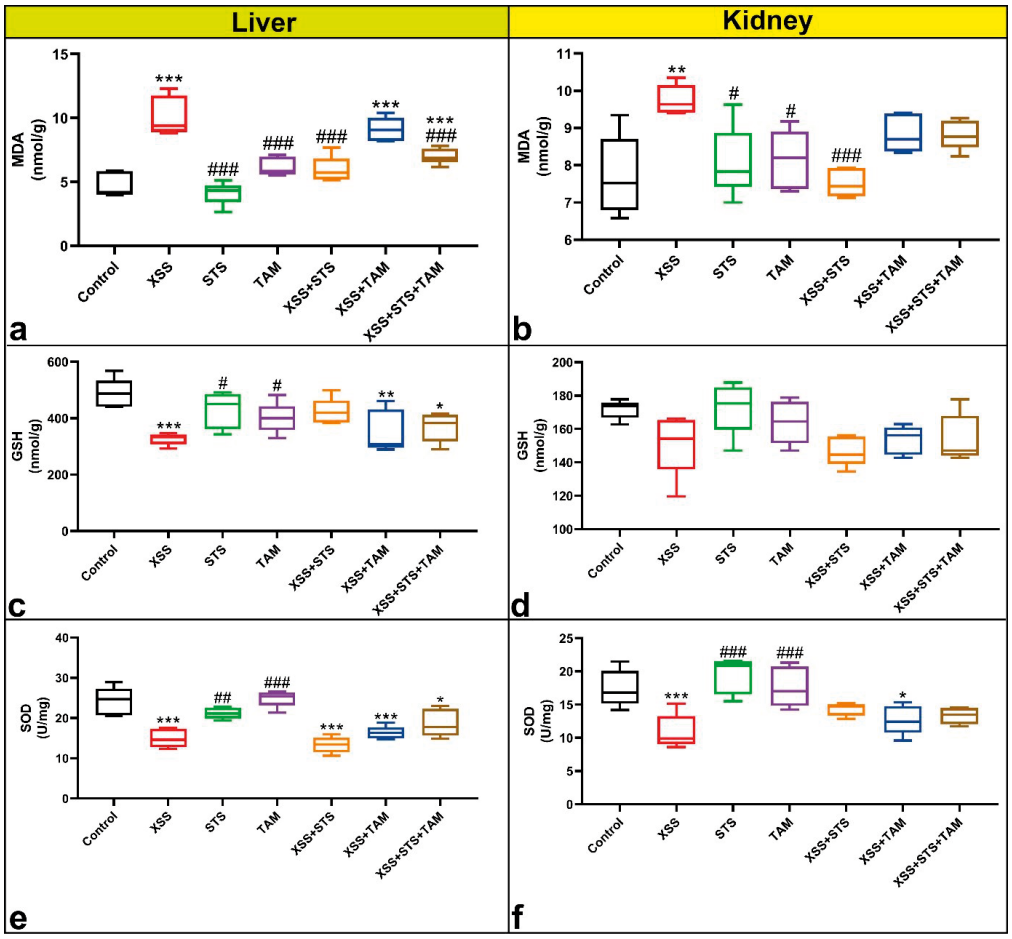

Fig. 1. Antioxidant effects of STS and TAM alone and in combination in liver and kidney tissues in XSS toxicity. (a) liver MDA, (b) kidney MDA, (c) liver GSH, (d) kidney GSH, (e) liver SOD, (f) kidney SOD.

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values are represented as mean ± SD. ***p < 0.001, **p < 0.01, *p < 0.05 compared to control; ###p < 0.001, #p < 0.5 compared to XSS. XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen; MDA – malondialdehyde; GSH – reduced glutathione; SOD – superoxide dismutase

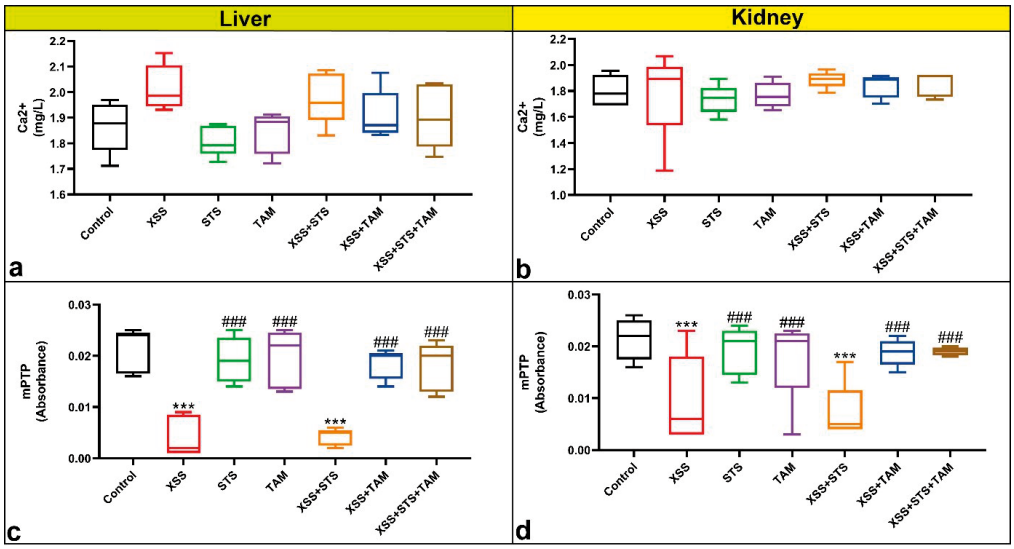

Fig. 2. Effect of STS and TAM on mitochondrial Ca^{2+} levels and mitochondrial permeability transition pore (mPTP) opening in XSS toxicity. (a) liver Ca^{2+}, (b) kidney Ca^{2+}, (c) liver mPTP absorbance, (d) kidney mPTP absorbance

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values are represented as mean ± SD. ***p < 0.001 compared to control; ###p < 0.001 compared to XSS. XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen

In contrast, although GSH levels increased in the treatment groups, this increase was not significant. Compared to the control group, XSS treatment significantly decreased SOD enzyme activities in both tissues ($p < 0.001$). Although all three treatment groups increased the decreased SOD enzyme activity in liver and kidney tissues, this increase was insignificant ($p < 0.05$).

Mitochondrial Ca^{2+} levels and mPTP opening. Although Ca^{2+} levels increased in mitochondria isolated from liver and kidney tissues of XSS-treated rats, this increase was not statistically significant ($p > 0.05$). However, mPTP absorbance values examined in liver and kidney tissues decreased in the XSS group compared to the control and this decrease was significant ($p < 0.001$). The mPTP absorbance values, which decreased in the XSS group, were significantly increased by the TAM-containing groups, whereas STS treatment alone did not change them (liver and kidney $p < 0.001$, Fig. 2).

Histopathology. Histopathologic findings were limited to liver tissue and no significant findings were found in kidney tissue. In microscopic examinations, lesions such as degeneration, single cell necrosis, Kupffer cell activation and periportal infiltration findings were found in the liver tissues of rats in XSS and some treatment groups and are presented in Table 4 and Figure 3. The most prominent histopathologic findings were found in the XSS group. In the XSS group, there was a cloudy appear-

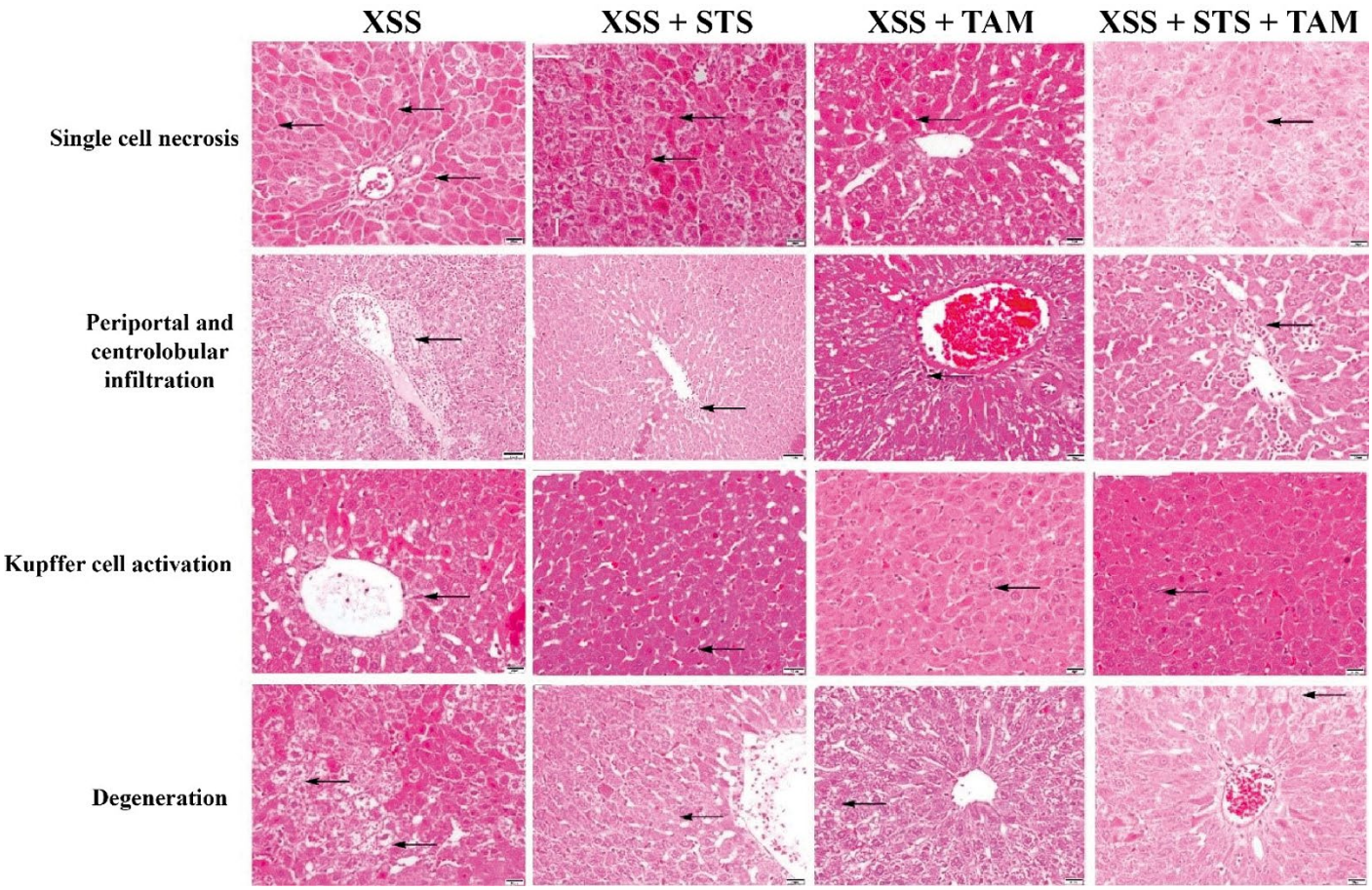

Fig. 3. The effect of STS and TAM alone and in combination on histopathological changes in the liver of rats with XSS toxicity (hematoxylin and eosin staining, Bar: 20 µm)
Explanations: XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen

Tab. 4. Grading of histopathological findings detected in the liver according to groups

Group	Degeneration	Single cell necrosis	Periportal infiltration	Kupffer cell activation
Control	1. 00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
XSS	2.00 ± 0.31^{b}	1.60 ± 0.24^{b}	0.80 ± 0.24^{a}	3.00 ± 0.20^{b}
STS	0.40 ± 0.24^{a}	0.40 ± 0.24^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
TAM	1.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.40 ± 0.24^{a}	0.20 ± 0.20^{a}
XSS + STS	1.40 ± 0.24^{abc}	1.60 ± 0.24^{b}	0.20 ± 0.20^{a}	1.20 ± 0.00^{abc}
XSS + TAM	1.20 ± 0.20^{acd}	0.60 ± 0.24^{a}	0.60 ± 0.24^{a}	2.20 ± 0.31^{bc}
XSS + STS + TAM	0.60 ± 0.24^{ad}	1.40 ± 0.24^{b}	0.40 ± 0.24^{a}	1.40 ± 0.22^{bc}

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values are represented as mean ± SD. a, b, c, d – differences between groups in the same column are statistically significant ($p < 0.05$). XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen

ance of cell cytoplasm in the liver, more prominent in the centrilobular region, Kupffer cell activation characterized by hypertrophy and hyperplasia of Kupffer cells, and cellular reactions consisting of lymphocytes and macrophages in the periportal region. In single cell necrosis, it was noted that cell cytoplasm was extremely eosinophilic and the nuclei of the cells were lysed. There was no inflammatory reaction against these necrotic hepatocytes. Lesions were found in almost all regions of the liver. Degeneration, single cell necrosis, periportal infiltration and Kupffer cell activation were significantly decreased ($p < 0.05$) in the groups given TAM and STS together with XSS compared to the XSS group.

Immunohistochemistry. Figure 4 shows that there was a statistically significant increase in ATP synthase immunoreactivity in the liver tissue of rats in the XSS group compared to rats in the control group ($p < 0.001$). However, ATP synthase activity, which increased due to XSS toxicity, decreased significantly in XSS + STS, XSS + TAM and XSS + STS + TAM treatment groups ($p < 0.001$).

Throughout history, plants have been widely used for their pharmacological effects. Given the long his-

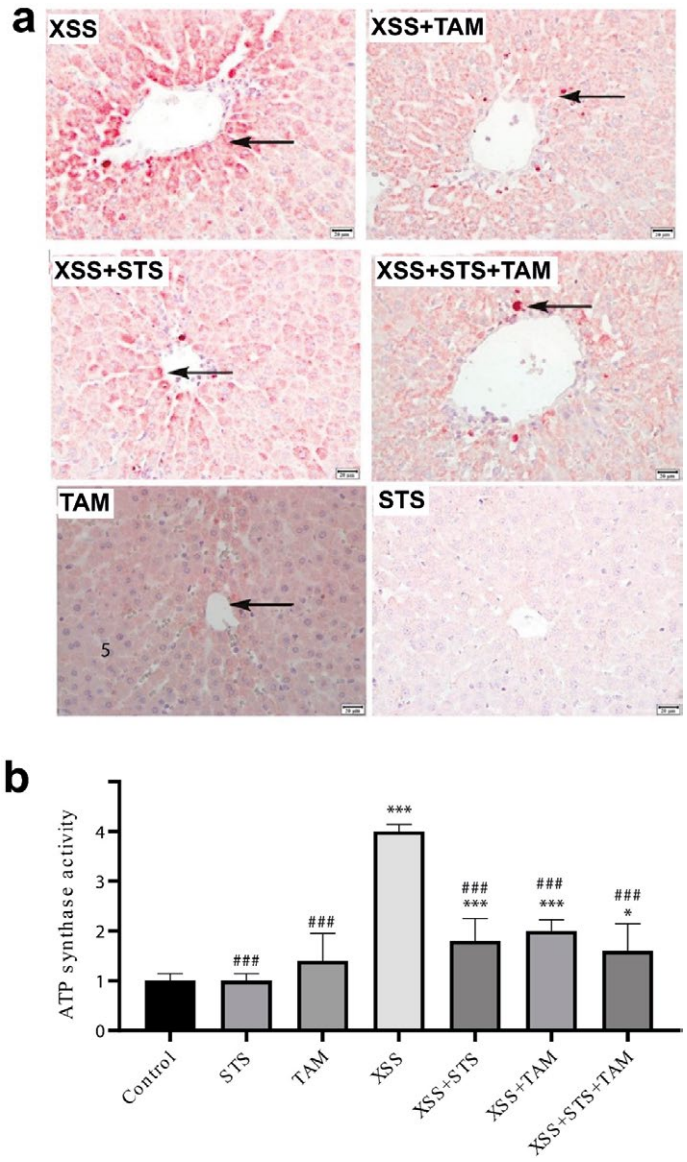

Fig. 4. Immunohistochemical analysis of ATP synthase expression in the liver tissues of the rats in the experimental groups after XSS, STS and TAM administration (horse radish peroxidase technique; amino-9-ethylcarbazole chromogen and hematoxylin counterstaining, Bar: 20 µm)

Explanations: One-way ANOVA and Tukey were performed for group comparisons. Values are represented as mean ± SD. ***$p < 0.001$, *$p < 0.05$ compared to control; $^{###}p < 0.001$ compared to XSS. XSS – *Xanthium strumarium* seeds; STS – sodium thiosulphate; TAM – tamoxifen

tory of consumption of plants in different societies, people can often be under the misconception that plants are completely harmless and non-toxic (9). However, plants contain many compounds and some of these compounds can be toxic (9). Herbal poisoning in humans occurs during therapeutic use or as a result of intentional and/or accidental consumption (27). In animals, this type of poisoning is usually caused by the consumption of feed mixed with the poisonous plant (23, 47).

In poisonings caused by the *Xanthium strumarium* plant, ATR, the toxic compound it contains, causes mPTP opening, disrupts mitochondrial integrity and causes cell death (48). Studies have shown that mitochondrial integrity is preserved when compounds that prevent mPTP opening are used (17, 44). TAM is known to be an important mPTP blocker (14). Some recent studies have indicated that STS may also reduce mitochondrial damage (18, 34). However, there are no studies on the efficacy of TAM and STS against *Xanthium strumarium* poisoning.

Increased mitochondrial Ca^{2+} levels in cells due to different toxic compounds or diseases, decreased membrane potential, ATP depletion and increased oxidative stress both in the cell and in the mitochondria increase the rate of mPTP opening (20). The opening of the mPTP leads to an increase in the matrix volume due to the entry of water and solutes from the cytoplasm into the mitochondria and ultimately to rupture of the outer mitochondrial membrane (35). Increased oxidative stress mediates both Ca^{2+} increase and direct mPTP opening in mitochondria (42). In this study, XSS toxicity increased mitochondrial Ca^{2+} levels non-significantly. This increase triggered mPTP opening. In addition, oxidative stress characterized by increased MDA and decreased antioxidants in liver and kidney also contributed significantly to mPTP opening. XSS also significantly increased ATP synthase activity examined immunohistochemically. ATP synthase is located in the inner mitochondrial membrane and catalyzes ATP synthesis from ADP and inorganic phosphate (Pi) under physiological conditions (26). In pathological conditions with mitochondrial dysfunction, ATP synthesis is disrupted and the existing ATP is transported to the mitochondrial matrix where it is degraded under the catalysis of ATP synthase enzyme and proton is supplied to the mitochondria (3, 10). Increased ATP-synthase activity in the XSS group is a result of ATP transport to mitochondria and degradation. This is an attempt to repair mitochondrial dysfunction. TAM and STS administered to treat XSS toxicity significantly reduced oxidative stress. TAM containing groups (XSS + TAM and XSS + STS + TAM) inhibited mPTP opening. However, all three treatment groups were effective in reducing the increased ATP synthase activity due to XSS toxicity. Numerous studies in recent years have reported that TAM is an effective antioxidant as well as a specific mPTP blocker (11, 31, 45, 46). The antioxidant effect of STS, known as the specific antidote of cyanide poisoning, has also been investigated and as a result of the research, it has been reported to be an important antioxidant that increases antioxidant enzyme activities (24).

Xanthium strumarium plant causes damage in liver and kidney tissues by disrupting mitochondrial integrity due to the toxic compounds it contains and AST, ALT, ALP, BUN, creatinine values, which are markers of this damage, increase in serum (49). In this study in which we induced toxicity by giving 75 g/kg XSS extract orally to rats; AST, LDH, CK and BUN levels

of rats in the XSS group were found to increase compared to the control group. However, serum glucose levels decreased (44, 48). The reason for the decrease in glucose levels is that ATR and CATR in the structure of *Xanthium strumarium* inhibit oxidative phosphorylation in cells and disrupt ATP production (28, 38). As a result of impaired ATP production, glucose is taken into the cell and broken down into purivic acid or lactic acid for the energy needs of the cells (28). When the results of this study were analyzed, in general, it was determined that the groups containing STS (XSS + STS, XSS + STS + TAM) were more effective in reducing the increased blood biochemistry parameters. STS has been proven to reduce elevated serum AST, ALT and ALP levels in cyanide poisoning, as well as elevated BUN levels in kidney damage due to various factors (6, 30). However, some studies have reported a protective role of TAM against drug and chemical induced hepatotoxicosis (25, 50).

XSS prevented mitochondrial function by altering energy metabolism, disrupting the lipid membrane of cells through oxidative damage, and altering cellular pressure and balance by opening mPTP channels, resulting in hepatocyte damage. Previous studies have reported that this hepatocyte injury is characterized by severe hepatocellular degeneration, necrosis, periportal cell infiltration, cytoplasmic vacuolation and loss of cell boundaries in liver tissue (1, 43, 49). In the present study, in parallel with the findings of other investigators, we found increased degeneration, single cell necrosis, periportal infiltration and Kuffer cell activation in the XSS group. All three treatment groups were effective in reducing the histopathologic damage caused by XSS in liver tissue. However, combined treatment (XSS + TAM + STS) was more effective.

In this study investigating different compounds for the treatment of XSS-induced poisoning, we obtained strong evidence that TAM and STS, with their antioxidant and mitochondrial damage reduction effects, can be used as therapeutic compounds in XSS poisoning cases. However, the effects of using these compounds in repeated doses over a longer period of time should also be investigated.

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Corresponding author: Assist. Prof. Zeliha Keskin Alkaç, Faculty of Pharmacy, Pharmaceutical Toxicology Department, Firat University, Elazig, Türkiye; e-mail: zkeskin@firat.edu.tr