Protective Effects of Xanthine Derivatives Against Arsenic Trioxide-Induced Oxidative Stress in Mouse Hepatic and Renal Tissues

Authors

Navid Omidifar[1, 2], Ahmad Gholami[1, 3], Mansoureh Shokripour[2], Mohammad Ali Nourani[2], Milad Mohkam[4], Seyyed Mojtaba Mousavi[5], Seyyed Alireza Hashemi[6], Bagher Khorram[7], Amir Nili Ahmadabadi[8], Mahintaj Dara[9]

Affiliations

1 Biotechnology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2 Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3 Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
4 Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
5 Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei City, Taiwan
6 Health Policy Research Center, Health Institute, Shiraz University of Medical Sciences, Shiraz, Iran
7 Student Research Committee, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
8 Department of Pharmacology and Toxicology, Medicinal Plants and Natural Products Research Center, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan
9 Stem Cells and Transgenic Technology Center, Shiraz University of Medical Sciences, Shiraz, Iran

Keywords

arsenic trioxide (ATO), kidney, liver, oxidative damage, pentoxifylline (PTX)

received 23.11.2023
accepted 15.01.2024
published online 13.02.2024

Bibliography

Drug Res 2024; 74: 133–144
DOI 10.1055/a-2247-5232
ISSN 2194-9379

Georg Thieme Verlag, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Dr. Amir Nili Ahmadabadi
Department of Pharmacology and Toxicology, Medicinal Plants and Natural Products Research Center, School of Pharmacy, Hamadan University of Medical Sciences, Shahid Fahmideh St.
6517838736 Hamadan
Iran
Tel.: 081-31310000
Amirnili54@yahoo.com

Dr. Mahintaj Dara
Stem Cells Technology Research Center,
Shiraz University of Medical Sciences, Zand St.,
7134814336 Shiraz
Iran
Tel.: (+98 71) 32 12 2 884, (+98 71) 32 35 69 96
dara.mahintaj@gmail.com

ABSTRACT

In this study, the protective efficacy of pentoxifylline (PTX) as a xanthine derivative against arsenic trioxide (ATO)-induced kidney and liver damage in mice was investigated. Thirty-six mice were divided into six groups, receiving intraperitoneal injections of saline, ATO, PTX, or a combination for four weeks. Blood samples were analyzed for serum biochemistry, while hepatic tissue underwent examination for histopathological changes and assessment of oxidative stress markers and antioxidant gene expression through Real-Time PCR. ATO exposure significantly increased serum markers (creatinine, ALT, BUN, ALP, AST) and induced histopathological changes in the liver. Moreover, it elevated renal and hepatic nitric oxide (NO) and lipid peroxidation (LPO) levels, and reduced antioxidant enzyme expression (CAT, GSR, GPx, MPO, SOD), total thiol groups (TTGs), and total antioxidant capacity (TAC). Conversely, PTX treatment effectively lowered serum hepatic and renal markers, improved antioxidant markers, and induced histopathological alterations. Notably, PTX did not significantly affect renal and hepatic NO levels. These findings suggest that PTX offers therapeutic potential in mitigating liver and acute kidney injuries induced by various insults, including exposure to ATO.

Introduction

Arsenic is a trace element found in the environment that has been linked to the development of several cancers [1]. As a metalloid, it can be found in natural and anthropogenic sources in water, air, and soil [2]. Arsenic can exist in different oxidation conditions and forms, comprising both inorganic and organic compounds. Arsenate and arsenite are the most common anionic species produced from arsenous and arsenic acids. [2]. Despite its long history of medicinal use spanning over 2,400 years, arsenic has both acute and chronic toxic effects [3]. The severity of arsenic toxicity is influenced by its oxidation state and chemical form. Acute arsenic poisoning can occur from exposure to high arsenic doses in a short time, whereas long-lasting exposure to inorganic arsenic affects multiple systems in the body [2]. Chronic inorganic arsenic exposure has been associated to health problems such as skin blemishes, cardiovascular illness, cancer, and neurological difficulties. The toxicity of arsenic in the body can cause oxidative stress, DNA damage, inflammation, and disruption of cellular signaling pathways. Arsenic compounds have been demonstrated to affect multiple organs in the human body, comprising the kidneys, liver, lungs, gastrointestinal tract, and respiratory tract [4]. The kidneys are a crucial organ system responsible for eliminating nitrogenous waste products, regulating acid-base balance, and maintaining fluid and electrolyte homeostasis. However, arsenic trioxide toxicity can lead to significant impairments of renal functions, resulting in noticeable changes in circulating blood composition [4]. Notably, chronic arsenic exposure through oral ingestion can cause hepatotoxicity, leading to liver dysfunction, hepatitis, cirrhosis, and liver cancer [1, 2]. Arsenic toxicity can activate various pro-inflammatory signaling pathways, resulting in the production of inflammatory cytokines and chemokines, which exacerbate liver injury [1, 3]. Several investigations have shown that arsenic-induced hepatotoxicity is exacerbated by oxidative stress in the liver, which is produced by lipid peroxidation, DNA damage, and mitochondrial dysfunction [3].

Pentoxifylline (PTX) is a derivative of methylxanthine with significant hemorheological features. Initially marketed in the US for the treatment of intermittent claudication, PTX has since been found to have broad immunomodulatory effects [5]. Animal studies have demonstrated that PTX and its metabolites can enhance neutrophil migration while providing protection in models of gram-negative sepsis, meningitis, and peritonitis [6–8]. PTX has also been shown to modulate platelet and leukocyte hyperreactivity in acute respiratory distress syndrome models [7]. These effects are thought to be due, at least in part, to the suppression of tumor necrosis factor-a (TNF-α) production by monocytes as well as interleukin 1 stimulation of leukocytes (IL-1) and prevention of TNF-α secretion [6, 7]. The therapeutic benefits of PTX extend beyond treating intermittent claudication, including improved in experimental models of infection using animals like as meningitis, gram-negative sepsis, and peritonitis, as well as in hypercoagulable states in humans [7–10]. PTX is a non-selective phosphodiesterase inhibitor (PDE) that may modulate the production of nitric oxide (NO) as well as epidermal growth factor (EGF) [9, 10]. In animal models of ischemic injury, PTX has been shown to lower levels of TBARS, and reduce myeloperoxidase and malondialdehyde levels in gut tissue, although it does not appear to have significant effects on mucosal healing [11, 12].

PTX has been shown to have antioxidant properties, however, its therapeutic efficacy against ATO-induced oxidative damage to the kidneys and liver has not been established. The aim of this study is to evaluate the influence of PTX upon ATO-induced oxidative injury in the kidney and liver tissues of mice, based on our previous research on PTX, ATO, and oxidative stress.

Materials and Methods

In-vivo Experimental Protocol

Thirty-six male albino mice of the BALB/c strain, aged 8 weeks, were prepared with the weight range of 25 ± 2.5 g. The animals were fed a standard laboratory diet and water ad libitum, and were placed randomly in 6 groups in laboratory conditions for 1 week (relative humidity of 50 %; equal dark/light cycle, at 24 ± 2 °C degree) to adapt to environmental conditions. All animal experiments were performed in accordance with the guidelines of the University of Helsinki and the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Animal Ethics Committee of the Shiraz University of Medical Sciences, under code number I.R.SUMS.REC.1399.748.For 4 consecutive weeks, the mice were treated as follows:

First Group (control group): Intraperitoneal injection of normal saline (300 µl) for single week.

Second Group: Daily intraperitoneal injection of the given dose (5 mg.kg^{-1}) of arsenic trioxide for single week.

Third Group: Daily intraperitoneal injection of PTX (100 mg.kg) for single week.

Forth Group: Daily intraperitoneal injection of PTX (25 mg.kg) plus arsenic trioxide (5 mg/kg) for single week.

Fifth Group: Daily intraperitoneal injection of PTX(50 mg.kg) plus arsenic trioxide (5 mg.kg^{-1}) for single week.

Sixth Group: Daily intraperitoneal injection of PTX(100 mg/kg) + arsenic trioxide (100 mg.kg) for single week.

It is worth noting that pentoxifylline (Sigma-Aldrich, MO, USA) was administered in groups 4-6 one hour before administration of arsenic trioxide. The dose of arsenic trioxide (Merck Darmstadt, Germany) and pentoxifylline was determined in terms of pilot studies. For biochemical tests, the blood samples were taken 24 h after the last dose. The specimens were kept at − 20 ° C after the separation of the serums.

Briefly, 100 mg of liver and right kidney tissue was thoroughly homogenized in phosphate-buffered saline (Merck Darmstadt, Germany) (50 mM, pH 7.2) followed by centrifugation at 8000 g for 12 minutes at 4 °C. Finally, the supernatant was collected for the further biochemical tests. After laparotomy, a portion of the right kidney and liver were surgically collected and maintained at − 80 ° C [13, 14].

Histopathological Analysis

Prior to histopathological examination, the liver organ underwent a fixation process by submerging it in a formaldehyde solution with a concentration of 10 % for a minimum duration of 24 hours. The

use of an automatic tissue processor was employed to create a paraffin-embedded block, which was then sliced into 4-6 µm pieces using a rotating microtome [15]. Afterward, the tissue samples underwent staining using hematoxylin and eosin (H&E) dye (Sigma-Aldrich, MO, USA), followed by observation under a light microscope (Olympus CX31 microscope). After initial screening using a 40x power lens, at least 20 LPF (low power field, 100x) from each slide were examined in search of necrotic, inflammatory, hemorrhagic, and hepatical degenerative areas. By visually assessing the percentage of anomalous findings in every LPF, an approximate estimate was obtained, and the final result was derived by calculating the average of the observations across the various fields. In case of any uncertainty, the anomalous findings were verified using HPF (high power field, 400x) assessment. The percentage of histopathological changes was scored as 0 (0 %), 1 (1–25 %), 2 (26–50 %), 3 (51–75 %), and 4 (76–100 %).

Measuring the biochemical parameters

Creatinine (Cr), aspartate aminotransferase (AST), Blood urea nitrogen (Bun), Uric acid (UA) alkaline phosphatase (ALP), alanine aminotransferase (ALT), and were measured by calorimetric method (Pars Azmoon Co., Tehran, Iran) according to manufacture instructions [16, 17].

Total Antioxidant Capacity Assay

The liver and kidney homogenate supernatants were assessed for total antioxidant capacity (TAC) by determining the Fe^{3+}-TPTZ complex (Sigma-Aldrich, MO, USA) reduction to the Fe^{2+}-TPTZ complex at acidic pH using a reductant. To prepare the reagent, TPTZ (10 mM), acetate buffer (300 mM, pH 3.6), and $FeCl_3$ (20 mM) (Sigma-Aldrich, MO, USA) were combined in a ratio of 10:1:1 in 40 mM HCl (Merck Darmstadt, Germany). Subsequently, 200 µL of the reagent was mixed with 20 µL of the sample and incubated for 15 minutes. The peak absorbance of the Fe^{2+}-TPTZ complex was determined at a wavelength of 593 nm, using the standard curve as a reference. The outcomes were expressed as nmol/mg protein [18].

Determining the Total Thiol Group (TTGs)

The DTNB reagent (Sigma-Aldrich, MO, USA) was used to determine the total amount of thiol groups in the supernatants of tissue homogenates. In brief, 10 µl of the supernatant was mixed with 200 µl of a buffer solution containing Tris base (0.25 M) (Sigma-Aldrich, MO, USA) and EDTA (20 mM) (Merck Darmstadt, Germany) at pH 8.2 in a 96-well plate (Hangzhou A-gen Biotechnology, China), and the initial absorbance was recorded at 412 nm. Next, 10 µl of DTNB reagent (10 mmol/l in methanol) was added and the plate was incubated at 37 °C for 15 minutes. The final absorbance of both the supernatant samples (A2) and the DTNB blank (B) was measured again at 412 nm. The thiol levels (nmol/mg of protein) were determined using reduced glutathione as a standard [19].

Nitric Oxide analysis

The supernatant of tissue homogenates was assayed for nitric oxide (NO) using a Griess reagent (FroggaBio Inc, Canada) consisting of NED (0.1 %), phosphoric acid (2.5 %), and sulfanilamide (1 %), according to the procedure described by Nili-Ahmadabadi et al. The reagent was mixed with the supernatant in a 1:1 ratio and incubated at 37 °C for 15 min. The absorbance was measured at 520 nm using various concentrations of sodium nitrate solution as a standard. The outcomes were expressed as nmol/mg protein [15].

Antioxidant gene expression analysis by means of quantitative polymerase chain reaction

The extraction of total RNA from tissue samples was carried out by employing the RNX-plus reagent (Cinnagen-Iran) in accordance with the guidelines provided by the manufacturer. To synthesize complementary DNA (cDNA), the YTA cDNA Synthesis Kit (Yekta-Tajhiz, Iran) was utilized, following the instructions from the supplier. This involved using 0.5 µl of oligo (dT) primer and 1 µg of total RNA. The reverse transcription (RT) reactions were performed at a temperature of 42 °C for a duration of 50 minutes. Subsequently, PCR (polymerase chain reaction) was conducted using specific primers (▶ **Table 1**), with the Actin-beta gene (Act-b) serving as an internal control standard. The PCR conditions were optimized to determine the appropriate number of cycles required for detecting the product during the linear phase of mRNA transcripts. The reaction conditions consisted of an initial denaturation step at 95 °C for 15 minutes, followed by cycling at 95 °C for 10 seconds, annealing and extension at 56 °C for 1 minute, and a total of 45 cycles. The obtained results were normalized using the $2-\Delta\Delta CT$ method, with the expression levels of the genes in normal samples serving as a reference.

Statistical Analysis

The GraphPad Prism software V6.0 was applied to analyze the data (mean ± SEM). One-way analysis of variance (ANOVA) was recruited to compare the statistical differences between the values. The post hoc Tukey test was performed on quantitative variables (P 0.05).

▶ **Table 1** The primers used for related antioxidant gene expression analysis via Quantitative technique.

Gene name	Forward Primer: 5′ → 3′	Reverse Primer: 5′ → 3′
SOD	CGGATGAAGAGAGGCATGTT	TGTACGGCCAATGATGGAAT
CAT	AGCTGACACAGTTCGTGAC	ATGGCATCCCTGATGAAGAAA
GPx	TCCACCGTGTATGCCTTCT	CATTCTCAATGAGCAGCACCT
GSR	CTTGCGTGAACGTTGGATG	GTGATCATGTATGAATTCCGAGTG
MPO	GGCACGGTGCTGAAGAA	CATCCAGATGTCAATGTTGTTGG
Act-b	CTGAGGAGCACCCTGTG	GGGTGTTGAAGGTCTCAAA

Results

Biochemical analysis

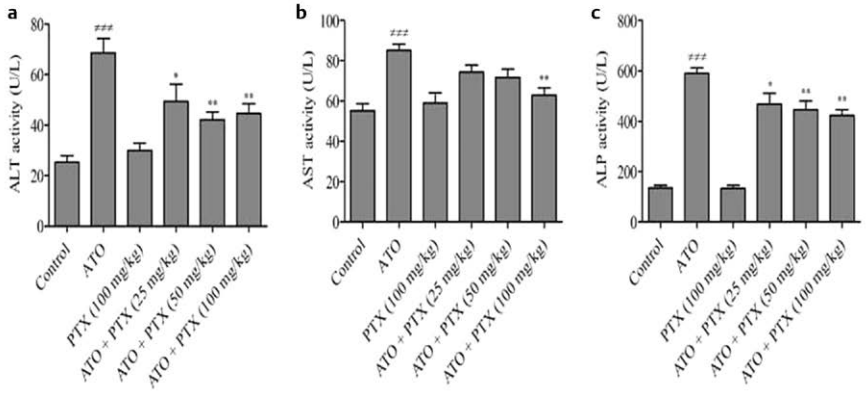
Serum levels of liver function tests (LFTs) including aspartate aminotransferase (AST), blood urea nitrogen (BUN), uric acid (UA), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and creatinine were measured. The results presented in ▶ **Fig. 1** and ▶ **2** demonstrate a substantial rise in the levels of creatinine, AST, BUN, ALP, and ALT in the liver serum following the administration of ATO, in comparison to the control group. These increases were found to be statistically significant ($P < 0.05$, $P < 0.001$, $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively). PTX administration at doses of 100, 50, and 25 mg/kg was capable to decrease the elevated levels of ALP and ALT ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively) as well as AST serum levels at the dosage of 100 mg/kg ($P < 0.01$). Nevertheless, the administration of PTX did not result in any significant alterations in the serum levels of creatinine and BUN. Furthermore, the treatment groups did not exhibit any significant changes in the levels of UA in the serum, as illustrated in ▶ **Fig. 1** and ▶ **2**.

Renal Oxidative Stress Biomarkers

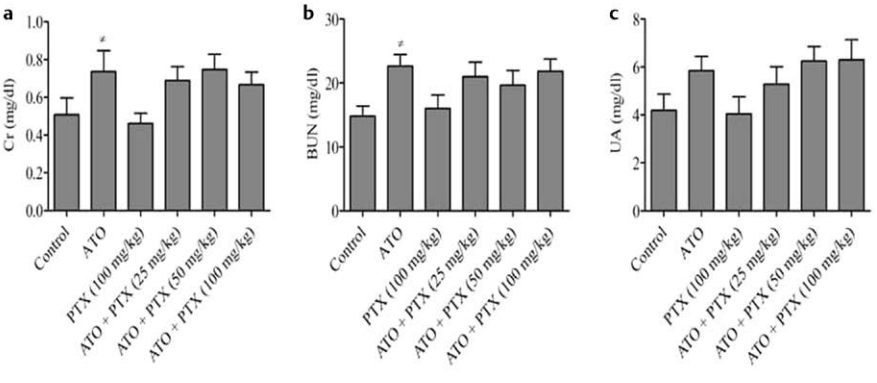
After the administration of ATO, the levels of lipid peroxidation (LPO) in renal tissue increased ($P < 0:001$), while the levels of total antioxidant capacity (TAC) ($P < 0:01$) and total thiol groups (TTGs) ($P < 0:01$) decreased significantly when compared to the control group. However, PTX treatment at a dosage of 100 mg/kg significantly reduced renal lipid peroxidation ($P < 0:05$), while no significant changes were observed in renal TTGs and TAC levels in the treatment groups when compared to the ATO group (▶ **Fig. 3**).

Liver Oxidative Stress Biomarkers

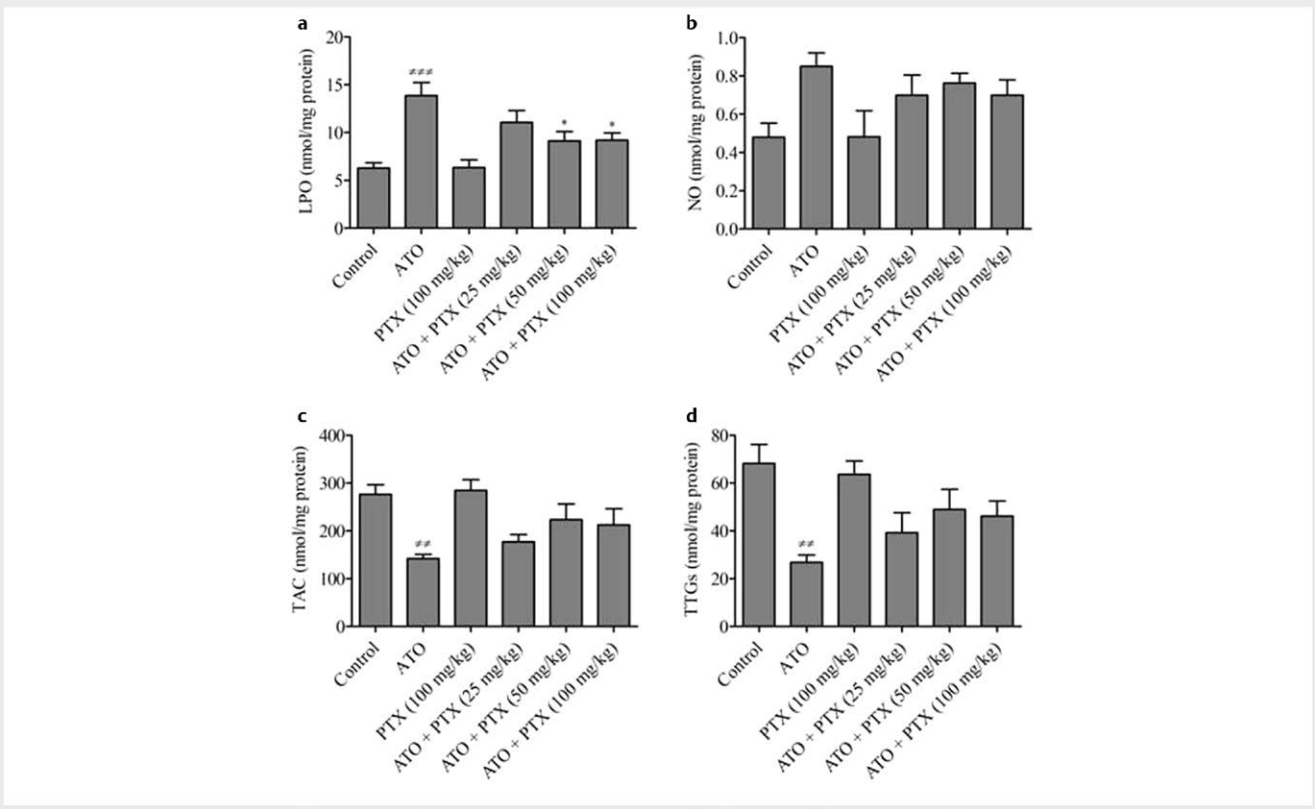
After administration of ATO, the levels of liver tissue NO ($P < 0:01$) and LPO ($P < 0:001$) increased, while the levels of TTGs ($P < 0:001$) and TAC ($P < 0:001$) decreased significantly when compared to the control group. However, when PTX was administered, it was observed that the liver tissue lipid peroxidation decreased significantly at dosages of 50 mg/kg ($P < 0.01$) and 100 mg/kg ($P < 0.05$). Additionally, PTX at doses of 50 mg/kg ($P < 0.01$) and 100 mg/kg ($P < 0.05$) exhibited significant improvement in the TTGs of liver tissue in comparison to the ATO group. Moreover, PTX at doses of



▶ **Fig. 1** Pentoxifylline's Effect on Hepatic Markers in ATO-Exposed Mice.



▶ **Fig. 2** The study employed a statistical analysis technique known as one-way ANOVA, which was followed by Tukey's test. The outcomes of the analysis are reported as means accompanied by standard error of the mean (SEM). The sample size for each group was six. The "≠" symbol was used to indicate significant differences at a level of $P < 0.05$ when compared to the control group. PTX: pentoxifylline; Cr: Creatinine; BUN: Blood urea nitrogen; ATO: arsenic trioxide (equal 5 mg/kg); UA: Uric acid.



▶ **Fig. 3** The impact of PTX on renal oxidative damage in mice exposed to ATO. The study employed a statistical analysis technique known as one-way ANOVA with Tukey's test. The results were reported as means accompanied by standard error of the mean (SEM). The size of the sample for each group was six. The statistical analysis revealed significant dissimilarities, as indicated by the"≠≠≠" symbol for P < 0.001and "≠≠" symbol for P < 0.01 in relation to the control group, and by the "*" symbol for P < 0.05 in comparison to the ATO group. LPO: lipid peroxidation; TAC: total antioxidant capacity; NO: nitric oxide; PTX: pentoxifylline; TTGs: total thiol groups; ATO: arsenic trioxide (equal 5 mg/kg).

50 mg/kg (P < 0.01) and 100 mg/kg (P < 0.001) displayed a significant enhancement in the TAC of liver tissue in comparison to the ATO group, as depicted in ▶ **Fig. 4**.

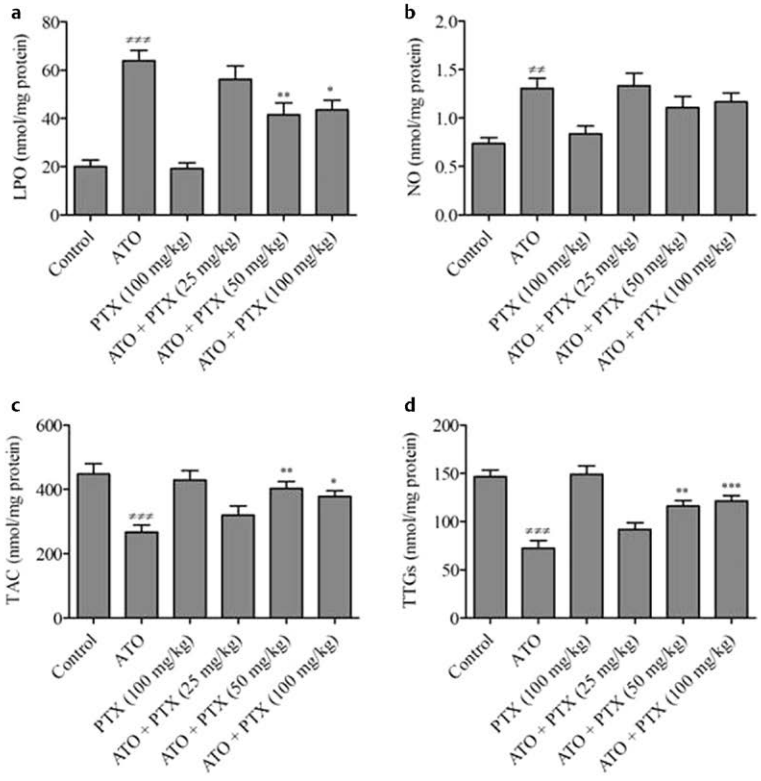
Antioxidant expression analysis

The study aimed to examine the abundance and distribution of antioxidant components in the liver and kidneys. To achieve this, we conducted an analysis of the expression levels of distinct mRNA markers, namely SOD, MPO, GPX, GSR, and CAT. The present study involved the examination of multiple groups, including Control, N/S, A, P25 + A, P50 + A, P100 + A, and P100. The findings of our study indicated that the group identified as N/S demonstrated the most significant levels of antioxidant factors, whereas the A group exhibited the least significant levels. Upon administration of pentoxifylline, particularly in the P25 + A, P50 + A, P100 + A, and P100 groups, a significant elevation in the levels of antioxidant factors was detected (p < 0.05). This augmentation was found to be more prominent as the concentration of pentoxifylline escalated. The study conducted a comparative analysis of all groups with respect to the control group (p < 0.05), as illustrated in ▶ **Fig. 5** and ▶ **6**.

Effects of ATO on Liver Histopathology

The aim of this research was to explore the potential protective properties of PTX against liver injury caused by arsenic trioxide (ATO) via the examination of pathological alterations in the liver. The results, depicted in ▶ **Fig. 7**, demonstrated that the livers of mice in the normal group and PTX single group exhibited normal lobular architecture and cell distribution. However, the livers of mice treated with ATO exhibited significant pathological changes, including vast necrosis, inflammatory cell infiltrations, and hemorrhage. On the other hand, mice that received pre-treatment with various dosages of PTX (25 mg, 50 mg, and 100 mg) showed varying degrees of improvement in comparison to the arsenic-induced hepatotoxicity group. Specifically, the treated groups exhibited varying degrees of necrosis and inflammatory cell infiltrations. Notably, the 100 mg PTX-treated group (I and J) showed almost normal architecture and cellular order, signifying the protective impact of PTX against ATO-induced liver impairment.

The study employed a statistical analysis technique known as one-way analysis of variance (ANOVA) in conjunction with Tukey's test. The statistical information is displayed in the form of means accompanied by standard error of the mean (SEM). The sample size for each group was six. The statistical analysis revealed notable dissimilarities, as evidenced by the symbols used. Specifically, three "≠" symbols were employed to indicate a statistically significant difference at P < 0.001 when compared to the control group, while one or two "*" symbols were used to denote P < 0.05 and P < 0.01, respectively, when compared to the ATO group. The study exam-



▶ **Fig. 4** The impact of PTX on hepatic oxidative damage in mice exposed to ATO. The data was subjected to statistical analysis using the one-way ANOVA with Tukey's test. The results are reported in the form of means with standard error of the mean (SEM) and a sample size of six for each group. The symbols "≠≠≠P<0.001" and "≠≠P<0.01" are employed to denote statistically significant differences in comparison to the control group, whereas the symbol "*P<0.05" is indicative of a statistically significant difference in comparison to the ATO group. LPO: lipid peroxidation; TAC: total antioxidant capacity; NO: nitric oxide; PTX: pentoxifylline; TTGs: total thiol groups; ATO: arsenic trioxide (equal 5 mg/kg).

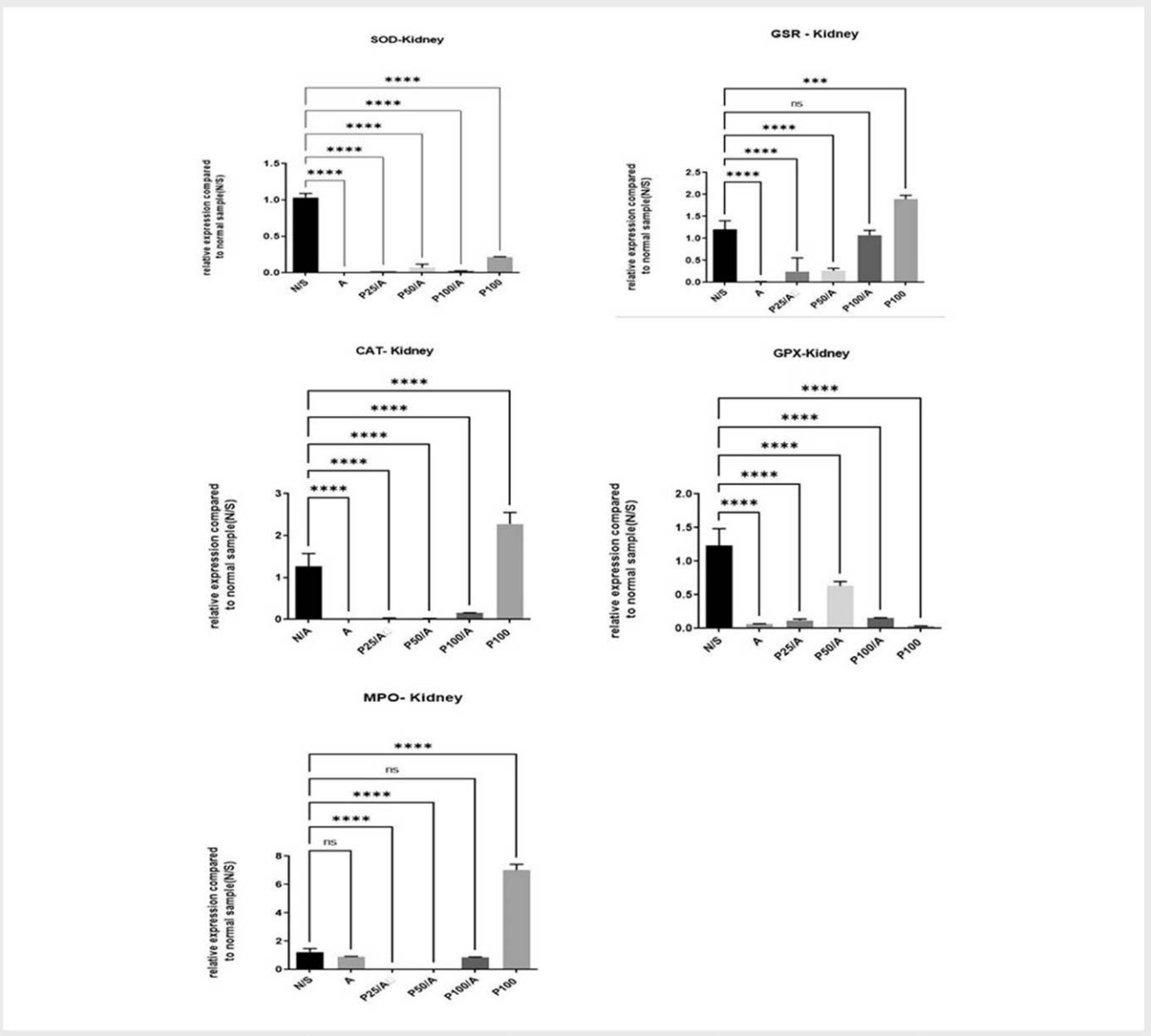
ined hepatic markers, specifically ALT (alanine aminotransferase), ALP (alkaline phosphatase), and AST (aspartate aminotransferase). The research conducted a comparative analysis of the impacts of arsenic trioxide (ATO) and pentoxifylline (PTX) at a dosage of 5 mg/kg for each treatment.

Discussion

Currently, the use of Arsenic in medicine has gained widespread recognition. However, it is important to be cautious about the potential harm that Arsenic and its derivatives including ATO can cause impairment to the liver and kidney. In this regard, various drugs including Pentoxifylline, shows significant anti-inflammatory impacts and anti-oxidative stress in various kidney and liver disorders. The objective of the current research was to examine the effects of pentoxifylline (PTX) on the development of acute kidney injury and liver damage caused by arsenic trioxide in mice. Furthermore, the findings demonstrated the correlation between the therapeutic ability of PTX against oxidative damage in the kidney and liver caused by ATO and its antioxidant properties.

Pentoxifylline (PTX) is a pharmacological agent derived from methylxanthine that has been employed for the treatment of diverse pathologies, such as peripheral arterial disease and inflammatory disorders. In recent years, the investigation of PTX's potential role in the prevention and treatment of acute kidney injury (AKI) and liver injury has been conducted. Several studies have shown that PTX can attenuate the development of AKI and liver injury induced by various insults, including sepsis, ischemia-reperfusion injury, and toxic agents. The protective properties of PTX are attributed to its ability to modulate the inflammatory reaction, improve microcirculatory function, and inhibit apoptosis. For instance, Zhu et al. (2020) investigated the effect of PTX on rats with sepsis-induced AKI. According to the study, the administration of PTX resulted in a significant enhancement of renal function, a reduction in inflammation, and a decrease in oxidative stress markers [20]. Similarly, Salam et al. (2005) demonstrated in their study that administration of PTX provided protection against liver injury resulting from an overdose of acetaminophen in rats [21]. These findings indicated that PTX exhibits favorable therapeutic prospects in the treatment of AKI and liver damage, possibly attributable to its anti-inflammatory and antioxidant characteristics.

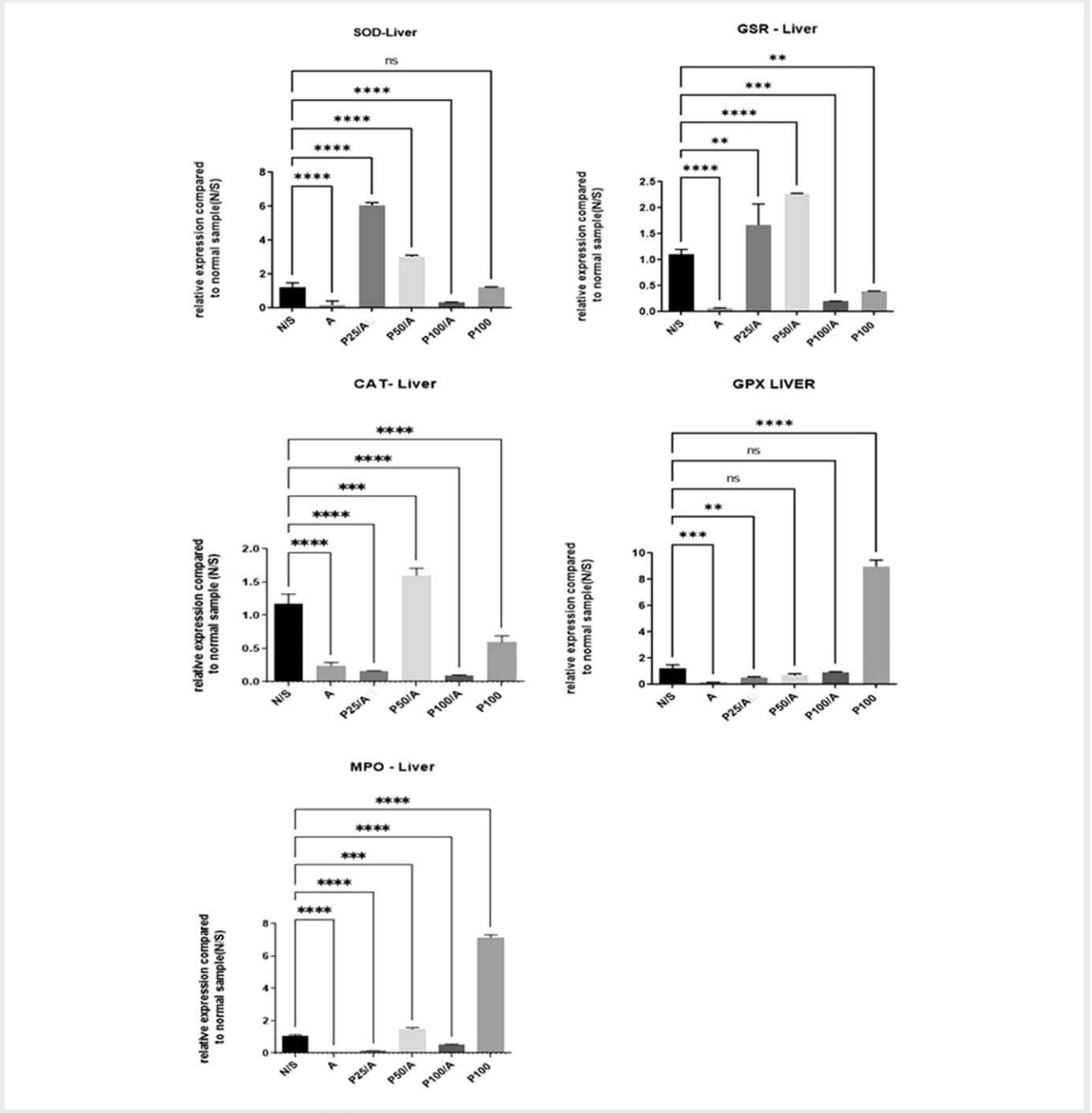
Arsenic is mainly metabolized in the liver, where it is transformed through biotransformation in response to thiol groups found in proteins or enzymes. This process can lead to the disruption of the hepatic plasma membrane's integrity, causing the release of enzymes like as alanine transaminase (ALT) and aspartate



▶ **Fig. 5** Kidney gene expression of SOD, GPX, GSR, MPO and CAT antioxidant factors. The elevation of antioxidant factors was found to be significant ($p < 0.05$) in groups exposed to arsenic upon an increase in the concentration of pentoxifylline. Statistical analysis was performed by comparing all groups by the normal group ($p > 0.05$).

transaminase (AST) into the bloodstream [22]. Elevated serum levels of these enzymes are typically indicative of liver inflammation or injury [22]. Our study demonstrated that the administration of ATO significantly increased serum levels of ALT, ALP, and AST in contrary to the normal group, indicating that ATO caused liver damage in mice. Nonetheless, administration of PTX at dosages of 100, 50, and 25 mg/kg exhibited significant reduction in elevated levels of ALT, ALP, and AST, which are corroborating with prior research studies [23, 24].

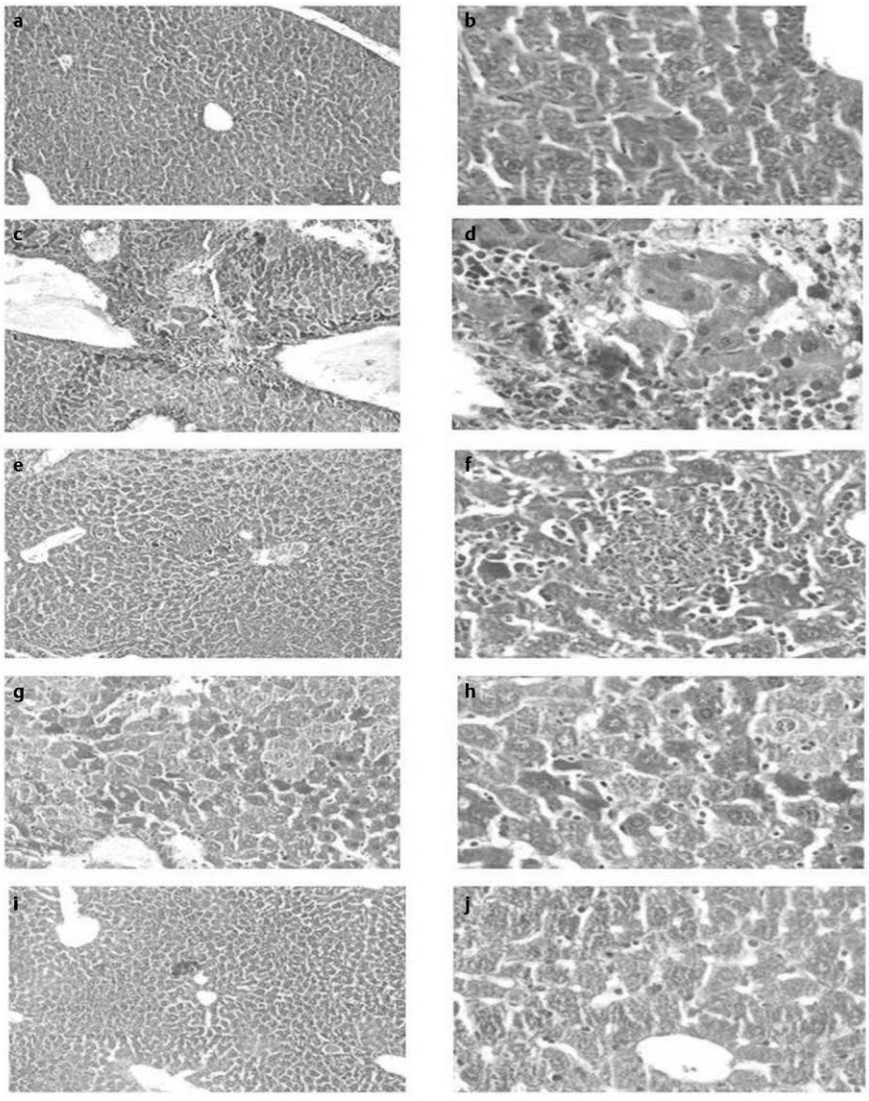
Arsenate, being a chemical analog of phosphate, can serve as a substitute for phosphate in various metabolic pathways, such as glycolysis and cellular respiration [25]. Conversely, arsenite has the ability to interact with sulfhydryl groups in proteins, disrupting glucose metabolism [26]. This interference can impact several biochemical processes including oxidation of fatty acid, glucose uptake, gluconeogenesis, and pyruvate dehydrogenase activity, ultimately leading to reduced cellular ATP production and citric acid cycle activity [27]. Furthermore, methylated arsenic compounds can cause DNA damage through multiple mechanisms, including chromosomal aberrations, DNA hypo- and hyper-methylation, and exchange of sister chromatids [27]. In the kidney, ATP depletion, mitochondrial membrane depolarization, and endothelial cell apoptosis can damage the glomerulus membrane, leading to histopathological changes in the liver and increased BUN and creatinine levels [28, 29]. As a major excretory organ with a high perfusion rate, the kidney is particularly vulnerable to the damaging effects



▶ **Fig. 6** Liver gene expression of SOD, GPX, GSR, MPO and CAT antioxidant factors. The elevation of antioxidant factors was found to be significant ($p < 0.05$) in groups exposed to arsenic upon an increase in the concentration of pentoxifylline. Statistical analysis was performed by comparing all groups by the normal group ($p > 0.05$).

of arsenic accumulation in renal tubular cells [30]. Our study revealed a considerable rise in serum levels of creatinine and BUN but not Uric acid (UA) following exposure to arsenic, suggesting renal damage. It should be noted that although PTX showed a protective effect against ATO-induced renal and hepatic damage, no considerable changes were noted in creatinine and BUN serum levels, indicating that PTX may not have a significant effect on kidney function. Moreover, UA serum levels did not change significantly in the treatment groups, suggesting that PTX does not affect uric acid metabolism.

The histopathological evaluation of liver tissue showed a variety of structural abnormalities following exposure to arsenic. Specifically, the observed histological changes in the liver, including vast necrosis, inflammatory cell infiltrations, and hemorrhage [31, 32], are in agreement with previous studies. Our findings suggest that PTX has the potential to ameliorate the detrimental ef-



▶ **Fig. 7** **a**) Negative control group. Power 100X H & E staining. Normal lobular and vascular architecture is seen. **b**) Negative control group. Power 400X H & E staining. Normal lobular and vascular architecture is seen. **c**) Arsenic induced hepatotoxicity group. Power 100X H & E staining. Vast Necrosis, inflammatory cell infiltrations as well as hemorrhage are evident. **d**) Arsenic induced hepatotoxicity group. Power 400X H & E staining. Vast Necrosis, inflammatory cell infiltrations and hemorrhage are evident. **e**) Treated group 25 mg pentoxifylline. Power 100X H & E staining. Inflammatory cell infiltrations and necrosis is still evident. **f**) Treated group 25 mg pentoxifylline. Power 400X H & E staining. Inflammatory cell infiltrations and necrosis is still evident. **g**) Treated group 50 mg pentoxifylline. Power 100X H & E staining. Inflammatory cell infiltrations and necrosis is still evident. **h**) Treated group 50 mg pentoxifylline. Power 400X H & E staining. Inflammatory cell infiltrations and necrosis is less evident. **i**) Treated group 100 mg pentoxifylline. Power 100X H & E staining. Treated group 100 mg pentoxifylline. Power 100X H & E staining.

fects of ATO on liver tissue. This observation aligns with the previous studies that highlighted the defensive properties of PTX in mitigating liver damage induced by oxidative stress [33–35].

Oxidative stress develops when there is a discrepancy between the rate at which reactive oxygen species (ROS) are produced and accumulated inside cells and tissues and the capacity of the biological system to clear the body of these harmful by-products. This inequity may also arise from discordance between the reactive oxygen/nitrogen species (ROS/RNS) and antioxidant systems responsible for cellular injury. Such a situation causes oxidative stress, which can ultimately lead to cellular damage and potential health complications. According to Sun et al. (2016) and Manna et al. (2008), various cells generate ROS/RNS amid the metabolism of inorganic arsenic [36, 37]. Our study found that ATO treatment resulted in increased production of LPO and NO in the kidney and liver, while levels of TTG and TAC were decreased. These findings aligned with previous studies by Wei et al. (2022) and Charles (2014), suggesting that ATO induces oxidative stress and impairs antioxidant defenses in the heart [38, 39]. In general, LPO is a distinguishing characteristic of Arsenic Toxicity in relation to oxidative stress. This phenomenon arises as a result of the oxidative degradation of polyunsaturated fatty acids within the cellular membrane

[40]. Arsenic exposure results in the release of iron from ferritin molecules, thus escalating the level of free iron inside the cell. The excess of free iron initiates a Fenton reaction, resulting in the excessive generation of Reactive Oxygen Species (ROS) and thereby exacerbating the degree of lipid peroxidation [41]. PTX has exhibited its ability to decrease the levels of LPO in the renal and liver tissue, potentially by scavenging ROS and enhancing the function of the antioxidant system within the tissue. The ability of PTX to hinder neutrophil activation can be associated with its antioxidant characteristics, given that activated neutrophils have the potential to generate superoxide radicals through NADPH oxidase [42, 43]. Furthermore, it is highly probable that PTX, a potent inhibitor of superoxide anion generation, exerts an influence on the onset and/or progression of lipid peroxidation (LPO) [44]. Moreover, this drug has the ability to decrease the formation of superoxide and hydroxyl radicals via impeding xanthine oxidase [45].

Nitric oxide (NO) is a pivotal signaling molecule that performs a critical function in the regulation of diverse cellular processes. However, the multifaceted functions of NO can result in nitrosative stress when produced excessively [46]. The administration of ATO may increase NO levels by activation of nitric oxide synthase, which is consistent with the reported outcomes by Kesavan et al. in 2014 [47]. In this context, the peroxynitrite radicals are generated as a result of the reaction between superoxide anion and NO that further exacerbate cellular damage by nitrating tyrosine residues on tissue proteins, leading to lipid peroxidation, necrosis, and apoptosis [48]. The impacts of PTX on NO production have been examined in numerous studies, and conflicting results have been reported. While some studies have suggested that PTX may induce NO production, others have demonstrated its ability to reduce the synthesis of NO. For instance, Beshay and colleagues showed that PTX inhibited the enzyme that produces nitric oxide in immune cells, and its effect was related to the amount of cAMP in the cells [47]. In contrast, Hoebe et al. reported that PTX potentiated NO production [49]. In this current research, we evaluated the influences of PTX on nitrosative stress provoked by ATO by measuring cardiac NO levels. Our findings indicate that PTX does not cause any reduction in the nitric oxide stress induced by ATO, as demonstrated by the evaluation of NO levels in the kidney and liver.

Antioxidant enzymes are essential components of the cellular defense system against free radicals that cause significant damage to cells. These enzymes include catalase (CAT), glutathione reductase (GSR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [50]. SOD helps to transform superoxide radicals into H_2O_2 and O_2, while CAT breaks down H_2O_2 into O_2 and H_2O. These enzymes play a critical function in defending cells from oxidative damage by free radicals. Moreover, GPx and GSR are also important antioxidant enzymes that have an essential function in safeguarding cells against oxidative injury. GPx helps to convert H_2O_2 and organic peroxides into H_2O and alcohols, respectively, using GSH as a donor of electrons. GSR, on the other hand, is responsible for regenerating reduced glutathione (GSH) from its oxidized form (GSSG) [51, 52]. Our study revealed differential gene expression profiles of antioxidant enzyme transcripts in response to the administration of ATO, as well as in the follow-up treatment with varying concentrations of PTX. In this context, previous studies have supported our findings, as our study demonstrates a significant decrease in the levels of GPx, SOD, CAT, GSR, and MPO in hepatic and renal tissues of mice treated with ATO, in contrast to the values seen in normal mice [53–55]. In contrast, the administration of PTX resulted in a significant upregulation of antioxidant enzyme gene expression, indicating its potential role in reducing ATO-induced oxidative stress in hepatic and renal tissues. These findings are consistent with previous reported studies [35, 56, 57]. In this regard, the antioxidant enzyme functions, including SOD, GSR, CAT, MPO, and GPx, were significantly increased in the hepatic and renal tissue of ATO-intoxicated mice following treatment with PTX. This rise in function may be due to the suppression of superoxide radical production and subsequent enhancement of the balance between oxidants and antioxidants [58].

Conclusion

The present study provides evidence that the administration of arsenic trioxide (ATO) to mice results in significant damage to the renal and hepatic tissues, characterize by broad necrosis, infiltration of inflammatory cell, and hemorrhage. However, intraperitoneal administration of pentoxifylline (PTX) can mitigate the nephrotoxicity and hepatotoxicity induced by ATO exposure and alleviates the resulting tissue damage. PTX administration enhances the antioxidant protection systems by upregulating the expression of key antioxidant enzymes, comprising catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), myeloperoxidase (MPO), and glutathione reductase (GSR). These findings suggest that PTX could be considered a valuable medicine that exhibits protective potential against ATO-induced nephrotoxicity and hepatotoxicity in renal and liver tissues.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Shiraz University of Medical Sciences, under code number I.R.SUMS.REC.1399.748.

Acknowledgment

This work was supported by the Research Council of Shiraz University of Medical Sciences, Shiraz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Hu H, Chen E, Li Y et al. Effects of arsenic trioxide on INF-gamma gene expression in MRL/lpr mice and human lupus. Biological trace element research 2018; 184: 391–397

[2] Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicology letters 2002; 133: 1–16

[3] Antman KH. Introduction: the history of arsenic trioxide in cancer therapy. The oncologist 2001; 6: 1–2

[4] Saxena PN, Anand S, Saxena N et al. Effect of arsenic trioxide on renal functions and its modulation by Curcuma aromatica leaf extract in albino rat. Journal of environmental biology 2009; 30; 527–531

[5] Samlaska CP, Winfield EA. Pentoxifylline. Journal of the American Academy of Dermatology 1994; 30: 603–621

[6] Mandell G, Novick W. Pentoxifylline and leukocyte function: foreword and overview. Pentoxifylline and leukocyte function. Somerville, NJ Hoechst-Roussel Pharmaceuticals; 1988

[7] Hakim J, Mandell G, Movick W. Pentoxifylline and analogues: effects on leukocyte function. Karger; Basel: 1990

[8] Ward A, Clissold SP. Pentoxifylline. Drugs 1987; 34: 50–97

[9] Abdollahi M, Simaiee B. Stimulation by theophylline and sildenafil of rat submandibular secretion of protein, epidermal growth factor and flow rate. Pharmacological research 2003; 48: 445–449

[10] Samardzic T, Stosic-Grujicic S, Maksimovic D et al. Differential regulation of nitric oxide production by increase of intracellular cAMP in murine primary fibroblasts and L929 fibrosarcoma cell line. Immunology Letters 2000; 71: 149–155

[11] Savaş Ç, Aras T, Çakmak M et al. Pentoxifylline inhibits overflow and reduces intestinal reperfusion injury. Journal of pediatric surgery 1997; 32: 905–910

[12] Hammerman C, Goldschmidt D, Caplan MS et al. Amelioration of ischemia-reperfusion injury in rat intestine by pentoxifylline-mediated inhibition of xanthine oxidase. Journal of pediatric gastroenterology and nutrition 1999; 29: 69–74

[13] Gheitasi I, Azizi A, Omidifar N et al. Renoprotective effects of Origanum majorana methanolic L and carvacrol on ischemia/reperfusion-induced kidney injury in male rats. Evidence-Based Complementary and Alternative Medicine 2020. doi 10.1155/2020/9785932

[14] Ansari S, Azarmehr N, Barmoudeh Z et al. Evaluation of the protective potential of hydroalcoholic extract of Thymus daenensis on acetaminophen-induced nephrotoxicity in rats. Heliyon 2020; 6: e03898

[15] Nili-Ahmadabadi A, Alibolandi P, Ranjbar A et al. Thymoquinone attenuates hepatotoxicity and oxidative damage caused by diazinon: an in vivo study. Res Pharm Sci 2018; 13: 500–508

[16] Sadeghi A, Bastin AR, Ghahremani H et al. The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Molecular Biology Reports 2020; 47: 3557–3566

[17] Bastin AR, Sadeghi A, Abolhassani M et al. Malvidin prevents lipopolysaccharide-induced oxidative stress and inflammation in human peripheral blood mononuclear cells. Iubmb Life 2020; 72: 1504–1514

[18] Doustimotlagh AH, Taheri S, Mansourian M et al. Extraction and identification of two flavonoids in phlomoides hyoscyamoides as an endemic plant of iran: the role of quercetin in the activation of the glutathione peroxidase, the improvement of the hydroxyproline and protein oxidation in bile duct-ligated rats. Current computer-aided drug design 2020; 16: 629–640

[19] Doustimotlagh AH, Kokhdan EP, Vakilpour H et al. Protective effect of Nasturtium officinale R. Br and quercetin against cyclophosphamide-induced hepatotoxicity in rats. Molecular biology reports 2020; 47: 5001–5012

[20] Alorabi M, Cavalu S, Al-Kuraishy HM et al. Pentoxifylline and berberine mitigate diclofenac-induced acute nephrotoxicity in male rats via modulation of inflammation and oxidative stress. Biomedicine & Pharmacotherapy 2022; 152: 113225

[21] Salam O, Baiuomy AR, El-Shenawy SM et al. Effect of pentoxifylline on hepatic injury caused in the rat by the administration of carbon tetrachloride or acetaminophen. Pharmacol Rep 2005; 57: 596–603

[22] Parker R, Armstrong M, Corbett C et al. Systematic review: pentoxifylline for the treatment of severe alcoholic hepatitis. Alimentary pharmacology & therapeutics 2013; 37: 845–854

[23] Das N, Paul S, Chatterjee D et al. Arsenic exposure through drinking water increases the risk of liver and cardiovascular diseases in the population of West Bengal, India. BMC public health 2012; 12: 1–9

[24] Barai M, Ahsan N, Paul N et al. Amelioration of arsenic-induced toxic effects in mice by dietary supplementation of Syzygium cumini leaf extract. Nagoya journal of medical science 2017; 79: 167–177

[25] Kulshrestha A, Jarouliya U, Prasad G et al. Arsenic-induced abnormalities in glucose metabolism: Bio- chemical basis and potential therapeutic and nutritional interventions. World Journal of Translational Medicine 2014; 3: 96–111

[26] Brazy P, Balaban R, Gullans S et al. Inhibition of Renal Metabolism. Relative effects of arsenate on sodium, phosphate, and glucose transport by the rabbit proximal tubule. The Journal of Clinical Investigation 1980; 66: 1211–1221

[27] Bergquist ER, Fischer RJ, Sugden KD et al. Inhibition by methylated organoarsenicals of the respiratory 2-oxo-acid dehydrogenases. Journal of organometallic chemistry 2009; 694: 973–980

[28] Schwerdtle T, Walter I, Mackiw I et al. Induction of oxidative DNA damage by arsenite and its trivalent and pentavalent methylated metabolites in cultured human cells and isolated DNA. Carcinogenesis 2003; 24: 967–974

[29] Delnomdedieu M, Basti MM, Otvos JD et al. Reduction and binding of arsenate and dimethylarsinate by glutathione: a magnetic resonance study. Chemico-biological interactions 1994; 90: 139–155

[30] Verma R, Vasu A, Saiyed AA. Arsenic toxicity in mice and its possible amelioration. Journal of Environmental Sciences 2004; 16: 447–453

[31] Mershiba SD, Dassprakash MV, Saraswathy SD. Protective effect of naringenin on hepatic and renal dysfunction and oxidative stress in arsenic intoxicated rats. Molecular biology reports 2013; 40: 3681–3691

[32] Zhang Z, Gao L, Cheng Y et al. Resveratrol, a natural antioxidant, has a protective effect on liver injury induced by inorganic arsenic exposure. BioMed Research International 2014; 2014: 1–7

[33] Luo M, Dong L, Li J et al. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. International journal of clinical and experimental pathology 2015; 8: 8990–8996

[34] Armagan I, Bayram D, Candan IA et al. Effects of pentoxifylline and alpha lipoic acid on methotrexate-induced damage in liver and kidney of rats. Environmental toxicology and pharmacology 2015; 39: 1122–1131

[35] Movassaghi S, Sharifi ZN, Mohammadzadeh F et al. Pentoxifylline protects the rat liver against fibrosis and apoptosis induced by acute administration of 3, 4-methylenedioxymethamphetamine (MDMA or ecstasy). Iranian journal of basic medical sciences 2013; 16: 922–927

[36] Manna P, Sinha M, Sil PC. Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Archives of toxicology 2008; 82: 137–149

[37] Sun T-L, Liu Z, Qi Z-J et al. (-)-Epigallocatechin-3-gallate (EGCG) attenuates arsenic-induced cardiotoxicity in rats. Food and Chemical Toxicology 2016; 93: 102–110

[38] Wei Z, Sun X, He Q et al. Nephroprotective effect of magnesium isoglycyrrhizinate against arsenic trioxide-induced acute kidney damage in mice. Experimental and Therapeutic Medicine 2022; 23: 1–11

[39] Charles CA. Effect of arsenic trioxide poisoning on hematological parameters, liver marker enzymes and kidney of male albino rats. pinnacle biological sciences 2014; 2: 1–4

[40] Kucukkurt I, Ince S, Demirel HH et al. The Effects of Boron on Arsenic-Induced Lipid Peroxidation and Antioxidant Status in Male and Female Rats. Journal of Biochemical and Molecular Toxicology 2015; 29: 564–571

[41] Mathews V, Paul M, Abhilash M et al. Myocardial toxicity of acute promyelocytic leukaemia drug-arsenic trioxide. Eur Rev Med Pharmacol Sci 2013; 17: 34–38

[42] Dávila-Esqueda M, Martinez-Morales F. Pentoxifylline diminishes the oxidative damage to renal tissue induced by streptozotocin in the rat. Experimental diabesity research 2004; 5: 245–251

[43] Karimia J, Ranjbarb A, Tavilania H. Hepatoprotective effects of pentoxifylline against acrolein-induced mitochondrial dysfunction in rat liver. Journal of Reports in Pharmaceutical Sciences 2014; 3: 19–27

[44] Gavella M, Lipovac V. Effect of pentoxifylline on experimentally induced lipid peroxidation in human spermatozoa. International journal of andrology 1994; 17: 308–313

[45] Eğin S, Açıksarı K, Ercan G et al. Effects of pentoxifylline on oxidative stress in rats with abdominal compartment syndrome model. International Journal of Surgery Open 2016; 5: 5–10

[46] Neri M, Riezzo I, Pomara C et al. Oxidative-nitrosative stress and myocardial dysfunctions in sepsis: evidence from the literature and postmortem observations. Mediators of Inflammation 2016; 2016: 1–12

[47] Kesavan M, Sarath TS, Kannan K et al. Atorvastatin restores arsenic-induced vascular dysfunction in rats: modulation of nitric oxide signaling and inflammatory mediators. Toxicology and applied pharmacology 2014; 280: 107–116

[48] Bektas S, Karakaya K, Can M et al. The effects of tadalafil and pentoxifylline on apoptosis and nitric oxide synthase in liver ischemia/reperfusion injury. The Kaohsiung Journal of Medical Sciences 2016; 32: 339–347

[49] Hoebe KH, Gonzalez-Ramon N, Nijmeijer SM et al. Differential effects of pentoxifylline on the hepatic inflammatory response in porcine liver cell cultures: Increase in inducible nitric oxide synthase expression. Biochemical pharmacology 2001; 61: 1137–1144

[50] Li S, Tan H-Y, Wang N et al. The role of oxidative stress and antioxidants in liver diseases. International journal of molecular sciences 2015; 16: 26087–26124

[51] Han K-H, Hashimoto N, Fukushima M. Relationships among alcoholic liver disease, antioxidants, and antioxidant enzymes. World Journal of Gastroenterology 2016; 22: 37–49

[52] Huchzermeyer B, Menghani E, Khardia P et al. Metabolic pathway of natural antioxidants, antioxidant enzymes and ROS providence. Antioxidants 2022; 11: 761

[53] Baş H, Kalender Y. Nephrotoxic effects of lead nitrate exposure in diabetic and nondiabetic rats: involvement of oxidative stress and the protective role of sodium selenite. Environmental Toxicology 2016; 31: 1229–1240

[54] Liu B, Zhang H, Tan X et al. GSPE reduces lead-induced oxidative stress by activating the Nrf2 pathway and suppressing miR153 and GSK-3β in rat kidney. Oncotarget 2017; 8: 42226–42237

[55] Apaydın FG, Baş H, Kalender S et al. Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats. Environmental toxicology and pharmacology 2016; 41: 219–224

[56] Xu D, Zhao W, Feng Y et al. Pentoxifylline attenuates nonalcoholic fatty liver by inhibiting hepatic macrophage polarization to the M1 phenotype. Phytomedicine 2022; 106: 154368

[57] Tanideh R, Farshad O, Jamshidzadeh A et al. Effects of Pentoxifylline in a Rat Model of Manganism: Evaluation of the Possible Toxicity. Journal of Chemistry 2021; 2021: 1–9

[58] Gholami A, Ataei S, Ahmadimoghaddam D et al. Pentoxifylline attenuates arsenic trioxide-induced cardiac oxidative damage in mice. Oxidative Medicine and Cellular Longevity 2021; 2021: 1–10