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Research Article

JNK- and Rel-Mediated Regulation of Inflammation and Neurotoxicity in *Nauphoeta cinerea* Exposed to Methylmercury and Monosodium Glutamate

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Food contaminants are closely monitored to establish food safety profiles. Previously, we used the lobster cockroach to delineate the oxidative and antioxidant activities that characterize exposure to the organometallic cation (methylmercury; MeHg) sometimes found in fish and the common cooking seasoning (NaCl and MSG). Here, we further examined the mechanism of inflammatory response to MeHg, NaCl, and MSG, in an attempt to establish the crosstalk between redox and inflammatory signaling during heavy metal exposure in food. The insects were exposed to diets contaminated with MeHg, NaCl, and MSG for 21 days. Subsequently, mRNA from head homogenate was used to quantify the expression of a member of the Nox/Duox NADPH oxidases that produce reactive oxygen species (DUOX), as well as target genes of the *JNK, TOLL*, and UPD3/JAK/STAT pathways. MeHg exposure deregulated redox and inflammation-related genes, including upregulated DUOX in the MeHg group, upregulated Reaper, UPD3, and SOCS36E in the MeHg + NaCl group, and downregulated Reaper in the MeHg + MSG group. Both MeHg + NaCl and MeHg + MSG exposures upregulated PVF, EGR, and REL and downregulated FOXO levels. Our results suggest a role for the activation of the Nox/Duox NADPH oxidases, alongside JNK and Rel signaling during MeHg and MSG exposure, therefore offering insights for handling heavy metal poisoning and showcasing *Nauphoeta cinerea* as a viable model for understanding redox and inflammatory signaling.

1. Introduction

Food materials are often complex matrices of nutrients and bioactive molecules. However, in the process of cultivation, harvesting, packaging, and preparation for consumption, additives and contaminants are incorporated in food. Additives such as nitrite and monosodium glutamate are added to improve preserved or processed food, while contaminants such as pesticides and heavy metals are either encountered in nature or inadvertently incorporated in food due to farming

procedures or the release of industrial waste [1, 2]. It is therefore important to not only understand the nutritive and functional content of food but also perform relevant toxicity tests on materials consumed by humans and animals. For example, in the case of Minamata disease where the Minamata Bay community on Japan's Kyushu Island had consumed fish and shellfish from MeHg-contaminated waters, their total exposure included the food contaminant—methylmercury (MeHg), as well as possible food additives such as sodium chloride (NaCl) and monosodium glutamate (MSG) [3].

Methylmercury bioaccumulation in fish can exceed million folds of methylmercury in surrounding water, a concerning concentration for both humans and animals that consume fish [4]. Mercury is a naturally abundant element in the earth's crust, existing as metallic mercury, as well as inorganic and organic compounds of mercury [5]. Natural occurrences such as volcanic eruptions and rock weathering, along with human activities such as mining, electricity generation, waste incineration, and industrial activities, emit mercury and its compounds into the environment, with major consequences for the food chain. Released mercury compounds then cycle through air, land, and water bodies, contributing significantly to the pool of toxic contaminants in food [6, 7]. The severity of MeHg poisoning is dose-dependent, with greater sensitivity in infants and developing embryos than in adults [8, 9]. Central nervous system (CNS) perturbations have been recorded in individuals with MeHg poisoning, including tingling and prickling sensations in the upper and lower extremities; impaired balance and coordination; visual, auditory, and gustatory impairments; and disorders of movement and muscle tone. There are also records of MeHg-related deaths [10, 11].

Redox imbalances that cause oxidative stress predispose to cellular and macromolecular damage; for example, xenobiotics that bioaccumulate in fish have been linked with organ damage via oxidative stress and inflammatory pathways [12]. Moreover, oxidative stress may develop from acute xenobiotic exposure [13, 14], but studies have also shown the delayed neurotoxicity of MeHg in humans, rodents, and insects [15–17]. Despite the wealth of knowledge about MeHg-related CNS disorders, little is known about the effect of MeHg on the crosstalk between redox and inflammatory signaling, especially during food poisoning. Moreover, food safety assessment entails monitoring both food contaminants and additives. We, therefore, modelled exposure to both the contaminant, MeHg, and the additive, monosodium glutamate (MSG). The sodium salts—NaCl and MSG—are the most widely used flavor enhancers, and they add to the unique tastes of food. Glutamate salts dissociate in aqueous environments, and majority of glutamate in the diet is used up by intestinal mucosa enterocytes for energy [18]. The body also synthesizes adequate amount of glutamate [18], hence, daily glutamate consumption of up to 16 mg/kg body weight is thought to be safe, despite concerns to the contrary [19–21].

Consequently, we used a known insect model to characterize MeHg- and MSG-mediated activation of redox and inflammatory signaling during food poisoning. *Nauphoeta cinerea* certifies the 3Rs principle (replace, reduce, and refine animal models) that ensures the ethical and conscientious use of animals in product testing. It has also been used for several toxicity testing exercises [15, 20, 22–25]. Importantly, the evolutionary conservation of metabolic, redox, and inflammatory signaling pathways has been demonstrated between the lobster cockroach and mammals [13, 14, 26], like other insect models of behavioral changes and energy metabolism [27].

2. Materials and Methods

2.1. Chemicals. Methylmercury, sodium chloride, ethanol, isopropanol, chloroform, and polymerase chain reaction kits were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monosodium glutamate (99% purity Ajinomoto®) was procured from a grocery in Santa Maria, Brazil.

2.2. Cockroach Experimental Protocol. The nymphs of *Nauphoeta cinerea* were collected from the Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria, Brazil. The cockroaches were reared in plastic boxes (45.5 cm × 40.2 cm × 29.5 cm) while the nymphs to be utilized for the experiment were selected and put in small transparent boxes (10.8 cm × 10.8 cm × 7.9 cm). The nymphs were adapted in the new box for 10 days before starting the treatment and were maintained at $25 \pm 2°C$ and 74% relative humidity with a photoperiod of 12 : 12 (light: dark) from April to May 2019. The insects had free access to water and standard dog food (Total Alimentos LTDA, Três corações, Minas Gerais, Indústria Brasileira) during rearing and acclimatization. During the experiment, the cockroaches were exposed to a formulated diet containing MSG, NaCl, or MeHg dissolved in ethanol. The basal diet contained (per 20 g of diet) 10 g milled corn flour, 7 g wheat flour, 2 g granulated sugar (sucrose), 0.5 g casein, 0.4 g powder milk, and 0.1 g table salt (NaCl), all mixed in a blender. Moreover, diets containing MSG and NaCl were added to the basal diet to a final concentration of 2%. MeHg is soluble in ethanol at room temperature; hence, we dissolved MeHg in ethanol, mixed it in the diet, and left the mixture to dry at room temperature. The control diet was also mixed with ethanol and left to dry to expose all animals to similar conditions, except the test compound. All diets were then stored at −20°C.

The cockroaches were randomly divided into six groups consisting of 60 nymphs each. The length from the beginning to the end of the experiment for each group included 1.4–1.8 cm for the Basal group, 1.14–1.62 cm for the NaCl group, 1.16–1.68 cm for the MSG group, 1.18–1.75 cm for the Basal + MeHg group, 1.25–1.76 cm for the MeHg + NaCl group, and 1.30–1.78 cm for the MeHg + MSG group. Cockroaches in different groups were fed with the basal diet alone, the basal diet with 2% NaCl, the basal diet with 2% MSG, the basal diet with 0.125 mg/g MeHg, the basal diet mixed with 0.125 mg/g MeHg and 2% NaCl, and the basal diet mixed with 0.125 mg/g MeHg and 2% MSG, respectively, for 21 days. The groups were repeated in duplicate. The subtoxic dose of MeHg was selected from the study

by Adedara et al. [28], the dose of MSG has been utilized in our previous studies, and a pilot test was carried out to ensure that the NaCl concentration in standard diet would enhance food palatability without affecting experimental results [20, 25, 29].

2.3. Total RNA Extraction and Complementary DNA (cDNA) Synthesis.

Total RNA from the cockroach head was isolated using TRIzol® reagent (Invitrogen Life Technologies, USA) following the manufacturer's instruction. DNase 1 reagent (Invitrogen Life Technologies, USA) was used to remove DNA contamination. The yield, quality, and purity of the isolated RNA were estimated spectrophotometrically by using a NanoDrop 2000™ spectrophotometer (NanoDrop Technologies, USA). The RNA samples with an absorbance ratio (260/280 and 260/230) between 1.9 and 2.0 were used for further analysis. RNA integrity was evaluated by agarose gel electrophoresis.

Reverse transcription of $1 \mu g$ total RNA was done to obtain the cDNA strand using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc., USA). The assay mixture for each tube consists of $10 \mu L$ of $1 \mu g$ RNA template, $1 \mu L$ of 5X iScript Reaction mix, $1 \mu L$ iScript reverse transcriptase, and $5 \mu L$ diethylpyrocarbonate (DEPC) water incubated at 25°C for 5 min. The tubes were continuously incubated at 42°C for 30 min and inactivated at 85°C for 5 min. The cDNA samples were chilled on ice and then stored at −20°C for future analysis. Each cDNA sample was diluted 10 times with nuclease-free water prior to the qRT-PCR analysis.

2.4. Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR).

qRT-PCR was conducted in a QuantStudio™ 3 system. Each well contained $20 \mu L$ mixture, 10 ng diluted cDNA, and $10 \mu L$ reaction mix (using the Platinum Taq DNA Polymerase kit (Thermo Fischer Scientific)) comprising 1 X PCR buffer, 3 mM $MgCl_2$, $0.2 \mu m$ each of the forward and reverse primers, 0.1 X of SYBR green (Invitrogen USA), 0.2 mM deoxyribonucleotide triphosphate (dNTP), and 0.25 U Platinum Taq DNA polymerase, made up with ultrapure deionized water. The amplification procedure used was as follows: 94°C for 5 min (1 cycle), 40 cycles of 94°C for 15 s, 60°C for 10 s, and 72°C for 30 s (fluorescence collection). A thermal denaturing cycle was included immediately to obtain the dissociation curve of PCR products (amplicons) and verify the amplification specificity using the procedure of 1 cycle at 94°C for 10 s, 55°C for 1 min, and 95°C for 15 s.

The reactions were carried out in two technical duplicates of each of the six biological replicates. The gene GAPDH was used as the reference gene for sample normalization, and the basal diet alone samples were used for calibrating calculations performed with the $2^{-\Delta\Delta CT}$ method.

2.5. Primer Design.

The NCBI platform (https://www.ncbi.nlm.nih.gov/) and FlyBase (specific for Drosophila) were used to search for gene sequences that code for proteins that function in certain inflammatory pathways in Drosophila melanogaster, Zootermopsis nevadensis, Cryptotermes secundus, and Blattella germanica. The obtained nucleotide sequence was queried against our Nauphoeta cinerea transcriptome with accession numbers SRR3581673 and SRR3581312 as published by our research team. Primer 3 input platform version 0.4.0 (http://primer3.ut.ee/) was used to design the primer of interest from the Nauphoeta sequences, and the quality of the primer sequence was tested with OligoAnalyzer 3.1 (https://www.idtdna.com/pages/tools/oligoanalyzer).

The primer details of the inflammatory genes and one oxidative stress gene are listed in Table 1. The qRT-PCR efficiencies in the exponential phase were calculated for each primer pair using standard curves (10-fold serial dilutions of the cDNA), and the mean threshold cycle (C_T) values for each serial dilution of each primer were plotted against the logarithm of the cDNA dilutions and calculated using the equation $E = 10^{(-1/\text{slope})} - 1$, where the slope is the gradient of the linear regression line, and the correlation coefficient (R2) shows the linearity of the standard curve for each gene.

2.6. Statistical Analysis.

Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 6 and Statistica software packages using a two-way analysis of variance (ANOVA), (2 (with and without 0.125 mg/g MeHg) by 3 (Basal/2%NaCl/2%MSG)) followed by Tukey's post hoc test. Significance was set at $P < 0.05$.

3. Results

3.1. Effect of MeHg and Food Additives (NaCl and MSG) in Diet on the Expression of N. cinerea c-Jun N-Terminal Kinase (JNK) Target Genes.

A two-way ANOVA showed a significant main effect of MeHg (F (1.66) = 6.38: $P = 0.01$) (Figure 1(a)) for the mRNA level of EGR. The mean of the MeHg-treated groups (1.64 ± 0.17, $n = 36$) was higher than that of the non-MeHg-treated groups (1.09 ± 0.37, $n = 36$). Two-way ANOVA showed a significant main effect of MeHg (F (1.66) = 5.85: $P = 0.02$) and diet (F (2.66) = 5.64: $P = 0.01$) (Figure 1(b)) for the mRNA levels of REAPER. The mean of the MeHg-treated groups (1.30 ± 0.42, $n = 36$) was higher than that of the non-MeHg-treated groups (0.97 ± 0.14, $n = 36$). With reference to the main effect of diet, the means are as follows: Basal groups (1.27 ± 0.35, $n = 24$), NaCl groups (1.32 ± 0.34, $n = 24$), and MSG groups (0.82 ± 0.01, $n = 24$). The NaCl groups had the highest means compared to the Basal and MSG groups. The main effect of MeHg for the mRNA levels of PVF was significant (F (1.66) = 32.24: $P \leq 0.001$) (Figure 1(a)). In addition, the mean of the MeHg-treated group (1.95 ± 0.14, $n = 36$) was greater than that of the non-MeHg-treated groups (0.81 ± 0.29, $n = 36$).

3.2. Effect of MeHg and Food Additives (NaCl and MSG) in Diet on the Expression of N. cinerea Toll and Rel Target Genes.

There was no significant interaction between MeHg exposure and mRNA levels of TOLL1 (Figure 2(a)). There was also a significant main effect of MeHg (F (1.66) = 8.06: $P \leq 0.001$) for the mRNA levels of REL (Figure 2(b)). The mean of the MeHg-treated group (2.16 ± 0.22, $n = 36$) was greater than that of the non-MeHg-treated groups (1.15 ± 0.13, $n = 36$).

TABLE 1: The primer features of the selected genes.

Target genes	Primer sequence (5′ > 3′)	Annealing temp (°C)
Gapdh	F-CCGTGTCCCTGTTCCTAATG R-GTCCAAGATGCCCTTCAGAG	62
EF1a1	F-CGTGTCTGTTAAGGAACTGC R-CAAGCAATGTGAGCTGTATG	61
Tbp	F-GGTGCGAATGTGGAGTACAG R-TAGTGGCTCCAGTGCAAGTC	64
Tubulin	F-TTGCCAGTGATGAGTTGCTC R-AGCATGTACCAAGGGCAGTT	63
TOLL1	F-TTGTGTTTCTGGACATCAGTCATAA R-CATGCAGATTGTTGTGTTCCA	60
FOXO	F-TCATTCACGGCACTCACCTA R-GGTGCTGAGTCACAAGTCCA	61
EGR	F-CACTTGTATGGCAGGTATTGGA R-GGATGAACACGATGTAAATGGA	63
REAPER	F-CGAAGAGCGAGAGAGGATTG R-CAGGATGGTCTGCTGAAGGT	60
PVF1	F-AGCATTCCACAGCCTCGTAT R-GTTGACTGGGAGTCTGAGCA	60
REL	F-TCGTTTGGAGAAGTTACGAAGC R-ATATTCTGGCAGGCAAATCG	61
UPD3	F-GGAACATCCACCTCTGATCG R-TAGGAGGACCCGAGAATGTG	60
SOCS36E	F-GGTGCTGGTGTCATATTTGAAG R-TCTGCTGGAGATTGAACTGC	60
DUOX	F-TGGGCCTTGGACTACAAAC R-AAATTGATACCAGTCCTGATGC	61

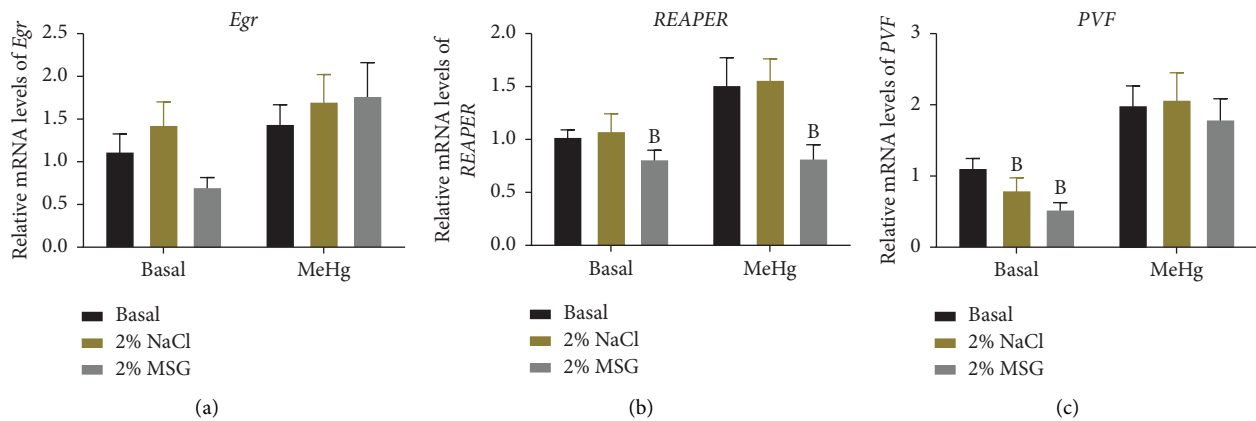F, forward primer; R, reverse primer.

FIGURE 1: Effect of the organometallic cation (MeHg) and food additives (NaCl and MSG) in diet on the expression of *N. cinerea* JNF/JNK target genes: (a) Egr: There was a main effect of MeHg. MeHg = F (1.66) = 6.38: P = 0.01; Diet = F (2.66) = 0.89: P = 0.42; MeHg *Diet = F (2.66) = 1.37: P = 0.26. (b) Reaper: There was a main effect MeHg and Diet. MeHg = F (1.66) = 5.85: P = 0.02; Diet = F (2.66) = 5.64: P = 0.01; MeHg *Diet = F (2.66) = 1.41: P = 0.25. (c) PVF: There was a main effect of MeHg. MeHg = F (1.66) = 32.24: P ≤ 0.001; Diet = F (2.66) = 1.33: P = 0.27; MeHg *Diet = F (2.66) = 0.41: P = 0.67. [b]P < 0.05 vs MeHg + Basal.

3.3. Effect of MeHg and Food Additives (NaCl and MSG) in Diet on the Expression of N. cinerea UPD3 Target Genes. A two-way ANOVA showed a significant main effect of MeHg (F (1.66) = 7.85: P = 0.01) (Figure 3(a)) and diet (F (2.66) = 5.07: P = 0.01) (Figure 3(a)) for the mRNA levels of UPD3. The mean of the MeHg-treated groups (1.48 ± 0.56,

n = 36) was higher than that of the non-MeHg-treated groups (0.99 ± 0.15, n = 36). With reference to the main effect of diet, the means are as follows: Basal groups (1.15 ± 0.16, n = 24), NaCl groups (1.61 ± 0.71, n = 24), and MSG groups (0.94 ± 0.17, n = 24). The NaCl groups had the highest means compared to the Basal and MSG groups.
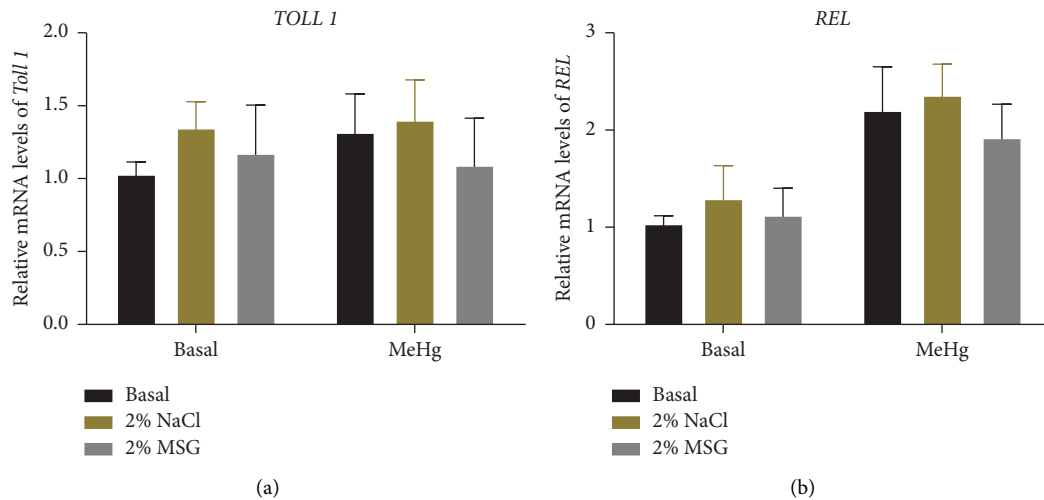
(a)

(b)

FIGURE 2: Effect of the organometallic cation (MeHg) and food additives (NaCl and MSG) in diet on the expression of *N. cinerea* Toll/NF-κB and Rel/NF-κB target genes: (a) Toll 1: MeHg = F (1.66) = 0.17: $P = 0.68$; Diet = F (2.66) = 0.50: $P = 0.61$; MeHg *Diet = F (2.66) = 0.26: $P = 0.77$. (b) Rel: There was a main effect MeHg. MeHg = F (1.66) = 14.79: $P \leq 0.001$; Diet = F (2.66) = 0.47: $P = 0.63$; MeHg *Diet = F (2.66) = 0.17: $P = 0.84$. $^bP < 0.05$ vs. MeHg + Basal.
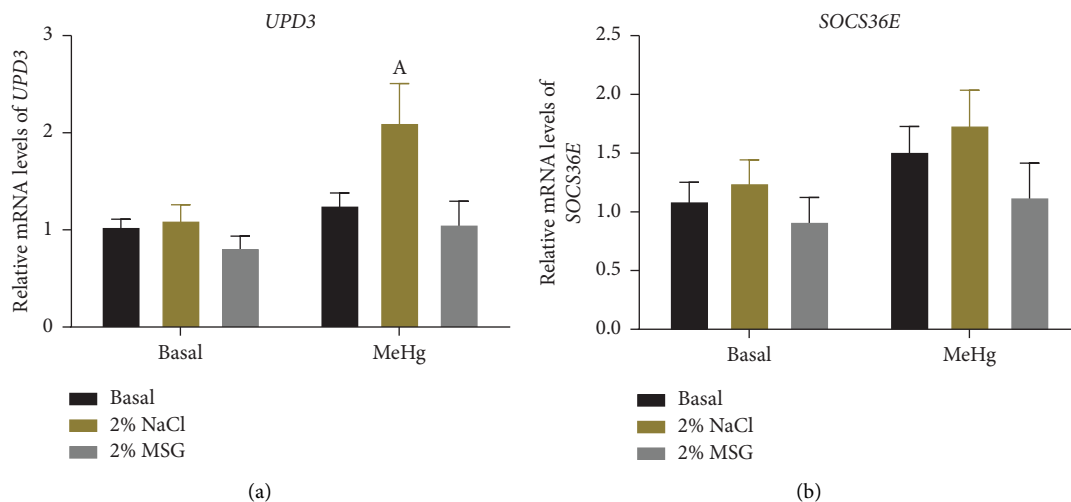

(a)

(b)

FIGURE 3: Effect of the organometallic cation (MeHg) and food additives (NaCl and MSG) in diet on the expression of *N. cinerea* UPD3/JAK-STAT target genes: (a) UPD3: There was a main effect of MeHg and Diet. MeHg = F (1.66) = 7.85: $P = 0.01$; Diet = F (2.66) = 5.07: $P = 0.01$; MeHg *Diet = F (2.66) = 2.19: $P = 0.12$. (b) SOCS36E: there was a main effect MeHg. MeHg = F (1.66) = 3.94: $P = 0.05$; Diet = F (2.66) = 2.12: $P = 0.13$; MeHg *Diet = F (2.66) = 0.20: $P = 0.82$. $^bP < 0.05$ vs. MeHg + Basal.

The main effect of MeHg for the mRNA levels of *SOCS36E* was significant (F (1.66) = 3.94: $P = 0.05$) (Figure 3(b)). Consequently, the mean of the MeHg-treated group (1.46 ± 0.31, $n = 36$) was greater than that of the non-MeHg-treated groups (1.09 ± 0.17, $n = 36$).

3.4. Effect of MeHg and Food Additives (NaCl and MSG) in Diet on the Expression of the N. cinerea DUOX and FOXO Transcription Factor. There was a significant main effect of MeHg (F (1.66) = 4.94: $P = 0.03$) for the mRNA levels of *DUOX* (Figure 4(a)). The mean of the MeHg-treated group (2.24 ± 0.20, $n = 36$) was greater than that of the non-MeHg-treated groups (1.42 ± 0.52, $n = 36$). Two-way ANOVA showed a significant main effect of MeHg (F (1.66) = 6.88:

$P = 0.01$) and diet (F (2.66) = 3.37: $P = 0.04$) (Figure 4(b)) for the mRNA levels of *FOXO*. The mean of the MeHg-treated groups (0.997 ± 0.15, $n = 36$) was lower than that of the non-MeHg-treated groups (1.383 ± 0.40, $n = 36$). With reference to the main effect of diet, the means were as follows: Basal groups (1.08 ± 0.01, $n = 24$), NaCl groups (1.46 ± 0.53, $n = 24$), and MSG groups (1.04 ± 0.30, $n = 24$).

4. Discussion

The crosstalk between redox and inflammatory signaling potentially explains several pathophysiological mechanisms associated with health and disease [12, 14]. Exogenous and endogenous stressors may destabilize local and systemic homeostasis, hence tilting redox-active molecules from their
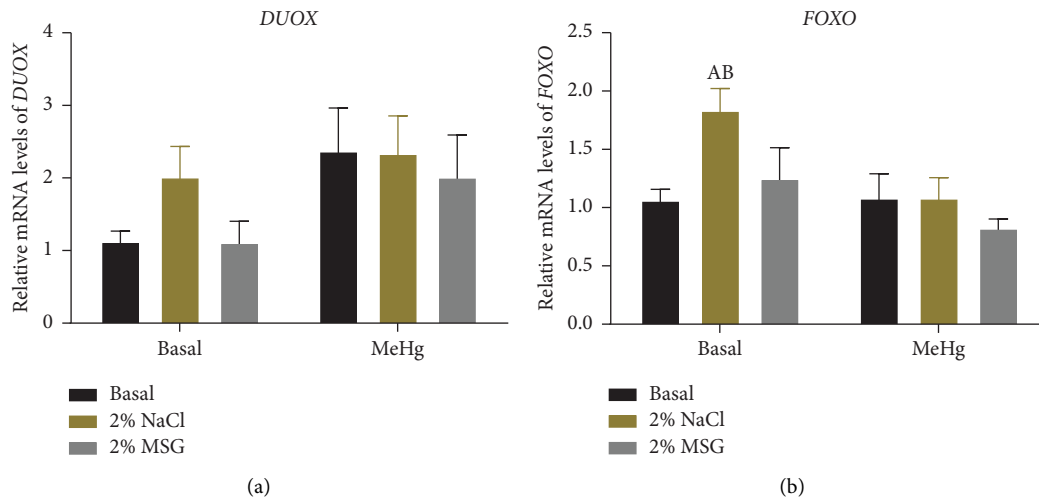
(a)

(b)

FIGURE 4: Effect of the organometallic cation (MeHg) and food additives (NaCl and MSG) in diet on the expression of the *N. cinerea* NOX enzyme (DUOX) and FOXO transcription factor. (a) DUOX: There was a main effect of MeHg. MeHg $= F\,(1.66) = 4.94$: $P = 0.03$; Diet $= F\,(2.66) = 0.97$: $P = 0.39$; MeHg *Diet $= F\,(2.66) = 0.53$: $P = 0.59$. (b) FOX0: There was a main effect MeHg and Diet. MeHg $= F\,(1.66) = 6.88$: $P = 0.01$; Diet $= F\,(2.66) = 3.37$: $P = 0.04$; MeHg *Diet $= F\,(2.66) = 2.30$: $P = 0.11$. $^a P < 0.05$ vs Basal, $^b P < 0.05$ vs. MeHg + Basal.

role in redox signaling to that of mediators of biological damage—–oxidative stress. For example, cellular perturbations from infection or disruption in tissue integrity predispose to inflammatory responses that upregulate redox-active molecules around the site of perturbation [30]. However, this active inflammatory response might disrupt homeostatic balance to become pathologically chronic, if unconstrained. Hence, redox-active molecules can exacerbate the inflammatory process, and inflammation can increase the levels of redox-active molecules, predisposing to oxidative stress [14, 31]. Here, we used the *Nauphoeta cinerea* model to examine the mechanisms of redox and inflammatory response to heavy metal contaminants and additives in food.

JNK is a mitogen-activated protein kinase that is conserved from insects to mammals [32]. JNK activation in response to stressors and as part of the regulatory framework for proliferation and apoptosis of cells or morphogenesis of tissue has been a well-documented [33, 34]. Likewise, several inflammatory mechanisms have been linked with JNK activation, including thymocyte proliferation, T-cell stimulation, and the secretion of interleukin-2 and tumor necrosis factor alpha [33–37]. We have recorded upregulated target genes of the JNK pathway (early growth factor and reaper) in insects exposed to both the food contaminant (MeHg) and the food additives (NaCl and MSG) that we have earlier shown to induce oxidative stress [25], thereby supporting findings of JNK-mediated crosstalk between oxidative stress and inflammatory response in insects, fish, and mammals [12, 14, 38, 39].

Moreover, regulatory proteins sharing sequence homology over amino acid regions called the rel domain are present in the *Drosophila melanogaster* morphogen—dorsal and the mammalian transcription factor-NF-κB [40]. Rel proteins bind cis-regulatory sequences after nuclear localization to upregulate the transcription of their target genes, including inflammation-related genes [41, 42]. Hence, our result of increased rel expression indicates possible rel-mediated amplification of the inflammatory response to MeHg, NaCl, and MSG coexposure, in line with reports of xenobiotic-induced rel inflammatory cascade in insects and fish [43, 44]. On the other hand, the cytokine, unpaired 3 (UPD3) which is homologous to the human interleukin-6 and the *Drosophila melanogaster* JAK/STAT pathway, has been shown to control wound repair, curtail inflammatory response, and enhance homeostasis and development, with negative regulation from the suppressor of cytokine signaling at 36E (SOCS36E) [45, 46]. Our report of UPD3 upregulation in cockroaches exposed to MeHg, NaCl, and MSG corroborates studies showing that UPD3 activation is a response to redox and inflammatory disruptions [14, 47, 48].

Oxidative stress and inflammation are common denominators across systemic pathologies, making it important to understand the crosstalk between both mechanisms [49–51]. We have previously shown increased oxidative stress in insects exposed to the food contaminant MeHg and the food additives NaCl and MSG [24]. Here, we buttressed those results with evidence of upregulation of a regulator of cellular reactive oxygen species generation—DUOX, alongside other results of upregulated inflammation-related genes (JNK and Rel), thereby corroborating mechanistic reports detailing inflammatory and oxidative stress responses to xenobiotic exposure via food [52, 53]. Besides, the O class of the forkhead transcription factors (FOXO) which have been shown to protect cells from oxidative stress via antioxidant regulation [54] was not significantly affected by MeHg, NaCl, and MSG exposure in diet, suggesting that the cockroach is unable to regulate MeHg-induced redox disruption via FOXO-mediated antioxidant signaling, similar to reports of downregulated FOXO signaling during exposure of insects to heavy metals [34].

5. Conclusion

Methylmercury contamination of food as well as the coexposure to food additives (NaCl and MSG) activates the redox-inflammation crosstalk in neural tissues of *Nauphoeta cinerea* via JNK, REL, and DUOX signaling.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Disclosure

This study is part of the doctoral thesis submitted by Blessing A. Obafemi to the Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul. The funding body has no role in the study, collection, analysis, and interpretation of data.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

B. A. Obafemi was responsible for conceptualization, data curation, investigation, formal analysis, supervision, preparation of original draft, resource validation, review and editing, methodology, software visualization, and funding acquisition. I. A. Adedara was responsible for investigation, review and editing, and methodology. A. L. A. Segatto was responsible for investigation, resource validation, review and editing, and methodology. D. O. Souza was responsible for conceptualization, investigation, supervision, resource validation, and funding acquisition. J. B. T Rocha was responsible for conceptualization, data curation, investigation, formal analysis, supervision, resource validation, review and editing, methodology, software visualization, and funding acquisition. O. C. Olagoke was responsible for conceptualization, investigation, resource validation, preparation of original draft, review and editing, methodology, and software visualization.

Acknowledgments

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