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Effect of exposure to fluoride and acetaminophen on oxidative/nitrosative status of liver and kidney in male and female rats

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Abstract:
Background: This study was undertaken to investigate, the effect of 6 weeks treatment with acetaminophen (AAP) and fluoride (F), administered either separately or together, on nitric oxide generation, lipid and protein peroxidation, total antioxidant status and level of reduced glutathione in the liver and kidney of male and female Wistar Han rats. Also, the influence of AAP on F excretion in urine was determined.
Methods: Thirty adult male and female rats were divided into five equal groups of six each: (I) controls drinking tap water; (II) controls drinking tap water and receiving 1 ml of tap water intragastrically; (III) animals receiving 12 mg F/L in drinking water; (IV) animals receiving 150 mg AAP /kg b.w./day; (V) animals receiving 12 mg F/L in drinking water and 150 mg AAP /kg b.w./day.
Results: F and AAP given separately and both together enhanced oxidative and nitrosative stress in investigated tissues. No gender differences were observed in oxidative/nitrosative stress parameters during treatment with F and/or AAP. Interestingly, the combined exposure to F and AAP resulted in an enhancement of oxidative/nitrosative stress in kidney of male and female rats compared to the group treated separately with F and AAP**.** No additive effect in the measured parameters in the liver during co-exposure to both xenobiotics was noticed.
Conclusions: As expected, the urinary F excretion increased in an exposure time-dependent manner in rats receiving F or a combination of F and AAP. The study also showed that AAP significantly decreased urinary F.

Key words:
fluoride and acetaminophen, male and female rats, oxidative and nitrosative stress, fluoride urinary excretion

Introduction

Normally humans are exposed to many substances concurrently. It is known that xenobiotics can interact significantly with each other, resulting in either an antagonistic or synergistic effect on the organism. Therefore, very important are interactions with substances, exposure to which is common, e.g., fluoride or acetaminophen. Exposure to fluoride can occur both in the environment and the workplace. It is a ubiquitous contaminant of the human environment (drinking water, dental prophylaxis, some drugs) and is utilized in a number of industrial practices. It is well known now that excessive fluoride intake can manifest not only as dental and skeletal fluorosis but can also affect soft tissues [8, 18, 24]. Fluoride can relatively easily cross the cell membrane and cause

structural and metabolic changes in the liver, kidney and brain [22, 24, 35]. Acetaminophen (AAP; chemically: *N*-(4-hydroxyphenyl)acetamide) is a widely used analgesic and antipyretic, and in most countries is available over the counter. AAP causes liver and kidney damage if taken in overdose [1, 15]. The initial step in AAP cytotoxicity is cytochrome P-450 dependent metabolism of the drug to the reactive derivative *N*-acetyl-*p*-benzoquinone imine – NAPQI [6, 16]. At therapeutic doses, however, NAPQI is efficiently detoxified being conjugated with glutathione (GSH) and thus excreted in urine. High doses of APP saturate the normal metabolic pathways of sulfation and glucuronidation. Excess APP is metabolized by P450 to deplete glutathione and cause liver necrosis, leakage of hepatocellular contents into the blood, and liver failure [12, 15, 16]. Although nephrotoxicity is less common than hepatotoxicity in APP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury [1, 2, 28, 29]. Many excellent reviews on acetaminophen and fluoride toxicity have been published [1, 6, 32, 35]. However, there seems to be no information available in the literature on the interaction between fluoride and acetaminophen. Moreover, the gender-dependent toxicity of acetaminophen is still controversial [6, 13, 28, 41].

In this study, we evaluated the influence of a 6-week co-exposure of male and female rats to fluoride and acetaminophen on oxidative and nitrosative stress parameters in liver and kidney. Additionally, the impact of AAP on F urinary excretion was investigated.

Materials and Methods

Treatment doses and animal species chosen for study

There is substantial information available regarding the mechanism of AAP hepatotoxicity following treatment with single toxic (high) concentration. However, much less information is available regarding the sub-injury dose effect of AAP during chronic treatment. An AAP dose of 150 mg/kg b.w. has been noted as a sub-toxic for rats [4, 27]. The AAP concentration used in this study was also established during a pilot study as the minimal dose causing changes in the concentration of oxidative stress parameters in a homogenate of liver and kidney.

Toxicity of acetaminophen depends on animal species, strain, age, gender etc. [6, 14, 33]. Rats, as well as rabbits and guinea-pigs, are less sensitive to AAP-induced liver and kidney injury than mice [14]. We selected to use rats in our experimental design since the rat model is a well established model of liver injury that exhibits effects similar to those observed in humans [45]. It is also known that Fischer-344 and Wistar rats are susceptible to AAP nephrotoxicity whereas Sprague-Dawley rats are not [28, 29, 32]. Wistar rats are widely used as a laboratory model in AAP-induced hepato- and nephrotoxicity [1, 2, 26, 33]. Taking into account the above information, and previously designed models of experimental fluorosis, our study was performed on male and female Wistar Han rats.

The concentration of fluoride in drinking water (12 mg/l) was carefully chosen according to results from our previous experiments as typical of different environmental exposures to F [41].

Animals and experimental design

The study was carried out on 30 male and 30 female Wistar Han rats (6-week old male and female rats weighing ~220 and ~170 g, respectively) obtained from the Gdansk University Experimental Animal Center. The animals were kept under standard laboratory conditions (temperature 20–22ºC, 12-h light/dark cycle, humidity 55–60%). All animals were fed standard laboratory chow "Labofeed B" containing water-soluble F at a concentration of 0.7 mg/kg, prepared by Feeds Production Plant A. Morawski, Poland (ISO PN 9001 and IQ Net Certificate). The study design was approved by the Medical University Bioethical Commission for Animal Studies.

The animals were kept for 10 days prior to the experiment in order to acclimatize. Then, both male and female rats were divided into five groups of six animals each:

I. Controls drinking tap water (containing F 0.3 mg/l).
II. Controls drinking tap water and receiving tap water intragastrically (1 ml once daily *via* stomach tube).
III. Exposed animals receiving F in drinking water 12 mg/l.
IV. Exposed animals receiving AAP 150 mg/kg b.w./day – sub-injury dose- (dissolved in 1 ml; once daily *via* stomach tube).

V. Exposed animals receiving F in drinking water 12 mg/l and AAP 150 mg/kg b.w./day (dissolved in 1 ml; once daily *via* stomach tube).

We did not observe any physiological signs of toxicity in the exposed animals. Different routes of drug administration (intragastrically *via* stomach tube or with drinking water available *ad libitum*) required two separate experimental control groups in the experiment. Fluoride and acetaminophen solutions were prepared daily from natrium fluoride (NaF, POCH, Poland, CAS: 7681-49-4) and acetaminophen ($C_8H_9NO_2$; Polfa Pabianice, CAS: 103-90-2), respectively, by dissolving in tap water. Water and chow consumption was measured daily; body weight was controlled once a week. Every week, 6 rats per sex per group were placed in metabolic cages and 24-h urine samples were collected. After 6 weeks of exposure, the animals were sacrificed and samples of blood, kidney and liver were collected. Any macroscopic changes in liver and kidney and statistical differences in tissue weight (normalized to change in body weight) between groups were observed. Blood samples were collected into tubes (without anticoagulant) by cardiac puncture, and then serum was obtained by centrifugation at 500 × g for 10 min. at 4°C. Serum samples were frozen at –20°C until analysis. Livers and kidneys were quickly removed, washed in cooled 0.9% NaCl and then homogenized in ice-cold buffer (100 mM KH_2PO_4/K_2HPO_4; pH 7.4 containing 1.15% KCl) using the homogenizer T10 basic Ultra-Turrax, IKA-WERKE. Then, homogenates were centrifuged at 1,500 × g for 15 min. at 4°C and the supernatant was collected. The postnuclear supernatant fractions were stored at –80°C. Total antioxidant status (TAS), advanced oxidation protein products (AOPP), thiobarbituric acid reactive substances (TBARS), nitric oxide (NO) and reduced glutathione (GSH) levels were assayed in the liver and kidney postnuclear supernatant fractions. In addition to that, level of protein was determined.

Analytical procedures

Determination of fluoride urinary concentration

The concentration of fluoride in urine was determined potentiometrically directly after dilution with equal volumes of TISAB (total ionic strength adjustment buffer) using a fluoride ion-specific electrode (Orion) and Ag/AgCl reference electrode [11]. Urine specific gravity was determined by means of a dedicated diagnostic test Multistix 10SG, BAYER. The accuracy of measurements was checked with reference samples – Seronorm Control Urine (Nycomed Pharma AC, Oslo, Norway). Mean recovery was 97.9%.

Determination of total nitric oxide level

NO levels were measured using the colorimetric assay (Stressgen Diagnostic Kit). The obtained results represented actually the sum of nitrite and nitrate levels. All the nitrates in a sample were initially converted enzymatically into nitrites by nitrate reductase, and then transformed according the Griess reaction into an azo dye allowing for colorimetric detection (λ = 540 nm) [10].

Determination of thiobarbituric acid reactive substances

Lipid peroxidation products were assayed by means of TBARS formation as described by Rice-Evans et al. [36]. Products of lipid peroxidation react with thiobarbituric acid at 100ºC under acidic conditions to form a pink-colored complex detectable at 540 nm. As malondialdehyde (MDA) accounts for ca. 99% of TBARS, the concentration of TBARS in tested samples was calculated using the MDA molar extinction coefficient equal to 1.56×10^5 M^{-1} cm^{-1}.

Assay of advanced oxidation protein products

AOPP levels were measured spectrophotometrically according to the method of Witko-Sarsat et al. [43]. AOPP concentrations were determined and expressed as micromoles per liter of chloramine-T equivalent whose absorbance at 340 nm was linear within the concentration range of 0–100 μM.

Determination of total antioxidant status

The total antioxidant potential in the liver and kidney was measured using a commercially available kit from SIGMA. 2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) was incubated with metmyoglobin (a peroxidase) and H_2O_2 to produce the radical cation $ABTS^+$, which had a relatively stable green color measured spectrophotometrically (405 nm). Antioxidants potentially present in a tested sample suppressed color development proportionally to their

concentration, thus allowing for quantitative assessment of free radical scavenging potential of the sample [34].

Determination of reduced glutathione

Tissue protein thiols were determined according to method of Sedlak and Lindsay [37]. The method is based on the reduction of Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] by SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitro-mercaptobenzoic acid has an intense yellow color and can be determined spectrophotmetrically at 412 nm. The results were expressed as mmol/g protein.

Protein assay

Protein content in postnuclear supernatant fractions of the liver and kidney was determined by the method of Lowry et al. [25].

Statistical analysis

The results were expressed as the mean ± SD. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. Differences with $p < 0.05$ were considered significant.

Results and Discussion

One of the main goals of the present study was to assess oxidative and nitrosative stress parameters as well as the total antioxidant status in the liver and kidney of male and female rats after a 6-week co-exposure to fluoride and acetaminophen. It has been well documented that gender is an important factor affecting the pharmacokinetic and pharmacodynamic properties of a drug [6, 33]. In the current study, gender-dependent difference in parameters of oxidative stress in the liver and kidney during exposition to acetaminophen and/or fluoride was examined. Liver and kidney extracts (postnuclear supernatant fractions) were assayed for TBARS, a lipid peroxidation indicator, AOPP, a marker of protein oxidation, NO, a free radical reactive nitrogen species, TAS and level of GSH as a significant part of the tissue antioxidative defense system. In addition, the effect of AAP on F excretion in the urine was evaluated.

A fluoride concentration of 12 mg/l in the drinking water that the rats were exposed to corresponds to human environmental exposure concentration in areas with high drinking water fluoride levels or under occupational conditions [42]. The acetaminophen dosing regimen was chosen carefully (150 mg/kg b.w./day) as being sub-toxic in rats [4, 27].

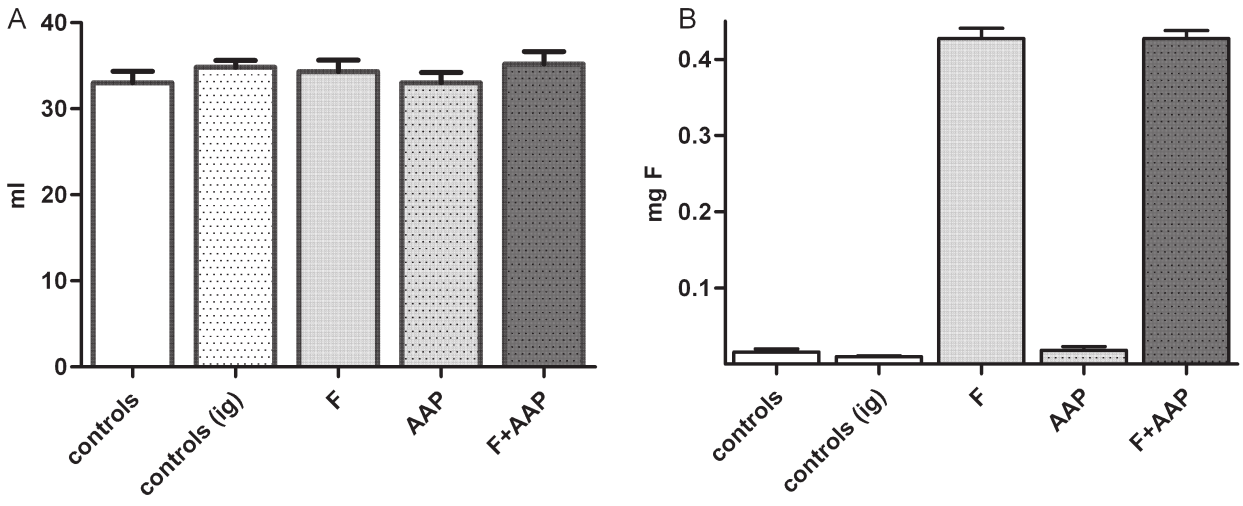

Fig. 1. (**A**) Water consumption (ml/24 h/rat) and (**B**) fluoride intake mg/24 h/rat. Data are the means ± SD; n = 12 per group (6 male and 6 female)

Tab. 1. Body weight of rats (g, mean ± SD)

Experimental group			Exposure time in weeks					
			1	2	3	4	5	6
I	controls	♀	170 ± 7.11	187 ± 7.10	202 ± 10.8	219 ± 9.18	234 ± 11.9	242 ± 9.09
		♂	220 ± 8.08	240 ± 6.09	259 ± 8.16	272 ± 8.22	290 ± 10.9	301 ± 9.14
II	controls (*ig*)	♀	166 ± 6.69	184 ± 8.71	200 ± 8.14	218 ± 9.13	233 ± 12.6	244 ± 8.81
		♂	228 ± 9.87	242 ± 12.5	258 ± 11.1	273 ± 12.7	287 ± 9.87	303 ± 6.17
III	F	♀	174 ± 6.14	190 ± 8.21	211 ± 7.27	226 ± 7.31	238 ± 9.54	252 ± 9.27
		♂	224 ± 8.15	238 ± 9.23	255 ± 9.37	269 ± 8.46	286 ± 10.9	302 ± 6.61
IV	AAP	♀	170 ± 7.17	189 ± 9.12	204 ± 11.1	216 ± 12.3	220 ± 9.78$^{\#}$	229 ± 8.41$^{\#}$
		♂	226 ± 9.16	241 ± 8.16	252 ± 8.21	268 ± 11.3	276 ± 8.96$^{\#}$	284 ± 11.9$^{\#}$
V	F + AAP	♀	167 ± 5.21	186 ± 6.23	200 ± 9.27	213 ± 9.26	221 ± 9.81$^{\#,*}$	227 ± 8.89$^{\#,*}$
		♂	220 ± 10.1	236 ± 9.29	253 ± 11.3	273 ± 9.51	278 ± 10.1$^{\#,*}$	285 ± 14.9$^{\#,*}$

# $p < 0.05$ as compared with controls II; * $p < 0.05$ as compared with group treated with F; n = 6 per group

Interestingly, among different animal models of acetaminophen-induced hepatotoxicity or nephrotoxicity, the rat model requires much higher doses of AAP, as calculated per body weight, to induce toxicity at the level comparable with that reported in other animals or humans. Therefore, a significantly higher dose of AAP is needed in order to induce organ damage or dysfunction in rats as compared with humans and other animals [14, 21].

In animals exposed to F and/or AAP no change in the intake of water was observed (Fig. 1). We observed a significant reduction of weight in both male and female rats treated with either AAP or AAP + F as compared with controls and the group exposed to F, respectively (Tab. 1). At the same time we have observed that daily food consumption (by the mentioned groups) decreased by about 10–15% (data not shown) as compared with the control. The difference in feed

Tab. 2. Fluoride concentration in urine (μg/ml, mean ± SD) adjusted to an average urine specific gravity of 1.024 g/ml

Animals		Exposure time in weeks				
Group	Treatment	1	2	3	4	5
I	controls	1.94 ± 0.16	1.99 ± 0.19	1.93 ± 0.06	2.04 ± 0.16	1.94 ± 0.09
II	controls (*ig*)	1.96 ± 0.11	1.95 ± 0.08	1.94 ± 0.12	2.02 ± 0.11	1.99 ± 0.13
III	F	2.46 ± 0.14	2.79 ± 0.14	3.21 ± 0.23	3.59 ± 0.33	3.67 ± 0.26
IV	AAP	2.01 ± 0.18	1.96 ± 0.16	1.90 ± 0.26	1.98 ± 0.26	1.96 ± 0.12
V	F+AAP	2.44 ± 0.20	2.93 ± 0.22	3.30 ± 0.21	3.11 ± 0.44	3.01 ± 0.66
	Statistical significance					
	I *vs.* III	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$
	II *vs.* IV	n.s.	n.s.	n.s.	n.s.	n.s.
	II *vs.* V	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$
	III *vs.* V	n.s.	n.s.	n.s.	$p < 0.01\downarrow$	$p < 0.001\downarrow$
	IV *vs.* V	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$	$p < 0.001\uparrow$

n = 12 per group (6 male, and 6 female); ↑ – increase F concentration in urine; ↓ – decrease F concentration in urine

intake could be a contributing factor to weight loss. The loss in body weight caused by APP was also reported by Manimaran et al. [26] in rats treated with 105 mg APP/kg b.w. Adeneye and Benebo [2] have observed significantly decreased body weight in rats exposed to APP (200 mg/kg, *ip*) for 14 days.

As expected, a significant increase in F urinary excretion occurred following administration of NaF in drinking water [8, 22]. The urinary F excretion increased in an exposure time-dependent manner (Tab. 2). However, from the fourth week of exposition, in rats co-exposed to F + APP a significant decrease in urinary F was observed in group relative to the group exposed to F. A decreased elimination of fluoride in urine might indicate a kidney failure, since the ability to excrete F markedly decreases when renal function deteriorates [40].

We have found no gender differences in oxidative/nitrosative stress parameters during treatment with F and/or AAP. Therefore, the female and male results are combined. Reactive oxygen species (ROS) exert detrimental effects and can damage cell macromolecules such as lipids, proteins or nucleic acids [17]. Nitric oxide is a gaseous molecule synthesized from L-arginine *via* two successive monooxygenation reactions catalyzed by nitric oxide synthases. Nitric oxide is a multifunctional messenger that plays a role in a variety of physiological processes. On the other hand, NO being a highly reactive free radical is responsible for various pathologies [9, 17]. In our study, the level of nitric oxide was elevated in the kidney and liver in all the groups of intoxicated animals compared to the controls (Fig. 2). An elevation in the level of NO level in rat tissues exposed to fluoride is consistent with our previous study results [23]. Sireli and Bulbu [39] observed an increase in nitric oxide in the liver of male albino rats exposed to NaF at a dose of 10.3 mg/kg b.w. Hinson et al. [19] found that NO level was increased in acetaminophen toxicity. Induction of hepatic nitric oxide synthase in the acetaminophen-treated rats was also reported by Gardner et al. [15]. Xu et al. [44] reported that NaF significantly increases NOS activity. This NOS increase releases NO, which combines with superoxide radicals to form peroxynitrites a highly toxic radicals responsible for tissues injury [9]. Moreover, no significant gender-dependent differences were observed in terms of nitrosative stress level, measured as NO production in the liver and kidney of male and female rats exposed to F, AAP or the combination of both. Interestingly, we found that co-exposure to F and AAP resulted in an increased level of NO in kidney when compared to the groups treated separately with F and APP.

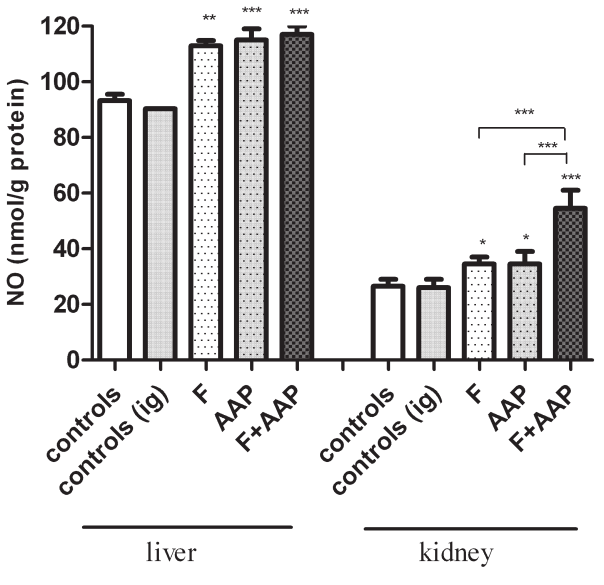

Fig. 2. Total nitric oxide levels in the liver and kidney (nmol/g) protein. Data are presented as the mean ± SD; n = 12 per group (6 male and 6 female). * p < 0.05; ** p < 0.01; *** p < 0.001 compared with control or as indicated

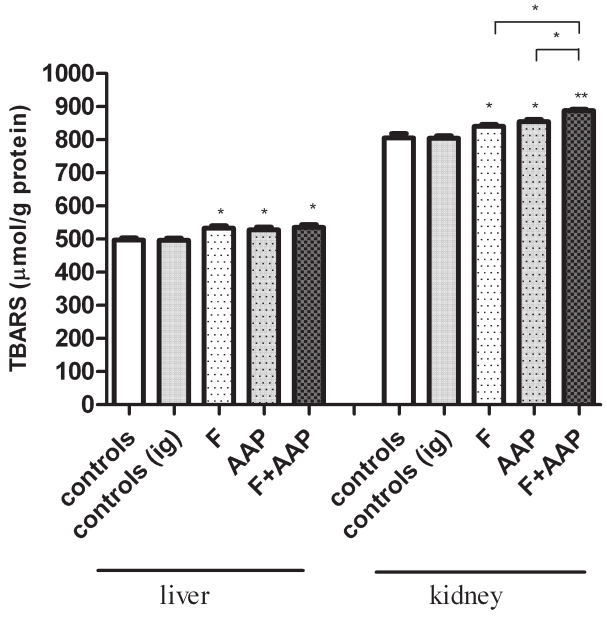

Fig. 3. TBARS levels in the liver and kidney (μmol/g protein). Data are presented as the mean ± SD; n = 12 per group (6 male and 6 female). * p < 0.05; ** p < 0.001 compared with control or as indicated

Fluoride also increased the activity of NADPH oxidase, another enzyme, contributing to ROS generation [8, 42]. Lipid peroxidation is one of the primary manifestations of oxidative stress induced by ROS, and has been linked to alterations in plasma membrane integrity. Consequently, increased fluidity and permeability of the membrane is observed [17]. The process of lipid peroxidation implicates complex chemical transformations, and is assessed by the characteristic end products such as TBARS, one of the most typical. Tissue levels of TBARS after AAP, F and F + AAP treatment were markedly elevated in both male and female rats compared to the group treated separately with F and APP (Fig. 3). Our results are consistent with those of other authors [1, 32, 38]. Fluoride is also known to affect the activity of membrane-bound enzymes, such as ATPase, by disturbing membrane integrity. No gender-dependent differences in lipid peroxidation were observed in the kidney and liver of exposed rats. However, co-exposure to both substances caused the elevation of lipid peroxidation in the kidney of male and female rats compared to the groups treated separately with F and APP.

ROS react with proteins directly or indirectly *via* sugar or lipid intermediates inducing subsequent alterations in protein conformation that cause aggregation, fragmentation, distortion of the structure, increased susceptibility to proteolysis, and thus dysfunction potentially resulting in cellular pathology. AOPP have been defined as a novel marker of oxidative stress-mediated protein damage [43]. AOPP were shown to be involved in the pathomechanism of coronary artery disease, uremia, diabetes mellitus, also in preterm neonates, and dentritic cell stimulation [17]. In our study, advanced protein oxidation was significantly marked in the liver and kidney of rats exposed to F and F + AAP as compared with the controls (Fig. 4). Although, the levels of AOPP in the liver and kidney of AAP-treated animals were also higher, they were not statistically significant. As opposed to these observations, Al-Belooshi et al. [3] reported that macrophages treated with AAP (10 μmol/ml) showed a significant increase in protein oxidation. On the other hand, Gibson et al. [16] reported that acetaminophen inhibited oxidation of bovine serum albumin in a concentration-dependent manner.

Oxidative stress occurs when the level of reactive oxygen species exceeds the antioxidative potential of the cell. Cells have developed various defense mechanisms protecting them against potentially deleterious ROS action. TAS is widely accepted as a good measure of tissue antioxidative potential [5]. In this study, we observed that TAS decreased significantly in the liver and kidney of both male and female rats after treatment with fluoride and/or acetaminophen (Fig. 5). There is a body of evidence that fluoride decreases the

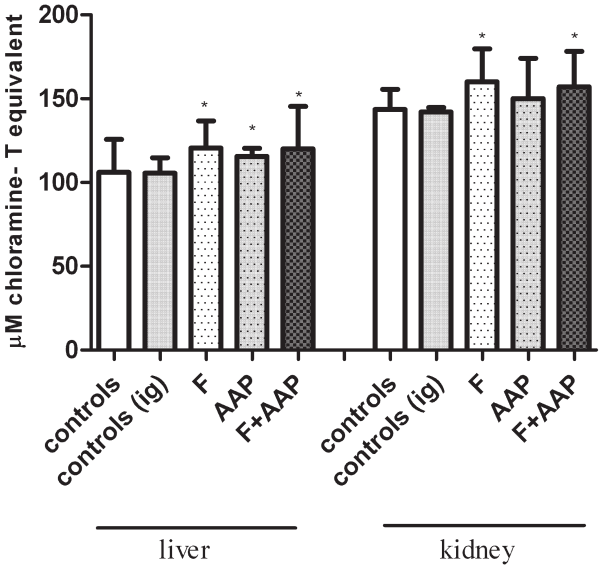

Fig. 4. AOPP levels in the liver and kidney (ĘM chloramine-T equivalents). Data are presented as the mean ± SD; n = 12 per group (6 male and 6 female). * $p < 0.05$ compared with control

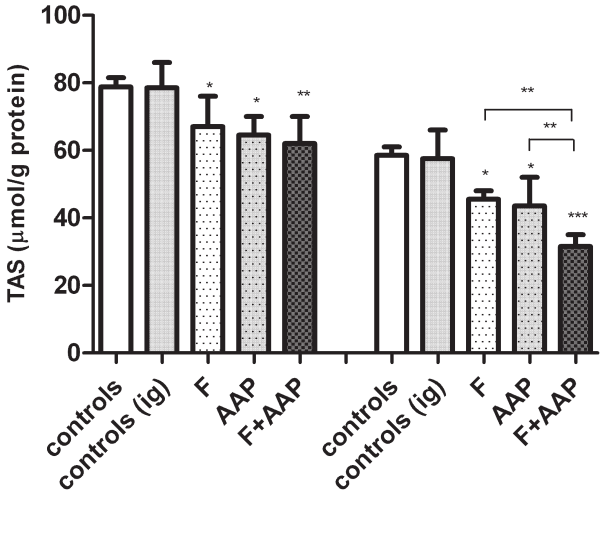

Fig. 5. TAS in the liver and kidney (μmol/g protein). Data are presented as the mean ± SD; n = 12 per group (6 male and 6 female). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with control or as indicated

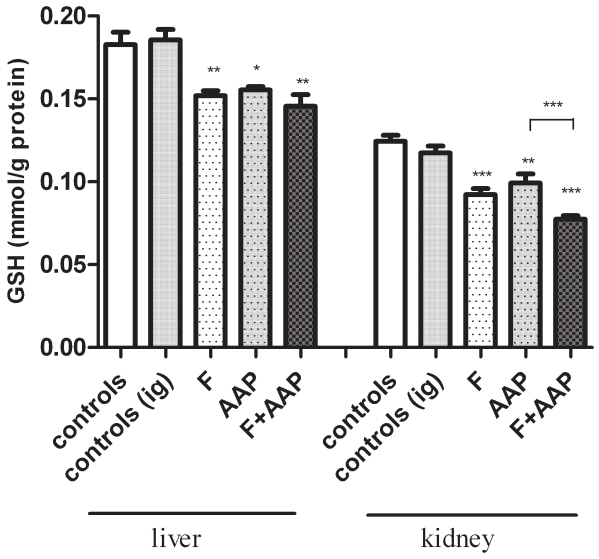

Fig. 6. GSH levels in the liver and kidney (mmol/g protein). Data are presented as the mean ± SE; n = 12 per group (6 male and 6 female). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with control or as indicated

enzymatic activity of glutathione peroxidase, catalase, superoxide dismutase, as well as cellular level of glutathione in rats exposed to sodium fluoride [8, 24, 38]. Hassan and Yousef [18] noticed a reduction of total antioxidant capacity in the liver of rats having been exposed to NaF at a dose of 10.3 mg/kg b.w. for 5 weeks. These results are in accordance with our previous study [23]. On the other hand, Reddy et al. [35] demonstrated that F does not impair antioxidant defense systems. O'Brien et al. [31] reported a significant decrease in total antioxidant status of the liver in rats exposed to various doses of AAP. Moreover, Nuttall at al. [30] observed that oral administration of the maximum therapeutic AAP doses to healthy volunteers caused a significant decrease in the serum TAS over time. Additionally, we have demonstrated, that co-exposure to F and AAP significantly decreased the content of TAS in kidney in both sexes as compared with the groups treated separately with F and AAP.

Cellular level of GSH is depleted in acetaminophen and fluoride intoxication [1, 8, 24, 28, 31]. In our study, GSH level was significantly decreased in the liver and kidney of rats exposed to F, AAP and F + AAP as compared with the controls (Fig. 6). Interestingly, that co-exposure to F and AAP significantly decreased the content of GSH in kidney in both sexes as compared with the groups treated separately with F. No gender-dependent differences in GSH concentration were observed in the kidney and liver of exposed rats. Dai et al. [13] suggested that gender-dependent AAP-induced hepatotoxicity may not be associated with gender-dependent basal glutathione levels.

In this study, we demonstrated that subchronic treatment of male and female rats with F and AAP, administered either separately or in combination, induced oxidative and nitosative stress phenomena in the liver and kidney that manifested as increased lipid peroxidation and a decrease in total antioxidant status and GSH level. Protein oxidation, as measured by AOPP, increased in all the treated groups compared to either control, however, the difference was statistically significant only in rats treated with F and a combination of F + AAP. Increased NO production was observed in the liver and kidney of all the treated animals. The combination of F and AAP was shown to have a synergistic effect in the kidney of both sexes, which resulted in TAS and level of GSH depletion, TBARS and NO elevation. No additive effect in the measured parameters in liver was observed during co-exposure to both xenobiotics in the concentrations investigated. No statistically significant gender-dependent differences were observed. Our findings are contrary to the results of Raheja et al. [33], most probably because of the much lower AAP doses used in our model. They observed that males are more susceptible than females to hepatic damage after treatment with 1 g APP/kg b.w. Chan et al. [6] ascertained a 6-fold higher survival rate in female rather than male mice 48 h after administration of AAP at a dose of 500 mg/kg b.w. Tarloff et al. [41] observed gender-dependent differences in AAP-induced toxicity only in 3-month-old rats, as opposed to adult or aging animals. They observed that some gender-dependent differences were organ-specific with male rats being more susceptible to hepatotoxic effect, and female rats more susceptible to the nephrotoxic effect of AAP. However, many studies ascertained no gender-dependent difference in AAP-induced toxicity [7, 20, 28].

Conclusion

In summary, our study demonstrated that subchronic treatment with fluoride and/or acetaminophen in subtoxic doses induced oxidative and nitrosative stress in

the liver and kidney of male and female rats. We did not find gender-dependent changes in the determined parameters or interaction between investigated xenobiotics in the liver.

The most important finding of our study is, demonstrated for the first time, synergism of fluoride and acetaminophen in the kidney of male and female rats as compared to groups treated separately with F and APP expressed as an enhancement of oxidative/nitrosative stress. Our results demonstrate a greater risk of kidney damage during co-exposure to both xenobiotics. Additionally, our experiment shows that acetaminophen significantly decreases urinary F excretion.

Conflict of interest:
The authors declare that there is no conflict of interest.

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