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INVESTIGATION OF THE EFFECT OF FORMALDEHYDE INHALATION ON TXNIP AND NF-KB LEVELS IN RAT KIDNEY

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ABSTRACT

Exposure to excessive formaldehyde (FA) has harmful effects on mammalian tissues. The aim was to investigate levels of oxidative stress in rat kidney due to chronic mild FA exposure. Sixteen female adult albino Wistar rats divided into control and experimental groups. The experimental animals were exposed to FA by inhalation to 10 ppm FA for 12 weeks, subchronic exposure. Kidney tissue Thioredoxin Interacting Protein and Nuclear factor-κB p105 levels were examined by enzyme-linked immunosorbent assay kit. The glutathione, malondialdehyde and advanced oxidation protein products levels were measured with the spectrophotometric methods. Serum albumin, creatinine, and urea concentrations were measured with enzymatic colorimetric assay. Compared to the control group, kidney GSH concentrations were significantly decreased in the FA group (P=0.015), while serum MDA concentration showed a significant increase (P=0.002). There was also a statistically insignificant decrease in tissue TXNIP and MDA and insignificant increase in tissue NF-κB p105 and AOPPs concentrations. Moreover, there was a strong positive correlation between tissue MDA and NF-κB p105 (r=0.841, P=0.001). Formaldehyde appears to cause cellular toxicity through oxidative damage because of disruption to oxidant/antioxidant system homeostasis. Subchronic administration of mild dose FA via inhalation to rats does not affect particular parameters on the apoptotic pathway.

Keywords: Formaldehyde, Thioredoxin-interacting protein, Nuclear factor-κB p105, AOPPs, Oxidative Stress, Kidney.

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INTRODUCTION

Formaldehyde (FA), systematic name methanal, is an organic, water-soluble, reactive compound with the formula CH_2O and one part per million (ppm) is equivalent to 1.23 mg/m^3 at one atmosphere pressure and 25 °C (WHO, 2010; Nielsen *et al.*, 2013). It is the simplest member of the aldehyde group of compounds.

Exogenous FA is absorbed into the body through the skin and through the digestive and respiratory tracts. FA is converted to formic acid in the liver and erythrocytes. The enzymes responsible for converting FA to formic acid are either formaldehyde dehydrogenase (FDH), which is now known as alcohol dehydrogenase 5 (ADH5) and S-formyl-glutathione dehydrogenase (International Agency for Research on Cancer, 2006). In the latter reaction, glutathione (GSH) acts as a cofactor. Formic acid may be excreted via the kidney, converted to CO_2 in the lung and exhaled, or condensed with tetrahydrofolate with subsequent entry into the mono-carbon pool. FDHs are found in all tissues. Transformation of FA is achieved by similar mechanisms in all species that have been studied. As the biotransformation of FA is rapid, it limits the detectability of FA in normal metabolism (Thompson *et al.*, 2008).

Exposure of the organism to FA occurs through both exogenous and endogenous sources. Some metabolic pathways yield FA intracellularly as a metabolite in the mono-carbon pool (Burgos-Barragan *et al.* 2017). FA may also be produced during the metabolism of some drugs and other exogenous compounds. Due to the volatility and reactivity of FA, measurement of tissue levels is difficult. Endogenous production of tissue FA vary and range from 3 ng/g to 12 ng/g wet tissue. Of this endogenous FA, 40% occurs in the free form, which has a very short half-life (t1/2=1.5 min) (Lunn *et al.*, 2010).

Formaldehyde is widely used in daily life and various industrial processes including the food industry (Reingruber and Pontel, 2018). In the medical field, FA has a long history of being used for cadaver and organ fixation in anatomy, histology, and pathology laboratories. FA has additional uses in medicine including in the structure of tooth coverings in dentistry, hemodialysis solutions, sterilization processes, and as a preservative in various medicines (Kundu *et al.,* 2015).

In 2006, FA was identified as a carcinogen by The International Agency for Research on Cancer (IARC), with genotoxic effects (IARC, 2006). In addition, several studies have shown that chronic exposure to FA may result in sensory irritation, salivation, dyspnea, headache, insomnia, seizures, and

neurodegenerative disorders (Gulec *et al*., 2006; Bakar *et al*., 2015). Chronic exposure to FA can also lead to the production and release of reactive oxygen species (ROS) (Birben *et al*., 2012). However, it is still unclear whether FA can induce toxic effects in distant organs such as the liver, kidney, and testes. Some studies have shown that prolonged exposure to FA can result in degeneration and necrosis of the renal proximal tubule and consequently impaired urinary system function (Woutersen *et al.*, 1989; Kum *et al*., 2007a; Zararsiz *et al*., 2007; Inci *et al*., 2013).

Thioredoxin-interacting protein (TXNIP, TBP-2), also known as vitamin D3 upregulated protein (VDUP1), has a range of functions and has shown to be involved in glucose and lipid metabolism, inflammation, and cancer biology (Alhawiti *et al.,* 2017). Endogenous TXNIP modulates the expression levels and activity of thioredoxin (TRX). The function of TRX is to scavenge reactive oxygen species and maintain redox balance (Li *et al*., 2014, Alhawiti *et al.,* 2017). In recent years, as a result of these important roles, TXNIP has attracted considerable interest as a potential endogenous target for medical and pharmaceutical manipulation.

The nuclear factor-κB (NF-κB) signaling pathway is involved in multiple cellular functions including transcription, inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis (Hayden and Ghosh, 2012). TXNIP and TRX can induce NF-κB activity. In addition TRX functions as a promoter of NF-κB-dependent transcription by reducing the reactive thiol (Matthews *et al*., 1992; Harper *et al*., 2001). NF-κB also regulates the expression of the immunoglobulin kappa (Iκ) chain in antibody producing B lymphocytes. The NF-κB pathway has been shown to be involved in the oncogenesis of many tumours (Basseres and Baldwin, 2006). Bi *et al.* demonstrated that the TXNIP/NF-κB pathway is involved in renal inflammation due to venenum bufonis, a toxin extracted from the skin or parotid glands of a number of frog and toad species (Bi *et al*., 2016).

Glutathione (GSH) is an important intracellular indicator of oxidative stress. Glutathione contains a tripeptide thiol (γ-glutamyl cysteinyl glycine) and is found in intracellularly in two forms: the oxidized glutathione disulfide (GSSG) and the reduced sulfhydryl form (GSH). Oxidative stress may be measured by analyzing the GSH/GSSG ratio in many tissues (Rahman *et al*., 2006). Therefore, the measurement of either GSH or the GSH/GSSG ratio has become a useful means of assessing oxidative stress experimentally.

Lipid peroxidation products are commonly measured as an indicator of ROS-mediated damage in tissues (Giera *et al*., 2012). It is important to measure malondialdehyde (MDA) as a marker of lipid peroxidation status in vivo because formaldehyde causes oxidative stress (Kum *et al.,* 2007a).

Advanced oxidation protein products (AOPPs) were reported for the first time by Witko-Sarsat *et al.* (1998) in the plasma of uremic patients and have been the subject of many studies investigating mechanisms of kidney damage. ROS directly oxidize tyrosine amino acid to form a dityrosine structure, leading to protein aggregation and fragmentation. The product formed by these cross-links is AOPPs but the mechanism has not been fully elucidated to date (Witko-Sarsat *et al*., 1998; Šebeková *et al*., 2012).

Routine biochemical assessment of renal function usually involves the measurement of albumin, creatinine and urea, and the calculation of blood urea nitrogen (BUN), which is derived by the formula Urea [mg/dL]/2.14, and the BUN/Creatinine ratio.

Despite the association between FA exposure and renal damage, there are few studies investigating the role of TXNIP-mediated NF- κB signalling in this process. The present study was undertaken to assess kidney toxicity due to FA by measuring changes in routine blood markers of renal function (albumin, creatinine, urea, BUN, BUN/creatinine ratio) and the effects of FA exposure on the TXNIP/NF-κB p105 pathway by measurement of TXNIP, NF-κB p105, GSH, MDA, and AOPPs concentrations in exposed experimental animals and comparison with controls. In addition this research was designed to investigate the toxic effects of subchronic, mild dose, FA exposure on a variety of tissues, excluding the lung.

MATERIALS AND METHODS

The experimental protocol of this study was approved by the Animal Experiments Local Ethics Committee of Kocaeli University (Project number: 2/5-2016). In order to protect animal rights, the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html) was followed. All experimental procedures were carried out strictly in accordance with the Declaration of Helsinki Principles (www.wma.net/e/policy/b3.htm) and 'Principles of laboratory animal care' (NIH publication No. 85-23, revised 1985).

Animals: Sixteen female adult albino Wistar rats from our own breeding colony, six months old were obtained from Kocaeli University Experimental Medicine Research and Application Unit. Animals were randomly divided into study and control groups at the beginning of the experiment. During the 12 weeks study period, all animals were housed under standard laboratory conditions with a 12/12 light - dark cycle, environment at 22- 24 °C, relative humidity of 40-70% and had free access to standard water and food pellets.

Experimental protocols: A specifically designed, handmade, glass cage was used for this subchronic

intoxication study (See Figure 1). The outer dimensions of the cage were 100x70x35 cm in total and was split into two chambers. A 20 cm chamber was used for FA vapor generation while the remainder of the cage (80 cm) contained the experimental animals. The two chambers were separated by a glass wall, with a 6 cm aperture, to allow FA vapor to pass from the generation chamber to the animal chamber. A similar aperture was created in the distal wall of the animal chamber to which an extraction mechanism was attached to facilitate a steady flow of gases through both chambers (Matsuoka *et al.*, 2010). Additionally, the cage had two separate lids for each chamber that could be separately opened from the top. This made it possible to place the test animals in the experimental chamber of the cabin and to replace formaldehyde source in the vapor chamber. A glass construction was preferred so that the behavior of the animals could be observed. In addition, the apparatus was not exposed to direct sunlight to avoid any additional heating effects on the experimental animals. A Toxi-RAE dosimeter (PGM-1860, Honeywell RAE Systems, USA) was placed in the larger chamber to measure FA vapor concentrations. Prior to the experiment, a series of tests were performed over the period of one week to ensure that the concentration of FA vapor in the test chamber could be maintained at an approximately constant concentration. FA solution (37%, 252549, Sigma-Aldrich, Missouri, ABD) was used as the FA source (Katsnelson *et al.*, 2013). After preliminary tests it was found that nine drops of FA solution placed on absorbable paper yielded a mean concentration in the experimental chamber of 10 ppm. When the dosing was confirmed to be consistent and replicable animal experiments were started.

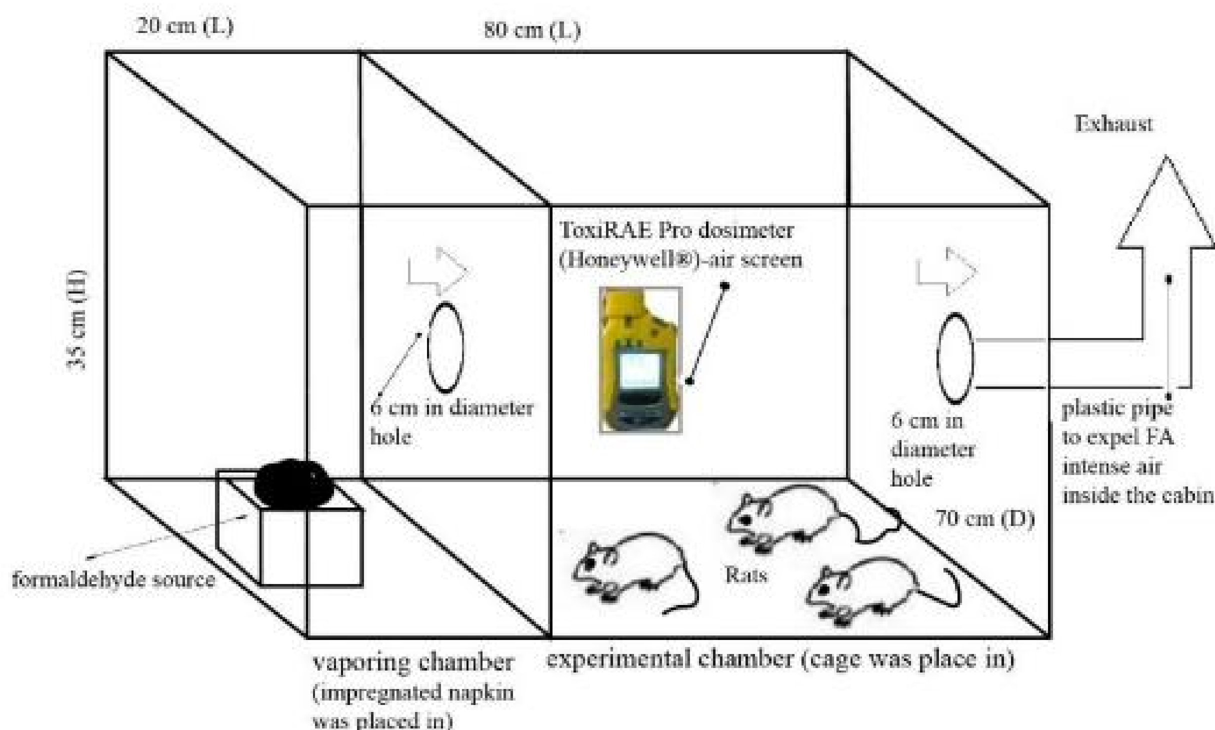

Figure 1. Diagram showing the design of the experimental chamber system for FA exposure. *created by Fatih Hunc

Normal air-breathing was provided to the control group (n=8) during the experimental period. Test animals were exposed to 10 ppm FA (mean actual concentration) for four hours per day, five days per week for a period of 12 weeks. Rats cages were put on the large section of the cabin. During the experiment, the animals continued to benefit from feed and water.

Sample collection: At the end of the 12 weeks experimental period, all animals were sacrificed under xylazine-ketamine anesthesia, the dose of which was adjusted according to individual animal weight. Blood samples (2.5 mL) were collected from each individual and centrifuged at 2200xg (NF 800R Nüve, Turkey) for 10 min to obtain serum and plasma samples. Serum and plasma samples were stored at -40°C. Kidney tissues were obtained for biochemical analysis. The kidneys were washed with saline solution (0.09% NaCl) to remove contaminating blood. Kidneys were weighed and homogenized with 1/10 phosphate buffered saline (PBS; 50 mM, pH 7.4) at 24,000 rpm with a homogenizer (T25 Basic Ultra Turrax, IKA WERKE,

Deutschland/Germany). The homogenates were then centrifuged for 15 min at 4°C at 10,000xg. The homogenate, aliquoted into small tubes, was retained for tissue-based measurements.

Kidney tissue analysis: Protein determination, which is essential for the proportional calculation of measurements on tissue homogenates, was performed according to the modified Lowry method (Hatree, 1972). Then tissue supernatant was deproteinized in a glacial metaphoshoric acid/di-Na EDTA/NaCl (in 100 mL distilled water, 1.67, 0.2, 30.0 g, respectively). Tissue MDA concentrations were determined by the method of Buege and Aust (Buege and Aust, 1978). Briefly, MDA was reacted with thiobarbituric acid by incubating for 15 min at 100°C. Kidney tissues MDA levels were measured spectrophotometrically in supernatant at 535 nm. The GSH method is based on the spectrophotometric measurement of the colored product formed by the reaction of the sulfidril groups with 5,5-dithio-bis-2-nitro benzoic acid (DTNB) at 412 nm (Ellman, 1959). GSH concentration was expressed as µmol/g wet tissue. AOPPs assay was performed as described by Witko-Sarsat *et al*. (1998) in tissue supernatant. 200 µL of supernatant, diluted 1:5 in PBS (0-100 µmol/L), was placed into each well of a 96-well microtiter plate. Then 10 µL of 1.16 M potassium iodide (KI) was added, followed by 20µL of acetic acid and the reaction mixture was immediately read at 340 nm in a microplate reader. The blank, containing 200 µL of PBS, 10 µL of KI, and 20 µL of acetic acid, was used as control. AOPPs concentrations were expressed in µmol/L of chloramine-T equivalents. The results of the AOPP assays were finally expressed as µmol/mg protein. Tissue rat TXNIP (Cat no: 201-11-1181) and NF-κB p105 (Cat no: 201-11-0288) concentrations were measured by sandwich enzyme-linked immunosorbent assay (ELISA) technique following the ELISA kits manufacturer's instructions (Sunred Biological Technology Co., China).

Serum analysis: Serum rat TXNIP concentrations were determined by sandwich ELISA technique, as per the analysis for tissue derived TXNIP, on the basis of the ELISA kits manufacturer's instructions (Cat no: 201-11-1181, Sunred Biological Technology Co., China). Albumin, creatinine and urea concentrations were measured with a Beckman Coulter Chemistry Analyzer AU5800 (Beckman Coulter, Tokyo, JAPAN). BUN and BUN/Creatinine ratio were calculated with related transformations based on creatinine and urea measurements.

Statistical analysis: Statistical analysis was performed with IBM SPSS version 20.0 (IBM Inc., Chicago, Ill., USA). Normality of distribution of data was tested with Shapiro-Wilk test. Results were presented as means ± standard deviation (SD) and median (25-75 %). Groups were compared with the non-parametric Mann Whitney-U test. Wilcoxon signed-rank test was also applied to compare the rat weights of two-paired samples. Spearman Correlation analysis was performed to evaluate the relationship between different continuous numerical data. Statistical significance was assumed when p was less than 0.05.

RESULTS

Descriptive analysis of all parameters is shown in Table 1. Since all parameters did not fit normal distribution, they were shown with median values.

Experimental and control animals were weighed before and after the experiment. The mean± standard deviation (SD) and median (25-75 percentile) of the control group was 268.38±17.83, 270.0 (253.2-278.2) grammes at the beginning of the experimental period. Prior to FA exposure the experimental group weights were 270.75±14.14, 269 (264.0-284.5) grammes which decreased significantly over the course of the experiment so that final weights in the experimental group were 239.75±19.88, 236.0 (221.0-260.7) grammes (P=0.011). There was no statistically significant decrease in weight of the control group during the course of the experiment [268.13±16.29, 266.5 (252-284.5), P=0.899] (Figure 2).

Table 1. Descriptive analysis of all parameters.

	Control group (n=8) **Mean ± SD** **Median (min.-max.)**	**10 ppm FA-exposed group (n=8)** **Mean ± SD** **Median (min.-max.)**	**P value**
Serum			
Albumin (g/dL)	3.06±0.57 2.98 (2.52-3.41)	2.70±0.51 2.77 (2.53-3.11)	0.723
Creatinine (mg/dL)	0.33±0.05 0.33 (0.30-0.38)	0.35±0.10 0.34 (0.27-0.36)	0.518
Urea (mg/dL)	43.69±6.52 42.35 (38.20-50.40)	41.27±3.36 42.54 (37.59-43.37)	0.355
BUN (mg/dL)	20.50±2.78 20.0 (18.25-23.25)	19.13±1.55 20.0 (17.25-20.00)	0.215
BUN/Creatinine ratio	62.75±7.38	57.96±14.39	0.355

	62.44 (55.61-68.75)	56.35 (54.80-71.29)	
TXNIP (ng/mL)	0.65±0.24 0.67 (0.45-0.84)	0.72±0.35 0.71 (0.39-0.95)	0.798
MDA (μm/mL)	1.96±0.31 2.18 (1.77-2.67)	2.51±0.25 2.39 (1.90-2.63)	**0.002***
Plasma			
GSH (μM)	5.73±6.62 3.74 (1.62-6.60)	2.02±1.46 1.48 (1.18-3.08)	0.161
Tissue			
TXNIP (ng/mg protein)	0.31±0.16 0.28 (0.18-0.43)	0.20±0.15 0.16 (0.11-0.30)	0.328
NF-κB p105 (ng/mg protein)	1.24±0.36 1.26 (0.95-1.59)	1.29±0.26 1.29 (1.02-1.45)	0.574
MDA (μm/mg protein)	0.33±0.10 0.35 (0.23-0.40)	0.33±0.09 0.33 (0.26-0.39)	0.878
GSH (ng/mg protein)	3.95±1.75 3.95 (2.51-5.25)	1.79±0.75 1.60 (1.43-1.79)	**0.015***
AOPPs (μmol/mg protein)	1.89±0.61 1.84 (1.33-2.38)	2.06±0.78 1.70 (1.54-2.97)	0.721

*P<0.05, mean ±SD (SD: Standard deviation). Compared with the non-parametric Mann Whitney-U test.

Figure 2. Serum albumin (g/dL) and pre- and post-experimental weight (grammes) of the control and FA-exposed groups are shown.

Serum MDA concentration increased significantly in the FA-exposed group compared to the control group (P=0.002). There was also a statistically insignificant increase in serum TXNIP concentration and an insignificant decrease in plasma GSH concentration (Figure 3).

Kidney tissue GSH concentrations significantly decreased in the FA-exposed group compared to the control group (P=0.015). There was also a statistically insignificant decrease in tissue TXNIP and MDA concentrations and a statistically insignificant increase in tissue NF-κB p105 and AOPPs concentrations (Figure 4).

There was no statistically significant difference in serum urea, BUN and creatinine concentrations nor BUN/creatinine ratio between the control and experimental groups (P>0.05) (Figure 5).

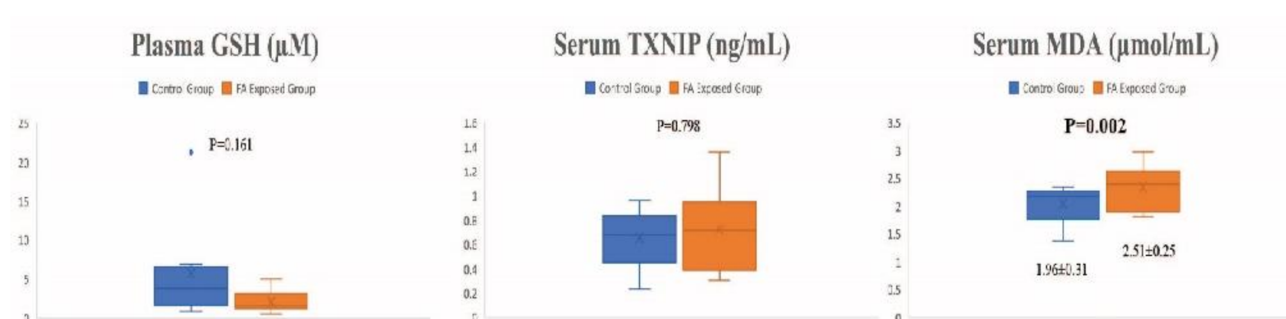

Figure 3. Comparison of plasma GSH and serum TXNIP and MDA concentrations in the experimental and control groups.

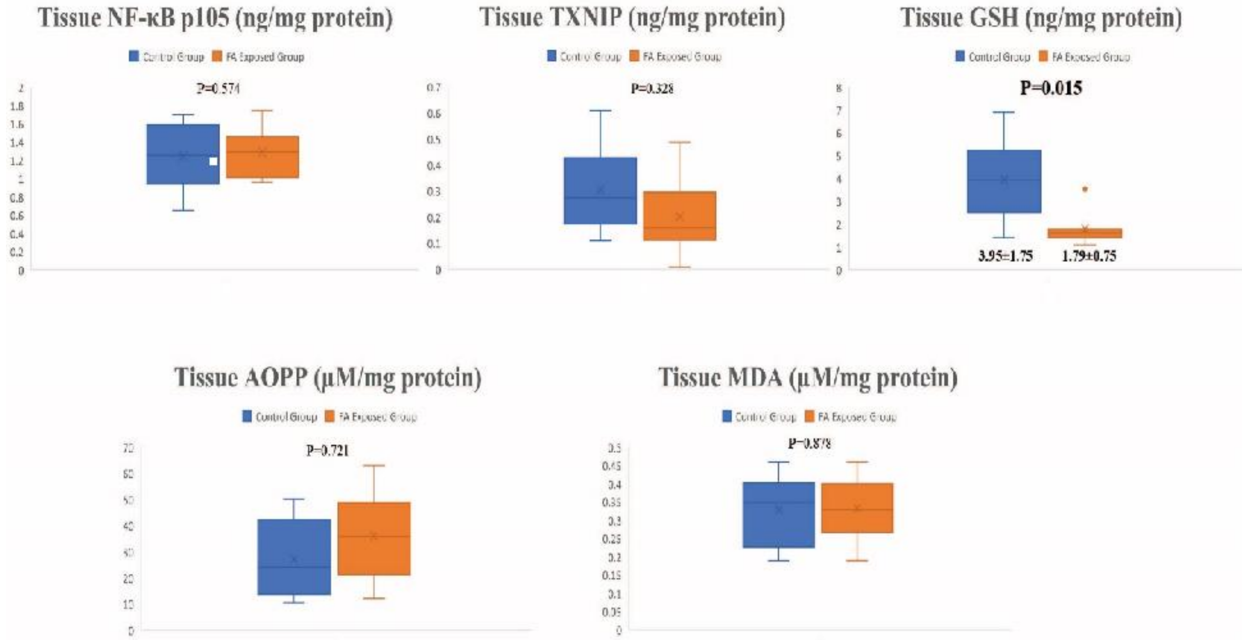

Figure 4. Comparison of tissue TXNIP, NF-κB p105, MDA, GSH and AOPPs concentrations between controls and FA-exposed animals.

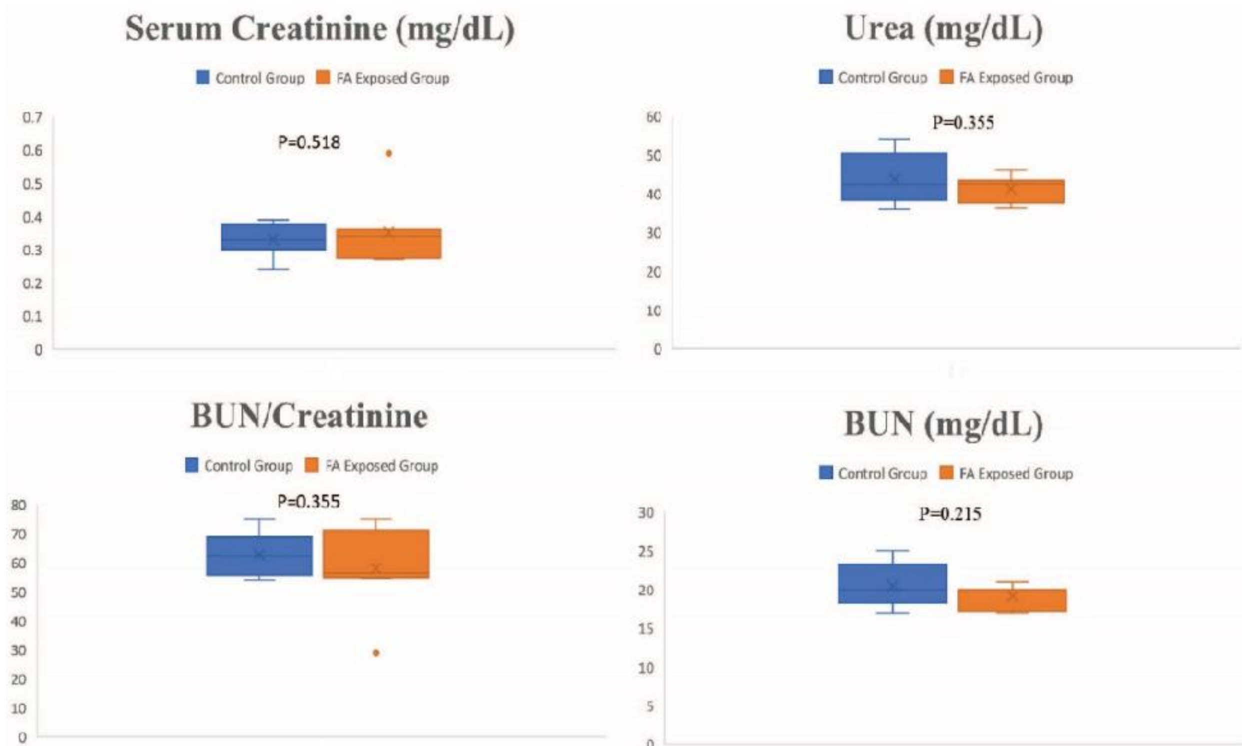

Figure 5. Serum albumin, creatinine, urea, BUN, and BUN/creatinine parameters were shown with P value.

Correlation analysis showed a strong positive association between tissue MDA and NF-κB p105 concentrations (r=0.841, P=0.001). A positive correlation was also detected between tissue GSH and NF-κB p105 concentrations (r=0.577, P=0.039). There was a weak correlation between tissue TXNIP and serum TXNIP concentration (r=0.555, P=0.049).

DISCUSSION

Animals exposed to chronic low grade (10 ppm) FA vapor for 20 hours per week had significant weight loss compared to healthy controls over a period of 12 weeks. Similar findings have been reported previously by Kerns *et al.*(1983) when animals were exposed to either 5.6 ppm or 14.3 ppm FA-exposed groups for 30 hours per week although the exposure periods were longer at up to 24 months (Kerns *et al.,* 1983). At lower exposure doses of 1, 2, and 4 ppm for 40 hours per week for periods of up to 13 months there was no significant difference between the weight changes in the experimental and control animals (Wilmer *et al.*, 1989). These findings suggest that higher exposure levels are responsible for the weight loss rather than length of time of the exposure.

Kum *et al.* (2007a) reported that blood urea concentrations increased significantly in all experimental groups (Inhalation of 6 ppm FA group; mean±SD 61.77±4.23) compared to the control group (mean±SD 37.82±1.79) ($P<0.001$) but there were no significant differences in total protein, albumin and creatinine concentrations. In the present study, a statistically insignificant decrease in albumin, urea, BUN concentrations and BUN/creatinine ratio values were found except creatinine ($P>0.05$) (see Table 1). Although blood urea concentrations tended to be lower in the exposed animals, it was not significantly lower than in the controls. There may be several reasons for this. FA was administered via inhalation and the vapor concentration was 10 ppm mild dose FA which may be too low to affect kidney function tests, especially over a shorter exposure period. In addition, inhalation of FA vapor will affect other tissues including the nasopharynx and upper respiratory tract damage and resulting in mild pulmonary irritation (Rana and Kumar, 1994; Bakar *et al*., 2015). FA inhalation has been shown to damage the oropharyngeal mucosa (David and Arkerman, 2016) which may cause poorer feding behavior in exposed animals, thus the 10 ppm FA-exposed group may have lost weight due to reduced calorie intake.

The toxic effects of FA on the urinary system have been demonstrated in a number of clinical and experimental studies. It has been reported that FA causes acute glomerular degeneration, tubular necrosis, and tubular dilatation in the kidney (Dixit *et al.,* 2005; Zararsiz *et al.*, 2006, 2007; Onyije and Avwioro, 2012). Studies have shown that a deficiency of TXNIP or suppressed TXNIP gene expression is protective against diabetic nephropathy, myocardial ischemia/reperfusion damage, affects regulation of the pro-apoptotic pathways and protects against kidney autophagy (Schulze *et al*., 2002; Saxena *et al*., 2009; Wu *et al.*, 2013; Huang *et al*., 2014; Shah *et al*., 2015). However TXNIP concentrations have not previously been investigated in kidney tissue in conjunction with markers of the oxidative stress that may increase due to FA. In this study, FA exposed animals tended to have higher blood concentrations of TXNIP with a concomitant decrease in tissue TXNIP, although neither of these findings was significantly different from controls. Increased serum TXNIP and a similar increase in tissue AOPPs was found in the FA-exposed animals suggesting a degree of suppression of the antioxidant system. The magnitude of kidney toxicity generated by short-term, mild dose inhaled subchronic FA may not be able to activate the TRX system. In addition, elevation of serum MDA and TXNIP concentrations may be an indicator of non-renal damage such as that occurring to the upper and lower respiratory tract, and other nearby organ pathology such as hepatotoxicity.

NF-kB is a ubiquitous transcription factor that interacts with over 200 genes. NF-κB has been shown to participate in many processes including apoptosis, cellular responses to oxidative stress, cytokine production and response to free radicals. The NF-κB protein complex has been shown to regulate cellular responses to various stimuli, including stress, cytokines, free radicals and bacterial or viral antigens. Studies have demonstrated that the TXNIP and TRX systems interact with NF-κB in response to inflammation, drugs, and oxidative stress (Hirota *et al*., 1999; Marshall *et al*., 2009; Kelleher *et al*., 2011). There are two important studies in the literature showing that TXNIP directly affects NF-κB (Kwon *et al*., 2010, Kim *et al*., 2017). These researchers showed in both studies that loss of TXNIP directly led to an increase in NF-κB concentration with different experimental models. In the first of these studies Kwon *et al.* (2010) used VDUP1(TXNIP)-KO mice and chemically induced hepato-carcinogenesis (Kwon *et al*., 2010). It was shown that VDUP1 has a crucial role in tumor formation and investigated the role of VDUP1 in the regulation of NF-κB activity. And they demonstrated that VDUP1 downregulated NF-κB activity used with NF-κB inhibitors. Because of this relationship between TXNIP and NFKB, we wanted to use these parameters in our experimental model. We only measured the levels of these parameters with ELISA methods compared to their advanced genetic experiments. The second study showing the relationship between NF-κB and VDUP1, Kim *et al.* (2017) investigated macrophage migration inhibitory factor (MIF), a cytokine that regulates the immune response and tumorigenesis under inflammatory conditions, in two cell lines, HeLa (human epithelial carcinoma) and 293T (human embryonic kidney fibroblast cell line) cells (Kim *et al.*, 2017). This study reported that MIF induces NF-κB activity by counteracting the inhibitory effect of TXNIP on the NF-κB pathway via direct interaction with TXNIP. In this study, we examined the levels of these two important proteins under oxidative stress conditions based on these two articles. It has been suggested that TXNIP plays a role in regulating NF-κB phosphorylation and

translocation, independent of activation of inflammatory cytokines (Park *et al.*, 2013). They investigated the interaction between the TXNIP and the NF-κB/iNOS pathways and concluded that this interaction may regulate disease states associated with sepsis. These researchers suggested that TXNIP has potential as a therapeutic target in the treatment of inflammatory diseases. Bi *et al*. (2016) found that NF-κB p65 was highly expressed in Venenum Bufonis-stimulated rats compared with controls. It was suggested that the TXNIP/TRX/NF-κB pathway may be activated because of the oxidative stress due to the Venenum Bufonis. We hypothesise that TXNIP concentrations did not increase sufficiently in our study to activate the NF-κB pathway in the FA-exposed group because of insufficient stimulus.

MDA induced injury in kidney tissue has been shown to be due to oxidative injury dependent on lipid peroxidation (Shah *et al*., 2015). In addition, potent antioxidant molecules, such as proanthocyanidins and vitamin E, significant decreased MDA concentrations in animals exposed to intravenous FA (10 mg/kg 14 days) compared to similarly exposed animals that did not receive the antioxidants (Bakar *et al*., 2015). In another study, Zararsiz *et al*. (2007) found significantly higher MDA concentrations in the kidney tissue of intra-peritoneally administrated FA group compared to the control group. In the present study exposure to FA was relatively mild compared to some of these previous studies, which did not result in a significant difference in tissue concentrations of MDA. However this degree of exposure was sufficient to cause a significant increase in blood MDA concentrations in the exposed animals. In addition to these studies, Zhou *et al.* (2006) demonstrated that MDA was markedly increased in the testes of FA-exposed rats that depending on the involvement of free-radical-induced oxidative cell membrane injury. However, concerning the MDA and GSH levels, Kum *et al*. (2007b) reported that there were no statistically significant differences between the only HCHO-exposed rats and control animals. Harris *et al.* suggested that GSH depletion may be the direct result of detoxication of FA in rat embryos (Harris *et al*., 2004). In our study, a statistically significant decrease in GSH concentrations was also observed in the FA-exposed group. This decrease may occur due to the oxidative stress of formic acid (formate$^+$ H$^+$), a major metabolite of FA (Liesivuori *et al*., 1991; Lunn *et al*., 2010). These studies showed an increase in MDA concentrations together with the decrease in GSH concentration in response to organic solvents, in agreement with the findings of our study, which showed that GSH concentrations decreased, probably as a result of chronic consumption of GSH in response to oxidative stress in kidney tissues.

The pathogenic role of AOPPs in renal injury is supported by a series of experimental observations. Cell culture and animal studies have shown that kidney cells are adversely affected by AOPPs (Guo *et al*., 2008; Wei *et al*., 2009; Zhou *et al*., 2009; Cao *et al*., 2013). In addition, AOPPs have been shown to stimulate NF-κB production. Shi *et al.* (2008) demonstrated that chronic loading of AOPPs caused NF-κB activation in rats with streptozotocin-induced diabetic or unilateral nephrectomy (Shi *et al*., 2008). Another *in vitro* study revealed that AOPPs induce vascular endothelial cell dysfunction by activating NF-κB and p38 mitogen-activated protein kinase signaling (Guo *et al*., 2008). In our study, there was a statistically insignificant increase in tissue AOPPs concentration (P=0.721). This may again have been due to either insufficient toxic stimulus or the short time frame of the experiment, or both.

Conclusion: We have shown that subchronic administration of a low dose FA via inhalation to rats did not affect appear to disturb markers of apoptosis in kidney tissue, whereas it diminished GSH, indicating a loss of oxidant/antioxidant system homeostasis. Further studies over the long term are needed to demonstrate FA toxicity and the mechanism of action of organ pathology in tissues distant from the respiratory tract. This is of particular importance for illuminating the role of mild, chronic exposure to FA in kidney tissues in terms of the risk of oxidative stress.

Conflict of Interest: No conflict of interest was declared by the authors.

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