AMELIORATIVE EFFECTS OF KAEMPFEROL AND ZINC GLUCONATE ON HAEMATOLOGICAL, NEUROBEHAVIOURAL AND OXIDATIVE STRESS CHANGES IN WISTAR RATS EXPOSED TO NOISE STRESS

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ZARIA, NIGERIA

JULY, 2017

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY,

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ZARIA, NIGERIA

JULY, 2017

DECLARATION

I declare that the work in the dissertation, entitled **"Ameliorative effects of Kaempferol and Zinc gluconate on Haematological, Neurobehavioural and Oxidative Stress Changes in Wistar Rats exposed to Noise Stress"** has been carried out by me in the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Isaac Oluwatobi AKEFE

Name of Student	Signature	Date

CERTIFICATION

This dissertation, entitled **"Ameliorative effects of Kaempferol and Zinc gluconate on Neurobehavioural, Haematological and Oxidative Stress Changes in Wistar Rats exposed to Noise Stress"** by Isaac Oluwatobi AKEFE, meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to God Almighty, my Help in ages past and in time to come.

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ABSTRACT

The aim of the study was to investigate effects of kaempferol and zinc gluconate administration on haematological, neurobehavioural and oxidative stress changes in Wistar rats, exposed to noise stress. Thirty (30) rats were randomly divided into five groups: Groups I and II were administered with deionised 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 on days 1, 8 and 15 after noise exposure. The effects of the different treatments on body weight change, open-field activities, neuromuscular coordination, motor strength, excitability scores, sensorimotor reflexes and learning and memory were assessed in the rats. Packed cell volume, haemoglobin concentration, total erythrocyte count, erythrocytic indices, platelet count neutrophil/lymphocyte ratio, total and differential leucocyte counts and erythrocyte osmotic fragility (EOF) test were determined using standard methods. The brain was used to evaluate noise stress-induced lipoperoxidative changes, through determination of brain malondialdehyde (MDA) concentration and activities of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase, using kits. The results of this study showed that noise stress-induced decrease in body weight gain was significantly ($P < 0.05$) ameliorated in the group treated with kaempferol + zinc (123.70 ± 0.99 g, 133.70 ± 1.59 g) than in the group treated with deionised water + noise (117.70 ± 1.02 g, 125.70 ± 1.20 g). There was a significant and consistent decrease in the EOF of rats treated with kaempferol + zinc. Kaempferol + zinc gluconate significantly ($P < 0.05$) ameliorated decrease in haemoglobin concentration (from 12.62 ± 0.12 to 14.32 ± 0.11 g/dL), packed cell volume (from 37.85 ± 0.35 to 43.47 ± 0.30 %) and erythrocyte counts (from 6.43 ± 0.04 to 7.20 ± 0.06× 10^{12}/L). Values of mean corpuscular haemoglobin (20.40 ± 0.33 ρg) and mean corpuscular haemoglobin concentration (33.20 ± 0.15%) were significantly ($P < 0.05$) higher in kaempferol + zinc treated rat compared to the deionized water + noise treated group (18.43 ± 0.34) and (29.65 ± 0.89) respectively. Rats treated with zinc had the highest mean corpuscular volume (61.35 ± 0.67 fL) compared to the deionized water + noise treated group (58.87 ± 0.29). Platelet counts were also significantly higher ($P < 0.05$) in rats treated with kaempferol + zinc (609.20 ± 6.90 x 10^6 g/dL) compared to the group treated with noise + deionised water (439.80 ± 7.91 x 10^6 g/dL). 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) than in the group treated with deionised water + noise (0.35 ± 0.03). Combination kaempferol + zinc significantly ($P < 0.05$) enhanced learning (from 2.33 ± 0.33 s to 1.17 ± 0.17) and memory (from 92.17 ± 4.08 s to 106.80 ± 2.14 s). Kaempferol + zinc significantly ($P < 0.05$) mitigated noise stress induced impairment in neuromuscular coordination neuromuscular coordination (from 45.0 ± 1.43^o to 65.06 ± 1.23^o), motor strength (from 51.68 ±229 s 78.33 ± 5.69 s), sensory motor reflex (from 2.88 ± 0.16 to 4.83 ± 0.17), and motor coordination on days 1, 8 and 15 (from 10.83 ± 1.40 cm, 11.5 ± 1.18 cm, 10.50 ± 0.923 cm to 5.33 ± 1.45 cm, 4.33 ± 1.41 cm and 4.83 ± 1.35 cm) respectively. Kaempferol + zinc exerted anxiolytic effects, demonstrated in treated rats subjected to open-field test. In addition, combined treatment significantly ($P < 0.05$) decreased malondialdehyde (MDA) concentration (from 1.10 ± 0.20 nmol/mg to 0.70 ± 0.20 nmol/mg) and enhanced activities of

antioxidant enzymes: catalase (from 32.50 ± 2.20 IU/L to 36.90 ± 2.00 IU/L), glutathione peroxidase (from 25.10 ± 1.10 IU/L to 29.40 ± 1.20 IU/L) and superoxide dismutase (from 1.80 ± 0.20 IU/L to 2.20 ± 0.30 IU/L). In conclusion, treatment with kaempferol and zinc singly and in combination ameliorated noise-induced haematological, neurobehavioural and oxidative stress changes in Wistar rats.

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LIST OF ABBREVATIONS

ACTH	Adenocorticotrpic Hormone
ANOVA	Analysis of Variance
CAT	Catalase
DW	Deionised Water
EDTA	Ethylenediaminetetraacetic Acid
EOF	Erythrocyte Osmotic Fragility
GABA	Gamma Amino Butyric Acid
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GSSG	Oxidised Glutathione
Hb	Haemoglobin
Ho	Null Hypothesis
HPA	Hypothalamic-Pituitary-Adrenocortical Axis
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
NaCl	Sodium Chloride
NIHL	Noise Induced Hearing Loss
N/L	Neutrophil/lymphocyte
SO^2	Superoxide Anion
OH^-	Hydroxyl

PCV	Packed Cell Volume
RBC	Red Blood Cell
RNA	Ribose Nucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Se	Selenium
SEM	Standard Error of the Mean
SOD	Superoxide Dismutase
Zn^{2+}	Zinc ion

CHAPTER ONE:

1.0 INTRODUCTION

1.1 Background of the Study

Noise is derived from the Latin term 'nausea' and has been defined as unwanted sound, which is a potential hazard to communication and health (Ismaila and Odusote, 2014). Stress refers to a non-specific response of the body to unpleasant stimuli, threatening homeostasis and the integrity of the organism (Wankhar *et al*., 2014; Munne-Bosch and Pinto-Marijuan, 2017). It is a state of threatened homeostasis provoked by psychological, physiological and environmental stressors (Zhang *et al.,* 2015). Noise is measured in decibel (dB) units (Ismaila and Odusote, 2014). Wang *et al*. (2016) 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 (Öhrström *et al.*, 2006; Michaud *et al.*, 2016; WHO, 2016) and ranks among the environmental stressors with the highest public health impact (WHO, 2016). 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 (Henderson *et al.*, 2006; Zhang *et al.*, 2015). The ROS also trigger apoptosis by activating proapoptotic mitogen activated protein (MAP) kinase-signaling pathways (Tao *et al*., 2015). Oxidative

destruction has been associated with ROS-induced diseases (Messarah *et al.,* 2011). Antioxidants are molecules that inhibit and scavenge ROS/RNS and convert them to less dangerous molecules (Michael and Peter, 2015).

1.2 Statement of Research Problem

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 (Tao *et al.,* 2015). Additionally, occupational noise accounts for 16% of the disabling hearing loss in adults world-wide, resulting in decreased economic production (Baliatsa *et al*., 2016). Nocturnal environmental noise also provokes measurable metabolic and endocrine perturbations, including secretion of adrenaline, noradrenaline, cortisol, increased heart rate and arterial blood pressure, and increased motility. While asleep, these biological responses to noise are mostly unnoticed (Basner and Samel, 2004, 2005; Selander *et al.,* 2009). Data confirm that exposure to traffic noise, not specifically at night, is associated with increased incidence of diabetes mellitus (Sorensen *et al.,* 2013), hypertension (Babisch and Kamp, 2009), stroke among the elderly (Sorensen *et al.,* 2011), and mortality from coronary heart disease (Selander *et al.,* 2009; Sorensen *et al.,* 2011; Floud *et al*., 2013; Baliatsa *et al*., 2016).

According to WHO (2016), noise causes health damage every day estimated at 4 million dollars. Moreover, psychoneurotic and psychosomatic complaints are also observed due to noise exposures (Martinho *et al*., 1999). Zheng and Ariizumi (2007) reported that noise exposure prolongor delays healing of surgical wounds, while three days of noise exposure increases immune function, but 28 days of its exposure suppresses it. In

addition, oxidative stress increases in rats subjected to 28 days of noise stress, suggesting that oxidative status at least partially accounts for the immune suppression (Cheng *et al.,* 2011). In farm animals, noise directly affects reproductive physiology and energy consumption (Brouček, 2014). Noise may also have indirect effects on population dynamics through changes in habitat use, courtship and mating, reproduction and parental care (Rabin *et al.,* 2003). Pigs exposed to 90 dB prolonged or intermittent noise resulted in decrease in growth (Otten *et al*., 2004). When poultry is exposed to intermittent loud noise, egg laying and growth rate decreases while mortality increases (Oh *et al*., 2011). Losses have been reported from mink farms in the form of premature births, insufficient lactation and females kill their offspring in connection with exposure to sonic booms (Algers *et al*., 1978). In cattle, exposure to a high-intensity noise (105 dB) results in decrease feed consumption (appetite reduction), milk yield, and effectiveness of the milk ejection reflex (Cwynar and Kolacz, 2011).

Noise-induced hearing loss is the greatest cause of sensory disability (WHO, 2016), affecting up to 1 in 6 of the population. It is estimated that approximately 20% of the burden is generated from excessive noise exposure in occupational and leisure settings (Thorne *et al*., 2008). Sustained exposure to sound pressure levels greater than 85 dB, or sudden exposure to impulse noise leads to irreversible damage to the sensory structures of the cochlea. Once damaged, the mammalian sensory hair cells do not regenerate, and the loss of hearing is permanent (WHO, 2016).

1.3 Justification

i. There is dearth of information in Nigeria on noise assessment and ameliorative strategies, including the use of specific antioxidant agents against noise-induced stress.

ii. The current study may provide valid information on adverse psycho-social effects of noise, which are greatly on the increase world-wide (Öhrström *et al.,* 2006; WHO, 2011), especially in Nigeria.

iii. The study may also shed light on the adverse effects of noise-induced stress on haematological and behavioural changes, and insights into the oxidative stress mechanism underlying the changes.

iv. The efficacy of kaempferol and zinc gluconate as effective natural antioxidants, to be evaluated in the current study, may provide beneficial data in mitigating noise-induced adverse stress changes.

v. The results may improve current therapeutic and prophylactic measures against noise stress.

1. 4 Aim of the Study

The aim of the study was to investigate effects of kaempferol and zinc gluconate administration on haematological, behavioural and oxidative stress changes in Wistar rats, exposed to noise stress.

1.5 Specific Objectives

The specific objectives of the study were to investigate the effect of noise-induced stress in rats on:

i. haematological changes of packed cell volume, haemoglobin concentration, erythrocyte and differential leucocyte counts, neutrophil:lymphocyte ratio and erythrocytic parameters.

ii. behavioural responses of rats to cognitive changes of emotion, locomotion and anxiety states.

iii. oxidative stress changes, including malondialdehyde concentration, haemolysis, activities of superoxide dismutase, catalase and glutathione peroxidase.

iv. ameliorative effects of kaempferol and/or zinc administration on adverse haematological, behavioural and oxidative stress changes of rats, exposed to noise-induced stress.

1.6 Statement of Research Hypothesis (H_o)

i. Noise stress does not significantly alter haematological, behavioural and oxidative stress changes in Wistar rats.

ii. Administration of kaempferol and zinc does not exert significant effects on haematological, behavioural and oxidative stress changes in Wistar rats, subjected to noise stress.

CHAPTER TWO:

2.0 LITERATURE REVIEW

2.1Stress Concept and Response

There are numerous negative factors that exert effects on the body, which are mediated by stress-induced neurochemical and hormonal abnormalities, often linked with oxidative stress (Kumar *et al.,* 2010). Stress entails physical and psychological modifications that disrupt the homeostasis of animals. (Ganesan *et al.,* 2011; Zhang *et al.,* 2015). It is a state manifested by a specific syndrome, which consists of all the changes that are not specifically induced within a biologic system. (Selye, 1977). Stress occurs when the animal's homeostasis is disrupted, and the response consists of a set of physiologic mechanisms, intended to return to homeostasis (Candiani *et al.,* 2008). The responses to stressors require a progression of events, beginning with sensing and signalling by an animal's biological mechanism of an existing threat. (Ewing *et al.,*1999). These events are seconded by activation of neurophysiologic mechanisms to prevent major damages. The sensory detectors receive information and transform it into neural signals, transmitted to cognitive and non-cognitive centres of the central nervous system to create a harmonised response to the stressor. These reactions may be behavioural, autonomic, neuroendocrine and immunological (von Borell *et al.,* 2007). The response of an animal to a stressor depends on the extent and intensity of the stressor, the animal's previous encounter, its physiological status and the immediate surrounding restraints (Fazio and Ferlazzo, 2003). The animal's perception of a stimulus as apparently stressful is essential

to initiate a stress response; and the activation of the stress response system does not occur, if an animal does not recoginse an event to be stressful (Minton, 1994).

2.2Mechanism of Stress Response

Although several hormones are released during stress, there are majorly two systems that afford a "fast" and "slow" response, which are the sympathetic (catecholamines) adreno-medullary (SAM) axis, and the hypothalamo-pituitary-adrenocortical (HPA) axis (glucocorticoids). The SAM axis involves the transmission of impulses from the hypothalamus, pituitary and the sympathetic neural pathways to the adrenal medulla, and the release of adrenaline by the adrenal glands. It is responsible for the short-term (acute) behavioural and endocrine responses that prepare the animal for an immediate response to environmental modification, referred to as the fear-fight-flight syndrome (von Borell, 2001). The HPA stress-response system represents a longer term, sustained response to stressors and was first conceptualised by Hans Selye as general adaptation syndrome (GAS; Selye, 1946).

2.3Stages of Stress

Selye (1977) classified stress response into three: viz, the alarm reaction; stage of resistance; and stage of exhaustion. The alarm stage is characterised by mobilisation of all defense mechanisms in the body to contend with the adverse effects of stress. When the stress is so severe that continued exposure is incompatible with life, death insues within few hours during this stage. Consequently, a stage of adaptation or resistance will result, since no animal can be maintained continuously in a state of alarm. The adaptive stage is characterised by the termination or diminishing of the initial symptoms. After a

more prolonged exposure to the stressor, this acquired adaptation vanishes and the third stage called stage of exhaustion ensues; progressing to death, unless the animal receives instantaneous aid from exterior source (Selye, 1946). The HPA axis responds to a variety of stressors by synthesising and releasing four main hormones: corticotrophin-releasing hormone (CRH), arginine-vasopressin (AVP), adrenocorticotrophic hormone (ACTH), and glucocorticoids. Glucocorticoids act as the final effectors of HPA axis and are essentially involved in modulating the response to any psychologic or physical stressor (Earley *et al*., 2010; Bellavance and Rivest, 2014).

2.4Noise as a Stress Factor

Noise is described as unsolicited sound, either chronic or intermittent, and it is described in terms which include its intensity, frequency, spectrum, and shape of sound pressure through time (Burn, 2008). Decibel (dB) is the unit for measuring the intensity of sound. Frequency implies the number of vibrations per second of the air through which the sound is propagating, and it is measured in Hertz (Hz) (Berglung *et al*., 1999; Broucek *et al.*, 2014).

The description of sound as noise, therefore, depends on the subjective notion as to whether listening brings about pleasant or unpleasant feelings. The condition under which a recipient is subjected to noise is imperative (Algers *et al*., 1978). The effects of noise on animal productivity and behaviour depend not just on its intensity or loudness (dB), frequency (Hz), duration and pattern, but also on the hearing ability of the animal species and breeds, the age and physiological state at the time of subjection. It also depends on the experience of the animal, the sounds the animal has been previously exposed to, and the predictability of the acoustic stimulus (Castelhano-Carlos and Baumans, 2009). The

effect of noise on the central nervous system is reliant on the state of the brain. In an exhausted individual the compensatory mechanisms are more susceptible than in a rested individual. Intense noise exposure mutilates the cochlea and inner ear and lead to a cascade of auditory effects along the entire central auditory cascade (Castelhano-Carlos and Baumans, 2009). A sudden and unexpected noise gives a dilatation of the pupil (Algers *et al*., 1978). Sound levels of approximately 40 dB are recommended as the appropriate level during the night. Sound levels above this have been shown to diminish the quality of sleep (Algers *et al.*, 1978). However, the noise levels in most husbandry buildings are significantly greater than 40 dB. Susceptibility to noise perception is species-dependent, and genetically determined (Lanier *et al.*, 2000; Seidman and Standring, 2010).

2. 5Sensitivity to Noise

Animals have a different spectrum of audible sounds with maximum sensitivity at inaudible frequencies to humans (Voipio, 1997). The peak of sensitivity in rats varies between about 8 and 50 kHz (Burn, 2008). The lowest frequency in rats have been reported to be 0.25 kHz, and the highest is 80 kHz. When comparing rat and human hearing sensitivity, human values are about 10- 20 dB greater than those of the rat. Humans are most sensitive to noise in the range of 500 Hz to 4 kHz, which includes the range of conventional speech (Castelhano-Carlos and Baumans, 2009).

Cattle hear high-frequency sounds better than humans and their high-frequency hearing limit being 37 kHz, compared with only 18 kHz for humans (Heffner, 1998). The best audible sound of cattle is also at a higher frequency, at about 8 kHz, compared with 4 kHz for humans (Phillips, 2009). However, threshold for discomfort for cattle was

recorded at 90-100 dB, with physical damage to the ear occurring at 110 dB (Phillips, 2009). Indeed, cattle, with an auditory range between 25 Hz and 35 kHz, can perceive lower pitched sounds than other farm animal species (Heffner and Heffner 1993). Dairy breeds are more sensitive to noise than beef breeds (Lanier *et al*., 2000).

The auditory range of sheep is 125 Hz to 40 kHz, with the most sensitive frequency slightly higher than in cattle and pigs at 10 kHz (Heffner, 1998). Sheep are most sensitive at 7 kHz (Ames and Arehart, 1972). Pigs' hearing range is akin to that of humans, but with a shift toward ultrasound (Kittawornrat and Zimmerman, 2011). The auditory range of pigs is between 55 Hz and 40 kHz and their sense of hearing is more sensitive in the range 500 Hz to 16 kHz, principally acute around 8 kHz (Heffner and Heffner, 1993). The frequency range for reasonable detection varies between 42 Hz and 40.5 kHz, with a region of best sensitivity from 250 Hz to 16 kHz (Heffner, 1998). Heffner and Heffner (1993) reported that the frequency range of pigs was an octave higher than the human frequency range, 40 Hz - 40 kHz. Furthermore, they found that the minimum sound which pigs can hear is 8 dB louder than the quietest sound level, which humans can hear. However, the sound pressure level at which sound becomes agonising to pigs is unknown. Algers *et al.* (1978) showed that birds had the greatest increase of auditory threshold in the higher frequency ranges following exposure to noise of 95-100 dB.

Animals not only have to accept the noise, but they also emit (Brumm *et al.*, 2009). Rodents not only produce audible sounds, but also generate and hear frequencies that are inaudible to humans (above 20 kHz), perceiving sounds up to 80 kHz stimulus (Castelhano-Carlos and Baumans, 2009). Vocalisations of animals are essential for communication and they also respond to the vocalisations of other species (Phillips,

2009) as might be anticipated in herd animals that evolved in multi-species grazing environments, which are prone to carnivores. Previous research with cattle and pigs has indicated that vocalisations are an indicator of stress. Cattle vocalisations commonly range between 50 and 1,250 Hz (Watts and Stookey, 2000). Weeks *et al.* (2009) recorded average levels of vocalisations from cattle in the range 80-90 dB and sheep vocalisations at about 70 dB. The use of high-frequency vocalisation is a potent indicator of behavioural thermoregulation in pigs (Hillmann *et al.*, 2004).

2.6 Pathophysiology of 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 HPA axis, resulting in the release of corticosteroid hormones from the adrenal gland into circulation, together with hearing loss and other health impairments such as headache, hypertension and coronary heart disease (Wankhar *et al*., 2014; Tao *et al.*, 2015).

Genetics has been identified to play a key role in the mechanisms of noise induced hearing loss (NIHL). It has been proven that certain patients are more vulnerable to permanent threshold shifts, based on auditory brainstem response (ABR) threshold measurements, when exposed to a similar noise experience. Loci of vulnerability to NIHL have not been identified in humans, but several mouse models have been shown to uphold NIHL (Seidman and Standring, 2010). Chen *et al.* (2002) showed that mice deficient in a gene Xp21.2, mapped in the dystrophin gene locus, is more vulnerable to NIHL, compared to control mice. Schick *et al*. (2004) proved that escalation in noise

sensitivity occurs at lower frequencies in vasodilator-stimulated phosphoprotein -/- mice. Fairfield *et al*. (2005) studied the relationship of NIHL and the function of heat-shock protein (HSP), molecules that enhance the survival of cells in response to stress, and heat shock factor-1 (HSF1), the key transcription factor that regulates HSPs expression in stress in mice. They reported that HSF1 mice have more hearing loss than normal mice, given the same noise exposure. Morita *et al*. (2007) studied the gene Ahl3, located in the 14-Mb region on the mouse chromosome 17 and its function in NIHL. They discovered a resistance to NIHL after prolonged noise exposure in mice, when the wild type Ahl3 allele was present, signifying a genetic variation in vulnerability to NIHL (Morita *et al*., 2007).

Acoustic trauma can instigate mechanical disruption of the cochlea and may initiate permanent hearing loss (Tao *et al.,* 2015). Lurie *et al*. (1994) described graduated degrees of acoustic trauma in guinea pigs, commencing with stripping of mesothelial cells under the basilar membrane, progressing to disruption of external hair cells and ultimately, separation of the organ of Corti from the basilar membrane as the duration of exposure increases. Oxidative stress has been acknowledged to play a key role in the initiation of noise-related health impairments.

2.7Role of Oxidative Stress in Mechanism of Noise Stress

Oxidative stress is a state of amplified generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which exceeds the scavenging competence of the antioxidant systems in the body. The shift in the balance between oxidants and antioxidants in favour of oxidants is termed oxidative stress (Puttachary *et al*., 2015). Noise stress instigates increased ROS and RNS generation, which are capable of

breaching lipid and protein molecules and damaging DNA; triggering loss of function and cell death following noise exposure (Henderson *et al.,* 2006; Zhang *et al.,* 2015). The ROS additionally trigger apoptosis by activating proapoptotic mitogen activated protein (MAP) kinase-signalling pathways (Tao *et al*., 2015). Oxidative destruction has been connected with ROS-induced diseases (Messarah *et al.,* 2011). Noise induced hearing loss may occur largely due to oxidative stress (Barzegar *et al.,* 2015). Exposure to noise has been proven to cause an initial boost in cochlear blood flow. Within a short period of time, there is an abrupt decline in cochlear circulation, evidenced by an aggregation of erythrocytes, capillary vasoconstriction, and stasis. This extreme metabolic activity and decreased cochlear blood flow from noise exposure alters cellular redox states and instigates the formation of ROS (Quirk and Seidman, 1995; Wright *et al.,* 2014). ROS may also be generated by diverse mechanisms. *In vivo,* they may be formed as a by-product of mitochondrial respiration. The ROS include the superoxide ion (O_2-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH−), hypochlorite (OCl−), and nitric oxide (NO−). (Munne-Bosch and Pinto-Marijuan, 2017). In excess, ROS damages cellular DNA, proteins, and lipids, as well as up-regulate apoptotic pathways, resulting in cell death, irreparable damage to eloquent hearing structures and other health correlated conditions, such as cardiovascular diseases, diabetes mellitus and sleep disturbances (Basner *et al*., 2014).

2.8Noise Stress on Rats

Noise unwaveringly affects reproductive physiology (Escribano *et al*., 2013). It also has indirect effects on population dynamics via changes in habitat use, courtship and mating, reproduction and parental care (Rabin *et al.,* 2003). Male rats exposed to noise showed

oligospermia and modifications of their testicle structure. The ovaries and the uterus diminished significantly in female rats following noise exposure of 110 dB for five minutes, 15 times per day for 11 days at 375-500 Hz. Remaining oestrus has likewise been reported to occur subsequent to noise exposure. Increase in abortion frequency and foetus resorption, or reduction of foetus weight have been likewise registered (Algers *et a*l., 1978). Geber (1966) reported that when noise is received by the mother‘s ear, the different brain cells integrate the signals. The hypothalamus and the hypophysis are activated; the adrenal cortex and medulla are stimulated and secrete their respective hormones. The uterine blood flow, gas-interchange, nutrition and swap of waste products between foetus and mother declines. Castelhano-Carlos and Baumans (2009) showed that exposure of rats to noise of 50-80 kHz at 80-90 dB within four days mating period reduced fertility by 73.2 %. Exposure to 100 dB of 3-12 kHz for one minute during the four days of copulation diminished fertility by 70-80 %.

2.9 Impact of Noise Stress on Health of Man and Livestock

Various documented evidences buttress a correlation between higher levels of environmental noise and several adverse health effects, including cardiovascular diseases (Münzel *et al*., 2014), poor sleep quality (de Kluizenaar *et al.,* 2009; Omlin *et al.,* 2011; van Kamp and Davies, 2013), annoyance (Miedema and Oudshoorn, 2001; van Kamp *et al.,* 2004; Frei *et al.,* 2014; Zhang *et al.,* 2015), impaired cognitive development and hyperactivity in susceptible younger population groups (van Kamp and Davies, 2013; Basner *et al*., 2014) and infertility (Algers *et al*., 1978). Noise-induced hearing loss has universal implications, with 10 million adults and 5.2 million children in the US, and 250 million people globally having a noise-induced hearing loss greater than 25 dB; a

clinically significant hearing loss (Tao *et al.,* 2015). Moreover, occupational noise accounts for 16% of the disabling hearing loss in adults universally, resulting in diminished economic production (Baliatsa *et al*., 2016). Nocturnal environmental noise also aggravates measurable metabolic and endocrine perturbations, including secretion of adrenaline, noradrenaline, cortisol, increased heart rate and arterial blood pressure, and augmented motility. While asleep, these biological responses to noise are mostly disregarded (Basner and Samel, 2004; 2005, Selander *et al.,* 2009). Data validate that exposure to traffic noise is linked with elevated incidence of diabetes mellitus (Sorensen *et al.,* 2013), hypertension (Babisch and Kamp, 2009), stroke among the elderly (Sorensen *et al.,* 2011), and mortality from coronary heart disease (Selander *et al.,* 2009; Sorensen *et al.,* 2011; Floud *et al*., 2013; Baliatsa *et al*., 2016). In addition, psychoneurotic and psychosomatic grievances are also expressed due to noise exposures (Martinho *et al*., 1999). Zheng and Ariizumi (2007) reported that noise exposure prolongs healing of surgical wounds; while three days of noise exposure enhanced immune function, but 28 days of its exposure suppresses immune function. Moreover, oxidative stress increased in rats subjected to 28 days of noise stress, suggesting that their oxidative status partly accounted for the immune suppression (Cheng *et al.,* 2011).

2.10Impact of Noise Stress on Livestock Production

There are several sources of harmful noise in farm animal housing: feeding 104-115 dB, mating 94-115 dB, high-pressure cleaning 105 dB and feed mixing 88-93 dB (Venglovský *et al*., 2007). Technical devices and routine activities (opening and closing doors, changing pens, washers and push carts), workers' speech, and animal

vocalisations have been identified as potential noise sources (Žitňák *et al*., 2011; Mihina *et al.*, 2012; Lendelová *et al.*, 2013).

Neural and neuroendocrine systems are probable mechanisms for the effects of noise on feed efficiency. Sound emission at the frequency of 2 kHz in noise of 75 dB, 85 dB, and 95 dB was found to aggravate appetite reduction of animals (Cwynar and Kolacz, 2011). Sudden noise of 105 dB decreased the quantity of milk at the next milking. An ejection in progress may even be interrupted (Algers *et al*., 1978). Feed intake was increased, milk yield was unaffected, and indices of the rate of milk-releasing was enhanced. However, immediate exposure to a high-intensity noise (105 dB) initiated decreased feed consumption, milk yield, and intensity of milk release. Steady increase of noise to 105 dB resulted in a less-negative response. Gygax and Nosal (2006) showed that somatic cell counts were increased with an increasing intensity of vibration, but not with acoustic noise. Unexpected high intensity noise (above 110 dB), such as low altitude jet aircraft over flights at milking time could reduce efficiency of the milk ejection reflex, decrease efficacy of milk removal, increase residual milk, and lead to overall reduction in milk yield. Noise at 75 dB increased average daily weight gain of lambs and improved their feed efficiency compared to control and the 100 dB groups (Ames, 1978).

Pigs exposed to 90 dB prolonged or intermittent noise reduced growth (Otten *et al*., 2004). Studies via simulated aircraft noise at levels of 100 dB to 135 dB found negligible effects on rate of feed utilisation, weight gain, food intake, and reproduction of boars and sows (Manci *et al*., 1988).

According to Campo *et al*. (2005), noise may adversely affect the productive performance of birds. When poultry are subjected to intermittent loud noise, rate of laying eggs and growth rate were diminished, while mortality was amplified (Oh *et al*., 2011; Broucek, 2014). Broiler chickens exposed to 110 dB aircraft noise for five minutes every 20 minutes each day and every three nights for nine weeks from birth revealed no difference in growth in comparison to a control group. Egg productivity was altered at exposure levels as high as 120 to 130 dB. Noise at 90 dB seemed not to affect productivity and egg quality of laying hens (Oh *et al*., 2011). In general, exposure to sudden, intense noise cause reduced egg production in fowls. Pheasants are reported to have broken their eggs, while suffocation in panic-struck was evident in fowls exposed to high noise intensity. Subjection to 120 dB for 84 days showed no significant influence in quantitative and differential sperm counts of roosters, but sperms used on insemination worsened the hatchability of eggs. Noise exposure for a long time reduces egg productivity (Algers *et al*., 1978). Losses have been reported from mink farms in the form of premature births and inadequate lactation in connection with exposure to sonic booms. There are reports that the females kill their own off-springs following loud noise exposures (Algers *et al*., 1978).

2.11Impact of Noise on Animal Behaviour

Mice and rats exposed to high noise intensity responded by freezing into a still stance. When rats were exposed to 95 dB at 0.5-5 kHz for two 5-minutes' periods per day for 28 weeks, their behaviour changed and over all, they became aggressive (Algers *et al*., 1978). Sudden sounds are possibly also more startling than those with steady onsets (Burn, 2008). In open field behaviour, incessant noise of 85 dB was shown to amplify

defaecation and decrease both social and non-social activities, such as sniffing, grooming or crawling of rats, compared with 50 dB, 65 dB or 75 dB. Noise-exposed rats committed fewer errors, explored less and completed their trials faster. Mammals particularly appear to respond to sudden high intensity noise, with responses including freezing, startle response and fleeing from the noise source. Compared with chronic background or recurring noise, this periodic or arbitrary noise is especially effective for instigating distress responses. Most animals become less responsive to sounds emitted for long periods or at conventional intervals. The degree of animal reaction differs with species of animal, age and individual (McGlone and Swanson, 2010; Broucek *et al.,* 2014). Animal activity increases at background noise. Animals are also liable to be more active in the morning periods than in the afternoon test periods, which might be related to the arrival of staff and beginning of the working day with a general rise in noise levels (Castelhano-Carlos and Baumans, 2009).

A comprehension of animal response to helicopters or aircrafts is imperative in predicting the consequences of disturbance on ecology, welfare and behaviour of exposed free range farming or wildlife animals (Tracey and Flem, 2007). Domestic animals present behavioural responses to over-flights, but commonly seem to habituate to the disturbances over a time period. Exposure of laboratory animals to noise initiates greater abnormal behaviour, suppresses exploratory behaviour, and impaires learning. It has been reported that guinea pigs jump when exposed to sudden extremely loud noise (139-143 dB), reduce activity and remain huddled for up to 30 minutes afterwards. Tooth grinding was also reported in male guinea pigs exposed to frequent loud noise over a period of 6 weeks (Johnson, 2006). When an aircraft was 152 m above ground level, cattle ran for

less than 10 meters and recommenced normal activity within one minute. Unanticipated high intensity noise, such as low-altitude jet aircraft overflights (above 110 dB), at milking parlour could induce adverse behaviours, such as kicking or stomping (Morgan and Tromborg, 2007; Broucek *et al.*, 2014).

The noise threshold anticipated to cause a behavioural response by cattle is 85 to 90 dB (Manci *et al.*, 1988). Noises higher than threshold have incited retreat, freezing, or strong startle response (Morgan and Tromborg, 2007). When the transmitter of ultrasound was switched on at a space of 1 m, calves got up and orientated towards the sound source. After 30 s, all calves had their ears oriented away from the sound source. After 10 min, some calves started to scratch their ears continually. During the 10-minute period of exposure, none of the calves laid down again (Algers, 1984).

It has also been reported that brief exposure to 90 dB of noise caused sheep to depart from the source of the sound and aggregation of a cohesive group of individuals in the lying stance (Algers, 1984). Bighorn sheep reacted to helicopter flights by reducing the time spent foraging and were the most sensitive to disturbance during winter (43 % reduction in foraging efficiency). Alteration in Behaviour adversely affect wildlife and reduce the efficiency of management operations. Bleich *et al*. (1994) reported that frequent disturbance by aircraft could cause animals to depart from their home territory. The distance from the source of disturbance is a key indicator of alert behaviour. Goats exhibited alert behaviour when the helicopter was directly overhead and alert response diminished exponentially with horizontal distance from the helicopter. Goats were frequently disoriented and ran for a distance up to 1.5 km in response to helicopter over-flights. Horses are also very receptive to noise. Algers (1984) suggested that after the

start of noise stimulus, horses turned their heads and oriented their ears in the direction of the sound source, and then immediately directed their ears away. At a new tone, the horses reacted with concentration briefly and then turned their ears away again. Observations revealed that horses galloped, moved haphazardly and exhibited biting/kicking behaviour in response to jet fly-overs (Gladwin *et al.*, 1988). No injuries or abortions were noted, and evidence proved that the mares adapted to the flyovers over the period of one month (Manci *et al*., 1988).

Auditory stimuli are used by pigs as a way of social communication (Gonyou, 2001). Pigs subjected to 90 dB protracted or discontinuous noise increased lying down time and diminished social interactions (Otten *et al*., 2004). According to Talling *et al.* (1996), pigs demonstrate aversion to abrupt loud noise when tested in an open-field. This response was more glaring for a frequency of 8 kHz than for 500 Hz and for an intensity of 97 dB than for 85 dB, though habituation occurred relatively swiftly (Kittawornrat and Zimmerman, 2011). Protracted sounds, for example incessant fan noise, can also affect pigs. Behaviour of piglets and sows during suckling in relation to sound levels were studied by Algers and Jensen (1985). Sows were subjected to a relatively silent background noise of 59 dB or exposed to fan noise at a level of 85 dB. In the noise-exposed environment, the piglets,appreciably decreased massaging of the udder and, hence, reduced milk production was observed. In the study of Algers (1984) with sudden noise exposure in 10-day-old pigs, an instantaneous attention and orientation response for about 10 s was reported. When suckling piglets were subjected to continuous loud noise, they paid less attention to the front teats and more regularly used the teats at the rear of the udder (Algers and Jensen, 1991).

Talling *et al*. (1996) showed that within a recurrent 15-minute exposure to noise, preliminary differences between treatment and pre-treatment locomotion in pigs declined over the course of the trial. Laboratory rabbits vary their behaviour when exposed to conventional laboratory sounds in non-sound isolated housing (Jildge, 1991). Effect of noise on rabbits instigates unpleasant effects including nervous and behavioural abnormalities and cause startled response and traumatic injuries to limbs and back (Marai and Rashwan, 2004). With regard to the noise, threshold areas in the sensitive range of rabbits lie between zero and 20 dB sound pressure, which implies a sensitive hearing.

The classic reaction of domestic fowls after exposure to abrupt, intense noise is a short-term startle response. The reaction ends as soon as the stimulus ceases, and within a few minutes all activity returns to normal. This suggests that birds habituate comparatively quickly (Gladwin *et al*., 1988). A significant negative effect of acute noise exposure at 80 dB and 100 dB on stressfulness and fearfulness in broilers was reported by Chloupek *et al*. (2009). Campo *et al*. (2005) found that laying hens subjected to noise at 90 dB (truck, train and aircraft noises) for 60 minutes were more fearful than control hens kept at 65 dB, caused by bird vocalisations and fans.

Algers *et al*. (1978) observed characteristic reactions to long lasting sound (95 dB, 500 Hz) as startle response, total immobility, small jerky head movements, latent period, running, and sleep-like behaviour in chickens. The response varied in form and strength in accordance to the age of the experimental birds and was strongest in 26-day-old chickens. Abrupt loud noises have also been observed to cause hysteria in various strains of chickens (Mitloehner *et al*., 2010). Book and Higher panic and aggression in turkeys in response to noise stimuli simulating aircraft overflights have been noted. Wild birds have

been reported to respond with disrupted sitting (Algers *et al.*, 1978). Behaviour of adult animals kept in captivity is also altered by noise. In zoos and aquaria, noise from visitors increases as visitor numbers increase, thus resulting in increased activity of the animals. Blaring sound has been shown to increase vigilance and activity and anxiety behaviours in pandas (Morgan and Tromborg, 2007; Broucek *et al.*, 2014).

2. 12Impact of Noise Stress on Behavioural Changes in Man

Noise is linked with complicated behavioural changes in humans. Subjects exhibited elevated psychological tension and diminished work quality after a 30-day exposure to 80 to 90 dBin their work environment (Ising *et al.*, 1980; Brouček, 2014). Exposure to background noise produces diverse antisocial behaviours in humans. Sauser *et al.* (1978) showed that exposure to a relatively low-level (70 to 80 dB) background noise caused study subjects to recommend lower salaries for fictitious employees. Test participants in a noisy environment will administer greater levels of shock and noise to another individual (Broadbent, 1957). Introduction of a background noise of 85 dB caused fewer persons to help pick up something that was dropped (Matthews and Canon, 1975). Noise also interferes with sleep by extending the time required to fall asleep, resulting in awakening once asleep, hampering return to sleep once awakened and initiating shifts from deeper to shallower stages. Noise causes reliable disruption in sleep patterns in humans (Thiessen and Lapointe, 1978; Boettcher *et al.*, 1995). In both rats and humans, noise seemed to diminish both slow-wave sleep (Gitanjali and Ananth, 2003) and paradoxical (or rapid eye movement; REM) sleep (Gitanjali and Ananth, 2003). Sound intensity as low as 60 to 65 dB was shown to consistently disrupt human sleep patterns (Thiessen and Lapointe, 1978). Women living with heavy snorers frequently report

insomnia, daytime sleepiness, and fatigue, which is not astonishing given that the average snoring male seeking treatment produces nearly 500 snores per hour with a maximum intensity range of 63 to 100 dB (Hoffstein *et al*., 1999). Moreover, an 80-dB intermittent noise has a greater undesirable impact on sleep quality, mood, and performance than does an 80-dB uninterrupted noise (Ohrstrom *et al.*, 1988). Lukas and Kryter (1970) and Broucek (2014) showed that older individuals are more likely to be awakened by noise.

2.13 Haemato-biochemical Changes in Noise Stress

Loud sound is well known for detrimental effects on blood pressure and heart rate in humans and animals (Tromborg, 2007). The most obvious effect is an overall stress reaction with higher secretion of ACTH resulting in an increase of adrenocortical hormones in the blood (Burrow *et al*., 2005). Long-term noise exposure results in a decrease in plasma glucocorticoids and a rise in plasma catecholamines, ACTH and cortisol concentrations (Otten *et al*., 2004; Kanitz *et al*., 2005).

Broucek *et al*., 1983 reported that sound from tractor engine (97 dB) substantially elevated glucose concentration and leucocyte counts and markedly reduced the level of haemoglobin in the blood of dairy cows. It has also been reported that primiparous cows treated individually by the 30-min noise of 110 dB, frequency 1 kHz in an open-field arena, showed significant increase in glycaemia, non-esterified fatty acids content and creatinine under the effect of acoustic exposition. Haemoglobin level declined significantly while increase in the count of eosinophils in blood, and immunosuppression has also been reported (Algers *et al*., 1978). 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 (Bedanova *et al*., 2010).

Pinar *et al*. (2011) reported an increase in erythrocyte and leucocyte counts except for monocytes in humans working in noisy environments. Waynert *et al.* (1999) reported that beef cattle subjected to noise exposure for 1 min per day over 5 days exhibited an overall steady decline in heart rate. Pigs exposed to 90 dB protracted or intermittent noise increased cortisol, ACTH, noradrenaline to adrenaline ratios (Otten *et al*., 2004). This response was stronger for a frequency of 8 kHz than for 500 Hz, and for an intensity of 97 dB than for 85 dB (Kittawornrat and Zimmerman, 2011).

2.14 Antioxidants

The body has numerous mechanisms to neutralise oxidative stress by producing antioxidants, which are either naturally produced *in situ* (endogenous) or supplied externally through foods and/or supplements (exogenous). Antioxidants are molecules that scavenge ROS/RNS and transform them to less dangerous molecules. They preclude and repair damages caused by ROS/RON (Munne-Bosch and Pinto-Marijuan, 2017).

Endogenous antioxidant compounds are categorised as enzymatic antioxidants and non-enzymatic (metabolic and nutrient) antioxidants. The major enzymatic antioxidants comprise superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GRx) (Gupta *et al.,* 2014). The catalytic activity of these enzymes permit the transformation of superoxide anion into hydrogen peroxide (H_20_2)

and water, thereby mitigating the harmful effects of ROS (Munne-Bosch and Pinto-Marijuan, 2017). SOD catalyses the dismutation of superoxide to $H_20_{2,}$ and it is the first defense against ROS (Molina *et al*., 2016). Catalase catalyses the break-down of H_20_2 to water and oxygen, and also plays a role in oxidative defense of the animal tissue by catalysing the reduction of hydrogen and lipid peroxides. This prevents oxidative damage in cell membranes. Increased activity of SOD results in increased production of H_20_2; and thus, a simultaneous rise in GPx and CAT activities is required for protection against heat stress-induced ROS (Zhang *et al.*, 2015; Munne-Bosch and Pinto-Marijuan, 2017).

Trace elements such as selenium (Se), zinc (Zn), copper (Cu) and manganese (Mn) perform important catalytic role in the enzymatic activity of GPx and SOD (Zn, Mn, Cu) (Molina *et al.*, 2016). Exogenous nutrient antioxidants are not produced in the body, but are provided via foods or supplements (El Erian *et al.*, 2015). They comprise vitamin E, trace elements (Se, Mn, Zn), flavonoids, carotenoids, omega-3 and omega-6 fatty acids (Asadi *et al.*, 2016).

2.15Mechanism of Action of Antioxidants

Antioxidants are compounds or systems that delay autooxidation by inhibiting formation of free radicals or by interrupting propagation of free radicals by one of the following mechanisms; scavenging species that initiate peroxidation, chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, breaking the autoxidative chain reaction and/or reducing localized oxygen concentrations (Jian-Ming *et al.*, 2010).

Antioxidants are molecules that can neutralize free radicals by accepting or donating electron(s) to eliminate the unpaired condition of the radical (El Erian *et al.,* 2015). The antioxidant molecules may directly react with the reactive radicals and destroy them, while they may become new free radicals which are less active, longer-lived and less dangerous than those radicals they have neutralised. They may be neutralized by other antioxidants or other mechanisms to terminate their radical status (Shih *et al.,* 2015). Another important function of antioxidants is to regulate ROS-related enzymes. Antioxidants may decrease the cellular level of ROS either by inhibiting the activities or expression of ROS-generating enzymes such as NAD(P)H oxidase and xanthine oxidase (XO), or by enhancing the activities and expressions of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Rizvi and Maurya, 2007).

2.15.1 Role of Antioxidants in ameliorating noise stress

The ameliorative effects of antioxidants for the treatment of structural and functional noise stress-induced pathologies in the auditory pathway have been reported: compounds such as mannitol (Dehne *et al.,* 2000), salicylates or vitamins A, C and E (Fetoni *et al*., 2015), N-acetylcysteine (Kopke *et al*., 2007), trolox (Yamasoba *et al.,* 1998), acetyl-l-carnitine combined with N-acetylcysteine (Kopke *et al*., 2005) and *Ocimum sanctum* –a medicinal herb popularly known as basil, attenuates cochlear oxidative damage following hearing loss (Alvarados *et al.,* 2015). Fetoni *et al*. (2013) reported that NIHL can be reversed by treatment with a natural antioxidant, CoQ10, which prevents cochlear oxidative stress through the decrease in $O_2^{\bullet -}$ production and lipid peroxidation.

Rosuvastatin, a lipid-lowering agent, has been verified to have antioxidant properties. Ersoy *et al.* (2014) reported that this agent decreases oxidative stress in the brain of adult rats, exposed to noise for 20 days. The up-regulation of the antioxidant defenses and the subsequent prevention of tissue damage undertaken by rosuvastatin in rats subjected to noise was confirmed by Koc *et al.* (2015). It is also worthy of note that inhaled hydrogen gas was reported by Kurioka *et al.* (2014) to be therapeutically effective in the deterrence of noise-induced oxidative damage in cochlear hair cells. In addition, Chen *et al. (*2014) reported that hearing loss and the underlying oxidative stress, triggered after exposure to 130 dB for 1 h was prevented through the use of hydrogen-saturated saline, which is capable of scavenging the OH• radical. Renexin, a derivative from cilostazol and *Ginkgo biloba* extract, has been shown to possess antioxidant properties (Park *et al.,* 2014). The irreversible hearing loss induced by exposure of adult mice to 110 dB of noise for 1 h was entirely prevented after the treatment with this compound. Rosmarinic acid, a polyphenol compound, commonly derived from culinary herbs, was able to mitigate the auditory function and assuage the loss of cochlear hair cells damaged by noise exposure, and also attenuated the noise-induced increase in lipid peroxidation and O_2•− production (Fetoni *et al*., 2015). The phenolic compound orientin, abundant in millet and juice of passion fruit, reduces brain oxidative stress markers and increases activities of antioxidant enzymes. It also improves spatial learning and memory acquisition of the passive avoidance response, altered after moderate noise exposure (Wang *et al.,* 2016).Administration of unitol, ascorbic acid and tocopherol has been used to enhance the non-specific body resistance to noise stress (Sokolovskii *et al.,* 1987). *Emblica officinalis* possesses antioxidant properties, effective against noise stress-induced

behavioural changes in Wistar rats (Wankhar *et al.,* 2014).Srinivasan *et al.* (2015) reported the neuroprotective effects of *Indigofera tinctoria* on noise stress affects Wistar rat brain. *Tualang* honey was also reported to improve memory performance and decrease depressive-like behaviour in rats exposed to loud noise stress (Azman *et al.,* 2015). Seidman *et al.* (2003) demonstrated the protective effect of resveratrol againt noise-induced hearing loss. Melatonin has also been shown to attenuate noise stress-induced gastro-intestinal motility disorders, gastric stress ulcer and oxidative stress in rats (Zhang *et al.,* 2015). These reports showed that antioxidants are potential agents that may be beneficial in the improvement of the current prophylactic and therapeutic interventions against noise-induced stress.

2.16 Kaempferol in Health and Disease

2.16.1 Physical properties of kaempferol

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) and commonly found in plant-derived foods and plants used in traditional medicine (Bigoniya *et al.,* 2013; Romanazzi *et al.,* 2017). Plants containing kaempferol belong to the family *Zingiberaceae* (Lee *et al.,* 2016),possessing the following physico-chemical properties (Haque*et al*., 2016; Romanazzi *et al.,* 2017):

Molecular weight: 286.24

Melting point: 276℃

Boiling point: 619.1°C at 760 mm Hg

Density: 6.38E-16 mm Hg at 25°C

Flash point: 240.7°C

Appearance: Yellow needle-shaped crystal

Molecular formular: $C_{15}H_{10}O_6$

Solubility: Soluble in hot alcohol, ether and alkalis

Particle size: 100% pass 80 mesh

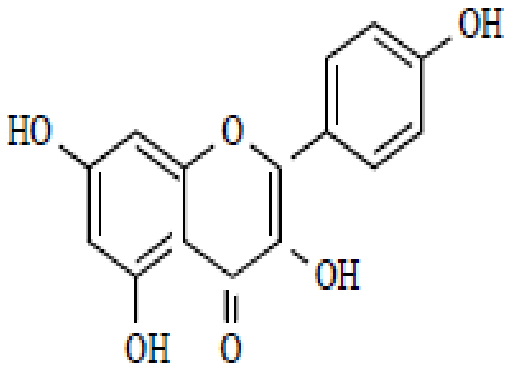


Figure 2.1: Structure of Kaempferol (Bigoniya *et al.*, 2013; Haque*et al*., 2016).

2.16.2 Sources of kaempferol

Flavonoids, including kaempferol encompass a set of plant secondary metabolites, distinguished by a diphenylpropane structure (Romanazzi *et al.*, 2017). They are widely distributed in the plant kingdom and are 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, *capparis spinosa*, endive squash, cluster bean, wall rocket, wide rocket, rocket salad, fennel, strawberry, fishwort, lettuce, goji berries, indian mulberry, gooseberries, rasberries, blackberries, elderberries, peach, pistachio and radishes, (Somerset and Johannot, 2008; Romanazzi *et al.*, 2017).

2.16.3 Biosynthesis and metabolism of kaempferol

Enzymes concerned with the biosynthesis of kaempferol are relatively common in the plant kingdom; thus, it is not surprising that this flavonoid is widely distributed in plants (Zhang *et al.*, 2017). Like other flavonoids, kaempferol has a diphenylpropane structure (C6-C3-C6) and is synthesised by condensation of 4-coumaroyl-CoA (C6-C3) with three molecules of malonyl- CoA (C6) (Winkel-shirley, 2002; Haque*et al*., 2016). This reaction, catalysed by the enzyme chalcone synthase (EC 2.3.1.74), results in the development of the flavonoid naringenin chalcone (C6-C3-C6). This chalcone is modified into the flavanone naringenin by the enzyme chalcone isomerase (EC 5.5.1.6), which catalyses the closure of the C3 ring. The enzyme flavanone 3-dioxygenase (EC 1.14.11.9) initiates a hydroxyl group in naringenin at C3 to form dihydrokaempferol. Finally, the enzyme flavonol synthase (EC 1.14.11.23) initiates a double bond in dihydrokaempferol at C2-C3 to yield kaempferol (Calderon-montano *et al.,* 2011). Sugars such as glucose, rhamnose, galactose and rutinose are typically bound to kaempferol to form glycosides. Some glycosides of kaempferol are very widespread in nature (for example, kaempferol-3-O-glucoside, also called astragalin) because their biosynthesis only requires additional enzymes that are universal in the plant kingdom, an example is flavonol 3-O-glucosyltransferase(EC 2.4.1.91) (Calderon-montano *et al.,* 2011; Haque*et al*., 2016).

2.16.4 Pharmacokinetics of kaempferol

Bioavailability and metabolism of kaempferol determines its bioactivities *in vivo*. Flavonols such as kaempferol are frequently ingested orally and as glycosides (Nwidu *et al.,* 2017). It is recognised that the high polarity of glycosides impede their absorption,

whereas the intermediate polarity of aglycones aids it. Although this suggests that the absorption of glycosides needs the previous hydrolysis to absorbable aglycones, studies have shown that glycosides may be absorbed without hydrolysis (Lehtonen *et al*., 2010). Like other flavonoids, kaempferol is primarily absorbed in the small intestine. The lipophilicity of kaempferol eases its absorption by passive diffusion, but evidence suggests that it may also be absorbed by facilitated diffusion or active transport (Crespy *et al.,* 2003; Nwidu *et al.,* 2017). Kaempferol is metabolised in the small intestine (to glucuronides and sulfoconjugates) by intestinal conjugation enzymes (Crespy *et al.*, 2003). Like other flavonoids, kaempferol and its glycosides are significantly metabolised by the colon microflora. The colonic bacteria can both hydrolyse the glycosides to aglycones and break the C3 ring of aglycones to form simple phenolic compounds such as 4-hydroxyphenylacetic acid, phloroglucinol and 4-methylphenol, which are either absorbed or excreted in faeces (Lehtonen *et al.,* 2010). After absorption, kaempferol is considerably metabolised in the liver to form glucurono-and sulpho-conjugated forms (Crespy *et al.,* 2003). The conjugated forms of kaempferol, some phenolic compounds produced by the colon microflora, kaempferol and some kaempferol glycosides can reach systemic circulation and tissues and are then excreted in urine. Several studies on human subjects have demonstrated that, after oral ingestion, kaempferol is detected in plasma at nanomolar concentrations (Barve *et al*., 2009).

Studies have shown interconversions between kaempferol (3,5,7,4’-tetrahydroxyflavone) and the flavonoids, galangin (3,5,7-trihydroxyflavone) and quercetin (3,5,7,3’,4’-pentahydroxyflavone) (Haque*et al*., 2016). Galangin can be changed to kaempferol by rat cytochrome P_{450} (CYP) viahydroxylation of the B-ring at C3’ (Silver *et al.,* 1997).

Nielsen *et al.* (1998) reported that kaempferol is metabolised to quercetin and that this effect may be mediated by the enzymes CYP1A. They suggest that the biological activities of kaempferol are observed after galangin ingestion, and that some of the effects induced by kaempferol *in vivo* are mediated in part by quercetin, which has a wide range of biological activities (Boots *et al.,* 2008).

2.16.5 Antixoxidant role of kaempferol

Kaempferol-containing plants exhibit *in vivo* and *in vitro* antioxidant activity (Aniya *et al.,* 2005; Zhang *et al.,* 2017a). Several studies reveal that the presence of a double bond at C2-C3 in conjugation with an oxo group at C4, and the presence of hydroxyl groups at C3, C5 and C4', are principal structural features, involved in the antioxidant activity of kaempferol (van Acher *et al*., 1996; Haque *et al*., 2016). Kaempferol has been proven to be a potent superoxide scavenger (Wang *et al.,* 2006; Septembre-Malaterre *et al*., 2016). The ability of kaempferol to diminish superoxide and peroxynitrite levels at low concentrations play a vital role in its antioxidant activity, as the formation of superoxide anion is requisite for the normal production of most reactive oxygen species involved in oxidative stress (Klaunig and Kamendulis, 2004; Zhang *et al.,* 2017a).

Wang *etal.* (2006) evaluated the antioxidant activity of several flavonoids and found that kaempferol is one of the strongest scavengers for the Fenton-generated hydroxyl radical, with an IC_{50} of 0.5 M. Similarly, Heijnen *et al.* (2001) observed that kaempferol is an effective peroxynitrite scavenger, with an IC_{50} of 0.35 ± 0.5 M. Kaempferol also exerts antioxidant effects through inhibition of activities of enzymes that generate ROS, such as xanthine oxidase (Wang *et al.,* 2006). Like other flavonoids, kaempferol can also ease the formation of hydroxyl radical through the Fenton's reaction by chelating ferrous or

cuprous ions (van Acher *et al.,* 1996). Kaempferol exerts its antioxidant effects by increasing the expression or activities of antioxidant enzymes such as superoxide dismutase, catalase, and haeme oxygenase-1 (HO-1) (Doronicheva *et al.,* 2007; Septembre-Malaterre *et al*., 2016). Kaempferol and its glycosides prevent lipid peroxidation (Hou *et al.,* 2004); the activity of kaempferol is higher than that of its glycosides because kaempferol has a higher lipophilia and capacity to penetrate into lipid bilayers to exert this activity. Kaempferol prevents the oxidation of low-density lipoprotein, which may play a protective role in atherosclerosis (Tu *et al.,* 2007; Zhang *et al.,* 2017a). The antioxidant activity of kaempferol has been observed in several *in vivo* studies (Aniya *et al.,* 2005). An extract of *Capparis spinosa,* containing kaempferol exerts a significant antioxidant activity, when applied topically in healthy human volunteers (Bonina *et al.,* 2002). Sanz *et al.* (1994) treated mice with kaempferol-3-O-galactoside afterbromobenzene intoxication.They observed that this flavonoidlessened hepatic lipid peroxidation products and increased the levels of reduced glutathione.

2.16.6 Anti-inflamatory effect of kaempferol

Leucocytes at injury sites secrete numerous cytokines, which help to recruit more leucocytes and promote the inflammatory response (Townsend *et al.,* 2016). Among the messengers are various interleukins (ILs) and tumour necrosis factor-alpha (TNF-α). Kaempferol has been discovered to correlate with diminished serum IL-6 levels, an inflammatory cytokine (Bobe *et al.,* 2010; Qin *et al*., 2016). In the same trial study, a decrease in IL-6 levels was shown to lower risk for advanced adenoma recurrence. *In vitro* studies have proven kaempferol to interfere largely in a number of inflammation mechanisms. In J744.2 macrophages, treatment with kaempferol prohibits expression of

both IL-1 beta and TNF-α (Kowalski *et al.,* 2005). The two cytokines promote the activation of specific enzymes and genes, essential for the inflammatory response. Kaempferol lowers the mRNA levels of both cytokines, inhibiting their transcription and subsequent effects. TNF-alpha and IL-1β are both linked to the development of a number of autoimmune disorders, such as multiple sclerosis. In addition, kaempferol has been reported to disrupt TNF-mediated operations (Lee *et al.,* 2009). It promotes activation of IL-8, another pro-inflammatory cytokine. In HEK 293 cells, kaempferol inhibits not only TNF-induced IL-8 promoter activation, but also IL-8 gene expression. The IL-8 is a potent promoter of angiogenesis, displaying the wide variety of proteins that kaempferol affects (Qazi *et al.,* 2011).

Among the functions of TNF-α is activation of NF-κB; a transcription factor, which prompts synthesis of inflammatory proteins. Administration of kaempferol prohibits migration of NF-κB into the nucleus, thereby averting induction of its gene targets (Lee *et al.,* 2009). IL-4 has been implicated in autoimmune disease and deregulation of inflammation (Finnegan *et al.,* 2002). It exhibits its effects through phosphorylating JAK3, a tyrosine kinase found in the cytoplasm. Predictably, kaempferol is proven to inhibit this signalling pathway, specifically by targeting JAK3 activity (Cortes *et al.,* 2007; Townsend *et al.,* 2016). Upon introduction of kaempferol, JAK3 is incapable of phosphorylating STAT6, effectively disrupting the secretion of STAT6-related inflammatory proteins. Since JAK3 is upstream of a number of transcription factors, kaempferol has potential to upset many JAK3-controlled processes. The inflammatory cytokine IL-2 also employs JAK3 to activate STAT5,therefore another transcription factor, kaempferol is a potent inhibitor of IL-2-mediated outcomes. Lastly, kaempferol

appears to exert therapeutic effects via the endocannabinoid system. It has been acknowledged to inhibit fatty acid amide hydrolase (FAAH) activity, allowing anandamide to continually exert its therapeutic effects (Thors *et al.*, 2008; Townsend *et al.*, 2016).

2.16.7 Neuroprotective activity of kaempferol

A number of *in vitro* and *in vivo* studies suggest that kaempferol and some of its glycosides and plants containing this flavonoid may possess neuroprotective property, and that they protect the body from the development of Alzheimer's disease, Parkinson's disease or Huntington's disease (Lopez-sanchez *et al.*, 2007; Dollahite *et al.*, 2016). Lopez-Sanchez *et al.* (2007) carried out an *in vivo* study to evaluate the neuroprotective effect of kaempferol and demonstrated that the intravenous administration of this dietary agent lessens ischaemia-induced brain damage in rats. This protective effect is associated with the capacity of kaempferol to diminish metalloproteinase activation, prevent protein nitrotyrosines accumulation and shield against apoptotic cell death, caused by oxidative stress (Lopez-Sanchez *et al.* 2007). It has been suggested that kaempferol and its glycosides, such as tiliroside and several kaempferol-containing plants (for example, *Tilia* species) have anxiolytic activity *in vivo* (Loscalzo *et al.*, 2009). *In vitro* and *in vivo* studies have also shown that kaempferol possess anti-allergic and anti-asthmatic activities, which may be mediated partly by inhibition of histamine release (Park *et al.*, 2008; Septembre-Malaterre *et al*., 2016).

2.16.8 Analgesic activity of kaempferol

Analgesic activity has also been observed in several kaempferol glycosides and kaempferol-containing plants in diverse *in vivo* studies (Kupeli *et al*., 2007). Okamoto *et*

al. (2002) reported data, suggesting that kaempferol may be useful for the treatment of cell- mediated immune diseases, such as acute graft-versus-host disease (GVHD). They also found that kaempferol acts directly on T-cells and prohibits Th1 cytokine production; thus, suppressing the expansion and generation of CD8+ cytotoxic T-lymphocytes *in vitro*. The administration of C57BL/6- into-BDF1 mice treated with kaempferol for 1 week after acute GVHD induction show reduced GVHD-associated anti-host CTL activity, induction of Th2 cell development, and host repopulation with donor lymphocytes, resulting in early restoration of body weight and increased survival (Okamoto *et al.,* 2002). Cortés *et al.* (2007) found that kaempferol inhibits IL-4-induced STAT6 activation by exclusively targeting the tyrosine kinase JAK3.

2.16.9 Side-effects and safety of kaempferol

Several reports regarding the safety of kaempferol exists. Some studies have shown that kaempferol induces antimutagenic activity (Francis *et al.,* 1989), others have reported that the flavonoid may induce genotoxic effects (Bestwick *et al.,* 2005). The prooxidant activity of kaempferol may play an important role in its genotoxic effects. Although the prooxidant effects of flavonoids have been attributed to their ability to autooxidise when they are dissolved in aqueous buffers, kaempferol does not show this capacity in a study performed by Canada *et al.* (1990). The prooxidant activity of kaempferol is also mediated by its ability to modulate the activities of antioxidant and prooxidant enzymes (Galatiel *et al*., 2002). Some reports suggest that the mutagenicity of kaempferol may also be due to its transformation, by CYP 1A1 enzymes, to the more potent genotoxicant, quercetin (Silver *et al.,* 1996). Although some *invitro* studies suggest that kaempferol and

quercetin may be genotoxic and carcinogenic, but the effects are yet to be demonstrated in animal studies (Takanashi *et al.,* 1983).

Kaempferol may react with iron and reduce its bioavailability (Hu *et al.,* 2006) or decrease the cellular uptake of folic acid (Lemos *et al.,* 2007); these effects may cause challenges in individuals with iron or folic acid deficiency. Kaempferol may also potentiate the bioavailalability of the anticancer drug, etoposide and increase its toxicity (Li *et al.,* 2009) or condense the anticancer activity of cisplatin (Gao *et al.,* 2010). It is imperative to mention that, like other antioxidant/prooxidant polyphenols (Lopez-lazaro, 2008), kaempferol can potentially increase or decrease the activity and toxicity of anticancer drugs with a prooxidant mechanism of action. At low concentrations, kaempferol exerts an antioxidant effect and reduces the activity of drugs required to generate ROS to kill cancer cells efficiently (Septembre-Malaterre *et al*., 2016).

2.17 Zinc in Health and Disease

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 (Andreini *et al.,* 2006; Prasad, 2013; 2014). Zn is required for the activity of over 300 enzymes; therefore, it partakes in many enzymatic and metabolic functions in the body (Andreini *et al.,* 2012). Zinc, a divalent cation with manifold biochemical and physiological functions, may play principal neuromodulatory roles in the central nervous system. Specific and non-specific neuronal carriers afford zinc entry into central neurones, where the metal ion accumulates in synaptic vesicles. Modulation of excitatory and inhibitory amino acid transmissions is amongst the many putative roles ascribed to brain zinc (Ghasem *et al*.,

2016). Indeed, it is co-released with glutamate, and modulates glutamatergic excitation by inhibiting N-methyl-D-aspartate (NMDA) receptors. It averts gamma-aminobutyric acid (GABA) inhibition by blocking GABA-A receptor function (Fregoneze *et al*., 1999). Zinc is also an indispensable component for DNA-binding proteins with Zn fingers, as well as copper/Zn superoxide dismutase and a variety of proteins, involved in DNA repair (Ho and Ames, 2002). Thus, Zn plays a vital role in transcription factor function, antioxidant defence and DNA repair.

2.17.1Biochemistry of zinc

Zinc, distinct from iron and copper, is redox neutral and reactive as a Lewis acid in biological reactions. Therefore, zinc plays crucial roles as a structural, catalytic, and signalling component. As a structural component in proteins, the involvement of zinc with proteins was first acknowledged by the crystallisation of insulin with zinc (Myers *et al.,* 2013). The "zinc finger" motif was first established in the transcription factor TFIIIA of *Xenopus* (Andreini *et al.,* 2011). Zinc fingers are now categorized into more than 20 classes of structurally distinct modules, and are recognised as a functional motif that interacts with a variety of proteins, lipids, and nucleic acids, clearly indicating the importance of zinc in cellular biochemistry. In catalytic functions, zinc can activate substrates in enzymes by stabilising negative charges, due to its strong Lewis acid property (Maret *et al.,* 2012). After the first identification of zinc in erythrocyte carbonic anhydrase as a zinc-dependent enzyme, the presence of zinc has been documented in many enzymes in all six classes, defined in the enzyme Commission (EC) system (Valle and Falchuk, 1993; Prasad, 2013, 2014).Within proteins, zinc can be coordinated by nitrogen, oxygen, and sulphur atoms and can have different coordination numbers

(Kochanczyk *et al*., 2015). The zinc proteome estimates that almost 9% of proteins are zinc proteins in eukaryotes with the number significantly greater in higher organisms. The number of zinc proteins encoded by humans is approximately 10%. In zinc proteomes, the number of zinc-binding motifs is counted predominantly, based on mining for intramolecular zinc-binding sites (Andreini *et al.,* 2006).

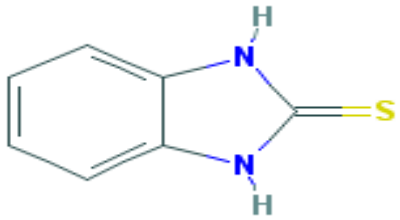


Figure 2: Structure of Zinc

2.17.2 Zinc homeostasis

2.17.3Zn distribution

The human body mass contains 2-3 g of Zn, and 57% is in skeletal muscle, 29%, 6% and 5% in bone, liver and skin, respectively (King *et al.,* 2000). There is only about 0.1% of total Zn circulating in plasma, and yet this minute fraction is essential to maintain Zn homeostasis at a systemic level. Plasma Zn turns over rapidly to meet tissue needs and has to be replenished daily from diet. Once getting into plasma, the dietary Zn is delivered to peripheral tissues. The systemic level of Zn distribution is closely regulated and the physiological concentration of plasma Zn is maintained within the range of 10-20 mmol/L (Meunier *et al.,* 2005; Disilvestrol *et al.,* 2015; Brnic *et al.,* 2016). The plasma Zn concentration is normally around 15 mmol/L in Zn adequate adults and about 11.0-13.5 mmol/L in Zn adequate children. Greater than 70% of plasma Zn is weakly bound to

albumin and is mostly derived from intestinal absorption with a destination of the liver and soft tissues (Rukganuer *et al.,* 1997; Disilvestrol *et al.,* 2015). The absorption of zinc in the duodenum and jejunum is strictly regulated; it increases up to 90% when dietary zinc is restricted (Taylor *et al.,* 1991), whereas zinc release, when in excess, is facilitated by the gastro-intestinal secretion, sloughing mucosal cells and integument, and renal excretion (Krebs, 2013).

2.17.4Zinc homeostasis in cells and in the body

After entry of Zn into cells, it is further distributed to diverse cellular organelles: about 30-40% to the nucleus, 10% to membranes and more than 50% to the cytoplasm. The intracellular Zn exists in three pools: 1) tightly bound to metalloenzymes, metalloproteins, and nucleoproteins; 2) loosely bound with diverse protein and amino acid ligands; 3) unbound as the free Zn^{2+} ion, albeit at very low concentrations (Rukganuer *et al.,* 1997; Disilvestrol *et al.,* 2015). While the total cellular Zn concentration is estimated to be in mmol/L range, the cytosolic concentration of free Zn^{2+} is predicted to be in the nmol/L range. The precised determination of free Zn^{2+} concentration is of importance to understand many Zn-mediated cellular events, yet the reliable measurement of free intracellular $Zn^{2+}$and visualisation of its dynamic changes is a technical challenge. This is partly a consequence of the selectivity and sensitivity of Zn probes, which is strongly influenced by the concentrations of Zn and additional cations in living biological systems (Disilvestrol *et al.,* 2015).

In general, more than 30 proteins, including ZnT and ZIP transporters, operate under strict coordinated regulation for the maintenance of systemic and cellular zinc

homeostasis in mammals. No short peptide hormone, hepcidin has been identified in zinc to play a vital role as seen in systemic iron homeostasis (Nameth *et al*., 2004; Golan *et al.,* 2015). Study findings reveal that a low-molecular-weight (2 kDa) zinc-regulated humoral factor, which is likely induced in zinc deficiency, controls gene expression. The expression is predominantly associated with immune functions and development in smooth muscle cells, suggesting that a short peptide functions as a humoral factor in systemic zinc homeostasis (Golan *et al.,* 2015).

The cellular and subcellular zinc homeostasis is achieved via sophisticated regulation of uptake, distribution, storage, and efflux, in which ZnT and ZIP transporters play primary roles. Zinc mobilisation between intracellular vesicles and organelles by these transporters also contributes to buffer perturbations of cytosolic zinc homeostasis, which is termed "buffering" and "muffling". Buffering and muffling are essential in situations of excess zinc conditions and also play a role in zinc ion fluctuations (Maret, 2013).

2.17.5Antioxidant role of zinc

There is dearth of information partaining to the role of zinc as an effective antioxidant, used in ameliorating noise stress-induced parturbations. However, Ghasem *et al*. (2016) reported that zinc supplementation greatly augments the fertilisation capacity of male rats, exposed to noise stress (Ghasem *et al.,* 2016). The effect of Zn on hepatic lipid peroxidation and activities of antioxidative enzymes in ethanol-fed rats has been demonstrated. Alcoholic liver disease may be caused by ROS-generated during the metabolism of ethanol by a microsomal ethanol-oxidising system. Increased formation of ROS and enhanced hepatic lipid peroxidation have been reported after acute and chronic

ethanol consumption, and are linked with the development of hepatic injury (Khalaf *et al.,* 2017). Feeding of ethanol to rats has been confirmed to augment the activities of SOD and GPx and diminishes the activity of catalase in rat liver. Zn supplementation to ethanol-intoxicated rats notably stabilises the increased activities of oxidative stress-related enzymes (viz. catalase, SOD and GPx) and lipid peroxidation; thus signifying its anti-peroxidative potential. The antioxidative role of zinc may underlie its fundamental ability to restore the structural as well as functional integrity of the liver exposed to ethanol toxicity, and to a large extent to regulate the antioxidant activities of enzymes as well as lipid peroxidation (Nunnes *et al.,* 2017). Ambali *et al*. (2010) reported that zinc exerts its antioxidant property in ameliorating the effect of chlorpyrifos-induced erythrocyte fragility in Wistar rats.

The deficiency of Zn aggravates the increase in ROS formation in ovariectomized rats, and its supplementation prevents ROS formation in ovariectomised rats (Baltaci *et al*., 2004).It has also been proposed that the augmentation of lipid peroxidation by cadium in rats is a result of a decrease in SOD and CAT activities. Zn supplementation prior to cadium administration averts several effects observed when cadium is administered alone (Jemaia *et al.,* 2007). Result indicates that one of the mechanisms involved in the protective role of Zn against Cd-induced toxicity is connected with inhibition of Cd-induced ROS formation. The role of Zn in protecting biological structures from ROS damage is due to several factors: first, by maintaining an adequate level of metallothionein which is also an ROS scavenger; then, as an essential structural component of Cu/Zn SOD; and finally, as a protective agent for thiols and additional chemical groups (Jemaia *et al.,* 2007). It has been shown that Cd induces a decline in

erythrocyte SOD and CAT activities and an increase in lipid peroxide concentration in plasma. Zn supply on exposure to Cd shields against the Cd-induced decrease in SOD activity and mightpartially protect against Cd-induced lipid peroxidation (Jemaia *et al.,* 2007). Zinc is also an incredibly essential supplement during administration of chelating agents in lead treatment, especially when using EDTA and dimacaprol as it has also been accounted to scavenge ROS released in the process (Flora and Tandon, 1990; Grogorescu *et al.,* 2015).

2.17.6Mechanism of action of zinc as an antioxidant

Zinc operates as an antioxidant by different mechanisms. Principally, zinc competes with iron (Fe) and copper (Cu) ions for binding to cell membranes and proteins, displacing these redox active metals, which catalyze the formation of $^{\cdot}OH$ from H_2O_2 (Bao *et al.,* 2013). Zinc also binds to sulfhydryl (SH) groups of bio-molecules, shielding them from oxidation. It increases the activation of antioxidant proteins, molecules, and enzymes, such as GSH, CAT, and SOD. It also attenuates the activities of oxidant-promoting enzymes such as inducible nitric acid synthase (iNOS) and NADPH oxidase, and inhibits the generation of lipid peroxidation products. Zn also instigates the expression of a metal-binding protein metallothionein (MT), which is very rich in cysteine and is an excellent scavanger of $^{\cdot}OH$ ions (Bao *et al.,* 2013; Gao *et al.,* 2014). Several studies have demonsrated that Zn may have a regulatory role in Nrf2. Zinc upregulates Nrf2 activity and attenuates oxidative stress (Bao *et al.,* 2013).

2.17.7Zinc signalling

The signalling functions of Zn is very crucial and occurs by increase in Zn^{2+} concentrations, triggered by stimuli. Zinc-activated signalling is associated with

pathophysiological functions (Fakada and Kambe, 2011); and thus, has therapeutic potential. Extracellular release of Zn acts as a signalling mediator in endocrine, paracrine, and autocrine systems. In the central nervous system, Zn, which is released from presynaptic neurons following excitation into synaptic clefts, modulates synaptic transmission by binding to various transporters and receptor channels on postsynaptic neurons (Takada *et al*., 2013). Zn, co-released from pancreatic β-cells along with insulin by glucose stimuli, can suppress hepatic insulin clearance and diminish insulin secretion from the β-cells (Tamaki *et al.,* 2013). Extracellular release of Zn binds to diverse cell surface proteins, and the most intriguing protein is the Zn receptor, the G protein-coupled receptor 39 (GPR39) (Sharir *et al.,* 2010). Zn plays vital signalling roles as a second messenger in the cytosol (Fakada and Kambe, 2011), where Zn signalling is instigated by Zn influx, which originates from extracellular sites and from intracellular organelles. "Zn wave" refers to Zn released from the perinuclear area, including the endoplasmic reticulum (ER) which has been shown to be important for cell signalling functions (Taylor *et al*., 2012). Moreover, Zn, released from cytosolic proteins with oxidation-sensitive Zn-binding sites such as MT via oxidative stimuli, is also involved in intracellular Zn signalling (Aras *et al.,* 2011; Nunnes *et al*., 2017). Intracellular Zn signalling is divided into several categories according to the time-scale in which it acts (Haase *et al.,* 2014). "Fast" or "early" Zn signalling occurs within seconds to minutes after stimulation and does not require transcription of proteins (Haase *et al.,* 2008). Conversely, "late" Zn signalling requires biosynthesis of proteins to control cytosolic Zn concentrations and occurs over a period of hours after stimulation (Kitamura *et al.,* 2006). The word "late" Zn signalling is commonly used, to contrast with the word "fast" or

"early" Zn signalling. This term portray the down-stream effects mediated through changes in gene expression, not signalling events. Prominently, intracellular Zn signalling targets a number of enzymes involved in cellular signalling, including protein tyrosine phosphatases (PTPs) (Wilson *et al.*, 2012), phosphodiesterases (PDEs), (Hajyo *et al*., 2011), calcineurin (Aydemir *et al*., 2009), caspases (Miyai *et al.*, 2014) and diverse kinases such as mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) (Hasson *et al.*, 1996).

Physiological levels of intracellular free Zn^{2+} concentrations regulate these enzymes. For example, inhibition of caspase-3, T-cell PTP, and PTP-1B is achieved with IC_{50} values below 10, 200, and 17 mmol/L, respectively (Haase and Maret, 2003). Inhibition of the activity of PTPs by Zn is commonly operative in intracellular Zn signalling. The enzymatic activity of caspase-9 is reversibly inhibited by Zn binding to cysteine and histidine residues in the active site, and similar reversible inhibition mechanisms operate in other enzymes such as PTPs (Haase and Maret, 2005). Specifically, inhibition of PTP-1B activity by Zn is possibly mediated via a cysteinyl-phosphate intermediate (Bellomo *et al.*, 2014).

2.17.8Effect of zinc on neuropsychological performance

Evidence of enhanced brain development attributable to improved Zn status has originated from studies of activity levels in young children in India (Sazawal *et al.*, 1996) and Guatemala (Bentley *et al.*, 1997). Although neuropsychological performance has been reported to improve with Zn supplementation in young Chinese children (Sandstead

et al., 1998), occurring only when other micronutrient nutrition is sufficient (Prasad, 2013,2014a).

2.17.9Zn action as anti-inflammatory agent

One of the major immune response transcription factors involved in molecular signaling NF-κB. Zn plays an essential role in activation of NF-κB. The regulation of NF-κB activation by Zn is however, cell-specific (Prasad *et al*., 2004; Prasad, 2014b). Report state that Zn is required for NF-κB DNA binding in purified or recombinant NF-κB p50 protein in T-helper cell lines (Prasad *et al.*, 2001). Prasad *et al.* (2001) reported that normal healthy volunteers, who were supplemented with 45 mg elemental Zn daily, had a significant reduction in TNF-α and IL-1β messenger RNAs and TNF-α induced NF-κB DNA binding in isolated peripheral blood mononuclear cells in comparison to placebo-treated subjects (Prasad *et al.*, 2004). Zn upregulated the expression of A20 in HL-60 cells. The study showed that Zn decreased ox-LDL-induced generation of TNF-α, IL-1β, and VCAM-1, oxidative stress markers in the plasma and activation of NF-κB, and increased A20 and PPAR-α protein in human monocytic and vascular endothelial cells (Bao *et al*., 2010; Prasad, 2014b). It is believed that Zn inhibited NF-κB activation via A20, a Zn finger transactivating factor that plays an essential role in down regulating IL-1β and TNF-α-induced NF-κB activation. A20 was originally thought to protect cells from TNF-α-induced cytotoxicity by inhibiting the activation of NF-κB resulting in lower IL-1β and TNF-α signalling in endothelial cells (Bao *et al*., 2010). It was shown that A20 inhibits NF-κB signalling by TNF-α and IL-1β via TNF-receptor-associated factor pathways in endothelial cells (Bao *et al*., 2010; Prasad, 2014b).

2.17.10Clinical manifestations of zn deficiency

The symptoms may be very severe and even fatal if not recognised and treated promptly by Zn administration as seen in patients with *Acrodermatitis enteropathica* (AE). AE is a genetic disorder mainly affecting infants of Italian, Armenian, or Iranian lineage (Bao *et al.,* 2010). The manifestations include bullous pustular dermatitis, particularly around the orifices. Ophthalmic signs include blepharitis, conjunctivitis, photophobia, and corneal opacities (Prasad, 2013, 2014b). Neuropsychiatric signs include irritability, emotional instability, tremors and cerebellar ataxia, weight loss, growth failure, and male hypogonadism are prominent features. Congenital malformation of foetuses and infants born of pregnant women with AE has been reported frequently (Cavdar *et al.,* 1980; Prasad, 2014a).

Acrodermatitis enteropathica patients are very susceptible to infections. Thymic hypolasia and plasmacytosis in the spleen are commonly seen in experiment animals. T-cell-mediated immune disorders are corrected by Zn supplementation. Without Zn supplementation, the clinical course is downhill with failure to thrive, and complicated by intercurrent bacterial, viral, fungal, and opportunistic infections. Gastro-intestinal manifestations include diarrhoea, malabsorption, steatorrhoea, and lactose intolerance. Zn supplementation in therapeutic doses (in excess of 50 mg elemental Zn daily, in humans) results in complete recovery (Prasad, 2013, 2014b).

2.17.11 Zn deficiency and growth.

The one clinical feature of Zn deficiency that is most studied is the impairment of physical growth. As with other features of Zn deficiency, the most definitive

investigations have been those based on randomised, controlled studies of dietary Zn supplementation. In the Colorado studies in the 1970s and 1980s, the principal focus was on physical growth. The principal reason for this focus was the earlier clear demonstration in animal models that Zn had little pharmacological effect on growth (Williams and Mills 1970; Bostanci *et al.*, 2015). Consequently, the demonstration of an increase in growth velocity associated with modest dietary Zn supplements under double-blind, controlled, randomised study conditions provided convincing evidence for a pre-existing growth-limiting Zn deficiency state. In all, four of such studies were undertaken in Colorado. Subjects ranged from healthy cow's milk formula–fed infants (Walravens and Hambidge, 1976), at a time before Zn fortification of cow's milk formulas became the norm, to older infants, toddlers or young children with non-organic failure to thrive. Consequently, many other studies of Zn supplementation have included growth measurements. These results have been subjected to rigorous and repeated meta-analysis with confirmation of the effect of Zn supplements in increasing height and weight velocity, when administered to children in many countries (Brown *et al.*, 1998).

In some cases, effects have been observed on body composition rather than on weight or on linear growth velocity (Kikafunda *et al*. 1998). These different responses serve as a reminder of how much there remains to be learned about variations in the presentation of Zn deficiency and the factors responsible. They also serve as a reminder that the biochemical and hormonal factors underlying these effects on growth remain unclear. One of the environmental issues of note is the total diet, including concurrent deficiencies or imbalances of other micronutrients (Solomons *et al*., 1999).

2.17.12Diagnosis of Zn deficiency

Recommended daily dietary allowances (RDA) for infants up to 1 year is 3–5 mg, for children from 1 to 10 years is 10 mg, and for adults (both males and females) it is 15 mg. For pregnant women, RDA is 20 mg and for lactating women RDA is 25 mg (Prasad, 2013, 2014a). Currently, the most widely used test for diagnosing Zn deficiency in humans is the determination of plasma Zn level. Normal levels of plasma Zn in adults and children are 100 ± 10 μg/dL. Values below 80 μg/dL will be considered to be in the deficient range. Measurement of plasma Zn by flameless atomic absorption specifically is useful, provided the sample is not haemolysed or contaminated. In patients with acute stress or infection, Zn from the plasma pool may redistribute to other compartments, thus making the diagnosis of Zn deficiency difficult. Intravascular haemolysis may increase the plasma Zn level, in as much as the Zn in the erythrocytes is much higher than in the plasma (Bao *et al*., 2010). Plasma Zn level of < 50 μg/dL may be indicative of severe deficiency of Zn (Prasad, 2013, 2014; Chavellet *et al.*, 2014).

Determination of Zn in the lymphocytes, plasma somatomedin activity, and measurement of IL-2 mRNA in PHA-stimulated mononuclear cells by reverse transcriptase (RT)-polymerase chain reaction (PCR) are the most sensitive tests for diagnosing Zn deficiency in humans (Prasad *et al.*, 1993).

2.17.13Prevention and management of Zn deficiency

Research studies have emphasised the ubiquity of Zn in biology and the dependence of a wide range of vital metabolic processes on an adequate supply of this metal. As observed in all nutrients, the challenge is to achieve intakes of bioavailable Zn and tissue levels

within a physiological range. Presently, this is commonly considered to be a total of 50 mg elemental Zn per day for adults, with levels of intake for children that are less clear (Prasad, 2013, 2014). It is imperative to be appropriately sensitive to this upper limit, even if a decision is made to temporarily exceed this for anticipated (but often uncertain) pharmacological benefits. It is even more essential to be aware that these upper limits are for total daily intake, which may be higher than anticipated due to the increasing trend to fortify foods with Zn. The use of Zn, as a single micronutrient supplement, for research purposes has proved priceless, and such studies will continue to have an important role in advancing our understanding of the prevalence and effects of human Zn deficiency. There is already, however, also an important role for studies that include arms for multi-nutrient supplements and, even more important, for studies of dietary modifications that affect mineral bioavailability, whether these are at a local community level or involve more global strategies. Meanwhile, wide-spread Zn supplementation either as a single micronutrient or as a component of a multi-micronutrient mix is a good preventive measure (Prasad, 2013, 2014).

CHAPTER THREE:

3.0 MATERIALS AND METHODS

3.1 Location of Experiment

The experiment was carried out in the laboratory at the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (11^0 $10^/$ N, 07^0 $38^/$ E), located in the Northern Guinea Savannah zone of Nigeria.

3.2 Animals

A total of 30 eight-week-old Wistarrats, 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*.

3.3 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 deionised water; Group III, kaempferol; Group IV, zinc gluconate; Group V, kaempferol + zinc gluconate for 36 days. Following 21 days of pretreatment, 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; that is, on days 22, 29, and 36of the experiment in the controls.

Table 1: Experimental Grouping

Group	n	Noise (100dB)	Treatment	Dose
I	6	-	DW	2 mL/kg
II	6	+	DW	2 mL/kg
III	6	+	K	5 mg/kg
IV	6	+	ZG	50 mg/kg
V	6	+	K+ZG	5 +50 mg/kg

Key: DW = Deionised Water, K = Kaempferol, ZG = Zinc gluconate. n = Number of animals

3.4Drug 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 (Bigoniya *et al.*, 2013) and 50 mg Zn/kg body weight (Ghasem *et al*., 2016), 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 rate of 2 mL/kg body weight.

3.5Noise Stress Induction

Noise stress was induced using a modification of the method described by Wankhar *et al.* (2014). 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 and other parts of the world (Ismaila and Odusote, 2014).

3.6 Evaluation of Neurobehavioural and Cognitive Changes

The neurobehavioural and cognitive changes in the rats were carried out on days 22, 29, and 36 as follows.

3.6.1 Open-field assessment

This was carried out on all experimental rats using the open-field apparatus as described by Zhu *et al*. (2001). It is a simple test used to evaluate the locomotion and emotional status of animals. The open-field apparatus was constructed using cardboard box with a dimension of 50 × 50 × 45 cm with a clear Plexiglass on the inner surface (base). The floor of the box was divided into 25 equal squares. The parameters analysed were locomotor activity and anxiety, evaluated using horizontal locomotion (number of crossings of the lines marked on the floor) and frequency of rearing. Locomotor activity involved placing an animal in the box and allowing it for a period of 3 minutes to walk freely and become familiarised with the environment. Thereafter and for the next 2 minutes, the number of squares crossed with all the paws was recorded. Rearing was measured by determining the frequency at which each rat stood on its hind-limbs, with the fore-paws lifted up freely or leaned against the wall of the apparatus during the next 2 minutes, after the initial 3 minutes of habitation. The frequency of grooming (protracted washing of the coat), number of faeces voided, and the number of puddles of urine voided were also observed and recorded.

3.6.2 Assessment of neuromuscular coordination

The effect of the regimen on neuromuscular coordination was assessed using the inclined plane (Abou-Donia*et al.*, 2001). Each rat was placed on an apparatus made with an angled rough wooden plank, with a thick foam pad at its base. Angles were measured and marked on the apparatus beforehand, and were obtained by propping the plank on a vertical bar with several notches. The plain was initially inclined at an angle of 35° and thereafter increased step-wise by 5° until the subject could no longer hold on horizontally

for 30 seconds without sliding down. The test was performed with the subjects facing left and then right across the plank. The highest angle at which the rat could no longer stay, standing horizontally and facing any of the directions was recorded. Two trials were performed for each testing period.

3.6.3 Assessment of motor coordination

The motor coordination was assessed using the beam-walk performance task as described by Abou-Donia*et al.* (2001). Briefly, the beam-walk performance was evaluated by allowing individual rat from each group to walk across a wooden black beam of 106-cm length, beginning at 17.2-cm width and ending at 1.0-cm width. Periodic widths were marked on the side of the apparatus. On each side of the narrowing beam, there was a 1.8-cm step-down to a 3.0-cm area, where each subject stepped, if necessary. As the subject walked across, the width of the beam at which it stepped down was recorded by one rater on each side. This was repeated twice and the average recorded during each trial session.

3.6.4 Assessment of motor strength

The fore-paw grip-time was evaluated using the method described by Abou-Donia*et al.* (2001). It was used to evaluate the motor strength of the rats hung from a 5-mm-thick diameter wood dowel, gripped with both fore-paws. The time spent by each rat before releasing its grip was recorded in seconds.

3.6.5 Assessment of excitability scores

The excitability score of each rat was evaluated using the method described by Ambali and Ayo,(2012). Briefly, each rat was held by the tail upside down and kept in that

position for 30 seconds. Response of each rat was rated using ordinal scale of 0-5 as follows:

Grade 0: Rats did not show any sign of wriggling at all.

Grade 1: Rat wriggling was low with feeble fore-paw movement.

Grade 2: Rat responded through a stronger wriggling and forepaw movement.

Grade 3: Rat vigorously wriggled and showed strong fore-and hind-limb movement.

Grade 4: In addition to the observations in grade 3 above, rat attempted unsuccessfully to climb on its tail.

Grade 5: In addition to the observations in grade 3 above, the rat successfully climbed the tip of its tail.

3.6.6 Evaluation of learning and short-term memory

The effect of the different regimens on learning was assessed using the step-down inhibitory avoidance task as described by Zhu *et al.* (2001). This was evaluated 48 hours to the termination of the experiment. It involved the use of an acrylic chamber 40 × 25 × 25-cm, consisting of a floor made of parallel 2-mm calibre stainless steel bars, spaced 1cm apart. An electric current (80 volts) was delivered through the floor bars and a 2.5 cm high, 8 × 25 cm wooden platform, placed on the left extreme of the chamber. Each rat was gently placed on the platform. The ability of each rat to remain on the platform, having received an initial foot-shock when it stepped down from the platform the first time, was the basis of this assessment. The number of times the rat stepped down with all its limbs after receiving an electric shock was used as an index of learning. The maximum learning ability was observed, when the rat stayed on the platform for 2 minutes without stepping down. Short-term memory was assessed 24 hours later using

the same procedure. In this case, each rat was again placed gently on the platform, and the period of time it remained on the platform without stepping down was recorded as an index of memory retention. The ceiling memory was observed, when the rat remained on the platform for 2 minutes.

3.7 Collection and Analyses of Biomarkers of Oxidative Stress

At the end of the experimental period, all the rats were exposed to mild ether 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 minutes and the supernatant was decanted into sterile test tubes, and stored in the refrigerator for further analysis.

3.7.1 Determination of haematological parameters

Blood (1 mL) was collected from each rat through jugular vrnrsection into sample bottles, containing ethelenediaminetetraacetic 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; Dacie and Lewis,1975).

3.7.2 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*. (2011). 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 (24-26 ^{o}C) for 30 minutes. The content of each tube was centrifuged at 1500 x g for 10 minutes 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%.

$$\text{Percentage haemolysis (\%)} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 100$$

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

3.7.3 Determination of brain lipid peroxidative changes

Lipid peroxidation was determined by measuring the malondialdehyde concentration in the brain using the method described by Kamiyama *et al.,* (1993) using trichloroacetic acid (TCA) and thiobarbituric acid (TBA). The principle was based on measurement of the color developed as a result of the reaction of TBA with MDA. Briefly, brain samples from animals in all the groups were individually weighed and then homogenized in 5 mL of ice-cold phosphate buffer to obtain a 10% homogenate. Exactly 2 mL of 15% TCA, 2 mL of TBA and 100 µL of the tissue homogenate were measured into a test tube. The mixture was incubated at 80°C for 30 minutes, in a water bath and allowed to cool for some time followed by centrifugation at 3000 rpm for 10 minutes. A clear supernatant

was collected and its absorbance was determined at 532 nm using a UV spectrophotometer (Spectro Lab S23A, Techmel, USA). Concentrations of TBARS calculated as follows were expressed in nmol/mg protein:

$$\text{Concentration (nmols/mg protein)} = \frac{\text{Absorbance of sample}}{1.56\times10^{-5}\times \text{protein concentration (mg)}}$$

3.7.4 Determination of brain superoxide dismustase activity

The activity of SOD was assessed using the NWLSS^{TM} using the SOD activity assay, which provides a simple rate method for determining SOD activity. This method is based on monitoring the auto-oxidation rate of haematoxylin as originally described by Martin *et al*. (1987), with modifications to increase robustness and fragility. In the presence of SOD enzyme at specific assay pH of 7.4, the rate of auto-oxidation rates is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity was determined by measuring ratios of auto-oxidation rates in the presence and absence of the sample, and was expressed as traditional McCord-Fridovich "cytochrome C" units. Briefly, 230 µL of assay buffer was added to each well, followed by addition of 10 µL of assay buffer (for blank) or 10 µL to the wells. The wells were shaken to allow for proper mixing and incubated for 2 minutes. Haematoxylin reagent (10 µL) was added to begin the reaction. It was mixed quickly using the instrument's shaker function, and immediately the absorbance was recorded at 560 nm every 10 seconds for at least 5 minutes using a microtitre plate reader. The SOD activity was calculated as: SOD U/mL = 1.25 x % inhibition.

3.7.5 Determination brain catalase activity

Catalase activity was determined using the method described by Abei (1974). The principle was based on monitoring and measuring the consumption of hydrogen peroxide (H_2O_2) substrate at 240 nm. Exactly 10 µL of the homogenate was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of freshly prepared 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity. Catalase Concentration = Absorbance/E

Catalase Activity (U/mg) = Catalase concentration/protein concentration (mg/mL)

3.7.6 Determination of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was determined using Palgia and Valentine (1967) spectrophotometry method, and based on NWLSS^{TM} glutathione peroxidase assay kits protocol. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucletide phosphate (NADPH), forming $NADPP^+$, resulting in decreased absorbance at 340 nm and recycling the GSH. The decrease in absorbance at 340 nm is directly proportional to the GPx concentration. Briefly, 50 µL of diluted sample was added to each well, followed by addition of 50 µL of working NADPH to the wells. Fifty (50) microlitre of working H_2O_2 was then added to each well and the absorbance read at 340 nm for 5 minutes, using a

spectrophotometer with recording interval of 30 seconds. The GPx activity was then calculated from the net rate.

$$[GPx] = \frac{2\,(mRate_s - mRate_b).V_{RKM}}{} \;.\; df$$

2.74. Vs

Where $mRate_S$ = -1,000 ΔA340/min of sample; $mRate_b$ = -1,000 ΔA340/min of blank; 2.74 = NADPH 340 nm millimollar absorbtion coefficient at 1 cm path length; V_{Rxm} = volume of reaction mixture; Vs = Volume of sample; 2 = correction for 2 moles reduced glutathione, oxidized to 1 mole glutathione disulphide (oxidized glutathione) per mole NADPH oxidized; df = sample dilution factor.

3.8 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. Excitability scores were analysed using Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's test. 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.

CHAPTER FOUR:

4.0 RESULTS

4.1 Effect of Treatments on Body Weight Changes in WistarRats exposed to Noise Stress

The effect of the various treatments on body weight gain of the rats is presented in Figure 3. There was no significant difference in the cumulative weight of Wistar rats across the treatment groups on day one. However, on days 8 and 15, the cumulative weight gain increased significantly ($P< 0.05$) in the group treated with deionised water only (126.80 ± 1.35 g, 138.30 ± 1.80 g), compared with those treated with deionised water + noise (117.70 ± 1.02 g, 125.70 ±1.20 g). There was also a significant decrease in the group treated with deionised water + noise (117.70 ± 1.02 g, 125.70 ± 1.20 g) compared with those treated with noise + kaempferol + zinc (123.70 ± 0.99 g, 133.70 ± 1.59 g). There was no significant difference between the group treated with deionised water + noise (117.70 ± 1.02 g, 125.70 ± 1.20 g), compared with those treated with noise + kaempferol (120.80 ± 1.56, 130.70 ± 1.45 g), and noise + zinc (116.00 ± 1.29, 126.00 ± 1.75 g).

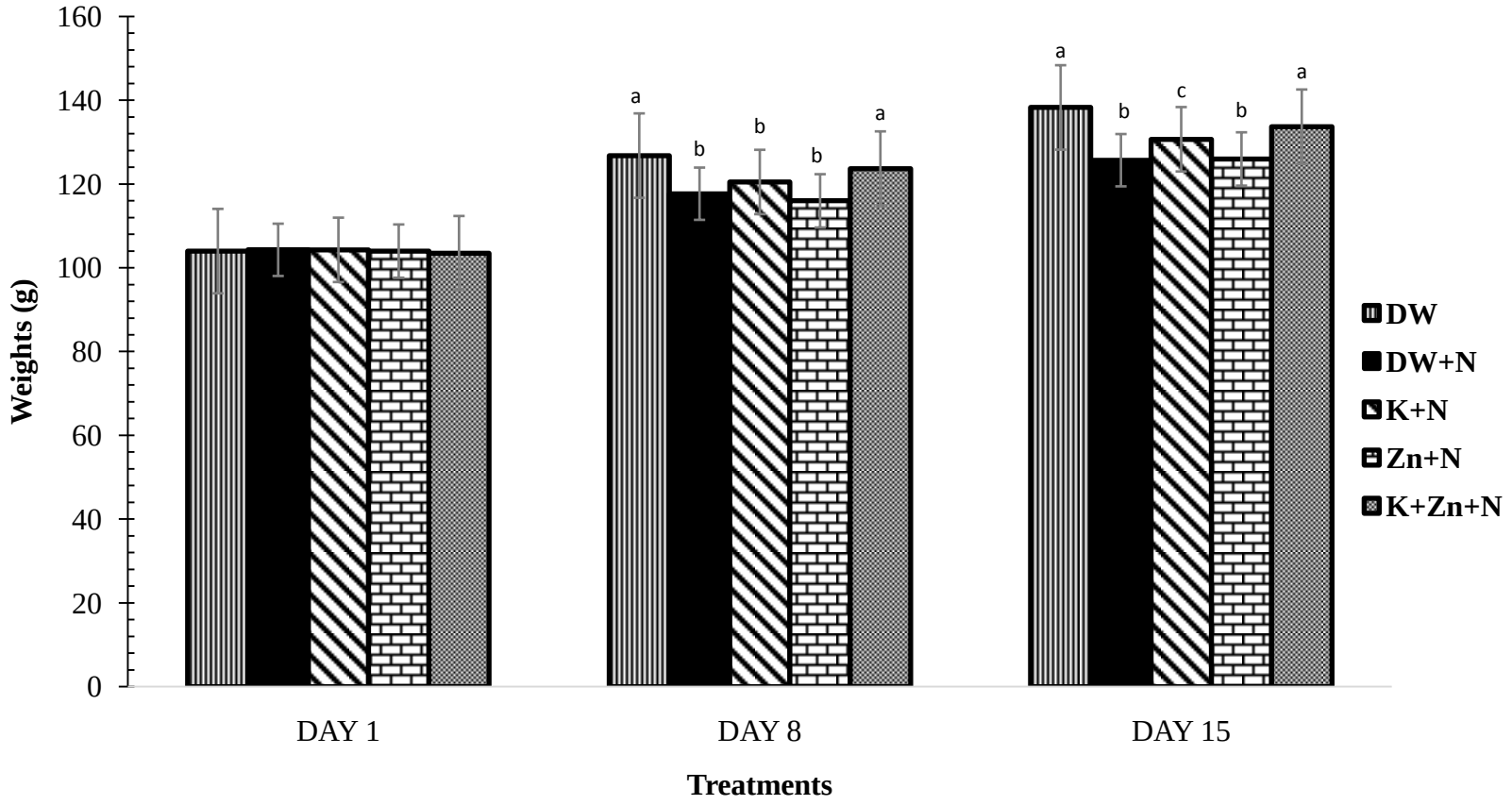


Figure 3: Ameliorative effect of kaempferol and zinc on body weight of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

4.2 Effect of Kaempferol and Zinc on Erythrocyte Osmotic Fragility in WistarRats exposed to Noise Stress

The effect of the various treatments on erythrocyte osmotic fragility of the rats is presented in Figure 4. The EOF across the treatment groups decreased as NaCl concentrations increased from 0 to 0.9%. There was a significant ($P < 0.05$) decrease in the EOF of groups treated with kaempferol + noise (76.78 ± 3.07, 43.02 ± 7.75, 4.88 ± 0.33, 6.22 ± 0.97), zinc + noise (83.80 ± 2.26, 33.12 ± 2.99, 5.13 ± 0.27, 6.83 ± 0.98) and kaempferol + zinc + noise (76.52 ± 2.00, 34.75 ± 3.73, 3.35 ± 0.30, 5.67 ± 1.12) compared to the group treated with noise + deionised water (95.42 ± 0.82, 74.73 ± 6.70, 12.00 ±1.56, 10.45 ±1.59) at 0.3 %, 0.5 %, 0.7% and 0.9 % concentration of NaCl respectively.

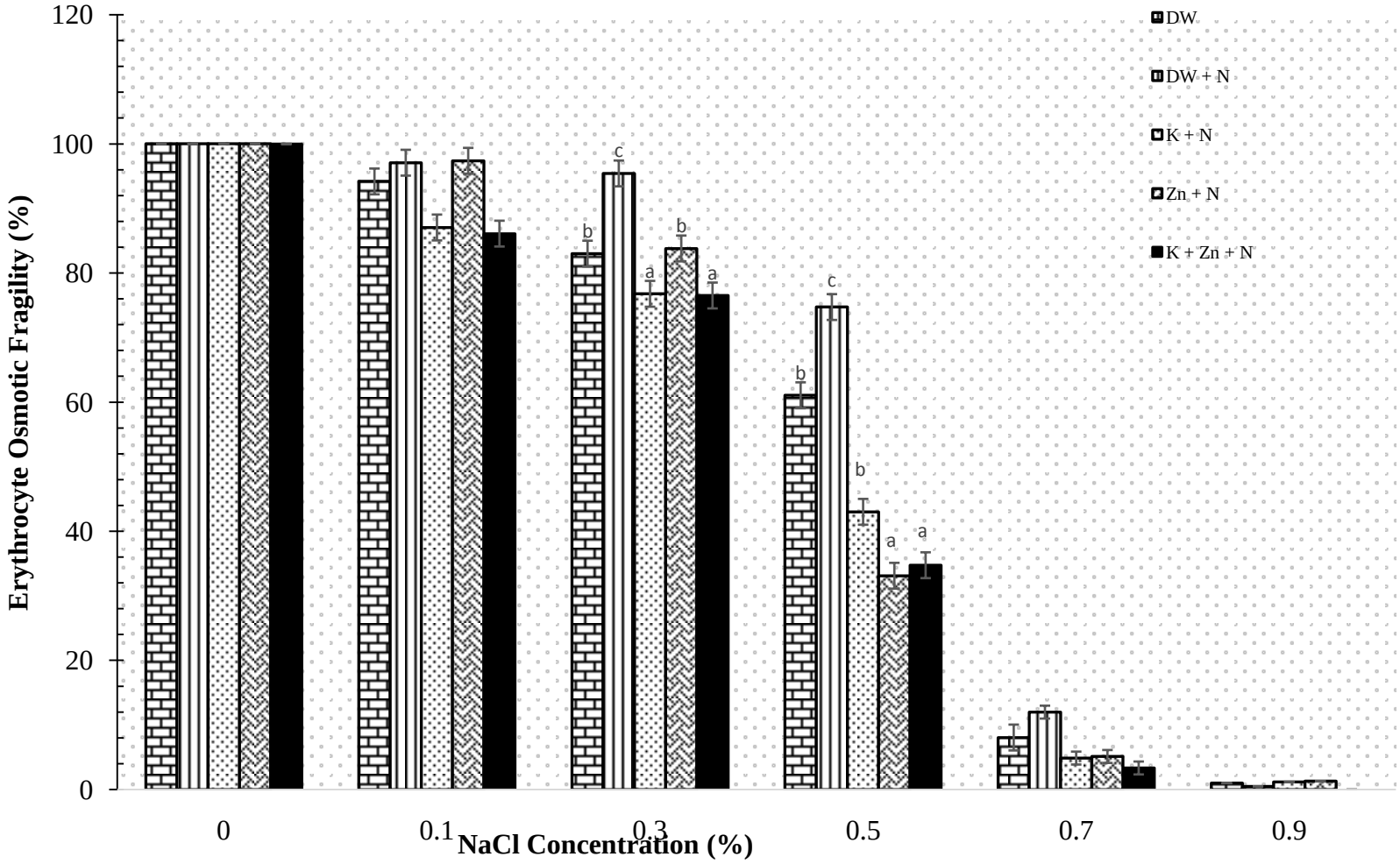


Fig 4: Ameliorative effect of kaempferol and zinc on erythrocyte osmotic fragility in Wistar rats exposed to noise stress. (Mean ± SEM, n = 6)

[a, b, c, d] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

4.3 Effect of Kaempferol and Zinc on Red Blood Cells and Erythrocytic Indices of WistarRats exposed to Noise Stress

4.3.1 Haemoglobin concentration

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; Table 2).

4.3.2Packed 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 2).

4.3.3 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 × 10^{12}/L) compared with counts obtained in the deionised water-treated group (7.19 ± 0.05 x 10^{12}/L) and kaempferol + zinc + noise treated group (7.20 ± 0.06× 10^{12}/L). The total erythrocyte counts obtained in

kaempferol + noise (6.90 ± 0.07 × 10^{12}/L) was not significantly (P< 0.05) higher than that obtained in zinc + noise treated group (6.65 ± 0.07 × 10^{12}/L; Table 2).

4.3.4Erythrocytic 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 ρg) 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 ρg) and mean corpuscular haemoglobin concentration (33.20 ± 0.15%). There was no significant difference between the group treated with kaempferol + noise and zinc + noise (Table 2).

4.3.5 Platelet count

Platelet count significantly (P< 0.05) decreased in the group treated with deionised water + noise (439.80 ± 7.91^6 g/dL), compared with the group treated with deionised water (637.30 ± 9.26^6 g/dL), kaempferol + noise + zinc (609.20 ± 6.90^6 g/dL), kaempferol + noise (532.70 ± 9.12) and zinc + noise (529.70 ± 5.43^6 g/dL). The group treated with deionised water had the highest platelet count (637.30 ± 9.26^6 g/dL) followed by the kaempferol + noise + zinc treated group (609.20 ± 6.90^6 g/dL). There was no significant difference in the counts between the groups treated with kaempferol + noise (532.70 ± 9.12^6 g/dL) and zinc + noise (529.70 ± 5.43^6 g/dL; Table 3).

4.3.6 Absolute and differential leucocyte counts

The total leucocyte count obtained in deionised water + noise-treated group (4.90 ± 0.06 × 10^6 g/dL) was significantly ($P< 0.05$) lower, when compared with the count recorded in the group treated with deionised water (6.26 ± 0.04 × 10^6 g/dL), kaempferol + noise (5.46 ± 0.02 × 10^6 g/dL), zinc + noise (5.29 ± 0.04 × 10^6 g/dL), and kaempferol + zinc + noise treated group (5.81 ± 0.09 × 10^6 g/dL; Table 3).

The number of neutrophils obtained in deionised water + noise-treated group (0.88 ± 0.02× 10^6 g/dL) was significantly ($P< 0.05$) lower than the counts recorded in the group treated with deionised water (1.11 ± 0.02× 10^6 g/dL), zinc + noise (0.93 ± 0.04× 10^6 g/dL) and kaempferol + zinc + noise treated group (1.41 ± 0.05× 10^6 g/dL). Similarly, the number of lymphocytes in the deionised water + noise-treated group (2.50 ± 0.01× 10^6 g/dL) was significantly ($P< 0.05$) lower, when compared with the group treated with deionised water (4.95 ± 0.01× 10^6 g/dL), kaempferol + noise (4.44 ± 0.02× 10^6 g/dL), zinc + noise (4.15 ± 0.03× 10^6 g/dL), and kaempferol + zinc + noise treated group (5.66 ± 0.01× 10^6 g/dL; Table 3).

The number of monocytes obtained in the deionised water + noise-treated group (0.98 ± 0.22× 10^6 g/dL) was significantly ($P< 0.05$) higher when compared with the group treated with deionised water (0.02 ± 0.01× 10^6 g/dL), kaempferol + noise (0.03 ± 0.03^6 g/dL), zinc + noise (0.04 ± 0.01^6 g/dL) and kaempferol + zinc + noise treated group (0.02 ± 0.02× 10^6 g/dL). There was no significant difference in the number of circulating basophils in all the treatment groups (Table 3).

The N/L ratio obtained in deionised water + noise-treated group (0.35 ± 0.03) was significantly ($P< 0.05$) higher, when compared with the group treated with deionised water (0.23 ± 0.01), kaempferol + noise (0.20 ± 0.02), zinc + noise (0.22 ± 0.03) and kaempferol + zinc + noise treated group (0.25 ± 0.01; Table 3).

Table 2: Ameliorative effect of kaempferol and zinc on erythrocytic indices of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

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

$^{a, b, c}$ = 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 3: Ameliorative effect of kaempferol and zinc on platelet and leucocyte counts of Wistar rats exposed to noise stress (Mean ± SEM, n = 6).

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

$^{a, b, c}$ = Values along the same row with different superscript letters are significantly ($P < 0.05$) different

DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

4.4 Effect of Kaempferol ond Zinc on Neuro-Behavioural Changes of WistarRats exposed to Noise Stress

4.4.1 Learning

The number of foot-shocks applied to the rats in the deionised water + noise group (2.33 ± 0.33) was significantly (P< 0.05) higher compared to that applied to those in the group treated with only deionised water (1.33 ± 0.21), kaempferol + noise (1.33 ± 0.21), zinc + noise (1.50 ± 0.22) and kaempferol + zinc + noise (1.17 ± 0.17; Figure 5).

4.4.2Memory

A significant (P < 0.05) difference was observed in memory between the groups. The amount of time (seconds) spent on the platform by the group treated with deionised water + noise group (92.17 ± 4.08 s) was significantly lower, compared to that recorded in the group treated with only deionised water (108 ± 2.08s, kaempferol + noise (103.70 ± 1.80 Secs) and kaempferol + zinc + noise (106.80 ± 2.14s; Figure 6).

4.4.3Motor strength

The effect of treatments on forepaw grip is shown in Figure 7. On day 8, there was no significant decrease in the time taken to hold onto a grip in rats treated with deionised water + noise group compared to groups treated with kaempferol + noise, zinc + noise, and kaempferol + zinc + noise. On day 15, there was a significant (*P*< 0.05) decrease in the group treated with deionised water + noise (51.67 ± 2.29 Secs) compared to group treated with kaempferol + zinc + noise (78.33 ± 5.69 Secs).

4.4.4 Sensorimotor reflex

There was no significant difference in the excitability scores of Wistar rats in all the treatment groups throughout the study period (Figure 8). On day 15, the excitability score

of the group treated with deionised water + noise was significantly lower (3.83 ± 0.40), compared to the group treated with only deionised water (4.84 ± 0.17) and kaempferol + zinc + noise group (4.83 ± 0.17)

4.4.5Neuromuscular performance

There was a relatively lower inclined plane performance in the group treated with deionised water and noise (58.33 ± 2.11^0) on day 15, compared with that of other groups, but the difference was not significant. On the overall, there was no significant difference in the inclined plane performance of Wistar rats across the groups (Figure 9).

4.4.6 Motor coordination

There was a significant ($P < 0.05$) difference in the step-down width of groups, exposed to noise stress and the unexposed group. The step-down width of the group treated with deionised water + noise was the highest on days 1, 8 and 15 (10.83 ± 1.40 cm, 11.5 ± 1.18 cm, 10.50 ± 0.923 cm), compared to the group treated with kaempferol + zinc + noise, which had the least width (5.33 ± 1.45 cm, 4.33 ± 1.41cm, 4.83 ± 1.35 cm) for the respective days (Figure 10).

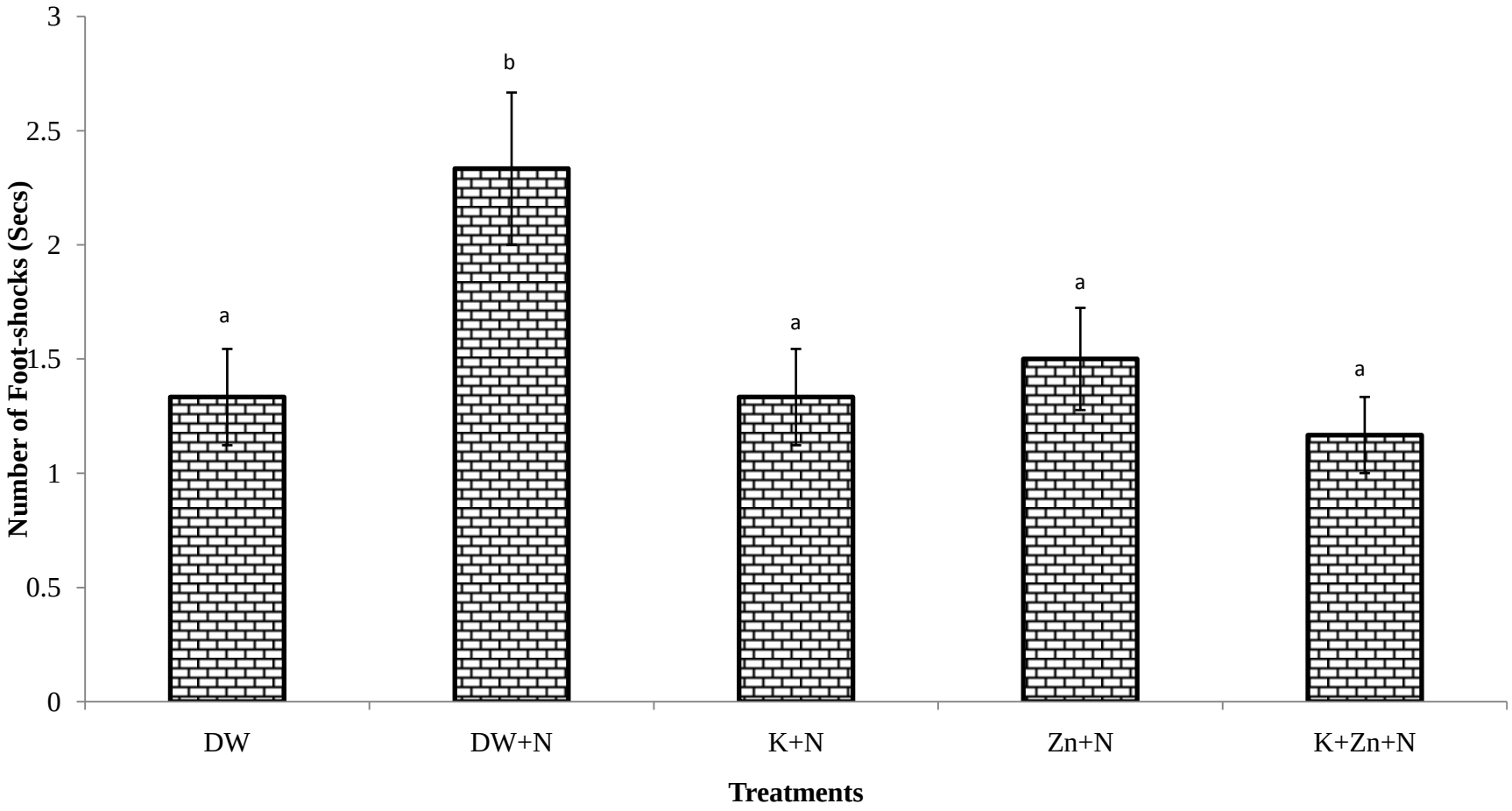


Figure 5: Ameliorative effect of kaempferol and zinc on learning of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b] = Values with different superscript letters are significantly ($P < 0.05$) different

DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

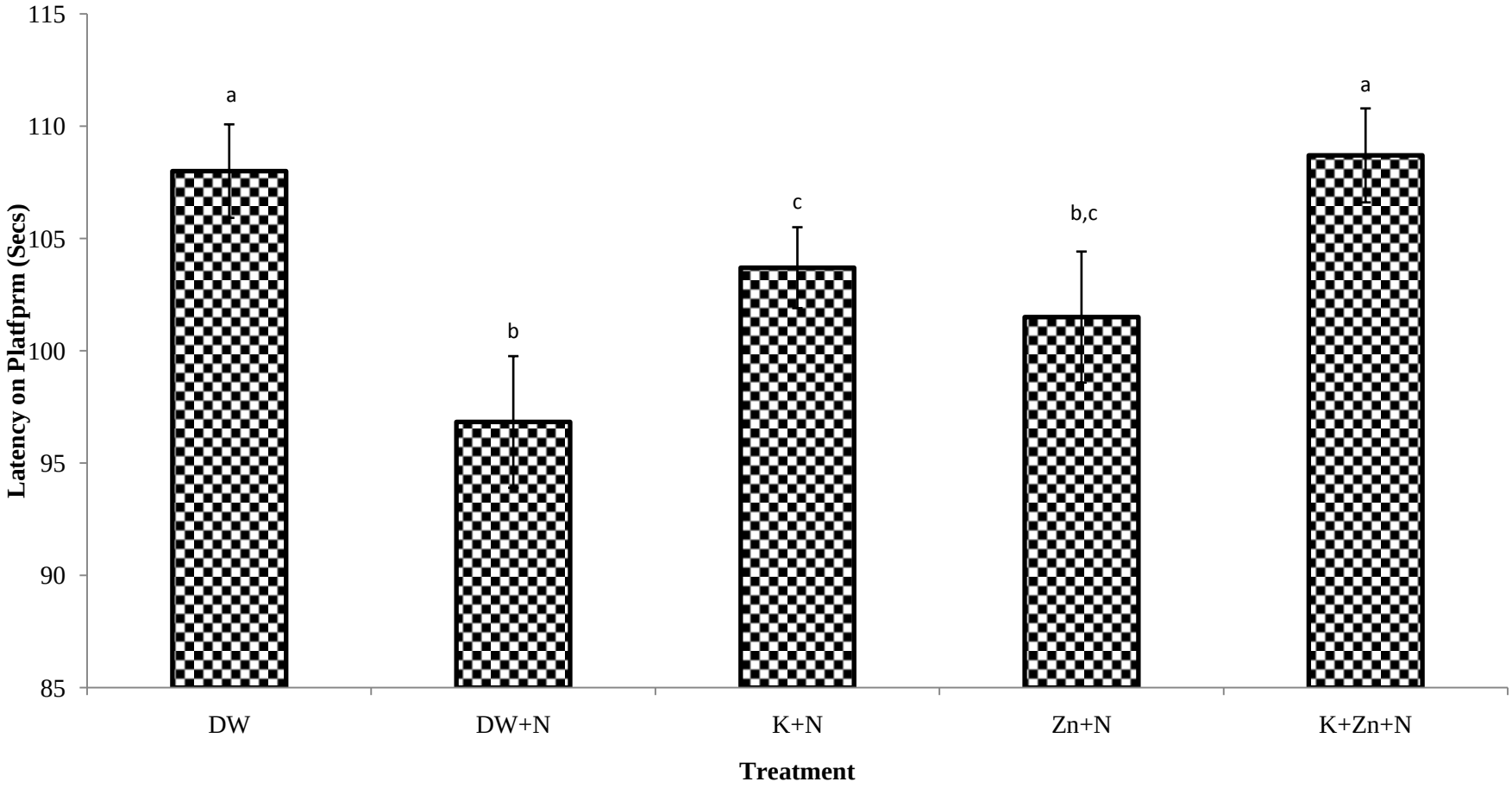


Figure 6: Ameliorative effect of kaempferol and zinc on memory of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

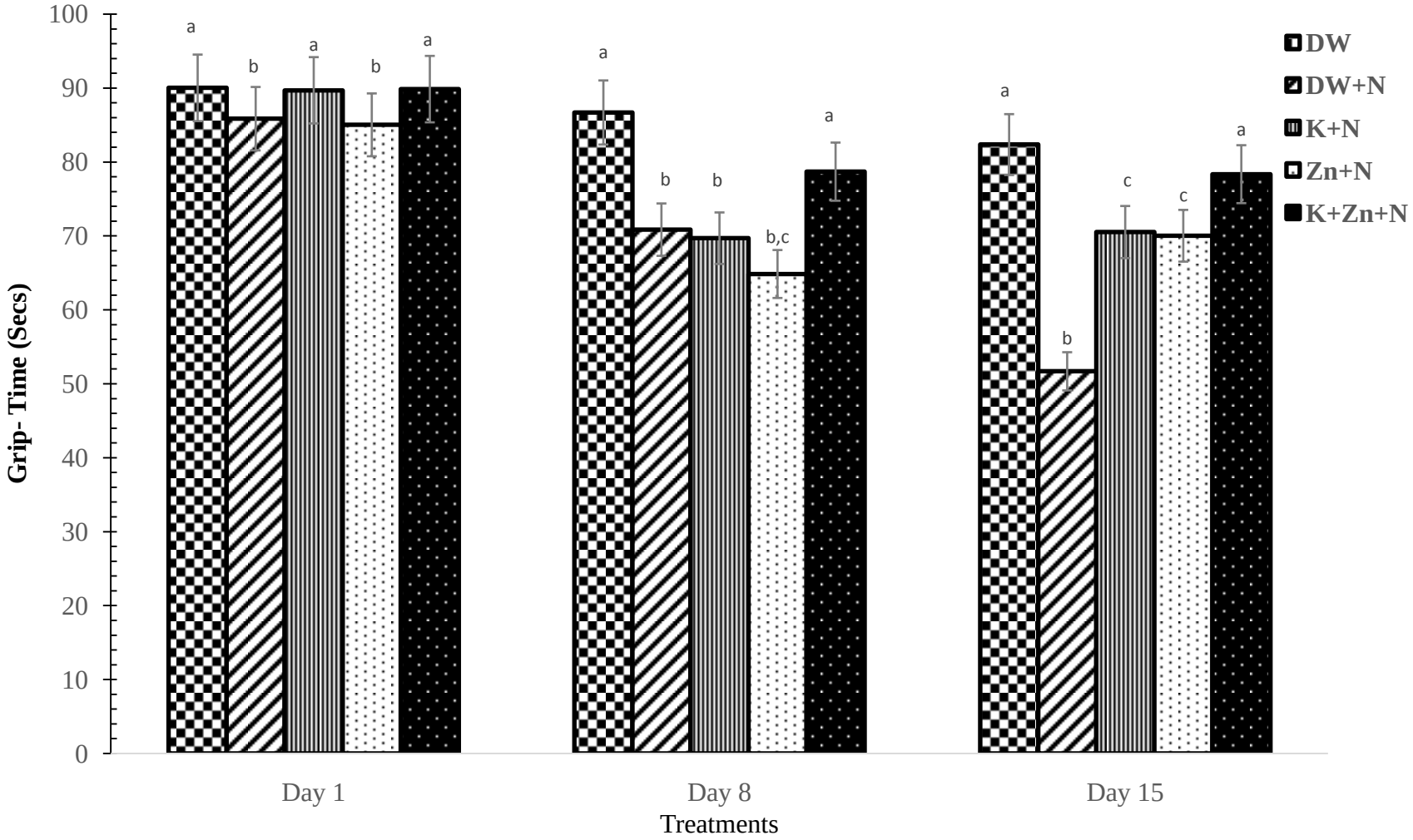


Figure 7: Ameliorative effect of kaempferol and zinc on motor strength (fore-paw grip) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water, K, Kaempferol, Zn, Zinc, N, Noise

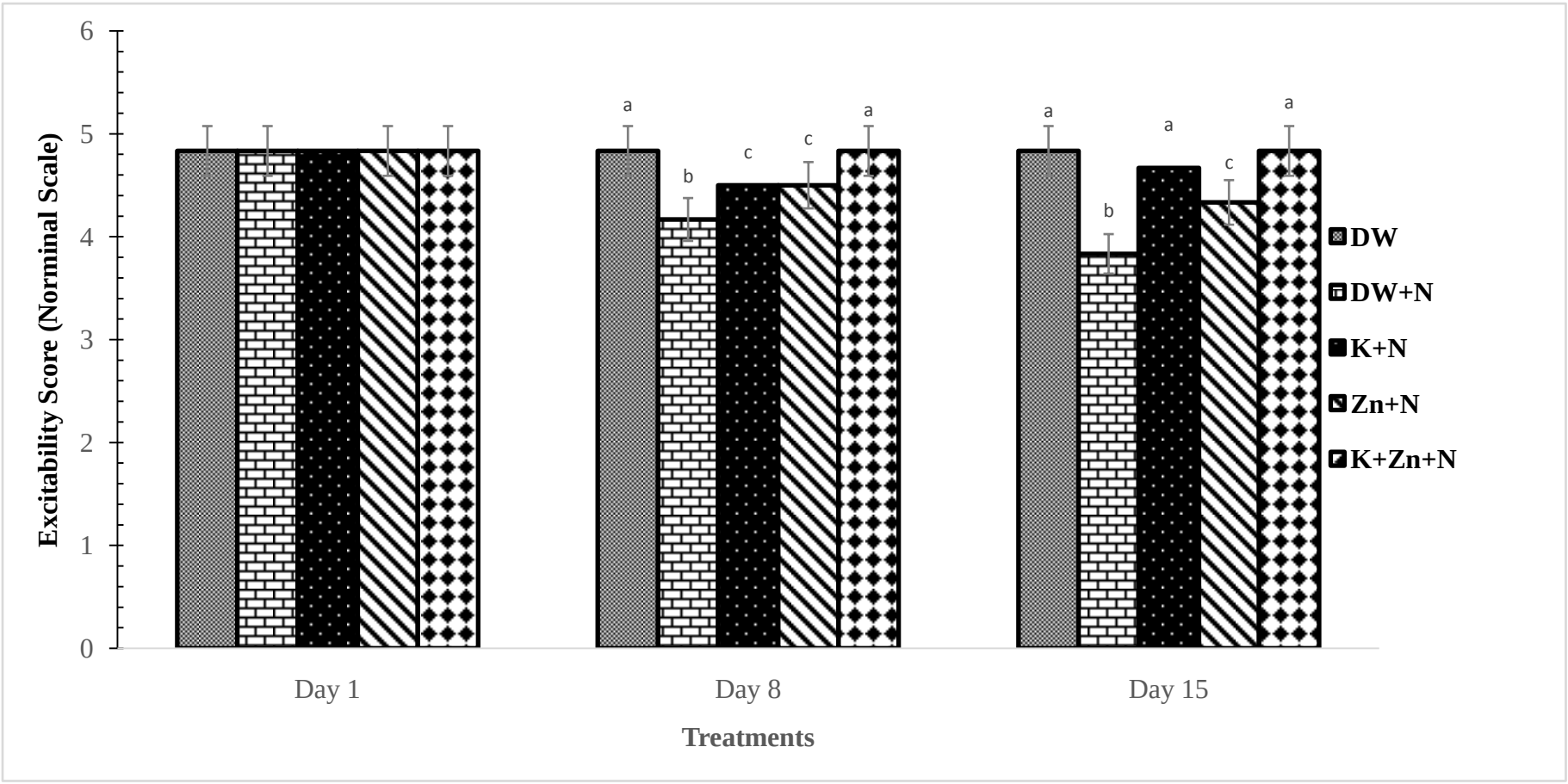


Figure 8: Ameliorative effect of kaempferol and zinc on sensorimotor reflex (excitability score) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise.

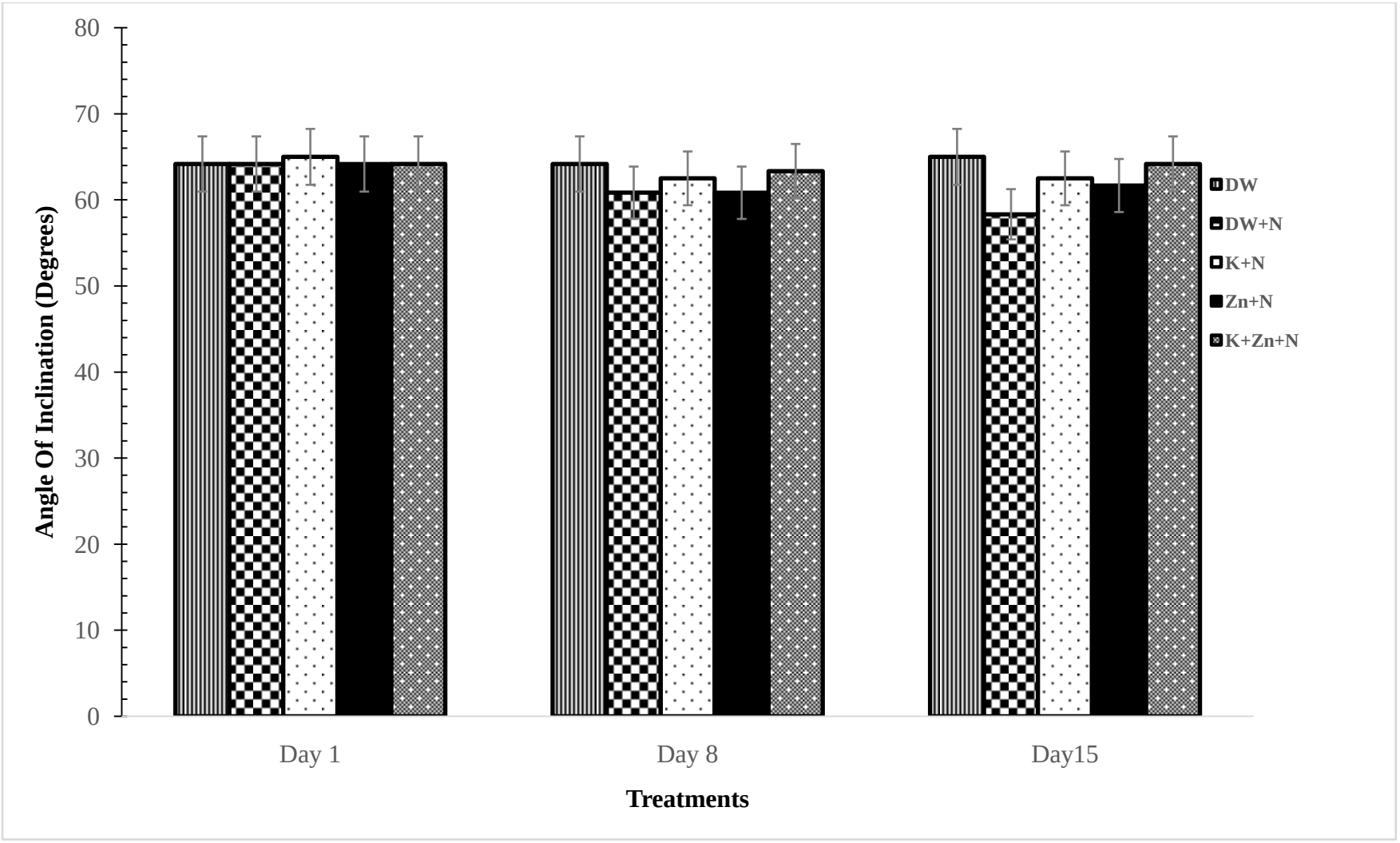


Figure 9: Ameliorative effect of kaempferol and zinc on neuromuscular performance (Inclined plane) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

DW, Deionised water, K, Kaempferol, Zn, Zinc, N, Noise

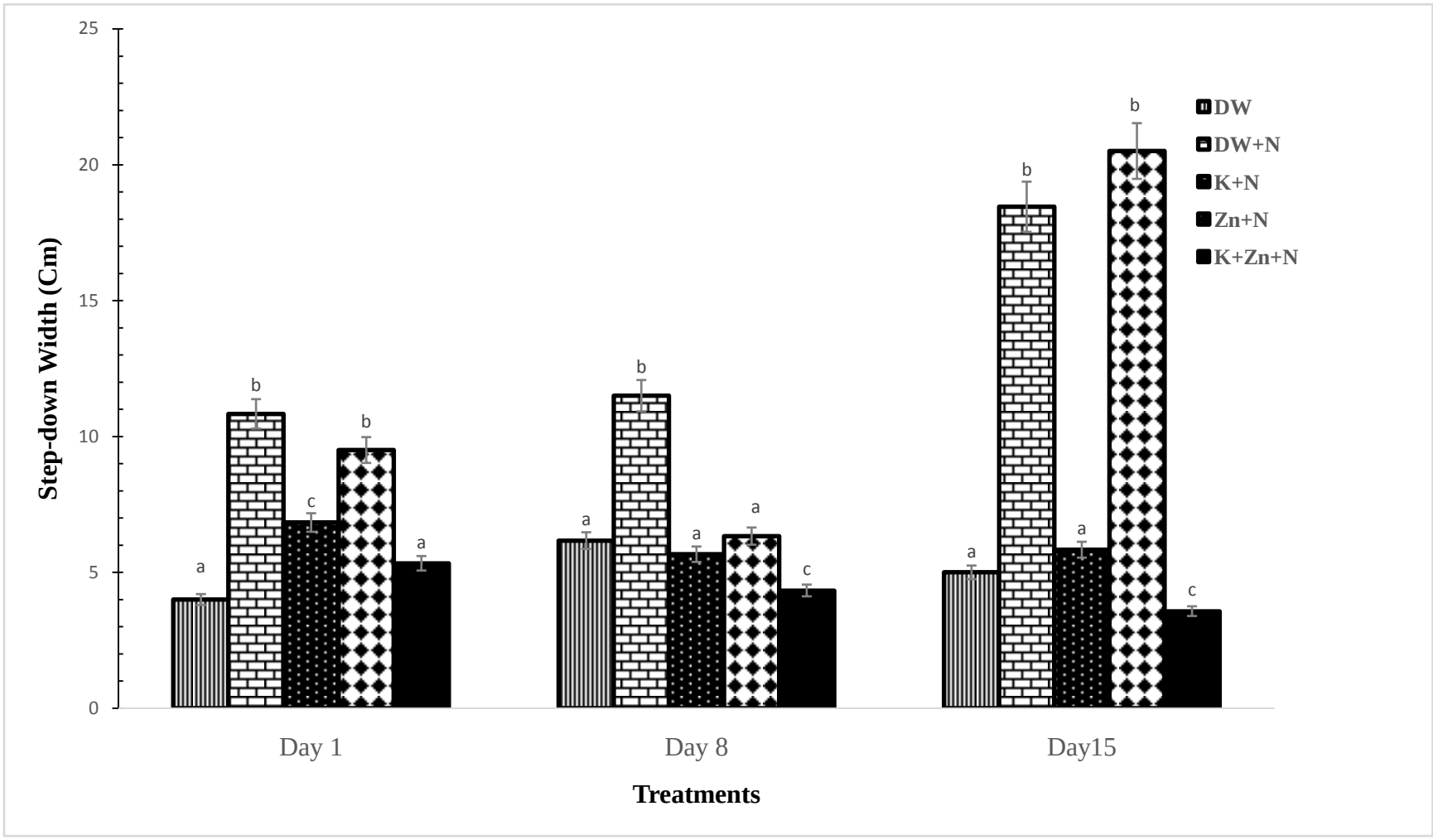


Figure 10: Ameliorative effect of kaempferol and zinc on motor coordination (beam-walk) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters are significantly ($P < 0.05$) different
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

4.5Open Field Parameters

4.5.1Frequency of rearing

As presented in Figure 11, the general pattern of the mean frequency of rearing across the treatment groups was that of a progressive decrease throughout the study period. On day 1 of the period, the mean frequency of rearing obtained in the deionised water + noise-treated rats (52.10 ± 0.60) was significantly ($P< 0.05$) higher than that recorded in other groups. However at day 8, the frequency in kaempferol + noise (36.00 ± 8.48) and kaempferol + zinc + noise (36.20 ± 6.44)-treated groups was significantly ($P< 0.05$) higher than the value obtained in deionised water + noise-treated group (30.00 ± 4.42). At day 15, in the group treated with zinc + noise, the frequency was significantly ($P< 0.05$) lower (15.40 ± 2.64) than in the group treated with deionised water + noise (27.00 ± 2.21). The frequency in the groups treated with kaempferol + noise and kaempferol + zinc + noise increased relatively compared to that of the group treated with deionised water + noise, but this increase was not significant.

4.5.2 Stretch-attend Postures

At day 1, a decreased number of stretch–attend postures was recorded in the group treated with deionised water + noise (4.10 ± 2.00), compared with the other groups. The highest number was observed in the group treated with kaempferol + noise (6.10 ± 0.40). On days 8 and 15, a significant increase ($P< 0.05$) in the number of stretch–attend postures was observed in the group treated with deionised water + noise (4.80 ± 1.04, 5.10 ± 0.50) respectively compared with the other groups. The lowest number was recorded in the group treated with kaempferol + zinc + noise (3.90 ± 0.48, 3.30 ± 3.10), respectively (Figure 12).

4.5.3 Defaecation

There was no significant difference in the defaecation pattern across the various groups on day 1. On day 8, there was a significant ($P< 0.05$) increase in the group treated with deionised water + noise (3.90 ± 1.46), compared to the group treated with kaempferol + zinc + noise (2.49 ± 1.20). On day 15, there was a significant ($P< 0.05$) increase (4.20 ± 2.24) in the group treated with deionised water + noise, compared to the group treated with kaempferol + noise (2.40 ± 0.90), zinc + noise (2.0 ± 0.64) and kaempferol + zinc + noise (2.0 ± 0.00; Figure 13).

4.5.4 Urination

There was no significant difference in the urination pattern across the various groups on day 1. On day 8, there was a significant ($P< 0.05$) increase (2.80 ± 0.82) in the group treated with deionised water + noise, compared to the group treated with kaempferol + noise (1.80 ± 0.55). On day 15, there was also a significant ($P< 0.05$) increase in the urination pattern of the group treated with deionised water + noise (2.80 ± 0), compared to the control group treated with only deionised water (1.20 ± 1.40), kaempferol + noise group (2.00 ± 0.40), zinc + noise group (2.20 ± 1.60) and kaempferol + zinc + noise group (2.0 ± 2.00; Figure 14).

4.5.5 Grooming

As shown in Figure 15, on day 1, there was a significant difference in the grooming pattern in the control group treated with deionised water (32.80 ± 0.16), compared to the other groups. The group treated with deionised water + noise also significantly ($P< 0.05$) recorded a higher grooming pattern (23.00 ± 0.50) than the group treated with zinc + noise (10.00 ± 0.80), and kaempferol + zinc + noise (15.00 ± 0.40). On day 8, the group

treated with kaempferol + zinc + noise had a significantly ($P< 0.05$) higher (28.10 ± 0.80) grooming pattern than the group treated with deionised water (15.00 ± 0.20), deionised water + noise (20.48 ± 0.82) and zinc + noise (11.40 ± 0.25). On day 15, the grooming pattern in the group treated with deionised water + noise was significantly ($P< 0.05$) higher (30.0 ± 3.20), compared to that of the control group (22.20 ± 4.80). However, the group treated with kaempferol + zinc + noise recorded a significantly ($P< 0.05$) higher grooming pattern (35.50 ± 0.70) than the group treated with zinc + noise (20.00 ± 2.10), kaempferol + noise (25.20 ± 5.20) and deionised water + noise (30.0 ± 3.20).

4.5.6 Locomotor activity

The general pattern of the locomotor activity of rats exposed to the different treatment groups is shown in Figure 16. On day 1, the locomotor activity in the group treated with deionised water + noise was significantly ($P< 0.05$) lower (4.20 ± 0.20) than that of the deionized water group (16.00 ± 0.40), zinc + noise group (16.80 ± 4.00) and kaempferol + zinc + noise group (12.40 ± 0.20). On day 8, there was a significant ($P< 0.05$) increase in the activity (43.60 ± 6.47) in the group treated with deionised water + noise, compared to the group treated with kaempferol + noise (32.10 ± 15.97) and zinc + noise (21.70 ± 5.52). On day 15, there was a significant ($P< 0.05$) decrease (20.50 ± 0.80) in the group treated with deionised water + noise, compared to the group treated with kaempferol + noise (28.10 ± 6.60) or kaempferol + zinc + noise (28.00 ± 4.40).

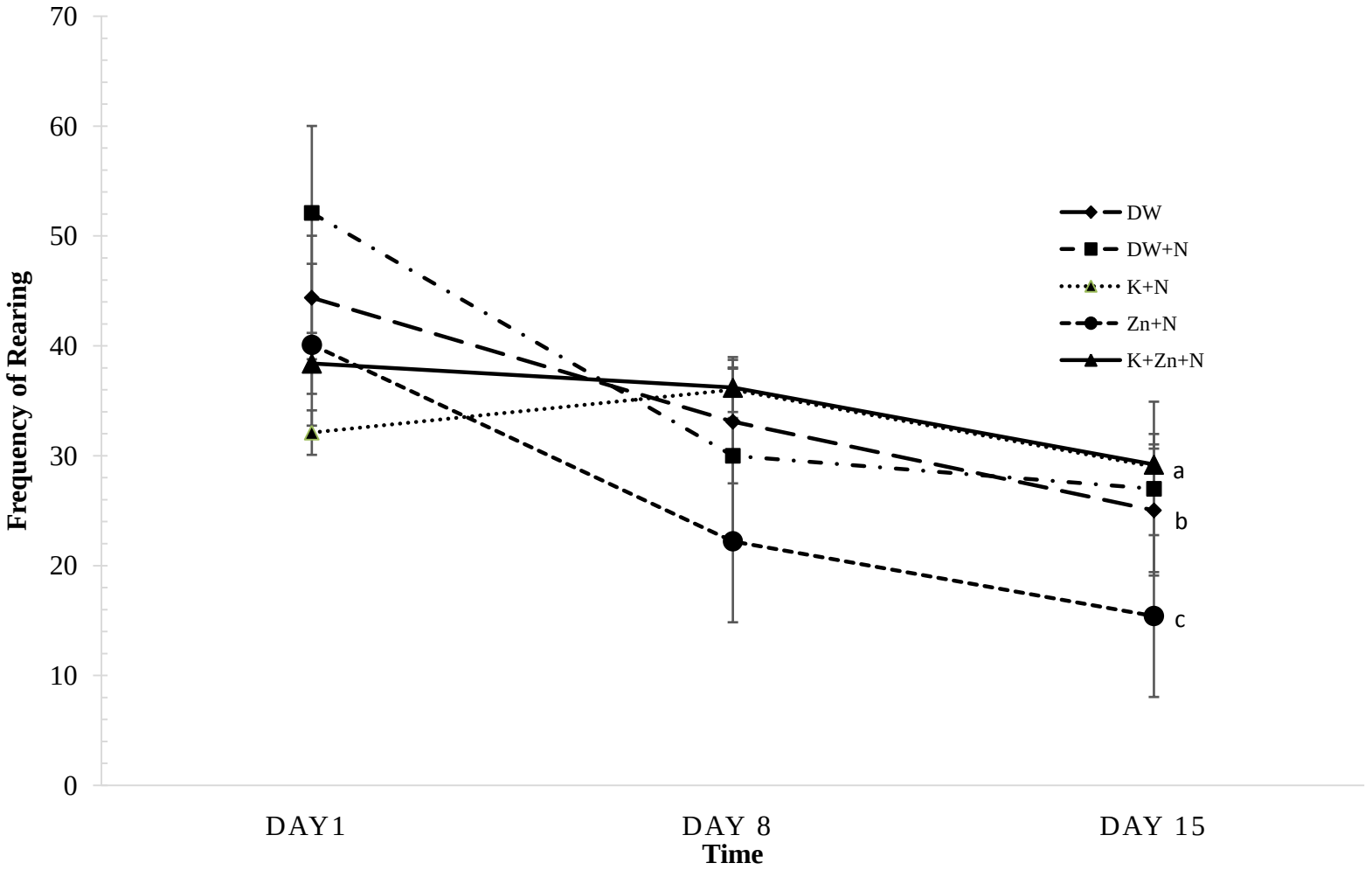


Figure 11: Ameliorative effect of kaempferol and zinc on frequency of rearing of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)

DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

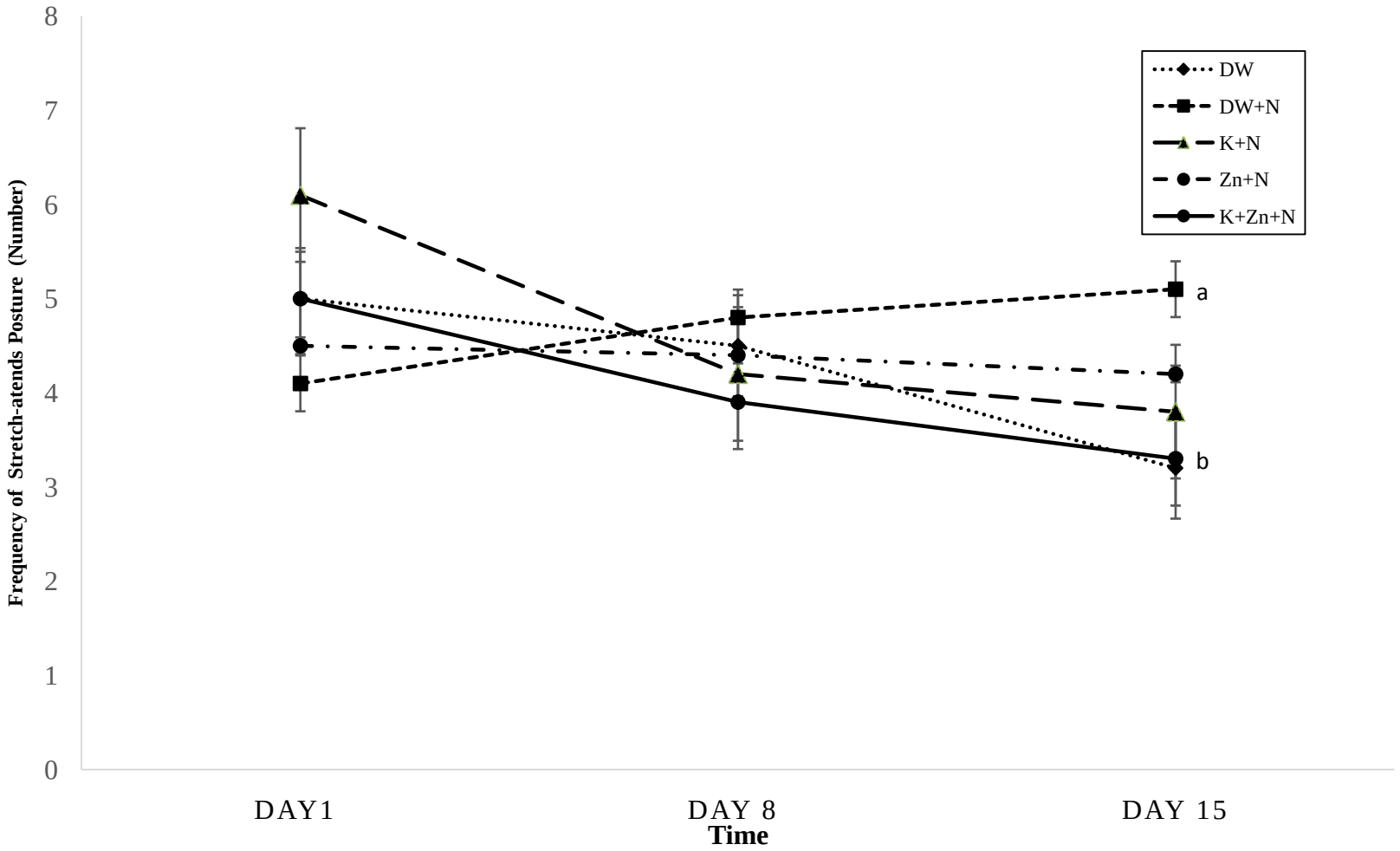


Figure 12: Ameliorative effect of kaempferol and zinc on frequency of stretch-atends postures (number) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

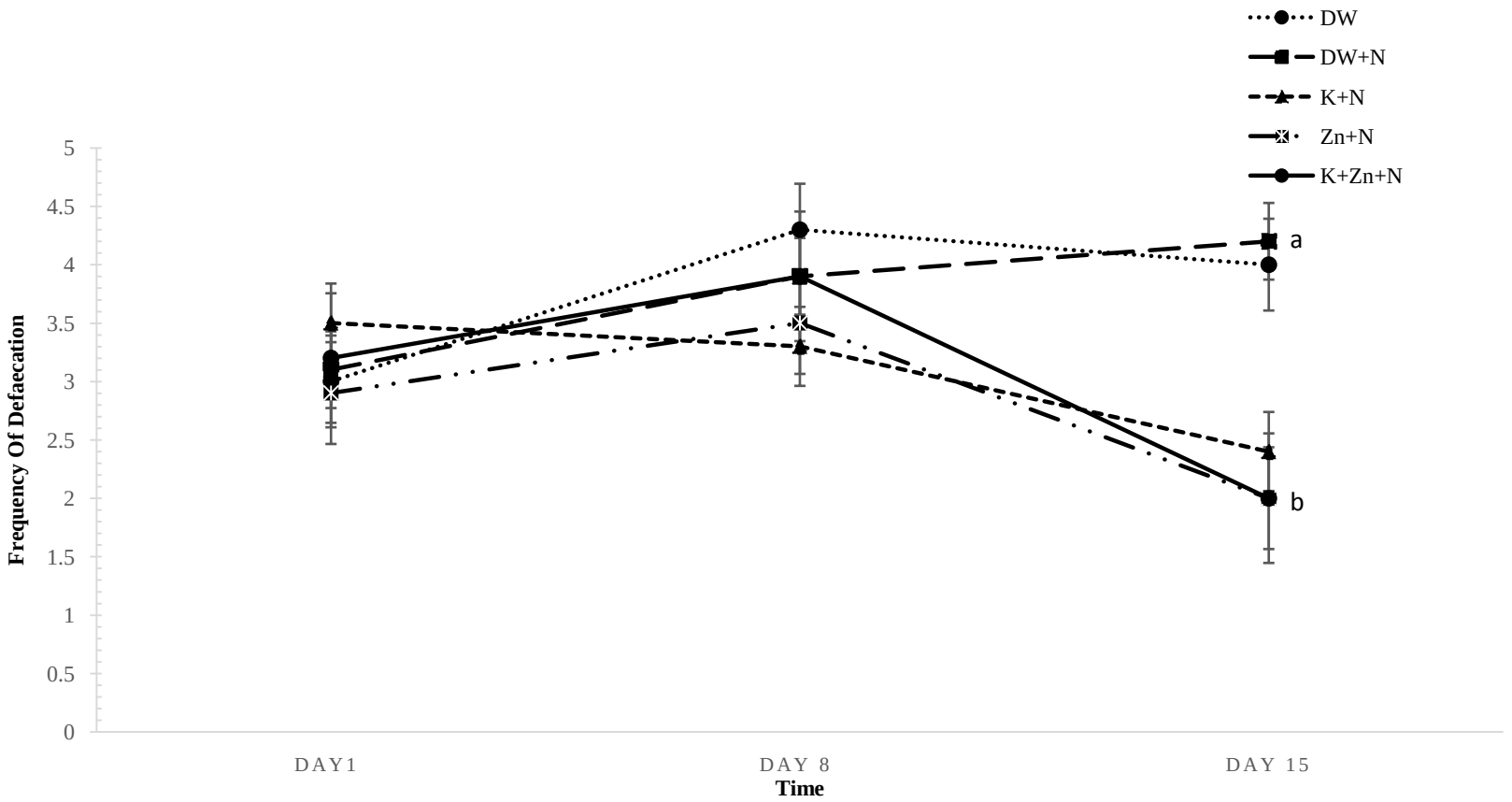


Figure 13: Ameliorative effect of kaempferol and zinc on frequency of defaecation of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water, K, Kaempferol, Zn, Zinc, N, Noise

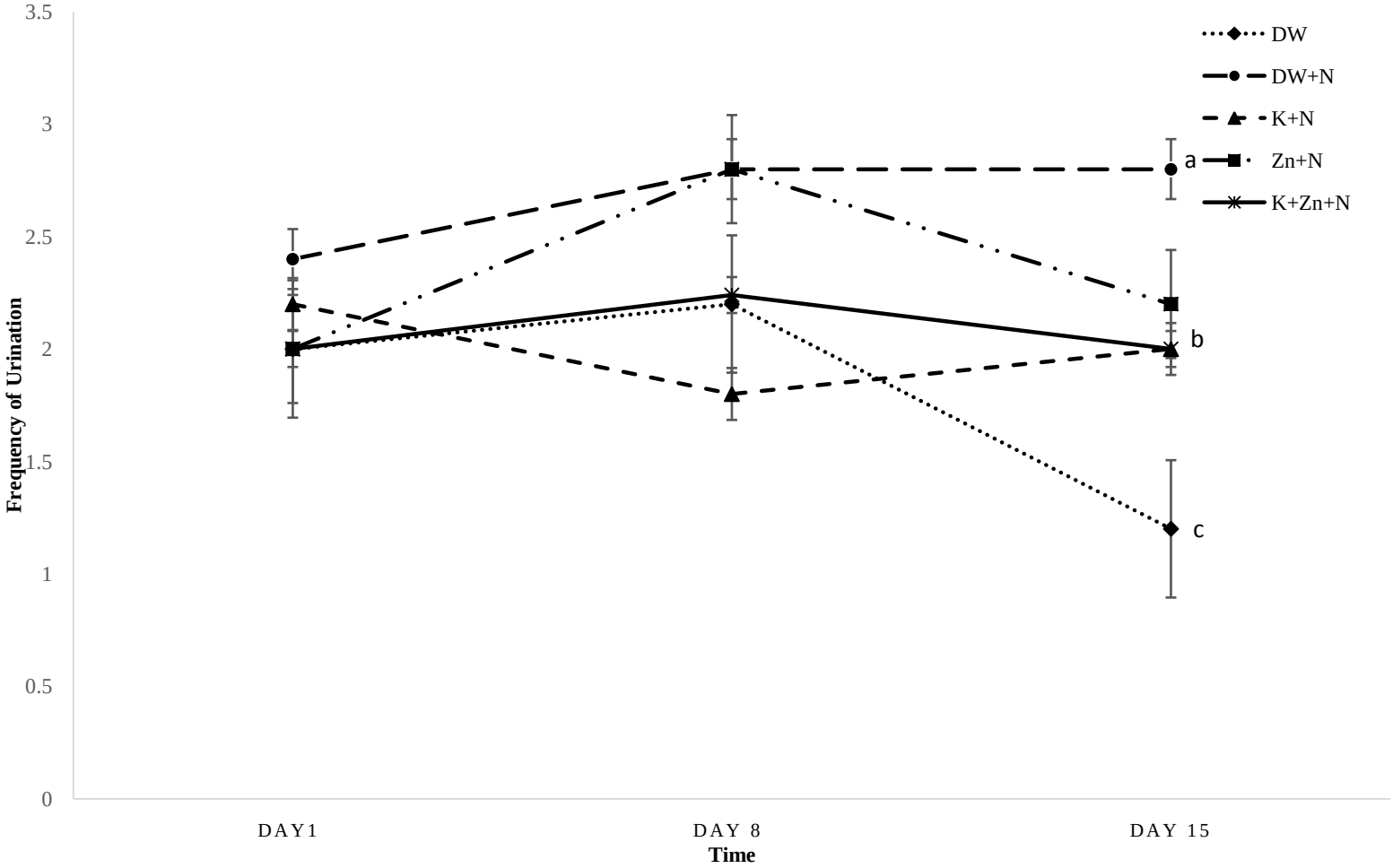


Figure 14: Ameliorative effect of kaempferol and zinc on frequency of urination of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

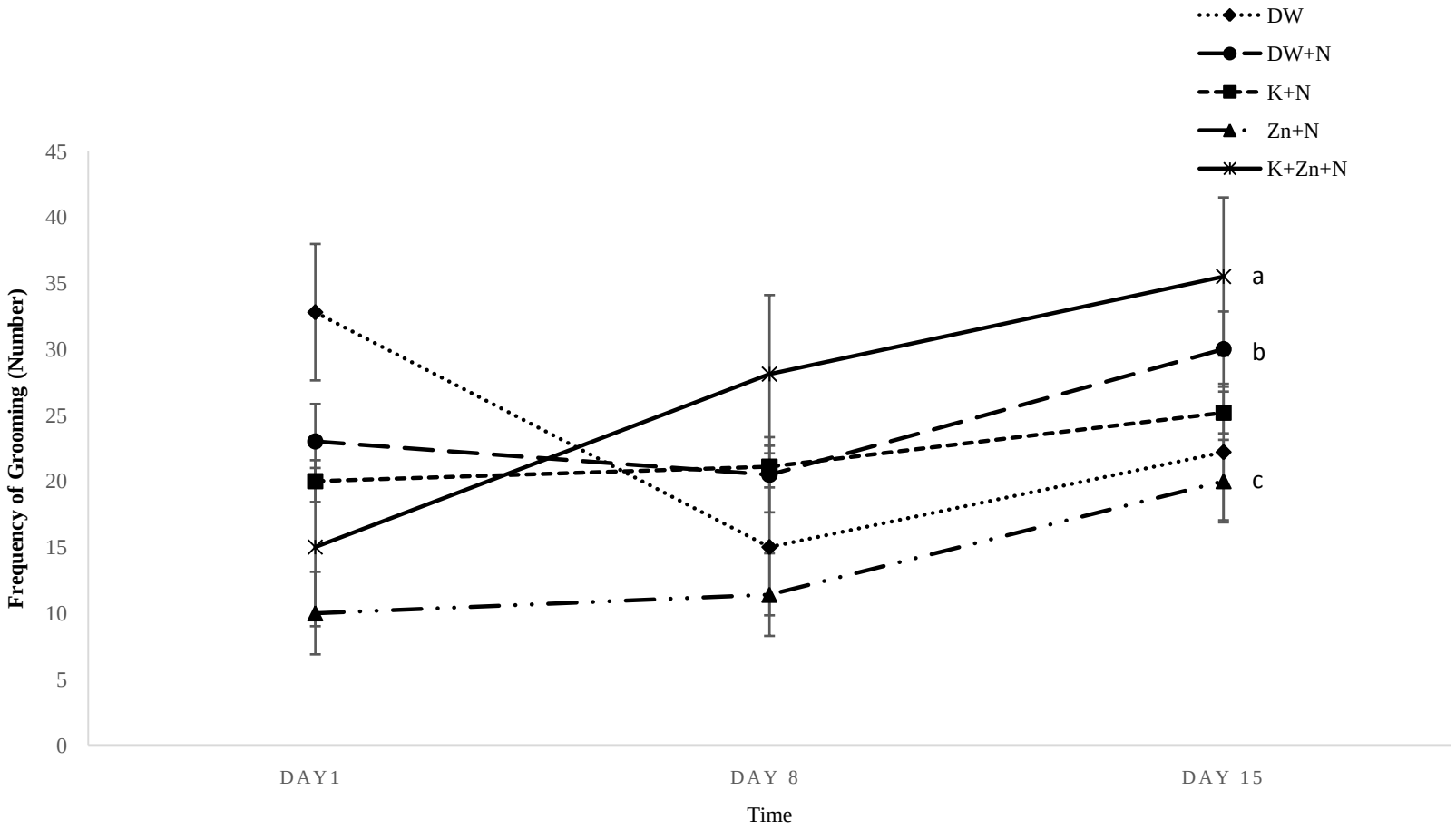


Figure 15: Ameliorative effect of kaempferol and zinc on frequency of grooming of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

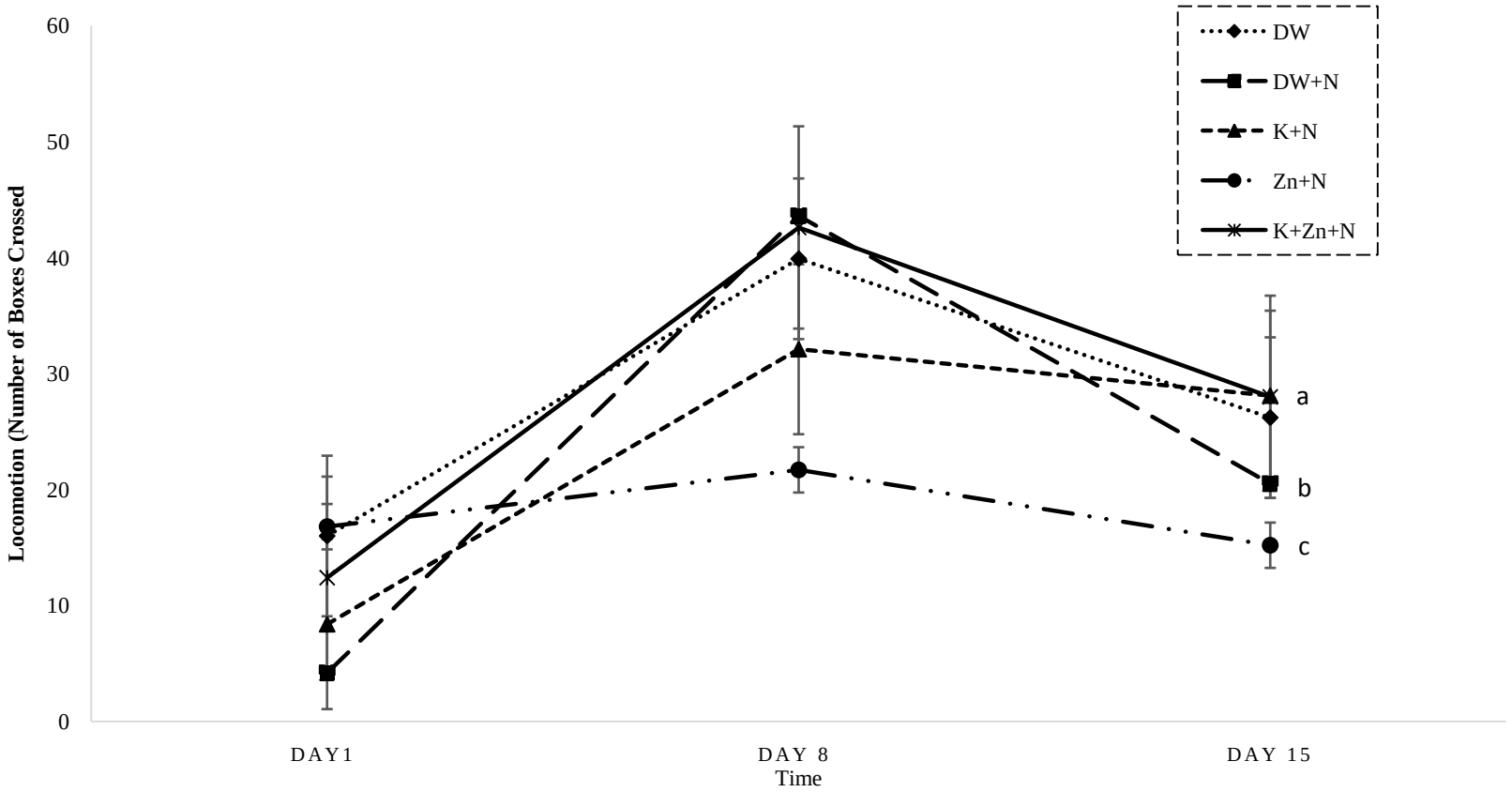


Figure 16: Ameliorative effect of kaempferol and zinc on locomotion of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

4.6 Effect of Kaempferol and Zinc on Oxidative Stress inWistarRats exposed to Noise Stress

4.6.1 Variation in malondialdehyde concentration

The effect of treatments on concentration of MDA is shown in Figure 17. There was a significant (P<0.05) decrease in the MDA concentration in rats treated with kaempferol + zinc + noise (0.70 ± 0.20 nmol/mg) compared to the deionised water + noise group (1.10 ± 0.20 nmol/mg) which was significantly (P<0.05) higher than the deionised water group (0.60 ± 0.10 nmol/mg) and kaempferol + noise group (0.80 ± 0.20 nmol/mg).There was no significant difference in MDA concentrations between the group, administered with deionised water and that treated with kaempferol + zinc + noise.

4.6.2 Variation in activities of antioxidant enzymes

4.6.2.1 Catalase activity

There was a significant (P < 0.05) decrease in the catalase activity in the group treated with deionised water + noise (32.50 ± 2.20 IU/L), compared to the group treated with deionised water only (41.30 ± 2.10 IU/L) and the kaempferol + zinc + noise group (36.90 ± 2.00 IU/L). The highest brain catalase activity was recorded in the group treated with deionised water only (41.30 ± 2.10 IU/L), while the lowest was recorded in the deionised water + noise group (Figure 18).

4.6.2.2 Superoxide dismutase activity

There was a significant (P < 0.05) decrease in the SOD activity of rats treated with deionised water + noise (1.80 ± 0.20 IU/L), which had the lowest activity compared to the group treated with kaempferol + zinc + noise (2.20 ± 0.30 IU/L). The highest activity was recorded in the group treated with deionised water. There was no significant

difference between the group treated with kaempferol + noise (2.00 ± 0.10 IU/L), zinc + noise (2.10 ± 0.10 IU/L)and deionised water + noise (1.80 ± 0.20 IU/L; Figure 19).

4.6.2.3Glutathione peroxidase activity

There was a significant ($P < 0.05$) reduction in the brain glutathione peroxidase activity in the group, treated with deionised water + noise (25.10 ± 1.10 IU/L), compared with the group treated with kaempferol + noise (29.10 ± 1.00 IU/L) and kaempferol + zinc + noise (29.40 ± 1.20 IU/L). The highest activity was recorded in the group treated with deionised water (30.20 ± 1.10 IU/L), while the lowest was obtained in the group treated with deionised water + noise (25.10 ± 1.10 IU/L; Figure 20)

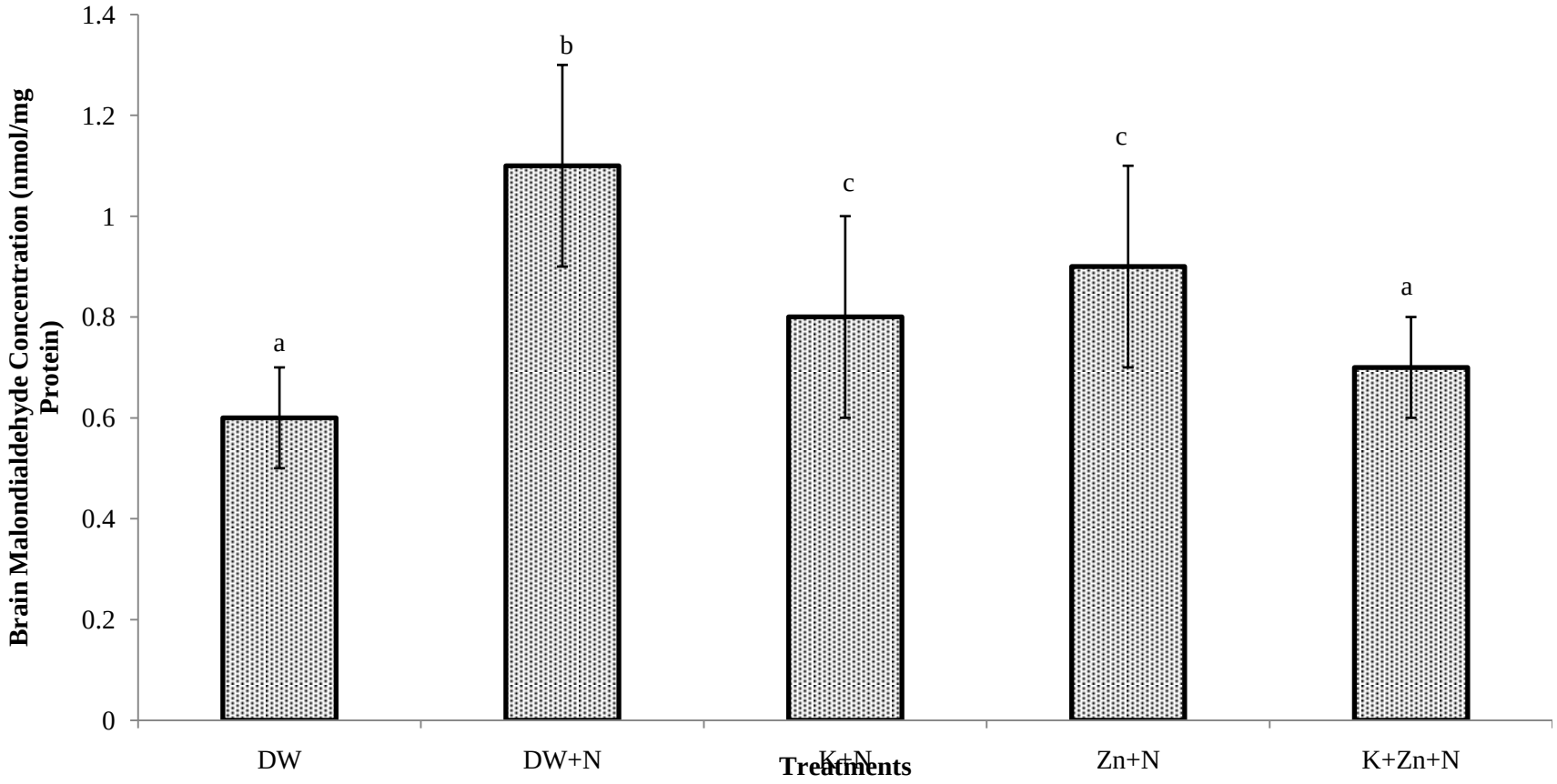
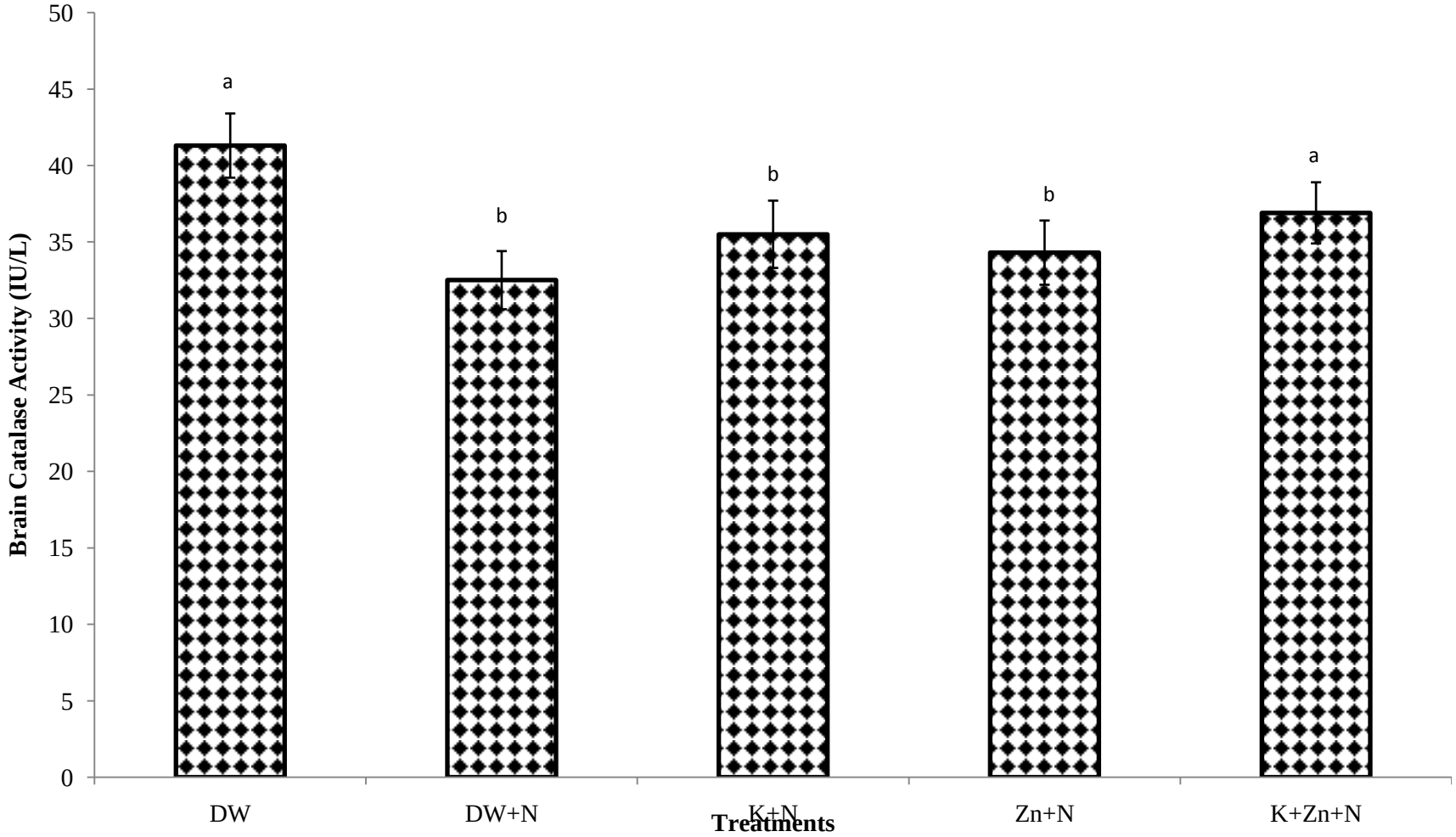


Figure 17: Ameliorative effect of kaempferol and zinc on brain malondialdehyde concentration (nmol/mg protein) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water, K, Kaempferol, Zn, Zinc, N, Noise



stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)
DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

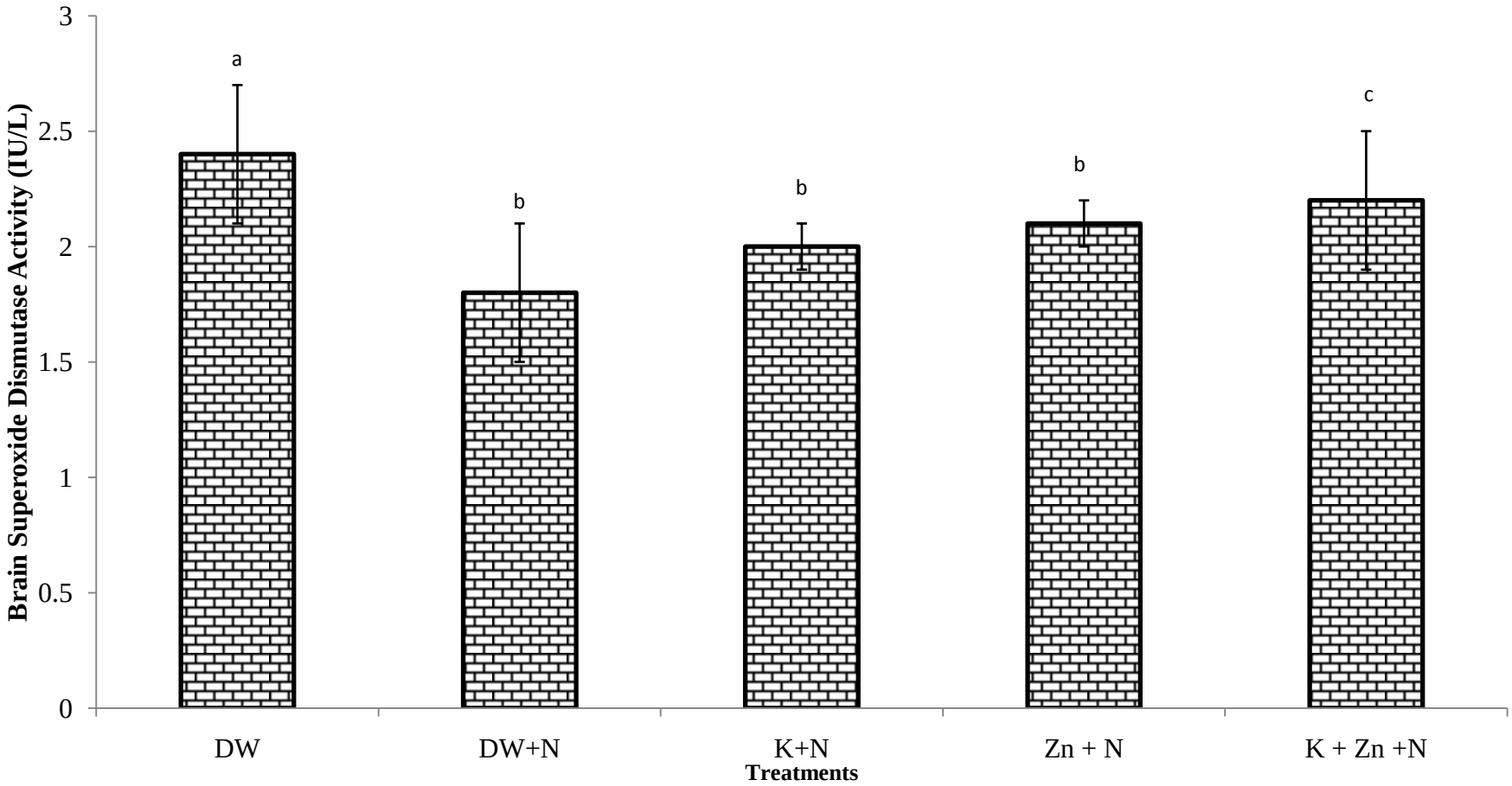


Figure 19: Ameliorative effect of kaempferol and zinc on brain superoxide dismutase activity (IU/L) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)

DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

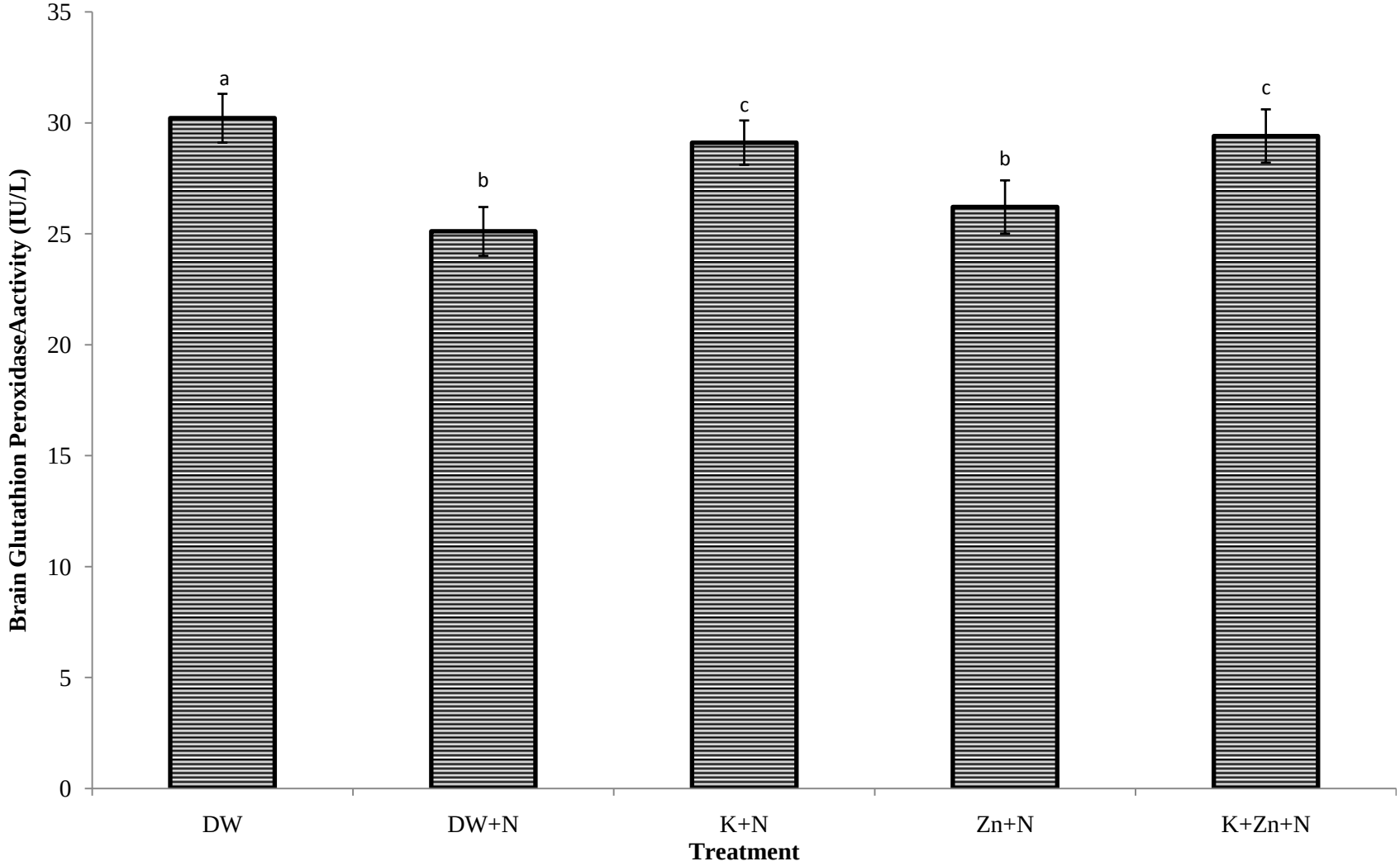


Figure 20: Ameliorative effect of kaempferol and zinc on brain glutathione peroxidase activity (IU/L) of Wistar rats exposed to noise stress (Mean ± SEM, n = 6)

[a, b, c] = Values with different superscript letters differ significantly ($P < 0.05$)

DW, Deionised water; K, Kaempferol; Zn, Zinc; N, Noise

CHAPTER FIVE:

5.0 DISCUSSION

Noise stress increases oxidative damage to cell as evidenced by decrease in weight gain, feed intake and feed efficiency (Del Vesco *et al*., 2014). The results showed that noise stress decreased body weight gain in rats evidenced by the significant decrease in the group treated with deionised water + noise compared with those treated with noise + kaempferol + zinc. Moreover, noise-stressed animals showed lower feed intake than those treated with the antioxidants. The findings agree with that of Alario *et al*. (1986), who reported that noise stress significantly decreased body weight of exposed rats. The decrease in body weight may be due to adrenal stimulation by noise stress which was ameliorated in the groups treated with kaempferol and zinc, singly and in combination (Alario *et al*., 1986). The suggested causes of decrease in weight gain in the group treated with deionized water and exposed to noise include distraction by noise from feeding, increase defaecation, increase urination, decreased locomotor activity and frequency of rearing. The increase in percentage weight gain in the treated rats may be due to the nutritive and appetizing qualities of kaempferol and zinc (Pérez, 1995), which enhanced feed consumption and increased weight gain. The greater weight gain observed in the group treated with kaempferol and zinc, compared to the groups treated separately with zinc or kaempferol indicates that kaempferol and zinc acted additively to ameliorate the decreased body weight gain induced by 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 (Asala *et al*., 2011).

Zinc promotes cell membrane integrity and its deficiency is associated with increased EOF (Xia *et al*., 1999). 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 additively to ameliorate noise-stress induced increase in erythrocyte osmotic fragility in Wistar rats. This finding affirms the role of edfm oxidative stress in the pathophysiology of noise stress. The result of this study is in agreement with that of Sharma *et al*. (2016), who reported that zinc ameliorates erythrocyte fragility in Wistarrats 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 promote cell membrane integrity. Besides, zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals (Sahin and Kucuk, 2003). Also, increase in erythrocyte fragility has been associated with decrease in zinc concentration in RBC membrane resulting in reduced plasma sulphydryl

concentration (Roth and Kirchgessner, 1991). Although, the present study did not measure erythrocyte zinc concentration, Kraus *et al*. (1997) have shown that 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 (Ruz *et al.*, 1992) and a greater susceptibility to oxidative damage from elevated peroxynitrite concentrations (Powell, 2000). Similarly, lowered zinc level alters the composition of erythrocyte membranes (Avery and Bettger, 1992) and also impairs the activity and function of Na+ K+ ATPase, which is important for cell membrane stability (O'Dell *et al*., 1990). Furthermore, decrease in cellular zinc concentration has been associated with apoptosis induction via the protein kinase C-dependent pathways (Chou *et al*., 2004) and activation of apoptotic protease, caspase-3 (Duffy *et al*., 2001). Therefore, the amelioration of noise stress-induced erythrocyte fragility by zinc may have partly resulted from its ability to inhibit caspase-3 (Perry *et al*., 1997) and the repletion of zinc deficit caused by oxidative stress (Chou *et al*., 2004). Kaempferol ameliorated EOF by scavenging free radicals which induce lipid peroxidation (Perry *et al*., 1997).Thus, kaempferol and zinc gluconate have antioxidant potentials in the amelioration of noise stress in Wistar rats suggesting that the metal zinc enhanced the breakdown of the kaempferol glycosidic bonds.

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 (Wankhar *et al*., 2014; Tao *et al.,* 2015). Haematology is important in evaluating the health and nutritional status of an animal (Gupta *et al*., 2007). In addition, the use of neutrophil-lymphocyte (N/L) ratio is a sensitive haematological indicator of stress response (Rajalekshmi *et al*., 2014). In the current study, haematological parameters of noise-exposed Wistar rats were significantly 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.* (2011) and Minka and Ayo, (2012) 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 (Minka and Ayo, 2012). 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 (El Hendy *et al*., 2001), 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 (Dacie and Lewis, 1995) and younger erythrocyte are larger than older ones (Nash and Wyard, 1981). 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 (Shrivastava *et al.*, 2017). 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 deionised 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 Broucek *et al*. (1983), who reported a significant decrease in leucocyte count of animals exposed to noise stress. Algers *et al*. (1978) 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 (Algers *et al*., 1978) has been reported, and oxidative stress has been implicated in noise-induced immunosuppression (Wankhar *et al.*, 2014).

The finding of the present study agrees with that of Minka and Ayo (2012) who obtained an increase in lymphocyte counts following ascorbic acid supplementation in transported goats exposed to heat stress. It also agress with the finding of Habibu *et al.* (2014) 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 of Bedanova *et al*.(2010) 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 (2012) 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, (2006) 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*. (2010), 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 mitigatenoise-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 deionised water only, may be attributed to the antioxidant role of the antioxidants that scavenge ROS, involved in lipid peroxidation within platelet membrane (Akande *et al*., 2014).

The result of the present study shows that noise induces impairment in learning and memory causing increased oxidative stress, which agrees with the finding of Tao *et al.* (2015) who showed that impairement in learning and memory increases due to oxidative stress induced neuronal degeneration and suppression of neurogenesis of auditory nuclei in Wistar rats exposed to noise stress. Results of this study revealed a significant decrease in the number of foot-shocks received by the group treated with kaempferol + zinc + noise, compared to the group treated with deionised water + noise. There was also a significant increase in the amount of time (seconds) spent on the platform by the group treated with kaempferol + zinc + noise, compared to the group treated with deionised water + noise. A highly significant increase was also observed in the group treated with kaempferol and zinc, compared to the groups separately treated with kaempferol and zinc, indicating that kaempferol and zinc acted additively to ameliorate impairment in learning and memory of Wistar rats exposed to noise stress. The result concurs with the report by Vauzour *et al.* (2014) that kaempferol and other flavonoids improve learning and memory in Wistar rats. Tao *et al.* (2015) also reported the use of orientin, a similar antioxidant, to mitigate noise-induced memory and cognition impairments. Thus, kaempferol and zinc,which are potent antioxidants, exert mitigative effects on noise-induced impairment in learning and memory by improving central oxidative stress and increasing the activation of antioxidant proteins, molecules and enzymes.

There was a significant increase in the excitability score of the group treated with kaempferol + zinc + noise compared to the group treated with only deionised water + noise. The progressive decrease in the excitability score of rats exposed to noise only reflected the state of physical and mental alertness of the animals, indicating poor sensorimotor reflex and neuromuscular coordination. This deficit may be due to impairment in antioxidant enzyme activity, hence neuronal activity in the nervous tissue and muscle. Treatment with kaempferol and zinc significantly improved the excitability score, apparently due to enhancement in the activities of the antioxidant enzymes in the brain (Shrivastava *et al.*, 2017; Nunnes *et al.*, 2017). The present finding corroborates that obtained by Ayo *et al*. (2006) who showed the ability of vitamin C to mitigate low excitability score, induced by road transportation stress in goats.

On the overall, there was no significant difference in the inclined plane performance of Wistar rats across the groups. The finding that the inclined plane apparatus showed no significant effect demonstrated that noise stress did not impair the coordination of neuromuscular activity of rats: Therefore, the treatment of noise-stress exposed rats with kaempferol and zinc gave no beneficial effect.

The fore-grip test was used to assess the motor strength and also the integrity of the muscle. It was observed that the time taken for a rat to fall off a grip on day 15 decreased in the group treated with deionised water + noisecompared to groups treated with kaempferol + zinc + noise. This finding corroborates the report of Wankhar *et al*. (2014),

who reported that noise stress is a potent stressor, capable of disrupting the physical, emotional and mental homeostatic behaviours. This is majorly due to the increased generation of ROS, involved in lipid peroxidation of cytomembranes, neuronal degeneration, DNA damage and decrease in antioxidant defence systems of cells, evidenced by decrease in the activities of antioxidant enzymes: superoxide dismutase, catalase and glutathione peroxidase (Minka and Ayo., 2014). The antioxidant role of kaempferol is indicated by its ability to increase fore-paw grip-time in the group treated with kaempferol by enhancing the activities of antioxidant enzymes.

Beam walking across bridges of different cross-sections provide a well-established method of monitoring motor coordination and balance in rats. The resultof the present study showed a significant decrease in the step-down width of the groups exposed to noise and treated with zinc and kaempferol separately and in the group given their combination, compared to the group treated with deionised water + noise. The increase in latency and number of foot slips in the exposed group treated with deionised water indicates a progressive increase in impairment of motor function. This study, therefore, demonstrated that noise stress actually caused muscular in-coordination, which was evident in rats treated with deionised water + noise only. The cerebellum plays a role in maintaining posture, through a servocomparator function and may have been affected by noise stress resulting in muscular incoordination (Wankhar *et al.*, 2014). Apparently, the distorting signals from the vestibular apparatus (non-auditory labyrinth) and propioceptive signals from different parts of the body abolished the corrective signals that adjust muscle tone, leading to poor posture and balance. The basal ganglia may also be

implicated in noise stress because it plays an important role in controlling the muscle tone and voluntary movements.The caudate nucleus stimulates muscle tone through stimulation of vestibular nucleus and inferior olive. It receives projection fibres from the motor cortex to corpus striatum, globuspallidus and to several nuclei in the brainstem. The neurones may also be implicated by their ability to interrupt the nervous pathway. With the administration of the antioxidants; kaempferol and zinc, there was a considerable amelioration or protection, which decreased the foot-slip width. This undoubtedly accentuatesthe role of oxidative stress in impairing motor coordination (Tao *et al.,* 2015).

It has been shown that an increased frequency of rearing is indicative of a normal animal, which is very familiar with its environment and has good habituation, learning and locomotor activity (Wankhar *et al.,* 2014). The general pattern of the mean frequency of rearing across the treatment groups was that of a progressive decrease throughout the study period. On day 8, there was a significant increase in rearing in the group treated with kaempferol and zinc compared to that treated with deionised water. In the absence of locomotor alterations, increased exploration as indicated by increased rearing is interpreted as anxiolytic effects, whereas decreased exploration as anxiogenic effect. Thus, exposure of rats to noise in the present study resulted in anxiogenic effects but anxiolytic effects was obtained in rats treated with kaempferol + zinc + noise. This finding is in agreement with the results of previous studies that established the anxiolytic effect of kaempferol (Vissiennon *et al.,* 2012). The mechanism of the anxiogenic effect of noise stress has been suggested to include the involvement of increased activity of the

hypothalamo-pituitary-adrenal axis, and alterations in dopaminergic and serotonergic neurotransmissions in the hypothalamus (Wankhar *et al.*,2014). The finding that treatment of rats with kaempferol and zinc resulted in the highest frequency of rearing, when compared with the control animals, showed that administration of kaempferol and zinc ameliorated the anxiogenic effect induced by noise stress. It has been shown that an increase in the frequency of stretch-atend posture is indicative of increased anxiety (Brown *et al.,* 2007). Thus in the present study, the exposure of Wistar rats to noise resulted in an increase in stretch-atend postures, demonstrating an anxiogenic effect. The result of the stretch-atend posture further buttressed the fact that noise produces an anxiogenic effect found to be ameliorated by treatment with kaempferol and zinc.

An increase in frequency of defaecation and urination is indicative of increased anxiety (Brown *et al.,* 2007). The frequency of defaecation and urination in the open-field apparatus was higher in the noise-exposed rats, when compared with that obtained in the control and kaempferol and zinc treated groups. This again shows that kaempferol and zinc ameliorated the anxiogenic effect caused by noise in the rats.

A significant increase in locomotion and grooming in the exposed groups treated with kaempferol and zinc compared to the group treated with deionised water was also observed in this study. This result corroborates the report of Wankhar *et al*. (2014) that the antioxidant effect of vitamin C content in *E.officinalis* results in alteration of locomotor activity in Wistar rats, exposed to noise stress. Evidence has been shown that stress induces an increase in corticotrophin releasing hormone and corticosterone which may be responsible for the decreased locomotor activity (Wankhar *et al.,* 2014). It is suggested that the antioxidant property of kaempferol and zinc may reduce the

corticotropin-releasing hormone into the blood stream, which may induce significant protection in the studied behavioural parameters. The mechanism by which kaempferol mitigated the alteration in the studied behavioural parameters may also be due to its antioxidation property. Kaempferol exerts antioxidant effects through inhibition of the activity of enzymes that generate ROS, such as xanthine oxidase (Wang *et al.,* 2006). Like other flavonoids, kaempferol may also ease the formation of hydroxyl radical through the Fenton's reaction by chelating ferrous or cuprous ions (van Acher *et al.,* 1996). Kaempferol also induces antioxidant effects by increasing the expression or activity of antioxidant enzymes such as superoxide dismutase, catalase, and haeme oxygenase-1 (Doronicheva *et al.,* 2007; Septembre-Malaterre *et al*., 2016). Kaempferol and its glycosides prevent lipid peroxidation of cytomembranes (Hou *et al.,* 2004); and the activity of kaempferol is higher than that of its glycosides because kaempferol has a higher lipophilia and capacity to penetrate into lipid bilayers to exert its activity.

The increase in MDA concentration, recorded in the present study demonstrated that noise stress instigated an increase in ROS generation, capable of breaching lipid and protein molecules and damaging DNA triggering loss of function and cell death (Henderson *et al.,* 2006; Zhang *et al.,* 2015) which agrees with the finding that noise stresscauses increased lipidperoxidation evidenced by increase in MDA concentration (Pinar *et al.,* 2011). The result of the present study showed that co-administration of kaempferol and zinc gluconate mitigated noise stress-induced increase in MDA concentration in Wistar rats. The finding that significant decrease in MDA was observed in the group treated with kaempferol and zinc, compared to the groups separately treated

with kaempferol or zinc indicates that kaempferol and zinc act additively to ameliorate adverse effects of noise stress, induced via increased lipid peroxidation in Wistar rats.The finding agrees with the result obtained by Shrivastava *et al.* (2017), who reported that kaempferol decreases MDA concentration in Wistar rats exposed to acrylamide intoxication. One of the major oxidation products of peroxidised polyunsaturated fatty acids is MDA. Increased MDA concentration is an indication of oxidative stress and lipid peroxidation. Lipid peroxidation leads to oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes engenders impairment of membrane fluidity and activation of several membrane bound-enzymes that are crucial for many biological processes (Tu *et al.,* 2007). However, kaempferol and its glycosides prevent lipid peroxidation (Hou *et al.,* 2004). Kaempferol prevents the oxidation of low-density lipoprotein (LDL), which may play a protective role in atherosclerosis (Septembre-Malaterre *et al*., 2016). The result of the present study is also in agreement with that of Khalaf *et al.* (2017) and Nunnes *et al*. (2017), who reported that zinc supplementation in ethanol-intoxicated rats considerably increases activities of oxidative stress-related enzymes and stabilises the concentration of MDA, thus signifying its anti-peroxidative potential.

The result of the present study showed that kaempferol and zinc gluconate increased the activities of serum antioxidant enzymes in the activities of the various antioxidant enzymes in the antioxidant treated groups, compared to the untreated groups. The finding that significant increase was observed in the group treated with kaempferol and zinc, compared to the groups separately treated with kaempferol or zinc, showed that

kaempferol and zinc act additively to ameliorate noise stress-induced oxidative stress in Wistar rats.The finding also agrees with the result obtained by Shrivastava *et al.* (2017), who reported that kaempferol restores acrylamide intoxication-induced decrease in glutathione peroxidase, superoxide dismutase and catalase activities in Wistar rats. Kaempferol exerts antioxidant effects by increasing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and haeme oxygenase-1. It also enhances the antioxidant status of animals by increasing the ROS-scavenging activities of antioxidant enzymes (Doronicheva *et al.*, 2007; Septembre-Malaterre *et al*., 2016). The finding of this study agrees with those of Khalaf *et al.* (2017) and Nunnes *et al*. (2017), who reported that zinc supplementation to ethanol-intoxicated rats increased considerably the activities of oxidative stress-related enzymes (glutathione peroxidase, superoxide dismutase and catalase) and lipid peroxidation thus, signifying its antiperoxidative potential.Zinc also plays a structural role in the maintenance of the integrity of Cu-Zn superoxide dismutase as a cofactor (Sahin and Kucuk, 2003; Ambali *et al*., 2010). Zinc is also involved in the regulation of glutathione that is vital to cellular antioxidant defense (Parrat *et al*., 1997). Furthermore, zinc stabilizes the thiol pool by protecting the sulfhydryl group, hence proteins from oxidation (Verhaegh *et al*., 1998). The combined effect of these properties coupled with its role in metabolic reactions as a cofactor for many enzymes involved in metabolic processes may have contributed to the mitigation of noise induced stress in rats.

Superoxide dismutase catalyses the dismutation of superoxide to $H_20_{2,}$ and it is deemed the first body defence against ROS (Molina *et al*., 2016). Catalase catalyses the breakdown of H_20_2 to water and oxygen, and also plays a significant role in oxidative

defence of the animal tissue, by catalysing reduction of hydrogen and lipid peroxides. Increased activity of Superoxide dismutase results in increased production of H_20_2; and, thus a simultaneous rise in glutathione peroxidase activity as seen in this present study (Zhang *et al.*, 2015; Munne-Bosch and Pinto-Marijuan, 2017). This result also affirms that trace elements such as selenium, Zinc, perform important catalytic role in the enzymatic activity of glutathione peroxidase and superoxide dismutase (Molina *et al.*, 2016).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The present study has shown that exposure of Wistarrats to noise stress caused:

1. an increase in erythrocyte osmotic fragility which made erythrocytes more susceptible to lysis.

2. a reduction in values of haematological parameters, including packed cell volume, haemoglobin concentration and total erythrocyte count. It also caused leucocytosis due to marked neutrophilia and lymphopaenia.

3. reduces weight-gain in rats which was ameliorated by kaempferol + zinc gluconate.

4.impairment in neurobehaviour and cognition.

5. an increase in oxidative stress and lipid peroxidation in the brain, evidenced by increased malondialdehyde concentration and decreased activities of antioxidant enzymes. This shows that lipid peroxidation plays an important role in the neurobehavioural cognitive and biochemical changes observed in rats exposed to noise

stress. Oxidative stress and lipid peroxidation are involved in molecular the mechanism underlying neurobehavioural and cognitive changes in rats exposed to noise stress.

The study has shown, for the first time, that treatment with the antioxidants, kaempferol and zinc improved deficits induced by exposure to noise stress in packed cell volume, haemoglobin concentration, total erythrocyte count, platelet count, and induced leucocytosis due to marked neutrophilia, as well as lymphopaenia in Wistar rats. In addition, kaempferol and zinc ameliorated noise-induced deficits in neurobehavioural and cognitive changes.

Furthermore, the study has, for the first time, revealed the ability of kaempferol and zinc to protect the integrity of erythrocyte membrane by ameliorating the noise-induced increase in EOF. Similarly, treatment with kaempferol and zinc protects the body against noise-induced oxidative stress and lipid peroxidation as demonstrated by reduced concentration of MDA and an increase in the activities of antioxidant enzymes in the kaempferol and zinc-treated groups.

6.2 Recommendations

i. Noise of up to 100 dB should be avoidedin animal farm premises and human environments.

ii. 5 mg of kaempferol and 50 mg of zinc gluconate may be beneficial to high risk population. Consumption of foods containing kaempferol and zinc antioxidants is encouraged.

iii. Region specific studies of the brain such as histopathology of the hippocampus and cerebrum, should be conducted to establish areas most susceptible to noise exposure and response to treatment with kaempferol and zinc.

iv. Other types of behavioural test models such as Morris water maze test and swimming test should be used in addition to the ones used in the present study.

v. Further studies should be carried out to ascertain the optimum dose of kaempferol and zinc for the mitigation of noise stress.

vi. Further studies should be carried out to determine the mitigative effect of kaempferol and zinc on acute and chronic noise exposure.

vii. Further studies are required to elucidate the mechanism by which kaempferol and zinc ameliorates haematological, neurobehavioural and oxidative stress changes in Wistar rats exposed to noise stress.

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