Journal of King Saud University – Science 35 (2023) 102575

HOSTED BY

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Autophagy activation, histopathological damage, and altered renal epithelial sodium channel and Na+,K+-ATPase gene expression in offspring kidney after *in utero* exposure to allethrin

Wadha Alqahtani [a], Afoua Mufti [b], Nouf Aldawood [c], Abdullah Alshamrani [a], Saber Nahdi [a], Waleed Aldahmash [a], Maroua Jalouli [d], Anouar Feriani [b], Lamjed Mansour [a], Nizar Tlili [e], Saleh Alwasel [a], Abdel Halim Harrath [a,*]

[a] Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[b] Laboratory of Biotechnology and Biomonitoring of the Environment and Oasis Ecosystems, University of Gafsa, 2112 Gafsa, Tunisia
[c] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, B.O. Box 84428, Riyadh 11671, Saudi Arabia
[d] Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia
[e] Institut Supérieur des Sciences et Technologies de l'Environnement Borj Cédria, Université de Carthage, Tunis, Tunisia

ARTICLE INFO

Article history:
Received 31 October 2022
Revised 17 January 2023
Accepted 19 January 2023
Available online 25 January 2023

Keywords:
Pyrethroid insecticide
Allethrin
Nephrotoxicity
Autophagy
Histopathology

ABSTRACT

The goal of the current study was to determine the effects of the allethrin, as a pyrethroid-based insecticide, on kidney function in rat offspring. Sixty-day-old Wistar albino female rats were randomly allocated to three groups: control group and two allethrin-treated groups that were intragastrically administered with 34.2 or 68.5 mg/kg b.w. of allethrin starting from the day 6 of pregnancy until delivery. The results revealed that the high dose of allethrin (68.5 mg/kg) reduced the number of glomeruli. Results showed that the glomeruli diameter in animals treated with 34.2 mg/kg of allethrin significantly decreased when compared to the controls ($p < 0.001$) whereas at the dose of 68.5 mg/kg the glomeruli diameter was significantly higher than those of the control group ($p < 0.05$). Interestingly, the expression of the autophagic markers Beclin-1 and LC-3 was increased when compared to control indicating an induction of the autophagic mechanism. Furthermore, allethrin administration downregulated in a dose-dependent manner the expression of the epithelial sodium channel (ENaC) gene. The mRNA levels of Na+/K+- ATPase gene were significantly higher in the group treated with the low dose and significantly lower with the high dose, when compared to control. However, there was no effect of allethrin on the gene expression of NHE3 in both treated groups. Taken together, the findings of this study suggested that the exposure to allethrin might contribute to the nephrotoxicity by affecting the number and the diameter of glomeruli, causing histopathological alterations and inducing autophagic-related markers and affecting the mRNA levels of ENaC and Na+/ K + ATPase genes.
© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

According to previous reports, prenatal growth determines a person's health and chances of survival in later life. (Barker 1994). Indeed, healthy behavior during pregnancy and the maternal feeding before and through pregnancy can affect and modify fetal growth and some physiological parameters (Cindy et al. 2016). It has been demonstrated that low weight at birth is an indicator of a non-optimal prenatal development, evidence known as "Barker hypothesis" suggesting the developmental origin of adult diseases (Osmond & Barker 2000). Indeed, any environmental disruptions are detrimental to embryonic development, which ulti-

Abbreviations: cDNA, Complementary DNA; ENaC, epithelial sodium channel; EDCs, endocrine disrupting chemicals; FBS, Fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H-E, hematoxylin-eosin; K, potassium; LD50, Lethal Dose 50; mRNA, Messenger RNA; Na, sodium; NBF, Neutral buffer formalin solution; NHE3, Na(+)/H(+) exchanger isoform 3; NOAELs, No Observed Adverse Effect Levels; PBS, Phosphate-buffered saline; RT-PCR, reverse transcriptase polymerase chain reaction.

* Corresponding author at: Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail address: hharrath@ksu.edu.sa (A. Halim Harrath).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2023.102575
1018-3647/© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

mately results in chronic disorders (Tamashiro & Moran 2010). At birth, low weight in general can be considered an indication of an increased risk of exposure to many diseases in the later life, including type 2 diabetes and obesity (Cottrell & Ozanne 2007). It is proposed that this response could be due to the so-called fetal programming, for example (de Boo & Harding 2006; Alshamrani et al. 2021). Since humans are most of the time being exposed to the effect of more than one chemical (Ijaz et al. 2021; Khaled et al. 2022), the development of any organ in the body can be affected by substantial mixture of endocrine disrupting chemicals (EDCs) including pesticides (Zaynab et al. 2021). The effect occurs even if the individual chemicals are present at doses at or less than their No Observed Adverse Effect Levels (NOAELs) of chemicals. A Danish study on women who were exposed to a mixture of pesticides when they worked in green houses showed that their children recorded low weight at birth (Wohlfahrt-Veje al., 2011). Moreover, it has been reported an accumulation of body fat was increasing from the time of birth to school age (Wohlfahrt-Veje et al., 2011).

Pesticides used to increase agricultural productivity have leaked into water supplies, the environment, and the food chain, posing a danger to all forms of life. Among the pesticides used, those based on pyrethroids are more popular because of their low toxicity on mammals, their high toxicity for insects and their biodegradability (Doherty et al. 1988). Allethrin, bifenthrin, pyrethrin, permethrin, cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, sumithrin, and fenvalerate are examples of pyrethroids, which are synthetic derivatives of pyrethrin (Song et al. 2022). Allethrin, may constitute a major problem for human health and could notably affect kidney structure and function. It is regarded as one of the synthetic chemicals that are dangerous to living organisms because it affects membrane phospholipids, damages tissues, and can cause cellular enzymes to leak (Taiwo Idowu et al. 2013). However, relatively few studies on the effects of high intake of pyrethroid-based pesticides on kidney function have been reported (Zain & Aitte 2022). In particular, and to our knowledge, this is the first study that investigated the *in utero* effect of allethrin on kidney function in female rat offspring.

2. Materials and methods

2.1. Animal treatment and sampling

This experiment was conducted on thirty pristine white Wistar rats (200–250 g of weight and 60 days old) that were housed in cages separately. The rats were left in a well-ventilated room at 21 ± 1 °C, humidity ranged between 60 % and 80 %, and a 12-hour: 12-hour dark cycle. The rats were fed and given tap water and were put in the experimental room for a week to acclimatization. They were weighed and randomly divided into three groups: two allethrin-treated groups (34.2 and 68.5 mg/kg BW, from day 6 of pregnancy, which corresponds to fetus implantation, until delivery); and one control group. The first and second groups treated with allethrin were given 34.2 mg/kg and 68.5 mg/kg of body weight based on the oral LD50 values of 1/20 and 1/10 of allethrin, respectively (Jalouli et al. 2022).

When the female progeny reached week eight (60 days) after parturition, they were euthanized, and their kidneys were taken out, cleaned, and identified according to where they came from (groups). Neutral buffer formalin solution (NBF) solution was used to fixed the kidneys to prepare it for histological and immunohistochemistry studies or in RNA later and kept at 80 °C for RT-PCR. All treatments were performed on animals after obtaining permission from the Ethical Committee for the Care and Use of Laboratory Animals at the University of Gafsa, Tunisia (Reference No: FSG-AE-20–23).

2.2. Histological preparation

After fixing the kidney in 10 % NBF, it was gradually dehydrated using an ascending concentration of ethanol and embedded in paraffin blocks. For the immunohistochemistry and histopathological (hematoxylin-eosin (H&E) staining) studies, the blocks were cut using a rotary microtome to thicknesses of 5 μm and 3 μm, respectively.

2.3. Immunohistochemistry

The kidney tissues sections were deparaffinized with xylene and exposed to a descending series of ethanol before being washed with distilled water and then with one PBS solution. After that, the sections were exposed to 0.1 % Triton X-100 with 0.1 % sodium citrate, blocked with Fetal bovine serum (FBS), and then incubated overnight at 4 °C with an anti-LC-3 (1:100 dilutions, Dg-Peptide Co., Hang Zhou City, China) and anti-Beclin-1 (1: 200, Dg-Peptide Co., Hang Zhou City, China) primary antibody. Thereafter, the sections were washed with 1 × PBS solution, and incubated with anti-rabbit secondary antibody conjugated with horseradish peroxidase (1:2000 dilutions, Abcam, USA) for 2 h and detected by diaminobenzidine tetrahydrochloride for 5 min. Slides were then stained with hematoxylin II as a counterstain, dehydrated and mounted with DPX mounting media. For quantitative analysis at percent of total tissue area, immunohistochemical photographs were analyzed using MacBiophotonics Image J 1.41a software. Data are expressed as a percentage of total tissue area.

2.4. Analysis of gene expression (RT–PCR)

The RNeasy Mini Kit (Qiagen, Westburg, The Netherlands) was used to isolate RNA from kidney. The NanoDrop machine was used to estimate the equality and integrity of extracted RNA using the 260/280 nm ratio. After that the iScript™ cDNA synthesis kit (Applied Biosystem, Carlsbad, CA) was used to reverse transcribed RNA into cDNA according to the manufacturer's instructions. Real-time PCR (RT- PCR) was carried out using SYPR green and gene-specific primers (Table 1) on an Applied Biosystems 7500 Fast RT-PCR system (Carlsbad, CA) using the following protocol: 1 cycle of initial denaturation at 95 °C for 2 min, followed by 40 cycles of 94 °C for 20 s, 58 °C for 20 s, and 72 °C for 20 s. The relative quantity of each gene transcript was estimated using the 2-ΔΔCT method, with GAPDH serving as the reference gene for normalization.

2.5. Statistics

GraphPad Prism (9.3.0) software was used to determine the statistical significance of the differences in mean values between the treatment and control groups using one-way ANOVA and Tukey's multiple comparisons test. The significance value was considered at $p < 0.05$. The mean and standard deviation (SD) are used to show all of the data.

3. Results

3.1. The effect of allethrin on the number and diameter of glomeruli

The number of glomeruli was estimated at the age of 60 days after birth. The results showed that allethrin decreased the number of glomeruli but this decrease was significant only at the high dose of treatment (68.5 mg/kg) compared with the control group (Fig. 1A). Moreover, a significant decrease was also found within

Table 1
Primers used in RT-PCR study.

Gene	F	R	Amplicon size (bp)
NHE3	GGAACAGAGGCGGAGGAGCAT	GAAGTTGTGTGCCAGATTCT	322
ENaC	TACCCTAAGCCCAAGGGAGT	TGTTCTGCAAGGACAGCATC	226
Na $^{+}$: K^{+} ATPase	TGCCTTCCCCTACTCCCTTCTCATC	CTTCCCCGCTGTCGTCCCCGTCCAC	323
GAPDH	TCCCTCAAGATTGTCAGCAA	AGATCCACAACGGATACATT	308

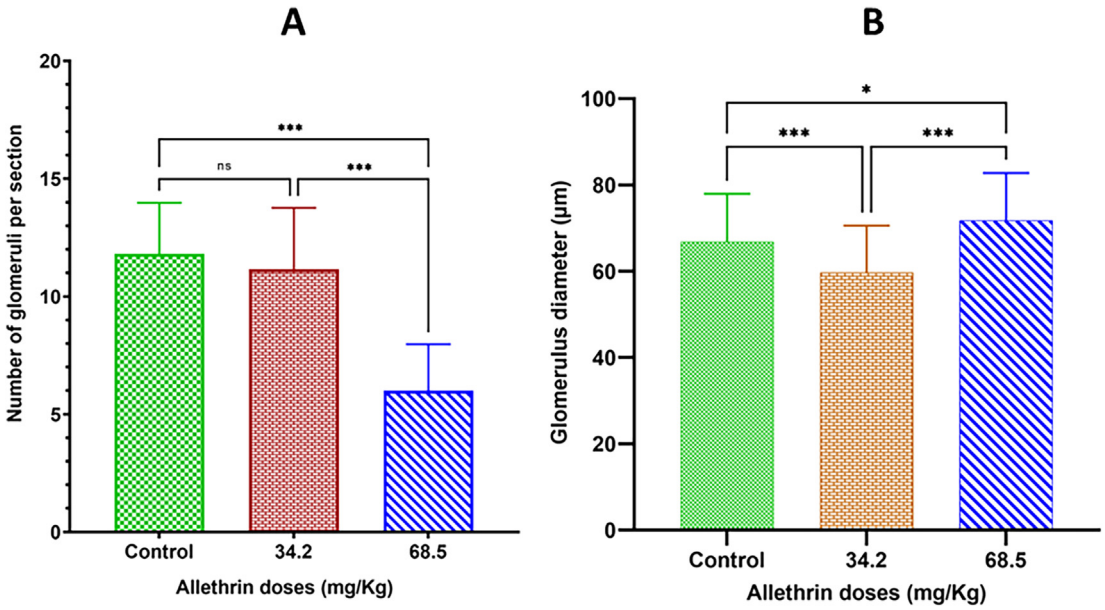

Fig. 1. (A) The effect of allethrin on the number of glomeruli in rat offspring. The decrease observed among the animals treated with the low dose (34.2 mg/kg) animals was nonsignificant compared to the control group, while a significant decrease was observed when using higher dose of allethrin (68.5 mg/kg) compared to the control group. (B) The *in utero* effect of allethrin on the glomeruli diameter. There was a significant decrease in the glomeruli diameter in animals treated with low dose (34.2 mg/kg) compared to control, while a significant increase was observed in animals of the second dose (68.5) when it was compared with the control group. Significantly different *p < 0.05, ***p < 0.001, compared with the control group. ns: nonsignificant decrease. (n = 8).

the kidney tissue of animals that were treated with the high dose when compared to those treated with low dose of 34.2 mg/kg.

The *in-utero* effect of allethrin on the glomeruli diameter is shown in the Fig. 1B. It was found that glomeruli diameter of the kidneys in the animal group treated with 34.2 mg/kg was significantly decreased compared to the controls (P < 0.001) whereas kidneys in 68.5 mg/kg treated group was significantly higher than the control group (P < 0.05).

3.2. Histopathological effect of allethrin on the kidney

The histological structures of the control group showed normal renal structures (glomeruli, Bowman's capsule, renal tubes, and collecting duct). The kidney cortex sections from control demonstrated typical appearance of renal corpuscles, glomeruli, surrounded by narrow Bowman's spaces. The cortical tubules with distal and proximal convoluted tubules are also typical. A large number of glomeruli have been observed, which are a bundle of looped capillaries located within Bowman's capsule and surrounded by the capsule space covered by a simple squamous parietal layer (Fig. 2A - C). In addition, the proximal convoluted tubules and the distal convoluted tubules, as well as the collecting duct were normal (Fig. 2B and D).

The histopathological study of the kidney in the group treated with the low dose of 34.2 mg/kg showed a periglomerular fibrosis and increased number of sclerosed glomeruli (Fig. 2E and F). Significant acute and chronic inflammation were detected as well (Fig. 2G). Interestingly, a higher number of foci of calcification was observed in kidney from 34.2 mg/kg allethrin-treated group in comparison with control (Fig. 2E-H). Furthermore, the presence of divergence in the simple squamous parietal layer of Bowman's capsule was also observed along with the presence of a large void in the capsule space (Fig. 2H). The histopathological study of the kidney's tubules showed severe tubular fibrosis, tubular dilatation and infiltration of immune cells that were detected in the tubular lumen from allethrin-treated rats' offspring (Fig. 2I-L).

The kidney tissue structure in the group treated with the high dose of 68.5 mg/kg was highly affected (Fig. 2 M–P). The presence of inflammatory cells was observed in the connective tissue and the collecting duct (Fig. 2M, N and P). In addition to the high level of calcification, the capsule space was very large compared to that from the control group and even to that from the first group of treatment (34.2 mg/kg) (Fig. 2O).

3.3. Effect of allethrin on the autophagic-related markers in kidney tissue

To study the involvement of autophagy mechanism in the effect of allethrin on kidney tissue, we examined the immunohistochemical expression of LC-3 and Beclin-1 proteins which are the main markers of this process. We found that immunohistochemical expression of Beclin-1 was significantly increased in both groups of allethrin-treated kidneys compared to the control group (Fig. 3-A-F). Quantitative analysis confirmed that Beclin-related intensities were significantly increased after allethrin exposure, indicating the occurrence of autophagy in the kidneys of both treated groups (Fig. 3M).

On the other hand, immunohistochemical examination revealed that the expression of LC-3 was localized on the glomeruli and on

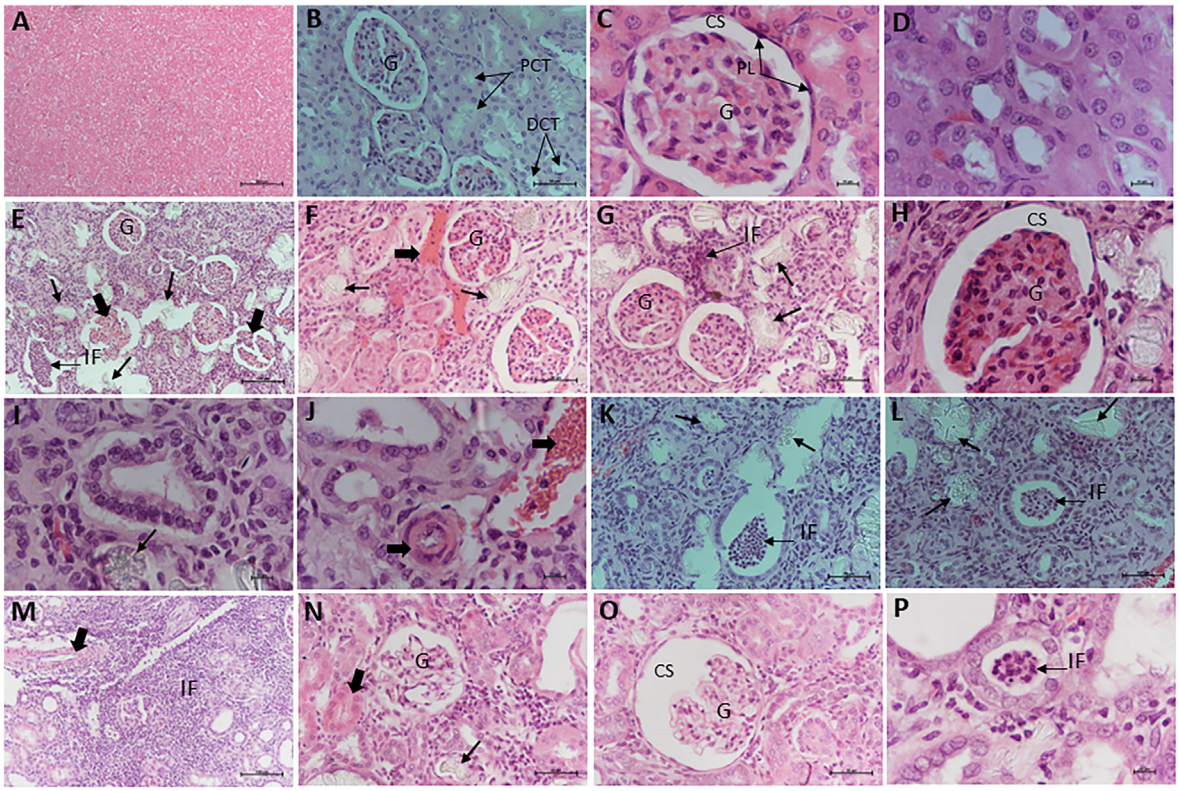

Fig. 2. Histopathological alterations in rat offspring kidneys after exposure to allethrin compared to control. The photomicrographs of the cortex of the kidney of control group stained with H&E (A-D) show a large number of glomeruli (A), normal glomeruli structure (B-C), and normal proximal and distal convoluted tubules (D). (E-L) Photomicrographs of renal histology stained with H&E in the group treated with the low dose of allethrin 34.2 mg/kg. (E): Abnormal features in the kidney tissue including atrophy of glomeruli, (F) calcification (arrow) and fibrosis (large arrow), (G) inflammation (IF), calcification (arrow) and fibrosis. (H) the capsule space went a dilation whereas the glomerulus shrank. (I-L) The collecting ducts have also been affected as a large number of inflammatory cells (IF), calcification (arrow), and fibrosis (large arrow) have been largely observed. (M–P) Photomicrographs of renal histology stained with H&E in the group treated with the high dose of allethrin (68.5 mg/kg). There are still more damages including (M) high inflammation (N and O) shrining of the glomeruli and dilatation of the capsule space, and (P) tubular inflammation. (n = 8).

collecting tubules (Fig. 3G-L). Immunoreactive cells were markedly increased in both groups of rats treated with allethrin compared with controls. Quantitative analysis showed a significant higher level of this protein in kidney tissue from treated rats compared with control groups (Fig. 3M). Interestingly, the immunopositivity was significantly higher in the kidney tissue from the high dose of treatment (68.4 mg/kg) compared to the lower one (34.2 mg/kg) (Fig. 3M). These findings demonstrate that allethrin triggered the mechanism of autophagy in kidney tissue.

3.4. Effect of allethrin on the gene expression of kidney- related markers

3.4.1. The epithelial sodium channel (ENaC)

Na + ions are transported across epithelia with high resistance by the epithelial sodium channel (ENaC). The results of RT-PCR showed that ENaC mRNA levels were significantly decreased in a dose-dependent manner compared to the control (Fig. 4A). Interestingly, the gene expression was significantly lower in the kidney tissue from the high dose of treatment (68.4 mg/kg) compared to the lower one (34.2 mg/kg).

3.4.2. The enzyme Na + K + ATPase

This enzyme helps in maintaining the homeostasis of the osmotic balance, including its important role in the kidneys, which is the waste filtration in the nephrons. It was found that there was an upregulation in the gene expression of Na + K + ATPase compared to control since we found an increased mRNA levels of this gene in the first dose -treated group compared to control. However, mRNA level of Na + K + ATPase was significantly decreased in the kidney of the groups that were treated *in utero* with the dose 68.4 mg/kg in comparison with the control (Fig. 4B).

3.4.3. Na+/H + Exchanger 3 (NHE3)

This gene has an important role in the kidney function as it is responsible for the reabsorption of NaHCO3 by the proximal tubules. There was no effect of allethrin on the gene expression of NHE3 in both treated groups (Fig. 4C).

4. Discussion

During the last years, an increased attention has been paid to the toxicity of pyrethroid insecticides on humans and animals due to the behavioral changes and other obvious pathological effects observed in exposed organisms (Khan et al. 2009). Allethrin, one of the pyrethroids, is regarded as a health risk because it may have an impact on many body organs, including the neurological system (Naz et al. 2019), the cardiovascular system (Naz et al. 2019), the male and female reproductive system (Madhubabu & Yenugu 2014; Jalouli et al. 2022), and the liver (Al-Mamun et al. 2017). Due to the fact that kidneys are particularly susceptible to the effect of toxic agents that can cause renal damage and even renal failure, the present study aimed to investigate the *in utero* effect of allethrin on kidney function of female offspring. Data from female rats whose mothers had consumed allethrin revealed that this pyrethroid had a significant impact on kidney tissue's structure and function, suggesting that allethrin exposure could be nephrotoxic.

The results indicated that the high dose of allethrin (68.5 mg/kg) significantly decreased the number of glomeruli, when compared to

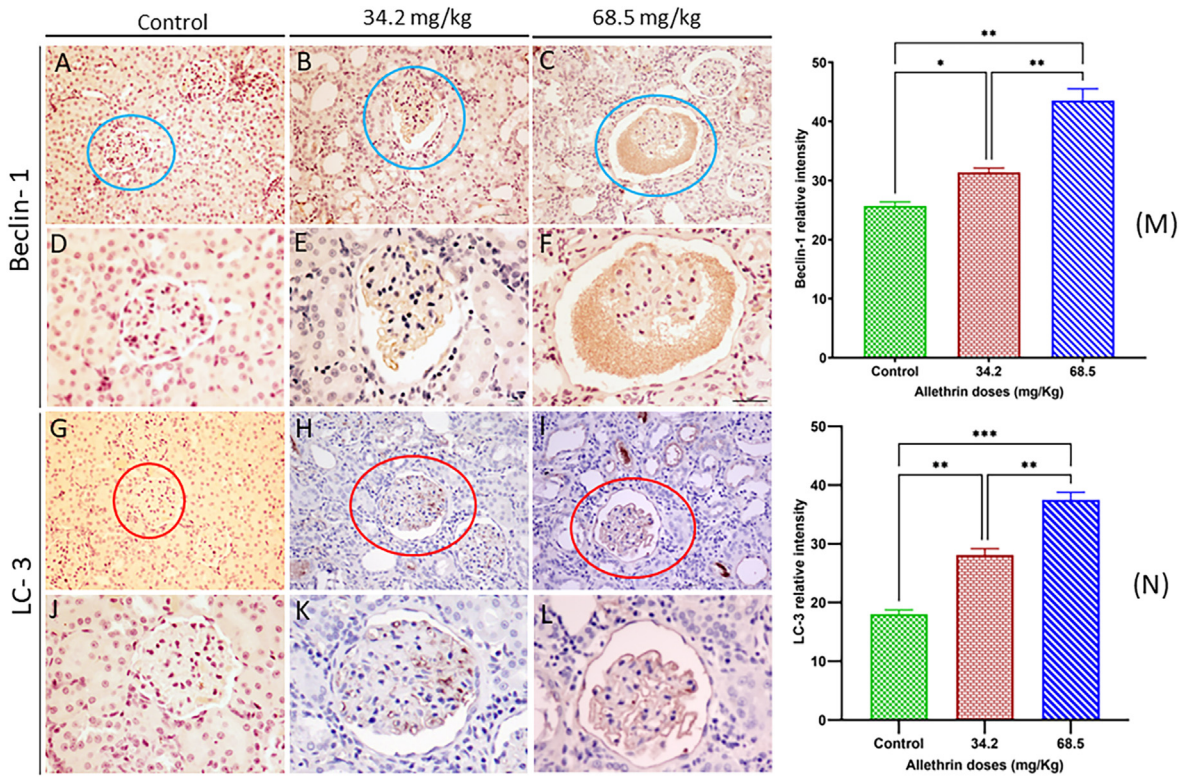

Fig. 3. Autophagy in kidney tissue of rat offspring was evaluated by immunohistochemistry staining of the autophagic markers, Beclin-1 and LC-3, in the control and exposed groups. Immunohistochemical analysis of Beclin-1 and LC-3 showed significant positive expression levels of these proteins in both treated groups compared to control group. All data were expressed as mean ± SD. *P < 0.05; **P < 0.001; ***P < 0.0001. (n = 8).

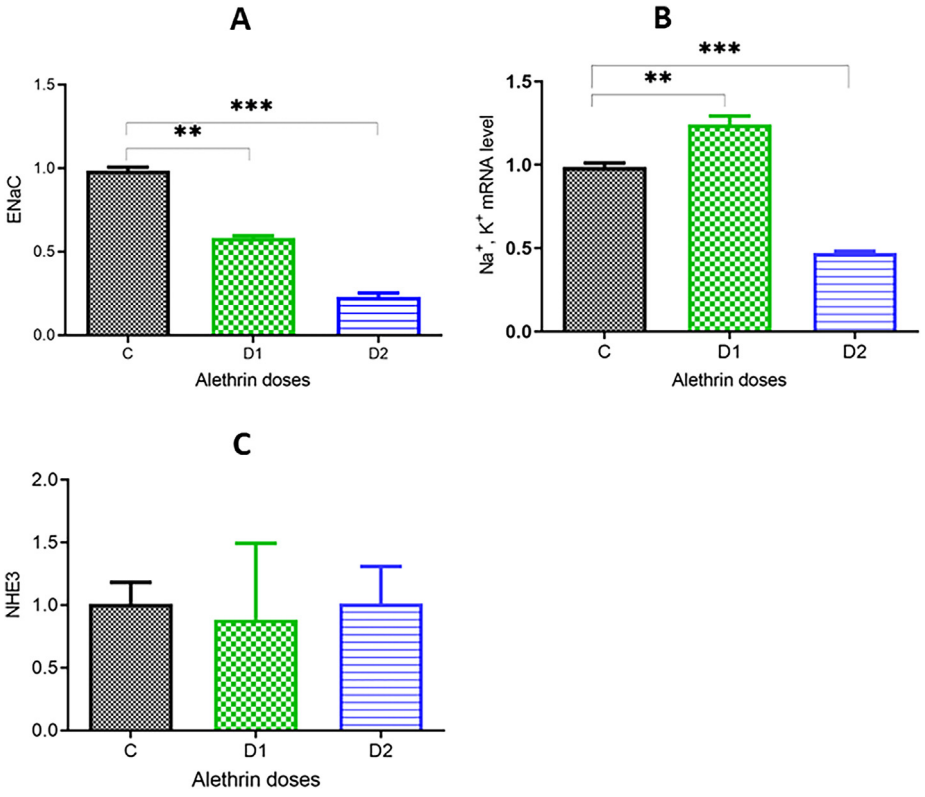

Fig. 4. Effects of allethrin on the expression of some kidney function genes in allethrin-treated groups of rat offspring compared to control. The mRNA expression levels of the genes ENaC, NHE3 and Na+, K + ATPase were detected by RT–PCR (A-C). (C) Control, (D1) = 34,2 mg allethrin /kg/day, (D2) = 68,5 mg allethrin /kg/day. All data are expressed as the means ± SDs. **P < 0.005; ***P < 0.001. (n = 8).

the control. Moreover, it was found that glomeruli diameter of the kidneys in the group treated with 34.2 mg/kg was significantly decreased when compared to the control group whereas the glomeruli diameter at the dose of 68.5 mg/kg was significantly higher than the control group. It can be concluded that the low dose of allethrin significantly impaired kidney structure through decreased the volume of glomeruli in rat kidney but not their number. However, the high dose of allethrin significantly decreased the number of glomeruli whereas their volume was significantly increased when compared to control and low dose.

This might be due to the fact that when the number of glomeruli decreased under the effect of allethrin toxicity, the fetal development of the kidney was reprogrammed through increasing the volume of the kidney as a recovery to the renal function. This result was confirmed by the histological results that reported the enlargements of the parietal layer of Bowman's capsule in allethrin-treated groups. The obtained results were in agreement with previous study showing that exposure to furan caused significant decrease in the average diameters of glomeruli and glomerular volume reflecting the reduction or failure of renal function (Selmanoğlu et al. 2012). Although very scarce studies reported the effect of pollutants on the number of glomeruli, it has been shown that the mean number of glomeruli has decreased in rats exposed to mercury vapor (Akgül et al. 2016).

The histopathological study showed an impaired kidney revealed by a periglomerular fibrosis, increased number of sclerosed glomeruli, significant acute and chronic inflammation, high number of foci of calcification, enlargement of the capsule space and severe tubular fibrosis and dilatation. These results are consistent with previous studies (Agarwal et al. 2013; Divakar et al. 2015) where mice were exposed to the mosquito coil and showed glomeruli degeneration, tubular necrosis and degeneration, tubular glomerulonephritis and leucocytic infiltration. Abnormalities that occurred in the tissues indicated the presence of a dysfunction in the kidneys, which led to the retention of stones occurring in the epithelial cells lining the renal tubules, where the crystals adhere to these cells (Asselman & Verkoelen 2002; Khan 2006). On the other hand, glomerular sclerosis and glomerular degenerations was seen in kidney rat that were exposed to mercury vapor (Akgül et al. 2016). It was observed that Bowman's space was dilated according to the control group and distal tubule cells showed damaged appearance which is in agreement with the current results. It has been described that exposure to pyrethroids leads to phospholipids, cholesterol, and fatty acids accumulation within the cells due to the inability of mitochondria to utilize them for ATP production, resulting in fatty degeneration of parenchymal cells, which induces tubular degeneration and tubular necrosis (Taiwo et al. 2008).

In the renal tubules, sodium is reabsorbed by ENaC, which helps regulate the volume of extracellular fluid and thus affects blood pressure by regulating osmolarity (Hanukoglu & Hanukoglu 2016). ENaC mRNA levels was significantly decreased in both groups of treatment with allethrin. Since the effect of allethrin on ENaC expression is significant, it ~~may~~ might disrupt sodium transport leading to the impairment of kidney function. Santesso et al. (2021) reported that, sodium fluoride exposition downregulated all three ENaC subunit genes at higher concentration (400 μM). In addition, NHE3 has an important role in the kidney function as it is responsible for the reabsorption of $NaHCO_3$ by the proximal tubules. Thus, it performs an essential physiological function in maintaining normal volume and acid-base balance in a wide variety of physiological conditions (Dynia et al. 2010). The present results showed that there was no effect of allethrin on the gene expression of NHE3. On the other hand, the Na+/K + ATPase is involved in various physiological processes of different organs and systems. In particular, the sodium gradient is necessary for the kidneys to filter wastes in the blood, to reabsorb amino acids, to help in osmotic balance, to reabsorb glucose and regulate sodium and potassium levels in the blood. That's why Na+/K + ATPase is expressed at high levels in the kidney (Pirahanchi & Aeddula 2019). Data of the current study showed an upregulation in the gene expression of Na+/K + ATPase in the kidney tissue of animals treated with allethrin at the dose of 34.2 mg/kg whereas a downregulating effect was detected in the renal tissue of the group treated with 68.2 mg/kg of allethrin. Thus, it seemed that a high dose of allethrin directly inhibited the ubiquitous cell 'sodium pump' (Na, K-ATPase). Similar study conducted to investigate the effect of mosquito coils containing pyrethroids on kidney of mice reported an impairment of ATP production in mitochondrial cells and changes in Na+/K + pump kinetics (Iqbal, n.d.; Agarwal et al. 2013). Mitochondria appear to be the major intracellular target for this toxicity as has been reported by previous studies (Johri et al. 2010). It may inhibit the respiratory chain (and electron transfer) resulting in the generation of reactive oxygen species (ROS) and mitochondrial disruption (Tang & Shaikh 2001) leading to caspase activation, causing cell death by apoptosis and necrosis (Tzirogiannis et al. 2003) which is in agreement with the present histopathological study.

Results showed also that *in utero* exposure to high dose of allethrin could induce autophagy in rat kidneys. Indeed, the expression levels of LC-3 and Beclin-1 were significantly increased in allethrin treated groups, mainly with the high dose. Prior research has reported that excess copper can trigger autophagy (Wan et al. 2020). These results are also consistent with those of a previous study that demonstrated an increased autophagic flux occurring in patients with chronic benzene exposure (Qian et al. 2019). Many pollutants that are released and accumulated in the environment have been described to promote cell apoptosis and autophagy simultaneously as a protective mechanism (Orrenius et al. 2013), which is in agreement with the results of the present study. Due to the fact that the two processes share a number of variables in response to various stimuli, there is currently much dispute on how apoptosis and autophagy interact (Glick et al. 2010).

According to Zhou et al. (2015), in general, autophagy is a type of stress mechanism that works to block apoptosis and avoid cell death. However, under particular stimuli conditions, autophagy will cause cell death or it is utilized as a method when the apoptosis mechanism is ineffective (Eisenberg-Lerner et al. 2009). By joining with Beclin-1 to create a Beclin-1-Bcl-2 complex, the anti-apoptotic protein Bcl-2 inhibits Beclin-1-mediated autophagy under normal circumstances (He & Klionsky 2009). However, excessive apoptosis causes the levels of Bcl-2 to drop and free Beclin-1 to rise, which activates the autophagy pathway, as was discovered in the current study.

5. Conclusion

This work proved that the chronic prenatal exposure ~~of~~ to two doses of allethrin (34.2 or 68.5 mg/kg b.w.) induced nephrotoxicity in rat offspring, especially at the high dose (68.5 mg/kg). Allethrin significantly decreased the number of glomeruli and induced histopathological alterations. It also increased the expression of the autophagic markers Beclin-1 and LC-3 and affected the mRNA levels of ENaC and Na + K + ATPase genes. These findings should be taken into account to evaluate the risk of this chemical on humans and animals given the potential for this pyrethroid to cause kidney damage ~~and~~ due its widespread use in residential insecticides.

Author contributions

A.H.H., A.F. and S.A. designed the experiments. W.K.A., A.M., A.F., and W.A. carried out the experiments. N.T., L.M., M.J. and A.H.H. analyzed the data. W.K.A, A.F., S.N. and A.H.H. wrote the first draft.

A.H.H and A.A. Approved the final version. All authors have read and agreed to the published version of the manuscript.

Funding

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. (IFKSURG-669).

Institutional review board statement

The study was conducted and approved according to the guidelines of the Institutional Review Board of Gafsa University (protocol code: FSG-AE-20–23).

Informed consent statement

This study was revised and approved by the Ethical Committee for the Care and Use of Laboratory Animals at the University of Gafsa, Tunisia (Reference No: FSG-AE-20–23).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. (IFKSURG-669).

References

Agarwal, A., Yadav, A.K., Singh, R., 2013. Ameliorating effects of garlic oil against mosquito coil smoke induced histopathological changes in rat kidney. Int. J. Pharm. Bio. Sci. 4, 1112–1116.
Akgül, N., Altunkaynak, B.Z., Altunkaynak, M.E., Deniz, Ö.G., Ünal, D., Akgül, H.M., 2016. Inhalation of mercury vapor can cause the toxic effects on rat kidney. Ren. Fail. 38, 465–473.
Al-Mamun, M.A., Rahman, M.A., Rahman, M.H., Hoque, K., Ferdousi, Z., Matin, M.N., Reza, M.A., 2017. Biochemical and histological alterations induced by the smoke of allethrin based mosquito coil on mice model. BMC Clin. Pathol. 17, 1–8.
Alshamrani, A., Aldahmash, W., Falodah, F., Arafah, M., Harrath, A.H., Alwasel, S., 2021. Long-term but not short-term maternal fasting reduces nephron number and alters the glomerular filtration barrier in rat offspring. Life 11, 318.
Asselman, M., Verkoelen, C.F., 2002. Crystal-cell interaction in the pathogenesis of kidney stone disease. Curr. Opin. Urol. 12, 271–276.
Barker, D., 1994. Mothers, babies, and disease in later life. BMJ Publishing Group, London.
Cindy, W., Elissa, S., Nicole, R., Kimberly, C., Nancy, E., Barbara, A., 2016. Maternal diet quality during pregnancy and fetal growth outcomes: a pilot study of lower-income pregnant women. FASEB J. 30, 671.20-.20.
Cottrell, E.C., Ozanne, S.E., 2007. Developmental programming of energy balance and the metabolic syndrome. Proc. Nutr. Soc. 66, 198–206.
de Boo, H.A., Harding, J.E., 2006. The developmental origins of adult disease (Barker) hypothesis. Aust. N. Z. J. Obstet. Gynaecol. 46, 4–14.
Divakar, D., Sasmal, D., Kumar, A., Sharma, N., 2015. Prophylactic role of piperine and curcumin in allethrin altered hematological and biochemical parameters in swiss albino mice. Pharmacologia 6, 396–412.
Doherty, J.D., Morii, N., Hiromori, T., Ohnishi, J., 1988. Pyrethroids and the striatal dopaminergic system in vivo. Compar. Biochem. Physiol. C, Compar. Pharmacol. Toxicol. 91, 371–375.
Dynia, D.W., Steinmetz, A.G., Kocinsky, H.S., 2010. NHE3 function and phosphorylation are regulated by a calyculin A-sensitive phosphatase. Am. J. Physiol. Renal Physiol. 298, F745–F753.
Eisenberg-Lerner, A., Bialik, S., Simon, H.-U., Kimchi, A., 2009. Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ. 16, 966–975.
Glick, D., Barth, S., Macleod, K.F., 2010. Autophagy: cellular and molecular mechanisms. J. Pathol. 221, 3–12.
Hanukoglu, I., Hanukoglu, A., 2016. Epithelial sodium channel (ENaC) family: phylogeny, structure-function, tissue distribution, and associated inherited diseases. Gene 579, 95–132.
He, C., Klionsky, D.J., 2009. Regulation mechanisms and signaling pathways of autophagy. Annu. Rev. Genet. 43, 67–93.
Ijaz, M.U., Ahmed, H., Ashraf, A., Aziz, S., Al-Ghanim, K., Akhtar, M., Riaz, M.N., Mahboob, S., 2021. Vitexin attenuates cisplatin-induced renal toxicity by reducing oxidative stress and inflammation. J. King Saud Univ.-Sci. 33, 101657.
Iqbal, Z. Histological Changes in Proximal and Distal Convoluted Tubules of Kidney of Albino Rats after Exposure to Mosquito Coil Smoke Inhalation.
Jalouli, M., Mofti, A., Elnakady, Y.A., Nahdi, S., Feriani, A., Alrezaki, A., Sebei, K., Bizzarri, M., Alwasel, S., Harrath, A.H., 2022. Allethrin promotes apoptosis and autophagy associated with the oxidative stress-related PI3K/AKT/mTOR signaling pathway in developing rat ovaries. Int. J. Mol. Sci. 23, 6397.
Johri, N., Jacquillet, G., Unwin, R., 2010. Heavy metal poisoning: the effects of cadmium on the kidney. Biometals 23, 783–792.
Khaled, I., Saidi, I., Ferjani, H., Ahmed, R.B., Alrezaki, A., Guesmi, F., Bouzenna, H., Harrath, A.H., 2022. BTEX induces histopathological alterations, oxidative stress response and DNA damage in the testis of the freshwater leech Erpobdella johanssoni (Johansson, 1927). J. King Saud Univ.-Sci. 34, 102196.
Khan, S.R., 2006. Renal tubular damage/dysfunction: key to the formation of kidney stones. Urol. Res. 34, 86–91.
Khan, A., Faridi, H.A., Ali, M., Khan, M.Z., Siddique, M., Hussain, I., Ahmad, M., 2009. Effects of cypermethrin on some clinico-hemato-biochemical and pathological parameters in male dwarf goats (Capra hircus). Exp. Toxicol. Pathol. 61, 151–160.
Madhubabu, G., Yenugu, S., 2014. Allethrin induces oxidative stress, apoptosis and calcium release in rat testicular carcinoma cells (LC540). Toxicol. In Vitro 28, 1386–1395.
Naz, M., Rehman, N., Ansari, M.N., Kamal, M., Ganaie, M.A., Awaad, A.S., Alqasoumi, S.I., 2019. Comparative study of subchronic toxicities of mosquito repellents (coils, mats and liquids) on vital organs in Swiss albino mice. Saudi Pharma. J. 27, 348–353.
Orrenius, S., Kaminskyy, V.O., Zhivotovsky, B., 2013. Autophagy in toxicology: cause or consequence? Annu. Rev. Pharmacol. Toxicol. 53, 275–297.
Osmond, C., Barker, D.J., 2000. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ. Health Perspect. 108 (Suppl 3), 545–553.
Pirahanchi, Y. & Aeddula, N.R. 2019. Physiology, sodium potassium pump (Na+ K+ pump).
Qian, S., Han, Y., Shi, Y., Xu, W., Zhu, Y., Jiang, S., Chen, Y., Yu, Z., Zhang, S., Yang, Y., 2019. Benzene induces haematotoxicity by promoting deacetylation and autophagy. J. Cell Mol. Med. 23, 1022–1033.
Selmanoğlu, G., Karacaoğlu, E., Kılıç, A., Koçkaya, E.A., Akay, M.T., 2012. Toxicity of food contaminant furan on liver and kidney of growing male rats. Environ. Toxicol. 27, 613–622.
Song, J., Ma, X., Li, F., Liu, J., 2022. Exposure to multiple pyrethroid insecticides affects ovarian follicular development via modifying microRNA expression. Sci. Total Environ. 828, 154384.
Taiwo Idowu, E., Aimufua, O.J., Yomi-Onilude, E., Akinsanya, B., Adetoro Otubanjo, O., 2013. Toxicological effects of prolonged and intense use of mosquito coil emission in rats and its implications on malaria control. Rev. Biol. Trop. 61, 1463–1473.
Taiwo, V., Nwagbara, N., Suleiman, R., Angbashim, J., Zarma, M., 2008. Clinical signs and organ pathology in rats exposed to graded doses of pyrethroids-containing mosquito coil smoke and aerosolized insecticidal sprays. Afr. J. Biomed. Res. 11.
Tamashiro, K.L., Moran, T.H., 2010. Perinatal environment and its influences on metabolic programming of offspring. Physiol. Behav. 100, 560–566.
Tang, W., Shaikh, Z.A., 2001. Renal cortical mitochondrial dysfunction upon cadmium metallothionein administration to Sprague-Dawley rats. J. Toxic. Environ. Health A 63, 221–235.
Tzirogiannis, K.N., Panoutsopoulos, G.I., Demonakou, M.D., Hereti, R.I., Alexandropoulou, K.N., Basayannis, A.C., Mykoniatis, M.G., 2003. Time-course of cadmium-induced acute hepatotoxicity in the rat liver: the role of apoptosis. Arch. Toxicol. 77, 694–701.
Wan, F., Zhong, G., Ning, Z., Liao, J., Yu, W., Wang, C., Han, Q., Li, Y., Pan, J., Tang, Z., 2020. Long-term exposure to copper induces autophagy and apoptosis through oxidative stress in rat kidneys. Ecotoxicol. Environ. Saf. 190, 110158.
Zain, M.H. & Aitte, S.A. 2022. Histopathological changes in the kidney tissues of white Swiss mice (Mus Musculus L.) exposed to alpha-cypermethrin.
Wohlfahrt-Veje, C., Main, K.M., Schmidt, I.M., Boas, M., Jensen, T.K., Grandjean, P., Skakkebæk, N.E., Andersen, H.R., 2011. Lower birth weight and increased body fat at school age in children prenatally exposed to modern pesticides: a prospective study. Environmental Health 10, 1–11.
Zaynab, M., Fatima, M., Sharif, Y., Sughra, K., Sajid, M., Khan, K.A., Sneharani, A., Li, S., 2021. Health and environmental effects of silent killers Organochlorine pesticides and polychlorinated biphenyl. J. King Saud Univ.-Sci. 33, 101511.
Zhou, J., Xi, C., Wang, W., Yang, Y., Qiu, Y., Huang, Z., 2015. Autophagy plays an important role in triptolide-induced apoptosis in cardiomyocytes. Toxicol. Lett. 236, 168–183.