

Ameliorative Effects of Kaempferol and Zinc Gluconate on Erythrocyte Osmotic Fragility and Haematological Parameters in Wistar Rats Exposed to Noise Stress

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Abstract

Noise pollution, especially in the urban environment, is on the increase and ranks among the environmental stressors with the highest public health impact. Oxidative stress and lipid peroxidation are involved in the molecular mechanism underlying increase in EOF of rats exposed to noise stress. The aim of this study was to investigate effects of kaempferol and zinc gluconate on neurobehavioural changes in Wistar rats exposed to noise stress. Noise stress Wistar rats were exposed to 100 dB (4 hr/day) for 15 days. Exposure of Wistar rats to noise stress caused significant increase in EOF but significantly decreased haematological parameters. Kaempferol + zinc significantly prevented decrease ($P < 0.05$) in haemoglobin (14.32 ± 0.11 g/dL), packed cell volume ($43.47 \pm 0.30\%$) and erythrocyte counts ($7.20 \pm 0.06 \times 10^{12}/L$). Values of mean corpuscular haemoglobin (20.40 ± 0.33 μ g) and mean corpuscular haemoglobin concentration ($33.20 \pm 0.15\%$) were highest in kaempferol + zinc treated rats. Administration of kaempferol + zinc caused leucocytosis due to neutrophilia and lymphocytosis as well as a significant ($P < 0.05$) decrease in N/L ratio (0.25 ± 0.01). In conclusion, treatment with kaempferol and zinc singly and in combination ameliorated noise-induced increase in EOF of Wistar rats. Singly, kaempferol was more potent than zinc. In combination, kaempferol and zinc acted synergistically to produce an additive effect.

Keywords: Kaempferol; Zinc; Antioxidant; Noise; Erythrocyte fragility; Oxidative stress

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Introduction

Noise was derived from the Latin term 'nausea' and has been defined as unwanted sound, which is a potential hazard to communication and health [1]. Stress refers to a non-specific response of the body to unpleasant stimuli, threatening homeostasis and the integrity of the organism [2,3]. It is a state of threatened homeostasis provoked by psychological, physiological and environmental stressors [4]. Noise is measured in decibel (dB) units [1].

Wang et al. [5] reported that noise exposure is a potent stressor as it increases the levels of the stress hormone, corticosterone. Noise pollution, especially in the urban environment, is on the increase [6-8] and ranks among the environmental stressors with the highest public health impact [8]. The auditory effects

include hearing impairment and permanent hearing loss due to excessive noise exposure. The non-auditory effects include stress-related, physiological and behavioural effects. Noise stress induces increased reactive oxygen and nitrogen species (ROS and RNS) generation, which are capable of breaking down lipid and protein molecules and damaging DNA; triggering loss of function and cell death [4,9]. The ROS also trigger apoptosis by activating pro-apoptotic mitogen activated protein (MAP) kinase-signaling pathways [10]. Antioxidants are molecules that inhibit and scavenge ROS/RNS and convert them to less dangerous molecules [11]. Noise-induced hearing loss has global implications, with 10 million adults and 5.2 million children in the US, and 250 million people world-wide having a noise-induced hearing loss greater than 25 dB; a clinically significant hearing loss [10]. Additionally, occupational noise accounts for 16% of the disabling hearing loss

in adults world-wide, resulting in decreased economic production [12]. Data confirm that exposure to traffic noise, not specifically at night, is associated with increased incidence of diabetes mellitus [13] hypertension [14] stroke among the elderly [15], and mortality from coronary heart disease [12,15-17]. According to WHO [8], noise causes health damage every day estimated at 4 million dollars. Moreover, psychoneurotic and psychosomatic complaints are also observed due to noise exposures [18].

Noise treatment of 80 dB resulted in a significant elevation of heterophil to lymphocyte ratio, signifying stress response of the broilers. Noise treatment of both 70 and 80 dB intensities also resulted in a significant upsurge of basophils [19] and leucocyte counts but markedly reduced the level of haemoglobin in the blood [20,21] reported an increase in erythrocyte and leucocyte counts except for monocytes in humans working in noisy environments.

The flavonoid kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a yellow compound with a low molecular weight (MW: 286.2 g/mol) which is commonly found in plant-derived foods and in plants used in traditional medicine [22,23]. It belongs to the family Zingiberaceae [24] and is frequently found in fruits, vegetables and some beverages. Abundant natural sources of kaempferol include apple, tea, tomatoes, green beans, green cabbage, olive oil, spinach, grape fruit, tree spinach, cucumber, sweet potatoes, broccoli, strawberries, cowpea, grapes, and aloe-vera, leek, onions, chives, amaranth, horseradish, chinese cabbage, mustard, broccoli, turnip greens and lettuce [25,26].

Zinc (Zn) is the next most abundant and essential trace element in the body after iron. It performs multi-functional biological roles, and up to 10% of all proteins in mammalian cells require Zn for their break-down, conformational modification or activity [26-28]. Zn is required for the activity of over 300 enzymes, and as such it partakes in many enzymatic and metabolic functions in the body [29]. Zinc, a divalent cation with manifold biochemical and physiological functions, may play principal neuromodulatory roles in the central nervous system. Indeed, zinc is co-released with glutamate, and it modulates glutamatergic excitation by inhibiting N-methyl-D-aspartate (NMDA) receptors, and averts gamma-aminobutyric acid (GABA) inhibition by blocking GABA-A receptor function [30,31] reported that zinc exerts its antioxidant property in ameliorating the effect of chlorpyrifos-induced erythrocyte fragility in Wistar rats. Neuropsychological performance has been reported to improve with Zn supplementation in young Chinese children, especially when other micronutrient nutrition is sufficient [27,28].

Materials and Methods

Location of experiment

The experiment was carried out in the laboratory at the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, (11° 10' N, 07° 38' E), located in the Northern Guinea Savannah zone of Nigeria.

Animals: A total of 30 eight-week-old Wistar rats, weighing

between 110 and 120 g and belonging to both sexes served as subjects. They were purchased from the breeding stock of the National Veterinary Research Institute, Vom, Nigeria, and maintained in metal cages under standard laboratory conditions in the animal holding facility of the Department. All efforts were made to minimise unwanted stress or discomfort to the animals during experimental procedures by ensuring minimal handling. The rats were given access to standard commercially-prepared rat pellet feeds (Vital Feeds, Jos, Nigeria) and water ad libitum.

Experimental groups: Rats were divided into five groups by simple randomisation, with each group consisting of six animals as follows: Groups I and II were administered with deionized water; Group III, kaempferol; Group IV, zinc gluconate; Group V, kaempferol + zinc gluconate for 36 days. Groups II, III, IV and V were subjected to noise stress of 100 dB dose for 15 days from day 22 to day 36. Behavioural activities were assessed in all groups on days 1, 8, and 15 after noise exposure and in the controls.

Drug and dosage: Kaempferol and zinc gluconate were purchased commercially (Aldrich Chemical Co. Ltd., Gillingham Dorset, England. Cat. No. 25,556-4) and administered orally at a dose rate of 5 mg/kg body weight [22] and 50 mg Zn/kg body weight [32] respectively. They were reconstituted in deionised water (50 mg mL⁻¹) prior to oral administration while the controls were given deionised water at a dose of 2 mL/kg body weight.

Noise stress induction: Noise stress was induced using a modification of the method described by Wankhar et al. [2]. Briefly, noise was produced by a loud speaker (15 W), installed at a distance of 30 cm above the cages for Groups II to V, and driven by a white noise generator (DT-8850; Shenzhen Technology Co. Ltd., Shenzhen, Guangdong, China), emitting frequencies in the range 0–20 kHz. A precision sound level meter was used to set the intensity of sound to 100 dB uniformly in the cage. During the experiment, the noise level peaked at 100 dB/4 h/day for 15 days. Noise intensity was continuously measured by a digital sound level metre (Voltcraft SL-200, Hirschau, Germany). The intensity of 100 dB was chosen because it reflects the common noise level in industrial workplaces and noisy environments, typical in Nigeria [1].

Collection and analyses of biomarkers of oxidative stress: At the end of the experimental period, all the rats were exposed to mild anaesthesia and 1.5 mL blood collected from the internal jugular vein of each. Thereafter, all the rats were sacrificed under deep anaesthesia and brain samples collected. The freshly collected brain samples were sonicated, suspended in phosphate-buffered saline and then vortexed for 5-10 seconds. The mixture was centrifuged at 1000 g for 15 min and the supernatant was decanted into sterile test tubes, and stored appropriately.

Determination of haematological parameters: Blood (1 mL) was collected from each rat into sample bottles, containing ethylenediaminetetraacetic acid (9:1) for the evaluation of haematological parameters. Haematological parameters of PCV, haemoglobin concentration, erythrocyte counts, erythrocytic indices, platelet count, absolute and differential leucocyte counts were determined using the automated haematologic analyser (Sysmex, KX-21, Japan).

Erythrocyte osmotic fragility: The erythrocyte osmotic fragility (EOF) of rats from each group (n = 6) was determined as an index of haemolysis according to the method described by Faulkner and King (1970) and modified by Oyewale et al. ([33]). Briefly, 0.02 mL of blood was added to tubes, containing increasing concentrations of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0.1%, 0.3%, 0.5%, 0.7% and 0.9% NaCl concentrations). The tubes were gently mixed and maintained at room temperature (24°C to 26°C) for 30 min. The content of each tube was centrifuged at 1500 x g for 10 min and the supernatant decanted. The haemoglobin content of the supernatant was determined spectrophotometrically at a wavelength of 540 nm, with distilled water serving as blank. The percentage haemolysis in each concentration of NaCl was evaluated, taking the tube with maximum haemolysis as 100%.

Percentage haemolysis (%) = Optical density of test × 100/ Optical density of standard

Graphs of percentage haemolysis against concentration was plotted to determine initial haemolysis, median corpuscular fragility, and maximum haemolysis values.

Data analyses: Data obtained from the study were expressed as mean ± standard error of mean (± SEM) and subjected to repeated-measures analysis of variance (ANOVA), followed by Tukey's *post-hoc* test to evaluate significant difference between the groups. Graphpad Prism, version 6.0 for Windows (Graphpad Software, San Diego, CA, USA, www. Graphpad.com) was used to analyse all the data. Values of $P < 0.05$ were considered significant.

Results

Effect of kaempferol and zinc on erythrocyte osmotic fragility in Wistar rats exposed to noise stress

The effect of the various treatments on erythrocyte osmotic fragility of the rats is presented in **Figure 1**. The EOF across the treatment groups decreased as NaCl concentrations increased from 0% to 0.9%. There was a significant decrease consistently in the EOF of groups treated with kaempferol + noise (6.22 ± 0.97, 4.88 ± 0.33, 43.02 ± 7.75, 76.78 ± 3.07), zinc + noise (6.83 ± 0.98, 5.13 ± 0.27, 33.12 ± 2.99, 83.80 ± 2.26) and kaempferol + zinc + noise (5.67 ± 1.12, 3.35 ± 0.30, 34.75 ± 3.73, 76.52 ± 2.00) compared to the group treated with noise + deionised water (10.45 ± 1.59, 12.00 ± 1.56, 74.73 ± 6.70, 95.42 ± 0.82) at 0.3%, 0.5%, 0.7% and 0.9% concentration of NaCl respectively.

There was a significant decrease ($P < 0.05$) in the haemoglobin concentration obtained in the group treated with deionised water + noise (12.62 ± 0.12 g/dL), compared to those treated with deionised water (14.27 ± 0.11 g/dL) and kaempferol + zinc + noise (14.32 ± 0.11 g/dL). There was no significant difference in haemoglobin concentration in the group treated with kaempferol + noise (13.72 ± 0.09 g/dL) and zinc + noise treated group (13.40 ± 0.12 g/dL). Kaempferol + zinc + noise group was significantly ($P < 0.05$) higher (14.32 ± 0.11 g/dL) compared to the group treated with kaempferol + noise (13.72 ± 0.09 g/dL) and zinc + noise treated group (13.40 ± 0.12 g/dL).

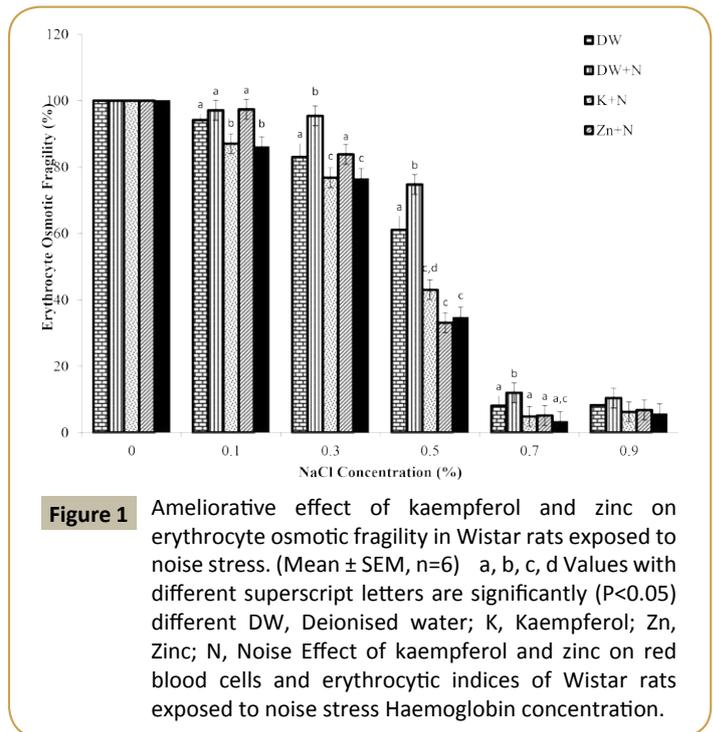


Figure 1 Ameliorative effect of kaempferol and zinc on erythrocyte osmotic fragility in Wistar rats exposed to noise stress. (Mean ± SEM, n=6) a, b, c, d Values with different superscript letters are significantly ($P < 0.05$) different DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise Effect of kaempferol and zinc on red blood cells and erythrocytic indices of Wistar rats exposed to noise stress Haemoglobin concentration.

Packed cell volume: The packed cell volume of rats in the deionised water + noise-treated group (37.85 ± 0.35%) significantly ($P < 0.05$) decreased compared with the values obtained in the deionised water-treated group (43.40 ± 0.29%), kaempferol + noise group (41.75 ± 0.24%), zinc + noise group (40.78 ± 0.33%) and kaempferol + zinc + noise treated group (43.47 ± 0.30%) (**Table 1**).

Total erythrocyte count: A significant ($P < 0.05$) decrease was observed in the total erythrocyte count of rats in the deionised water + noise-treated group (6.43 ± 0.04 × 10¹²/L) compared with counts obtained in the deionised water-treated group (7.19 ± 0.05 × 10¹²/L) and kaempferol + zinc + noise treated group (7.20 ± 0.06 × 10¹²/L). The total erythrocyte counts obtained in kaempferol + noise (6.90 ± 0.07 × 10¹²/L) was not significantly ($P < 0.05$) higher than that obtained in zinc + noise treated group (6.65 ± 0.07 × 10¹²/L).

Erythrocytic indices: In the group treated with deionised water + noise, the erythrocytic indices of mean corpuscular volume (58.87 ± 0.29), mean corpuscular haemoglobin (18.43 ± 0.3 µg) and mean corpuscular haemoglobin concentrations (29.65.20 ± 0.89%) were significantly ($P < 0.05$) decreased compared to the group treated with zinc + noise which had the highest mean corpuscular volume (61.35 ± 0.67 fL) and the group treated with kaempferol + zinc + noise which had the highest mean corpuscular haemoglobin (20.40 ± 0.33 µg) and mean corpuscular haemoglobin concentration (33.20 ± 0.15%). There was no significant ($P < 0.05$) difference between the group treated with kaempferol + noise and zinc + noise.

Platelet count: Platelet count significantly ($P < 0.05$) decreased in the group treated with deionised water + noise (439.80 ± 7.91 × 10⁶ g/dL), compared with the group treated with deionised water

Table 1 Ameliorative effect of kaempferol and zinc on erythrocytic indices of Wistar rats exposed to noise stress (Mean \pm SEM, n=6).

Parameters	Group				
	DW	DW+N	K+N	Zn+N	K+Zn+N
Hb (g/dL)	14.27 \pm 0.11 ^a	12.62 \pm 0.12 ^b	13.72 \pm 0.09 ^c	13.40 \pm 0.12 ^d	14.32 \pm 0.11 ^e
PCV (%)	43.40 \pm 0.29 ^a	37.85 \pm 0.35 ^b	41.75 \pm 0.24 ^c	40.78 \pm 0.33 ^d	43.47 \pm 0.30 ^e
RBC(X10 ¹² /L)	7.19 \pm 0.05 ^a	6.43 \pm 0.04 ^b	6.90 \pm 0.07 ^c	6.65 \pm 0.07 ^d	7.20 \pm 0.06 ^e
MCV(fl/Cell)	60.38 \pm 0.36 ^b	58.87 \pm 0.29 ^a	60.52 \pm 0.35 ^b	61.35 \pm 0.67 ^b	60.37 \pm 0.18 ^b
MCH (pg)	19.85 \pm 0.13 ^a	18.43 \pm 0.34 ^b	20.30 \pm 0.21 ^c	20.17 \pm 0.21 ^d	20.40 \pm 0.33 ^e
MCHC (g/dL)	32.88 \pm 0.03 ^a	29.65 \pm 0.89 ^b	32.92 \pm 0.04 ^c	32.85 \pm 0.02 ^d	33.20 \pm 0.15 ^e

^{a-e} Values along the same row with different superscript letters are significantly ($P<0.05$) different
Hb: Haemoglobin; PCV: Packed Cell Volume; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; DW: Deionised Water; K: Kaempferol; Zn: Zinc; N: Noise

Table 2 Ameliorative effect of kaempferol and zinc on platelet and leucocyte counts of Wistar rats exposed to noise stress (Mean \pm SEM, n=6).

Cells (X10 ⁶ g/dL)	Group				
	DW	DW+N	K+N	Zn+N	K+Zn+N
Leucocytes	6.26 \pm 0.04 ^a	4.90 \pm 0.06 ^b	5.46 \pm 0.02 ^b	5.29 \pm 0.04 ^b	5.81 \pm 0.09 ^a
Neutrophils	1.11 \pm 0.02 ^a	0.88 \pm 0.02 ^b	0.89 \pm 0.03 ^b	0.93 \pm 0.04 ^a	1.41 \pm 0.05 ^a
Lymphocytes	4.95 \pm 0.01 ^a	2.50 \pm 0.01 ^b	4.44 \pm 0.02 ^c	4.15 \pm 0.03 ^c	5.66 \pm 0.01 ^a
Monocytes	0.02 \pm 0.01 ^a	0.98 \pm 0.02 ^b	0.03 \pm 0.03 ^a	0.04 \pm 0.01 ^a	0.02 \pm 0.02 ^a
Basophils	0.00 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.02	0.01 \pm 0.01	0.01 \pm 0.02
N/L Ratio	0.23 \pm 0.01 ^a	0.35 \pm 0.03 ^b	0.20 \pm 0.02 ^a	0.22 \pm 0.03 ^a	0.25 \pm 0.01 ^a
Platelets	637.30 \pm 9.26 ^a	439.80 \pm 7.91 ^b	532.70 \pm 9.12 ^c	529.70 \pm 5.43 ^c	609.20 \pm 6.90 ^a

^{a-e} Values along the same row with different superscript letters are significantly ($P<0.05$) different
DW: Deionised Water; K: Kaempferol; Zn: Zinc; N: Noise

(637.30 \pm 9.26 \times 10⁶ g/dL), kaempferol + noise + zinc (609.20 \pm 6.90 \times 10⁶ g/dL), kaempferol + noise (532.70 \pm 9.12) and zinc + noise (529.70 \pm 5.43 \times 10⁶ g/dL). The group treated with deionised water had the highest platelet count (637.30 \pm 9.26 \times 10⁶ g/dL) followed by the kaempferol + noise + zinc treated group (609.20 \pm 6.90 \times 10⁶ g/dL). There was no significant difference in the counts between the groups treated with kaempferol + noise (532.70 \pm 9.12 \times 10⁶ g/dL) and zinc + noise (529.70 \pm 5.43 \times 10⁶ g/dL).

Absolute and differential leucocyte counts: The total leucocyte count obtained in deionised water + noise-treated group (4.90 \pm 0.06 \times 10⁶ g/dL) was significantly ($P<0.05$) lower, when compared with the count recorded in the group treated with deionised water (6.26 \pm 0.04 \times 10⁶ g/dL), kaempferol + noise (5.46 \pm 0.02 \times 10⁶ g/dL), zinc + noise (5.29 \pm 0.04 \times 10⁶ g/dL), and kaempferol + zinc + noise treated group (5.81 \pm 0.09 \times 10⁶ g/dL).

The number of neutrophils obtained in deionised water + noise-treated group (0.88 \pm 0.02 \times 10⁶ g/dL) was significantly ($P<0.05$) lower than the counts recorded in the group treated with deionised water (1.11 \pm 0.02 \times 10⁶ g/dL), zinc + noise (0.93 \pm 0.04 \times 10⁶ g/dL) and kaempferol + zinc + noise treated group (1.41 \pm 0.05 \times 10⁶ g/dL). Similarly, the number of lymphocytes in the deionised water + noise-treated group (2.50 \pm 0.01 \times 10⁶ g/dL) was significantly ($P<0.05$) lower, when compared with the group treated with deionised water (4.95 \pm 0.01 \times 10⁶ g/dL), kaempferol + noise (4.44 \pm 0.02 \times 10⁶ g/dL), zinc + noise (4.15 \pm 0.03 \times 10⁶ g/dL), and kaempferol + zinc + noise treated group (5.66 \pm 0.01 \times 10⁶ g/dL).

The number of monocytes obtained in the deionised water + noise-treated group (0.98 \pm 0.22 \times 10⁶ g/dL) was significantly

($P<0.05$) higher when compared with the group treated with deionised water (0.02 \pm 0.01 \times 10⁶ g/dL), kaempferol + noise (0.03 \pm 0.03 \times 10⁶ g/dL), zinc + noise (0.04 \pm 0.01 \times 10⁶ g/dL) and kaempferol + zinc + noise treated group (0.02 \pm 0.02 \times 10⁶ g/dL). There was no significant ($P>0.05$) difference in the number of circulating basophils in all the treatment groups (**Table 2**).

The N/L ratio obtained in deionised water + noise-treated group (0.35 \pm 0.03) was significantly ($P<0.05$) higher, when compared with the group treated with deionised water (0.23 \pm 0.01), kaempferol + noise (0.20 \pm 0.02), zinc + noise (0.22 \pm 0.03) and kaempferol + zinc + noise treated group (0.25 \pm 0.01) (**Table 1**).

Discussion

Effect of treatments on erythrocyte osmotic fragility in wistar rats exposed to noise stress

Erythrocyte osmotic fragility (EOF) which measures the resistance of erythrocyte to intracellular pressure is known to be a biomarker of oxidative stress or lipid peroxidation [34].

Zinc promotes cell membrane integrity and its deficiency is associated with increased EOF [35]. The present study demonstrated an increase in erythrocyte osmotic fragility following noise stress exposure in rats, apparently due to increased oxidative damage resulting in the impairment of erythrocyte membrane integrity and haemolysis. The result showed that administration of kaempferol and zinc gluconate protected the integrity of erythrocyte membranes of rats, exposed to noise stress as indicated by the significant decrease in erythrocyte osmotic fragility of rats treated with the antioxidants.

The finding that greater membrane stability was observed in the group treated with kaempferol and zinc, compared to the groups separately treated with kaempferol and zinc indicates that kaempferol and zinc act synergistically to ameliorate noise-stress induced increase in erythrocyte osmotic fragility in Wistar rats. This finding affirms the role of oxidative stress in the pathophysiology of noise stress. The result of the current study is in agreement with that of Sharma et al. [36] who reported that zinc ameliorates erythrocyte fragility in Wistar rats' deficient of zinc. The improved erythrocyte membrane integrity observed in treated rats in the current study may have been mediated by the antioxidant activity of kaempferol and zinc which could have elevated the levels of SOD, GPx, reduced ROS production and promotes cell membrane integrity. Besides, zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals [37]. Also, increase in erythrocyte fragility has been associated with decrease in zinc concentration in RBC membrane resulting in reduced plasma sulphhydryl concentration [38]. Although, the present study did not measure erythrocyte zinc concentration, [39] increased oxidative demand leads to lowered erythrocyte zinc concentration. This may have accentuated the erythrocyte fragility in the untreated noise group. This is because zinc deficiency results in lower superoxide dismutase activity [40] and a greater susceptibility to oxidative damage from elevated peroxynitrite concentrations [41]. Similarly, lowered zinc level alters the composition of erythrocyte membranes [42] and also impairs the activity and function of Na⁺ K⁺ ATPase, which is important for cell membrane stability [43]. Furthermore, decrease in cellular zinc concentration has been associated with apoptosis induction via the protein kinase C-dependent pathways [44] and activation of apoptotic protease, caspase-3 [45]. Therefore, the amelioration of noise stress-induced erythrocyte fragility by zinc may have partly resulted from its ability to inhibit caspase-3 [46] and the repletion of zinc deficit caused by oxidative stress [44]. Thus, kaempferol and zinc gluconate have antioxidant potentials in the amelioration of noise stress in Wistar rats.

Effect of zinc and kaempferol on haematological indices of wistar rats exposed to noise stress

In the central nervous system, sound proceeds in two directions; one to the auditory centre where it is perceived and interpreted; and the other to the deep parts of brain, where it activates the autonomic nervous system and is liable for a wide range of monaural effects. Noise is stressful and activates the hypothalamo-pituitary-adrenal (HPA) axis, resulting in the release of corticosteroid hormones from the adrenal gland into circulation, and may cause hearing loss and other health impairments such as headache, hypertension, coronary and other cardiovascular diseases [2,10]. Haematology is important in evaluating the health and nutritional status of an animal [47]. In addition, the use of neutrophil-lymphocyte (N/L) ratio is a sensitive haematological indicator of stress response [48]. In the current study, haematological parameters of noised-exposed Wistar rats were significantly ($P < 0.05$) affected. The PCV, Hb concentration and total erythrocyte count were decreased. The findings from this study are in agreement with the results obtained

by Kumar et al. [49] and Minka and Ayo [50] who reported. That the decrease observed in the PCV, Hb and total erythrocyte count in the stressed rats may be due to increased lipid peroxidation in erythrocyte cell membranes and subsequent haemoglobin oxidation [50]. Treatment with kaempferol and zinc singly and in combination ameliorated the decrease. Zinc deficiency has been reported to cause mild to moderate anaemia in growing rats [51], which is probably due to impeded erythropoiesis and protein synthesis. This is connected to the involvement of zinc in a wide spectrum of biological activities. MCV refers to the average size of individual erythrocyte [52] and younger erythrocyte are larger than older ones [53]. Therefore, the presence of high MCV may indicate an active erythropoiesis. The significantly higher MCV and MCHC coupled with the increase in PCV demonstrate a more efficient erythropoiesis in rats treated with both antioxidants.

Kaempferol has been shown to inhibit peroxidation of membrane phospholipids and acts as ROS scavenger [54] The ameliorative effect of kaempferol may also be due to its ability to improve the erythrocyte membrane integrity by mitigation of oxidative damage to the erythrocyte membrane.

The present study also revealed that repeated exposure of Wistar rats to noise may adversely affect the immune cells. The significant increase in neutrophils and lymphocytes count in the groups treated with kaempferol and zinc, compared to the exposed group treated with deionized water + noise indicates that kaempferol and zinc protected neutrophils and lymphocytes from noise-induced destruction. Circulating neutrophils are committed to apoptosis by ROS, which is produced by activated cells. Kaempferol and zinc may protect neutrophils by exerting antioxidant activity. In addition, the amount of circulating lymphocytes and haemoglobin decreased in noise-exposed animals. This report concurs with that of [20], who reported a significant decrease in leucocyte count of animals exposed to noise stress. Algers et al. [55] also reported decrease in haemoglobin counts of animals exposed to noise stress. This further shows that noise plays a role in the homeostasis of the immune system. Evidence that noise stress induces immunosuppression [55] and oxidative stress has been implicated in noise-induced immunosuppression [2]. The finding of the present study agrees with that of Minka and Ayo ([50] who obtained an increase in lymphocyte counts following ascorbic acid supplementation in transported goats exposed to heat stress. It also agrees with the finding of [56] that the zinc content of malases ameliorates EOF in chickens exposed to heat stress. This finding also further confirms that oxidative stress plays an important role in immunosuppression due to noise exposure. The increase in neutrophil/lymphocyte ratio observed in the noise-exposed group was in agreement with earlier findings [19] that noise stress cause significant increase in neutrophil/lymphocyte ratio. Thus, the administration of kaempferol and zinc decreased the neutrophil/lymphocyte ratio in the present study. The increase in neutrophil/lymphocyte ratio observed in the present study is also in agreement with the findings of Minka and Ayo [50] that the ratio increases in stress situations, especially in those induced via ROS mechanism. Thus, the administration of kaempferol and zinc

decreased the ratio in the present study. This finding confirms the previous result obtained by Ayo and Minka [57] that ascorbic acid ameliorates the risk of adverse effects due to ROS-induced cell damages and destruction. This study therefore postulates that kaempferol and zinc prevent the risk of noise stress-induced adverse effects due to ROS-evoked cell damages and destruction in Wistar rats.

The finding that there was no effect of the treatments on circulating basophils in the rats exposed to noise, apparently, implies that inflammatory response was not a sequelae of noise stress in this study. This result contradicts the report of Bedanova et al. [19], who reported that noise exposure of both 70 and 80 dB intensities in broilers result in a significant upsurge of basophil counts. The reason for this variation may be due to difference in species. The decrease in the monocyte count observed in the kaempferol and zinc treatment group indicates that the antioxidants may synergistically mitigate noise-induced stress in Wistar rats. The significant increase in platelet count observed in the kaempferol and zinc-treated group, compared to the group

treated with deionized water only, may be attributed to the antioxidant role of the antioxidants that scavenge ROS, involved in lipid peroxidation within platelet membrane [58].

Conclusion

Exposure of Wistar rats to noise stress caused significant increase in EOF and alteration in haematological parameters. Oxidative stress and lipid peroxidation are involved in molecular the mechanism underlying EOF of rats exposed to noise stress. Treatment with kaempferol and zinc singly and in combination ameliorated noise-induced increase in EOF alongside decrease in haematological parameters of Wistar rats exposed to noise stress. Singly, kaempferol was more potent than zinc. In combination, kaempferol and zinc acted synergistically to produce an additive effect. Further studies of specific regions of the brain and optimum dose of kaempferol and zinc for the mitigation of noise stress should be ascertained. High risk population are advised to increase consumption of foods containing kaempferol and zinc antioxidants.

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