

## Original Research Article

### **Effects of *Zingiber officinale* and Seeds of *Aframomum melegueta* Extract on the Biochemical and Immunological Indices of Stress-Induced Rats**

#### **Abstract**

**Objective:** This study investigated the effects of *Zingiber officinale* and the seeds of *Aframomum melegueta* extracts on the biochemical and immunological indices of stressed-induced Wistar rats.

**Methods:** Forty-five Wistar rats were randomized into nine groups (A-I) of five rats per group. Group A served as standard control and was not induced with electric shock, group B served as negative control hence they were induced and untreated, group C served as the positive control and was administered 2.9 mg/kg magnesium while groups D and E were administered 100 and 200 mg/kg body weight of *Z. officinale* extract and groups F and G were administered 100 and 200 mg/kg body weight of *A. melegueta* extract respectively, while co extract of *Z. officinale* and *A. melegueta* was administered to group H and I at a dose of 100 and 200 mg/kg body weight respectively. At the end of the experimental period, the animals were sacrificed and the sera obtained were used for bioassay analysis.

**Results:** There was significant decrease ( $P>0.05$ ) in the cortisol levels of extract-treated groups from week 1 ( $2.56\pm 0.55$   $\mu\text{g/dl}$ ) to week 4 ( $1.85\pm 0.30$   $\mu\text{g/dl}$ ) compared to the untreated group ( $3.98\pm 0.69$   $\mu\text{g/dl}$ ) (week 1) and ( $8.57\pm 1.31$   $\mu\text{g/dl}$ ) (week 4) as well as the kidney function test. The liver function test revealed a significant decrease in liver biomarkers (ALT, AST, ALP, direct bilirubin, and total bilirubin) in the extract-treated groups compared to the untreated control group. The extract of the plants impacted the WBC and its differentials while there was a significant decrease in the CRP in the extract-treated group ( $2.38\pm 0.22$  mg/l) compared to the untreated control ( $9.93\pm 1.50$  mg/l).

**Conclusion:** The extracts of *Z. officinale* and *A. melegueta* affects some biochemical and immunological parameters and hence could serve as a potential therapeutic agent to fight against stress and its related disorders.

**Keywords:** *Aframomum melegueta*, *Zingiber officinale*, c- reactive protein, electric shock, kidney function, liver function, stress

#### **Background**

Stress is considered to be physical and psychological tension resulting from an adverse situation or environment to which an individual struggle to adapt and maintain homeostasis [1]. According to the type and severity of the stimulus, stress can not only disrupt homeostasis but may also cause various diseases and can even prove fatal [2]. Stress is associated with diseases, such as ischemic stroke, cardiovascular disease, inflammatory bowel disease, and atopic dermatitis as well with psychiatric disorders [3]. The hypothalamic-pituitary-adrenocortical axis is an important neuroendocrine system that moderates responses to psychological and physical stress and inflammation [4]. Stress represents the main environmental risk factor for mental illness. Exposure to stressful events particularly early in life has been associated with increased

incidence and susceptibility of major depressive disorders as well as other psychiatric illnesses [4]. Among the key players in these events are glucocorticoid receptors. Dysfunctional glucocorticoid signaling may indeed contribute to psychopathology through a number of mechanisms that regulate the response to acute or chronic stress and that affect the function of genes and systems known to be relevant for mood disorders [5].

Electric foot shocks have been widely used for the development of various animal models of human disorders such as hypertension, anxiety, depression, and post-traumatic stress disorder (PTSD) by introducing subtle variations in current intensity, duration, number of shock exposures, and post-exposure treatment [6]. In the animal model of stress-induced hypertension, rats received intermittent electric shocks, but behavioral changes of rats exposed to this chronic foot shock stress (CFSS) paradigm and nutritional treatment to manage the level of stress induced is been explored in this work.

Overproduction of free radicals, such as reactive oxygen species (ROS) in the body plays an important part in the development of many chronic diseases. A variety of natural products possess antioxidant potential, such as vegetables, fruits, edible flowers, cereal grains, medicinal plants, and herbal infusions [7]. Traditionally many plants have been used successfully for medicinal purposes [8].

A great number of the world's population; particularly Nigerians, rely on traditional medicines for their primary healthcare needs [9]. These medicinal plants contain substances used for therapeutic purposes or are precursors for the synthesis of useful drugs [9, 10]. Ginger also has high antioxidant activity [8]. The rhizomes of ginger are the most widely used spice and condiment [11]. The consumption of ginger has been claimed to be useful in many oxidative stress-related medical conditions because of its anti-inflammatory effects, some of these medical conditions include hypertension, and diabetes-induced pancreatic and renal derangements [12].

Several medicinal importance has been attributed to ginger and alligator pepper plants; they include their therapeutic properties against a wide range of infections and health conditions such as cancer, cold, fever, coughs, nausea, arthritis, Alzheimer's disease, inflammations, and rheumatism [13]. Gingerol, one of the constituents of *Zingiber officinale* has been shown to be useful in treating inflammatory diseases such as arthritis and asthma [13]. Over the years, ginger has been highly regarded in the health and wellness product market as an economically important herb and their demands have continued to rise in many parts of the world [14].

*Aframomum melegueta* (Alligator pepper) also known as grains of paradise, is a tropical perennial herb of the genus *Aframomum* and the family, Zingiberaceae also known as the ginger family [15]. Alligator pepper is greatly utilized by many countries including Nigeria for diverse purposes, *A. melegueta* is a cosmopolitan plant employed in the Federal Republic of Nigeria as a spice in foods. It is locally called 'atare' in Yoruba and 'ose-oji' in Igbo [16]. It is a common item used for traditional sacrifices and other religious rites. Also, several uses of alligator pepper have been reported in the literature and these include its efficacy in controlling Egyptian cotton leaf worm and also important crop pests such as the diamondback moth [17]. Alligator pepper has immense medicinal importance; the seed extract of alligator pepper heals wounds and invigorates the immune system against diseases [17]. Studies have shown that seeds contain important phytochemicals namely, alkaloids, glycosides, tannins, flavonoids, sterols, triterpenes,

and oils, some of which are responsible for their antimicrobial properties [18].

There is no defined cure for stress, though there appear to be a drugs that have been used to manage it but these drugs are usually costly and could cause adverse effects, hence the need for plant-based remedies with reduced or no adverse effects. This study, therefore, investigated the effects of *Z. officinale* and seeds of *A. melegueta* extract on the biochemical and immunological indices of stress-induced rats.

## **Methods**

### **Sample Collection and Identification**

The rhizomes of *Z. officinale* and *A. melegueta* were purchased from Eke Awka market in Awka South Local Government Area of Anambra state. The samples were identified and authenticated by a Botanist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The voucher number of *Z. officinale* is NAUH-26A (Rhizome) while that of *A. melegueta* is NAUH-13<sup>A</sup>.

### **Preparation of Ethanol extracts of *Zingiber officinale* and *Aframomum melegueta* Sample**

Fresh *Z. officinale* rhizomes were thoroughly washed, peeled, and chopped while the seeds of *A. melegueta* were carefully selected and washed, both samples were air-dried at room temperature for four weeks and then ground into powder using Corona manual grinding machine.

Exactly 500g of the pulverized dried *Z. officinale* and *A. melegueta* seeds were macerated in 2 Litres of 70% ethanol. The ethanol extraction was allowed to stand for 48 hours for complete extraction after which it was separated, sieved using sieve cloth (Muslin cloth), and filtered using Whatman filter paper. The filtrate was separated and concentrated using a water bath at 500C [19].

### **Experimental Animals**

A total of 45 Male Wistar Albino rats were procured from Chris Animal Farm and Research Laboratory Mgbakwu Awka, Anambra State and used for the experiment. They were maintained and housed in cages at the Chris Animal Farm and Research Laboratory Mgbakwu Awka, according to the Institutional Animal Care and Use Committee (IACUC) guidelines on the care and handling of experimental animals. The animals were allowed to acclimatize for 7 days, and fed *ad libitum* with vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state. At the end of the 7 days' acclimatization period, the animals were weighed and then randomized into 9 groups of 5 rats each.

### **Experimental Procedure**

Group A was the normal control, group B was the stressed without treatment, group C was stressed and treated with standard drug (2.9 mg/kg body weight magnesium supplement) for 21 days , groups D, E, groups F, G and groups H, I were stressed and treated with 100 mg/kg and 200mg/kg body weight of the ethanol leaf extract of *Z. officinale*, *A. melegueta* and the combination of both *Z. officinale*, *A. melegueta* at 1:1 respectively for a period of 21 days after the induction of electric shock- induced stress to check the ability of the extracts in preventing or managing stress. Stress was induced using the electric shock maze at 110volts, 0.2 seconds shock

was induced four times to the rats at an interval of 5 minutes daily. Bodyweights, Cortisol levels of the rats was determined before initiating the daily treatment with 100mg/kg and 200mg/kg of the ethanol extracts of *Z. officinale* and *A. melegueta* for a period of 21 days. After 28 day of treatment, the animals were sacrificed and a total number of 45 blood samples were collected for biochemical and immunological analysis.

### **Induction of Acute Stress by Electric Foot Shock**

Stress was induced using the electric-foot shock maze/ chamber at 110volts. The induction was done four times daily at 5minutes interval for 28 days, after the induction Treatment was done daily immediately after induction for 28days.



**Figure 1:** Stress induction process

**Photographed by:** Nwarienne. M. Chiamaka, 2022

### **Sacrifice and Sample Collection**

After 28 days of stress induction and treatment, the animals were sacrificed by first using formalin as anesthetic and then their blood was collected by cardiac puncture for biochemical analysis.

### **Biochemical Assay**

#### **Determination of Cortisol Levels**

Their Cortisol levels were checked at about 8.00am in the morning before the induction, during the process of induction and after the completion of the treatment of the stressed animals. It was measured using a Cortisol ELISA Kit (Fine Test, Wuhan, China) and Corticosterone ELISA Kit (Enzo Life Sciences, Ann Arbor, MI, USA) according to the manufacturer's instructions.

#### **Determination of Kidney function test**

Urea and creatinine was analysed using the Limdi and Hyde method [20].

#### **Determination of liver function test**

The liver function test was conducted using the method of Limdi and Hyde method [20].

#### **Determination of White Blood Cell Differentials**

The haematological parameters were carried out using 5-part Auto Haematology Analyzer (BC-5300)

### **Determination of C- reactive protein**

**The determination of C- reactive protein was carried out using an essential reagent required for an immune enzyme metric assay which include high affinity and specificity antibodies (enzyme and immobilized) with different and distinct epitope recognition in excess and native antigen, as described by [21].**

### **Data Analysis**

The results were analyzed using the IBM SPSS version 25 (SPSS Inc., Chicago, Illinois, USA). All the numerical values were expressed as Mean  $\pm$  SEM. Statistical analysis of the results obtained was performed by using ANOVA Tests to determine if a significant difference exists between the mean of the test and control groups. The level of significance was set at  $p < 0.05$ .

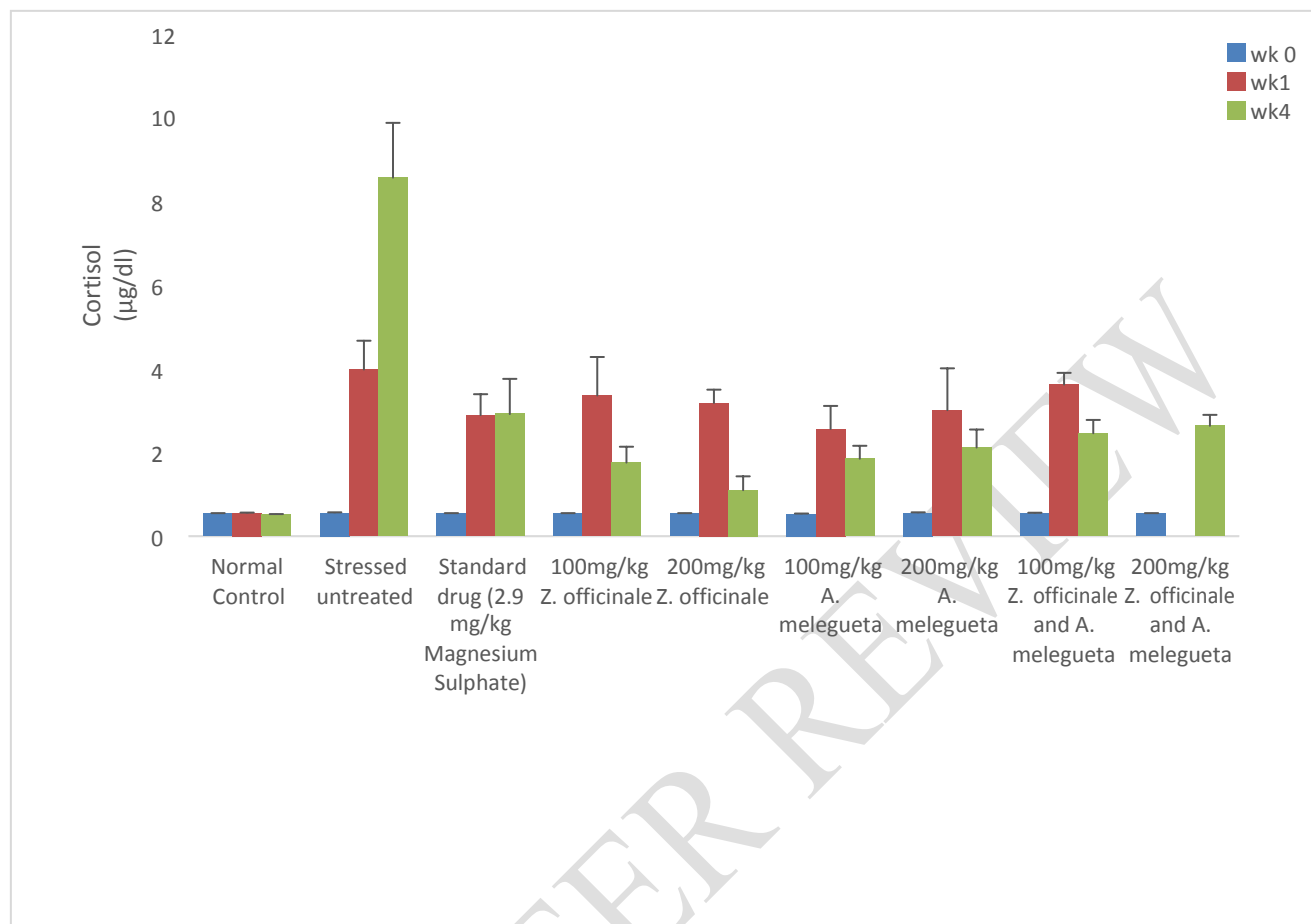
### **Ethical Approval**

The study protocol was approved by Nnamdi Azikiwe University Animal Research Ethics Committee in accordance with the Institutional Animal Care and Use policy in Research, Education, and Testing. Issued reference number NAU/AREC/2023/00059.

## **Results**

### **Results for Cortisol**

Figure 2 shows the effect of ethanol extract of *Z. officinale* and *A. melegueta* on the Cortisol level of electric shock-stressed induced rats before, during, and after the treatment period. The result showed a significant decrease ( $P > 0.05$ ) in the cortisol levels of extract-treated groups compared with the untreated control, with co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight bringing the cortisol level to nearly zero.



**Figure 2:** Shows the effect of ethanol extract of *Z. officinale* and *A. melegueta* on Cortisol level of electric-shock stressed-induced rats before, during and after treatment period

### Result for Kidney function test

The result of the kidney function test is presented in Table 1, it showed a significant decrease in the urea and creatinine levels of the extracts treated groups compared with the untreated control.

**Table 1: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Urea and Creatinine concentration of Electric-shock stress-induced rats.**

Groups	Urea (mg/dl)	Creatinine (mg/dl)
	Group A: Normal Control	0.48 ± 0.06
Group B: Stressed untreated	0.69 ± 0.12	0.19 ± 0.01
Group C: Standard drug (2.9 mg/kg)	0.66 ± 0.12	0.17 ± 0.01

Magnesium Sulphate)

Group D: 100mg/kg <i>Z. officinale</i>	0.61 ± 0.07	0.17 ± 0.01
Group E: 200mg/kg <i>Z. officinale</i>	0.63 ± 0.13	0.18 ± 0.02
Group F: 100mg/kg <i>A. melegueta</i>	0.41 ± 0.07	0.18 ± 0.01
Group G: 200mg/kg <i>A. melegueta</i>	0.60 ± 0.11	0.17 ± 0.01
Group H: 100mg/kg <i>Z. officinale</i> and <i>A. melegueta</i>	0.51 ± 0.10	0.19 ± 0.05
Group I: 200mg/kg <i>Z. officinale</i> and <i>A. melegueta</i>	0.49 ± 0.11	0.18 ± 0.01

**Result for Liver Function Test**

Table 2 shows the effect of ethanol extract of *Z. officinale* and *A. melegueta* on liver function tests in electric-shock stress-induced rats. The result showed a significant decrease in the activities of ALT, AST, ALP, direct bilirubin, and total bilirubin in the extract-treated groups compared with the untreated control.

**Table 2: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Liver Function Test in Electric- shock stress induced rats.**

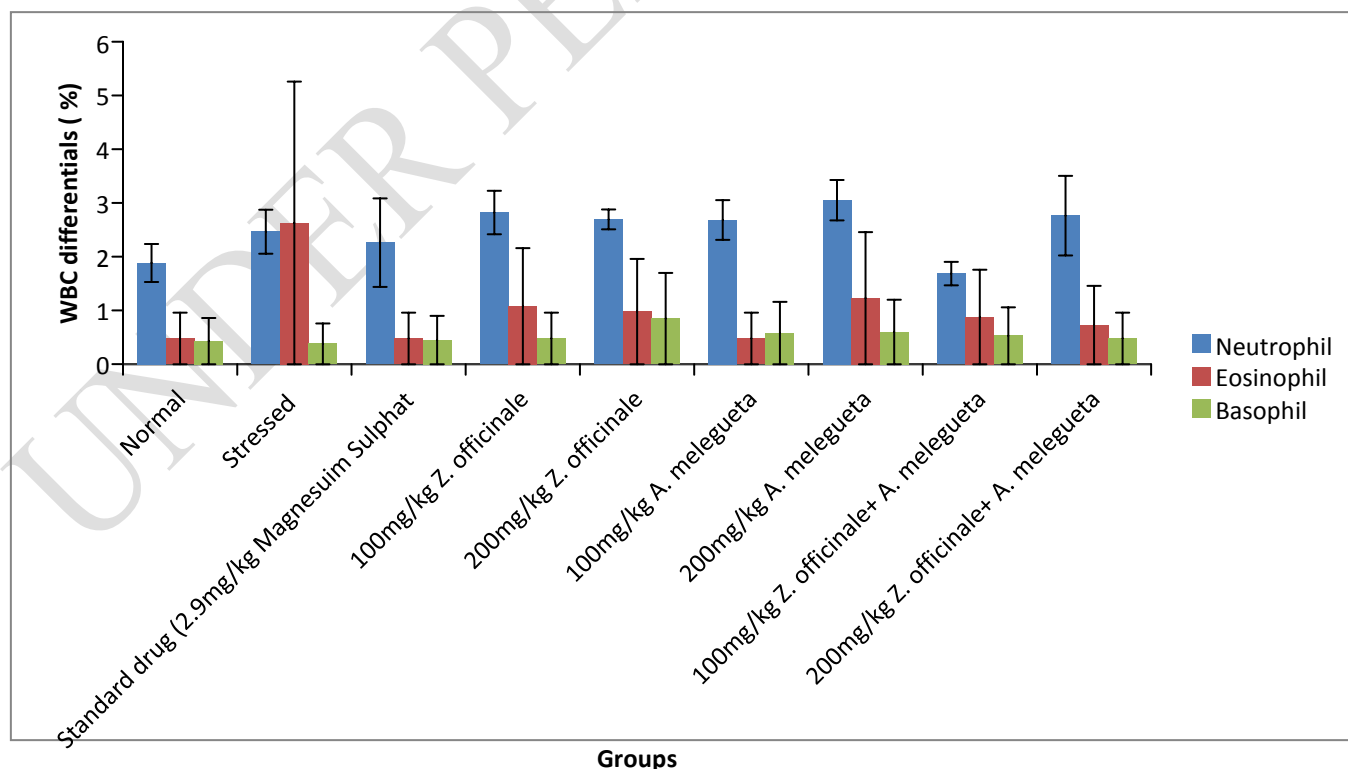
Groups	Liver Function Test					
	ALT (U/L)	AST (U/L)	ALP (U/L)	D. (mg/dl)	BIL T. (mg/dl)	BIL
Group A: Normal Control	20.75 ± 3.84	14.50 ± 2.60	89.01 ± 12.36	0.31 ± 0.06	0.96 ± 0.32	
Group B: Stressed untreated	42.25 ± 3.47	26.75 ± 4.94	166.98 ± 34.52 a	1.09 ± 0.16 a	1.97 ± 0.30a	
Group C: Standard drug (2.9 mg/kg Magnesium Sulphate)	33.00 ± 5.67	11.50 ± 0.87	120.75 ± 2.84	0.62 ± 0.13	1.14 ± 0.18d	
Group D: 100mg/kg <i>Z. officinale</i>	2.25 ± 3.07	13.00 ± 2.12	119.37 ± 5.68	0.75 ± 0.17	1.05 ± 0.14 d	
Group E: 200mg/kg <i>Z. officinale</i>	19.75 ± 4.03	16.25 ± 2.93	111.78 ± 14.88	0.55 ± 0.09	0.99 ± 0.09 d	
Group F: 100mg/kg	24.75 ± 7.75	18.25	77.97 ± 8.15	0.39 ± 0.04	1.56 ± 0.22	

<i>A. melegueta</i>		4.48	d	d		
<b>Group</b>	<b>G:</b>	38.75 ± 1.84	15.25	± 119.37	± 0.74 ± 0.06	0.95 ± 0.01
<b>200mg/kg</b>	<b>A.</b>		1.44	17.72		d
<i>melegueta</i>						
<b>Group</b>	<b>H:</b>	30.00 ± 6.45	13.75	± 107.64	± 0.56 ± 0.14	1.05 ± 0.01
<b>100mg/kg</b>	<b>Z.</b>		1.89	14.12		d
<b>officinale</b>	<b>and A.</b>					
<i>melegueta</i>						
<b>Group I: 200mg/kg</b>		22.25 ± 7.43	13.50	± 116.61	± 0.52 ± 0.17	0.77 ± 0.07
<b>Z. officinale</b>	<b>and A.</b>		4.72	6.70		d
<i>melegueta</i>						

Note a=Sig increase with respect to group A; d =Sig decrease with respect to group B.

### Result for White Blood Cell Differentials

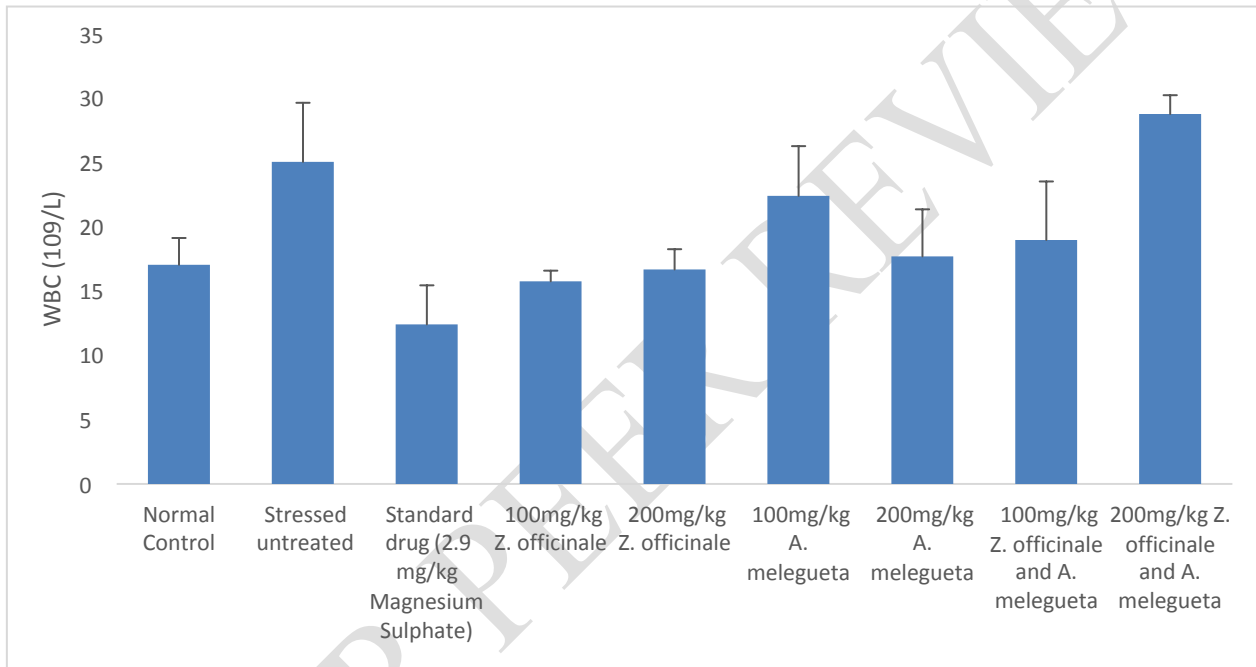
Figure 3 presents the effect of ethanol extract of *Z. officinale* and *A. melegueta* on the differentials of white blood cells (neutrophil, eosinophil and basophil) in electric shock stressed induced rats before, during and after treatment period. The result showed a significant increase ( $P < 0.05$ ) in the level of eosinophil and basophil levels of extract-treated groups compared to the untreated control while there was a significant decrease in the eosinophil level of the extract-treated group compared to the untreated control.



**Figure 3:** Effect of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cells differentials of electric-shock stressed-induced rats before, during and after treatment period

**Result for White Blood Cell Count**

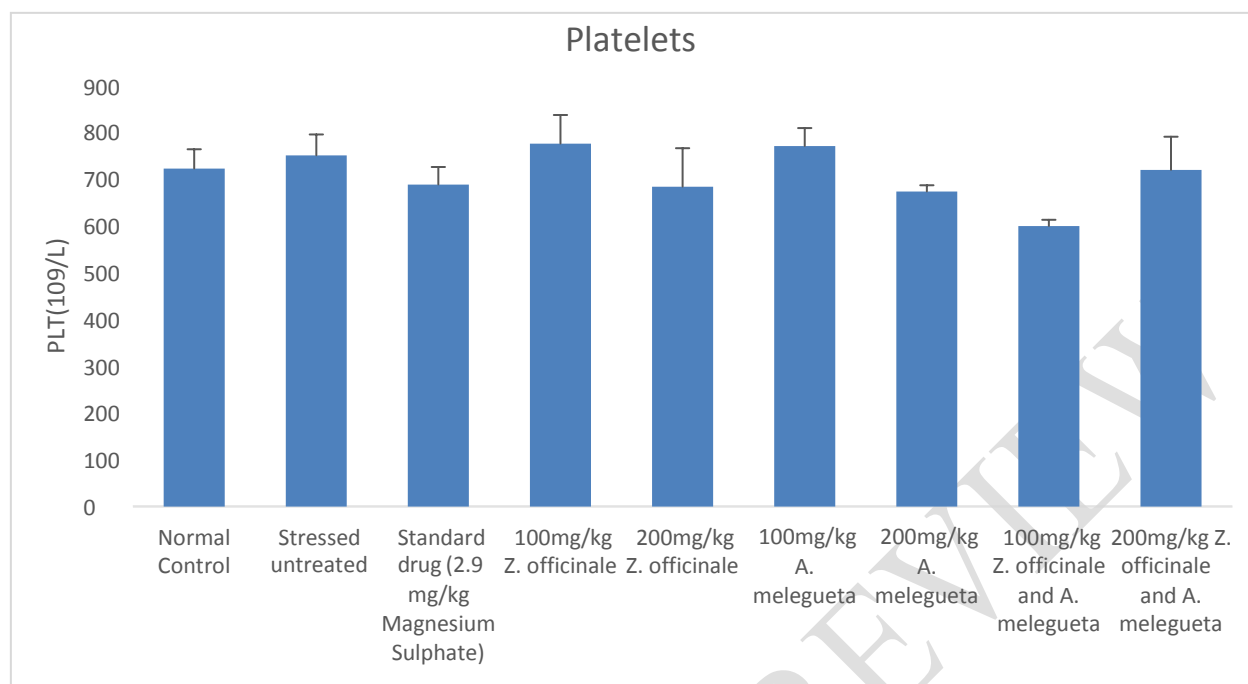
Figure 4 showed the effects of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cell concentration of electric-shock stress induced rats. The result showed a significant decrease ( $P>0.05$ ) in the white blood cell of extract-treated groups compared with the untreated control. However, co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight showed an increase in the white blood cell compared with the untreated group.



**Figure 4:** Effects of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cell concentration of Electric-shock stress-induced rats.

**Results for Platelet**

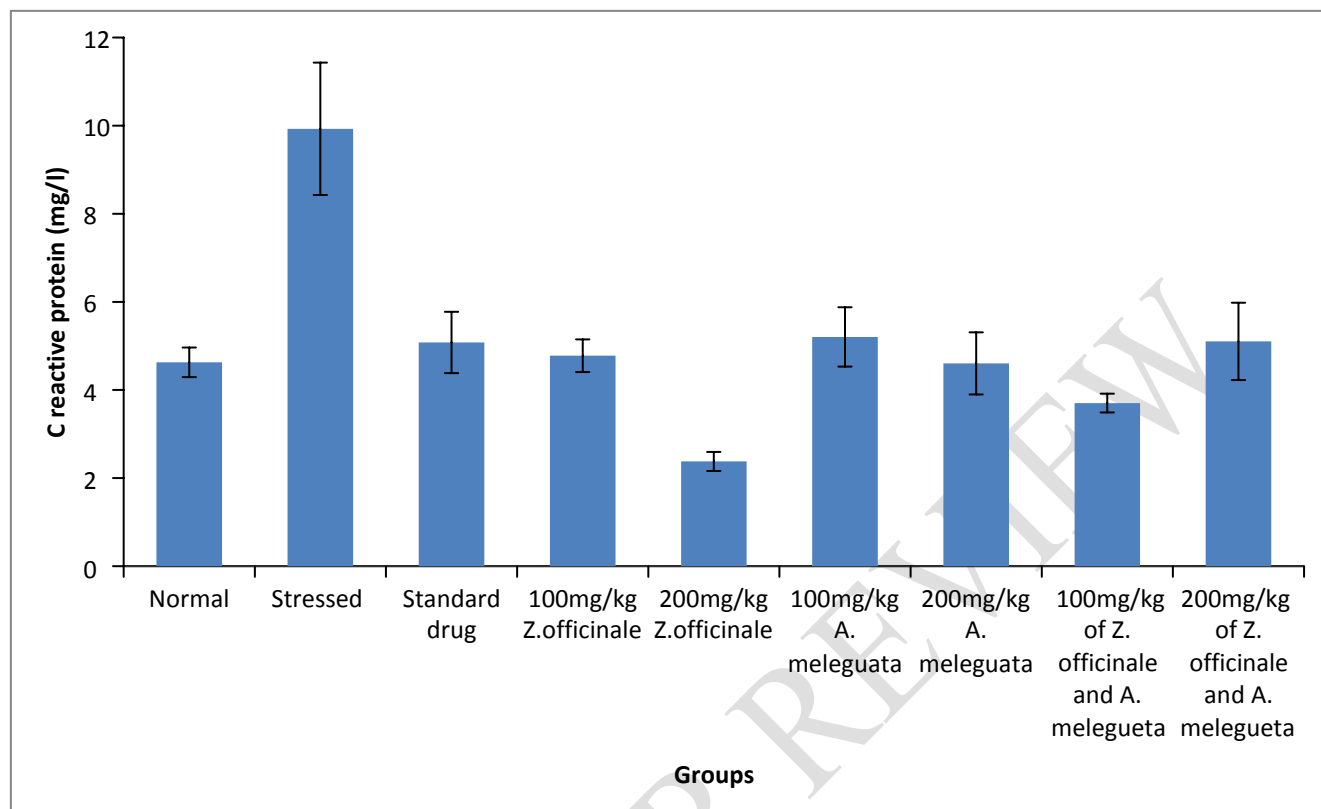
Results on the effects of ethanol extract of *Z. officinale* and *A. melegueta* on Platelets concentration of electric-shock stress-induced rats are presented in Figure 5. Oral administration of 100 mg/kg body weight of *Z. officinale* and co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg significantly increases the platelet level of the treated groups when compared with the control group.



**Figure 5:** Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Platelets concentration of electric-shock stress-induced rats.

### Result for C - reactive Protein

Results on the effects of ethanol extract of *Z. officinale* and *A. melegueta* on C-reactive protein concentration of electric-shock stress-induced rats are presented in Figure 6. The result showed a significant decrease ( $P > 0.05$ ) in extract-treated groups compared to the untreated group.



**Figure 6:** Effect of ethanol extract of *Z. officinale* and *A. meleguata* on c-reactive protein concentration of electric-shock stress-induced rats

## Discussion

Stress has been defined to be a feeling of emotional or physical tension which could arise from any event or thought that makes one feel frustrated, angry, or nervous. It is often a normal reaction the body has when changes occur, resulting in physical, emotional, and intellectual responses. As depicted in this study (Figure 2), there was a significant decrease ( $p > 0.05$ ) in the cortisol levels of the extracts treated groups compared to the untreated control. It is worth noting that co-administration of *Z. officinale* and *A. meleguata* at 200 mg/kg body weight decreases the cortisol levels to nearly zero at week 1 of administration. This is an important observation since cortisol has been implicated in stressful conditions. Cortisol is a steroid hormone that is produced by the adrenal glands, which sit on top of each kidney and it is released into the bloodstream in a stressed state. This result is consistent with the findings of O'Connor et al. [1], who explained that when the homeostasis of the body is altered, the body releases hormones such as cortisol switches on the autonomic nervous system (ANS) which allows the body to adapt and respond to day-to-day activities. Since the induction of electric foot shock has been implicated in the pathogenesis of inflammatory response, the ability of these extracts to cause a decrease in the cortisol level supports its anti-inflammatory potentials Brody et al. made a similar assertion [22].

Assessment of renal function is vital in the management of patients with kidney disease. Test of renal function has utility in identifying the presence of renal disease monitoring the response of kidneys to treatment and determining the progression of renal disease [23]. The result showing the effect of ethanol extract of *Z. officinale* and *A. melegueta* on urea and creatinine concentration in electric-shock stress-induced rats is presented in Table 1, it showed a significant decrease in the urea and creatinine levels of the extracts treated groups compared with the untreated control, which is consistent with the findings of Okeke et al [24]. Urea is a naturally occurring molecule that is produced by protein metabolism and found abundantly in mammalian urine. The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl. Accumulation of urea above this reference range has been implicated in several health conditions, especially uremia. The levels of urea concentrations in the extract-treated groups, as observed in this study implies that the administration of the extract does not pose any threat to the proper physiological functions of the kidney. Similarly, the normal reference range of creatinine has been reported to range between 0.7 to 1.3mg/dl for adult males and 0.5 to 1.1 mg/dl for adult females. The observed creatinine level in this study falls within the reference range of apparently healthy patients. Creatinine is a waste product made by the muscles as part of regular, everyday activity. The kidneys filter creatinine from the blood and send it out of the body via urine. Hence, any impairment in the proper physiological functions of the kidneys could plausibly lead to the accumulation of creatinine in the blood and consequently impair health.

Liver function tests are blood tests that measure different enzymes, proteins, and other substances made by the liver. As shown in Table 2 above, there was a significant decrease in the serum activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the extract-treated groups compared with the untreated stressed animals. AST and ALT are important liver biomarkers whose activities are significantly expected to be high in the liver. Impairment in the liver causes these enzymes to leak out of the hepatocyte into the bloodstream. The observed activity of the enzymes in this study showed the protective effect of the plant extracts on the hepatocytes which agrees with the report of Onwubuya and Oladejo [25].

The evaluation of hematological parameters is a piece of useful information in determining the effect of foreign substances including plant extracts in vivo [10]. They are used to ascertain any possible changes in the levels of biomolecules such as enzymes, metabolic products, hematology, normal functioning, and histopathology of the organs. In this study, the effect of the extract of *Z. officinale* and *A. melegueta* on white blood cells and its differentials showed that there was a significant increase ( $p \geq 0.05$ ) in the levels of the WBC in Figure 4 in the group treated with 200mg/kg of the combination of *Z. officinale* and *A. melegueta* gave a value of  $28.72 \pm 1.46$  which is almost double of the value of stressed untreated  $25.00 \pm 4.59$  and that of the normal group  $17.01 \pm 2.08$ , although the dose-dependent value of *A. melegueta* at 100mg/kg gave a high value of  $22.36 \pm 3.86$  but was suppressed at 200mg/kg, the combination of both extract at 200mg/kg proved to be more effective in producing more WBC in the process of treating stressed related inflammation. In Figure 3 there was a significant increase of neutrophils in groups treated with 200mg/kg of both extracts it gave a value of  $27.65 \pm 7.41$  which was higher than the stressed untreated group and those treated with standard drug, neutrophils are the first responders as they are swiftly recruited, constituting about 50% of all cell at the inflammation site [26]. The primary function of neutrophils at the site of inflammation includes compacting invading pathogens via various antimicrobial responses involving phagocytosis, therefore the

groups treated with 100mg/kg and 200mg/kg of *Z. officinale*, 100mg/kg and 200mg/kg of *A. melegueta* were also effective in mobilizing the neutrophils but the most effective dose was that of 200mg/kg of the combination of the extracts. While Eosinophil levels were significantly reduced as seen in Figure 3, Eosinophils less commonly called acidophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates [26]. They form about 2 to 3% of WBC and are responsible for tissue damage, inflammation, and allergy, from the result seen in Figure 3, the eosinophils levels were not significantly increased, but rather there was a decrease as compared to the stressed untreated group, therefore this depicts that there was no presence of allergy or tissue damage, this is consistent with the findings of Oladejo and Osukoya [27]. Neutrophils and monocytes as well as other hematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function [10]. Neutrophils are important phagocytic cells normally elevated at early stages of stressed conditions [28]. Monocytes are known to be the largest type of leucocyte and can differentiate into macrophages and myeloid lineage dendritic cells, hence, they help in fighting bacteria, viruses, and other infections in the body, thereby influencing the process of adaptive immunity [27].

The platelets level is known to aggregate where there is wound healing or inflammation it also has diagnostic and prognostic roles in certain diseases [29], from the results the groups treated with 200mg/kg of ginger and Alligator pepper combinations suppressed the platelets aggregation while 100mg/kg of ginger and Alligator pepper enhanced platelets aggregation comparing it to the groups treated with the standard drug, thereby enhancing the immune system. Thus the maximum efficacy of platelets is at 100mg/kg of both extracts than the groups treated with 200mg/kg of both extracts and the standard drug.

C-reactive protein (CRP) is a pentameric protein synthesized by the liver, whose level rises in response to 9 of 11 inflammation. CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process. A high level of CRP in the blood has been implicated in the pathogenesis of inflammation and inflammatory diseases. As depicted in this study (Figure 6), induction of stress in the experimental animals significantly caused an increase in their serum CRP in the stressed untreated group, the CRP levels rose from  $4.63 \pm 0.33$  to twice its normal level of  $9.93 \pm 1.50$ . However, the administration of extracts of *Z. officinale* and *A. melegueta* was able to mitigate this increase, bringing the serum CRP to nearly normal. This could plausibly be linked to the presence of bioactive compounds such as 6-gingerol and 6-shogaol in *Z. officinale*. 6-gingerol and 6-shogaol have been shown to have an anti-inflammatory effect by inhibiting the production of inflammatory mediators, such as prostaglandin E<sub>2</sub>, NO, inflammatory cytokines (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ )), and pro-inflammatory transcription factor (NF- $\kappa$ B) [28]. Boarescu et al. [30] have earlier reported that root capsules of *Z. officinale* enhance the analgesic and antioxidant efficacy of diclofenac sodium in experimental animals induced with acute inflammation.

## Conclusions

The abundance of these nutritional fruits *Z. officinale* and *A. melegueta* makes it easily accessible and affordable by all; these plants have shown anti-inflammatory abilities and have proven to be

useful in the management and treatment of the silent pathway to many severe illnesses which are Stress. However, from the studies, a combination of the two extracts showed some favorable increase in some biochemical parameters such as the neutrophils and white blood cells, but the treatment with the extracts separately at 100mg/kg bw and 200mg/kg bw dose was also highly beneficial in reducing immunological indices such as the CRP comparing to the stressed untreated group, from time immemorial, medicinal plants especially *Z. officinale* and *A. melegueta* have been used in the treatment of different diseases. From the results of this study, the administration of extract of *Z. officinale* and *A. melegueta* showed that the plants could be used to relieve stressed conditions, tackle inflammation and consequently help to fight various pathological conditions.

## Abbreviations

PTSD, Post-traumatic stress disorder; CFSS, Chronic foot shock stress; ROS, Reactive oxygen species; IACUC, Institutional Animal Care and Use Committee; ELISA, Enzyme-linked immunosorbent assay; CRP, C-reactive protein; ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; ANS, Autonomic nervous system; WBC, White blood cells; *Z. officinale*, Zingiber officinale; *A. melegueta*, Aframomum melegueta.

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