Original Research Article

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

Abstract

Objective: This study investigated the effects of *Zingiber officinale* and the seeds of *Aframomummelegueta* extracts on the biochemical and immunological indices of stressed-induced Wistarrats.

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 asnegative control hence they were induced and untreated, group C served as the positive controland was administered 2.9 mg/kg magnesium while groups D and E were administered 100 and200 mg/kg body weight of *Z. officinale*extract and groups F and G were administered 100 and200mg/kgbodyweightof*A.melegueta*extractrespectively,whilecoextractof*Z.officinale*and *A. melegueta* was administered to group H and I at a dose of 100 and 200 mg/kg body weightrespectively. At the end of the experimental period, the animals were sacrificed and the seraobtained were used for bioassay analysis.

Results: There was significant decrease (P>0.05) in the cortisol levels of extract-treated groupsfrom week 1 (2.56±0.55 µg/dl) to week 4 (1.85±0.30 µg/dl) compared to the untreated group(3.98±0.69 µg/dl) (week 1) and (8.57±1.31 µg/dl) (week 4) as well as the kidney function test.The liver function test revealed a significant decrease in liver biomarkers (ALT, AST, ALP,directbilirubin,andtotalbilirubin)intheextract-treatedgroupscomparedtotheuntreatedcontrol group. The extract of the plants impacted the WBC and its differentials while there was asignificant decrease in the CRP in the extract-treated group (2.38±0.22 mg/l) compared to theuntreated control (9.93±1.50 mg/l).

Conclusion:TheextractsofZ.*officinale*and*A.melegueta*affectssomebiochemicalandimmunological parameters and hence could serve as a potential therapeutic agent to fight againststress and its related disorders.

Keywords:*Aframomummelegueta,Zingiberofficinale*,c-reactiveprotein,electricshock,kidney function, liver function, stress

Comment [a1]: Please mention full name beside abbreviation for first time.

Comment [a2]: Please check , the level of significance was set at p<0.05

Comment [a3]: Please insert the full name for any abbreviation when mentioned for first time in abstract section.

Comment [a4]: Please check all title and subtitles formats to follow the instruction of journal

Background

Stress is considered to be physical and psychological tension resulting from an adverse situationor environment to which an individual struggle to adapt and maintain homeostasis [1]. Accordingto the type and severity of the stimulus, stress can not only disrupt homeostasis but may alsocause various diseases and can even prove fatal [2]. Stress is associated with diseases, such asischemic stroke, cardiovascular disease, inflammatory bowel disease, and atopic dermatitis aswellwithpsychiatricdisorders[3].Thehypothalamic-pituitary-adrenocorticalaxisisanimportant neuroendocrine system that moderates responses to psychological and physical stressand inflammation [4]. Stress represents the main environmental risk factor for mental

illness.Exposuretostressfuleventsparticularlyearlyinlifehasbeenassociatedwithincreasedincidence and susceptibility of major depressive disorders as well as other psychiatric illnesses[4].Amongthekeyplayersintheseeventsareglucocorticoidreceptors.Dysfunctionalglucocor ticoidsignalingmayindeedcontributetopsychopathologythroughanumberofmechanisms that regulate the response to acute or chronic stress and that affect the function ofgenes and systems known to be relevant for mood disorders [5].

Electric foot shocks have been widely used for the development of various animal models ofhuman disorders such as hypertension, anxiety, depression, and post-traumatic stress disorder(PTSD)byintroducingsubtlevariationsincurrentintensity,duration,numberofshock 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 chronicfootshockstress(CFSS)paradigmandnutritionaltreatmenttomanagethelevelofstressinduced is been explored in this work.

Overproduction of free radicals, such as reactive oxygen species (ROS) in the body plays animportantpartinthedevelopmentofmanychronicdiseases.Avarietyofnaturalproductspossess antioxidant potential, such as vegetables, fruits, edible flowers, cereal grains, medicinalplants,andherbalinfusions[7].Traditionallymanyplantshavebeenusedsuccessfullyformed icinal purposes [8].

Agreatnumberoftheworld'spopulation;particularlyNigerians,relyontraditionalmedicinesfortheirpri maryhealthcareneeds[9].Thesemedicinalplantscontainsubstancesusedfortherapeutic purposes or are precursors for the synthesis of useful drugs [9, 10]. Ginger also hashighantioxidantactivity[8].Therhizomesofgingerarethemostwidelyusedspiceandcondiment [11]. The consumption of ginger has been claimed to be useful in many oxidativestress-related medical conditions because of its anti-inflammatory effects, some of these medicalconditions include hypertension, and diabetes-induced pancreatic and renal derangements [12].

Several medicinal importance has been attributed to ginger and alligator pepper plants; theyinclude their therapeutic properties against a wide range of infections and health conditions suchascancer,cold,fever,coughs,nausea,arthritis,Alzheimer'sdisease,inflammations,andrheumatis m [13]. Gingerol, one of the constituents of *Zingiber officinale* has been shown to beuseful in treating inflammatory diseases such as arthritis and asthma [13]. Over the years, gingerhas been highly regarded in the health and wellness product market as an economically importantherb and their demands have continued to rise in many parts of the world [14].

Aframomummelegueta(Alligatorpepper)alsoknownasgrainsofparadise,isatropicalperennial herb of the genus Aframomum and the family, Zingiberaceae also known as the gingerfamily [15]. Alligator pepper is greatly utilized by many countries including Nigeria for diversepurposes, *A. melegueta* is a cosmopolitan plant employed in the Federal Republic of Nigeria as aspiceinfoods.Itislocallycalled‘atare’inYorubaand‘ose-oji’inIgbo[16].Itisacommonitem used for traditional sacrifices and other religious rites. Also, several uses of alligator pepperhave been reported in the literature and these include its efficacy in controlling Egyptian cottonleafwormandalsoimportantcroppestssuchasthediamondbackmoth[17].Alligatorpepperhasi mmensemedicinalimportance;theseedextractofalligatorpepperhealswoundsandinvigorates the

immune system against diseases [17]. Studies have shown that seeds containimportant 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 tomanage it but these drugs are usually costly and could cause adverse effects, hence the need forplant-based remedies with reduced or no adverse effects. This study, therefore, investigated theeffects of *Z. officinale* and seeds of *A. melegueta*extract on the biochemical and immunologicalindices of stress-induced rats.

Comment [a5]: Please unit the use these abbreviations for extracts in all manuscript

Methods

Comment [a6]: Materials and methods

Sample Collection and Identification

The rhizomes of *Z. officinale* and *A. melegueta* were purchased from Eke Awka market in AwkaSouth Local Government Area of Anambra state. The samples were identified and authenticatedby 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* isNAUH-13[A].

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 temperaturefor 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 2Litres of 70% ethanol. The ethanol extraction was allowed to stand for 48 hours for completeextraction after which it was separated, sieved using sieve cloth (Muslin cloth), and filtered usingWhatman 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 ResearchLaboratory 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 careand handling of experimental animals. The animals were allowed to acclimatize for 7 days, andfed *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 andthen randomized into 9 groups of 5 rats each.

Comment [a7]: Please insert the weight and age of used rats.

Comment [a8]: Please mention the diet given for rats and determine the availability of water for them.

Experimental Procedure

Group A was the normal control, group B was the stressed without treatment, group C wasstressed and treated with standard drug (2.9 mg/kg body weight magnesium supplement) for 21days , groups D, E, groups F, G and groups H, I were stressed and treatedwith 100 mg/kg and200mg/kgbodyweightoftheethanolleafextractof*Z.officinale*,*A.melegueta*andthecombination of both *Z. officinale, A. melegueta* at 1:1 respectively for a period of 21 days afterthe induction of

Comment [a9]: Please insert reference for used dose.

Comment [a10]: Please insert references for used doses of both extracts or mention causes for choosing these doses.

electric shock- induced stress to check the ability of the extracts in preventing ormanaging stress. Stress was induced using the electric shock maze at 110volts, 0.2 seconds shockwas induced four times to the rats at an interval of 5 minutes daily. Bodyweights, Cortisol levelsof the rats was determined before initiating the daily treatment with 100mg/kg and 200mg/kg ofthe ethanol extracts of *Z. officinale* and *A. melegueta* for a period of 21 days. After 28 day oftreatment, the animals were sacrificed and a total number of 45 blood samples were collected forbiochemical 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 wasdone four times daily at 5minutes interval for 28 days, after the induction Treatment was donedaily immediately after induction for 28days.

Figure 1: Stress induction process

Photographed by: Nwarienne. M. Chiamaka, 2022

Sacrifice and Sample Collection

After28daysofstressinductionandtreatment,theanimalsweresacrificedbyfirstusingformalin as anesthetic and then their blood was collected by cardiac puncture for biochemicalanalysis.

Comment [a11]: Please insert the dose and rout of anesthetic agent used.

Comment [a12]: Please mention the type of sample serum of plasma and mention the method of collection with reference please.

Biochemical

AssayDeterminationofCortisolL

evels

Their Cortisol levels were checked at about 8.00am in the morning before the induction, duringthe process of induction and after the completion of the treatment of the stressed animals. It wasmeasured 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

ThedeterminationofC-reactiveproteinwascarriedoutusinganessentialreagentrequiredforan immune enzyme metric assay which include high affinity and specificity antibodies (enzymeand immobilized) with different and distinct epitope recognition in excess and native antigen, asdescribed 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 ± SEM. Statistical analysis of the resultsobtained was performed by using ANOVA Tests to determine if a significant difference existsbetween the mean of the test and control groups. The level of significance was set at $p<0.05$.

Ethical Approval

ThestudyprotocolwasapprovedbyNnamdiAzikiweUniversityAnimalResearchEthicsCommitteeina ccordancewiththeInstitutionalAnimalCareandUsepolicyinResearch,Education, and Testing. Issued reference number NAU/AREC/2023/00059.

Results

~~**Results for Cortisol**~~

Figure2showstheeffectofethanolextractof*Z.officinale*and*A.melegueta*ontheCortisollevel of electric shock-stressed induced rats before, during, and after the treatment period. Theresult showed a significant decrease (P>0.05) in the cortisol levels of extract-treated groupscompared with the untreated control, with co-administration of *Z. officinale* and *A. melegueta* at200 mg/kg body weight bringing the cortisol level to nearly zero.

Comment [a13]: Why the authors not performed the GC MS analysis for both extracts to identify the valuable components?

Comment [a14]: Please check , the level of significance was set at p<0.05

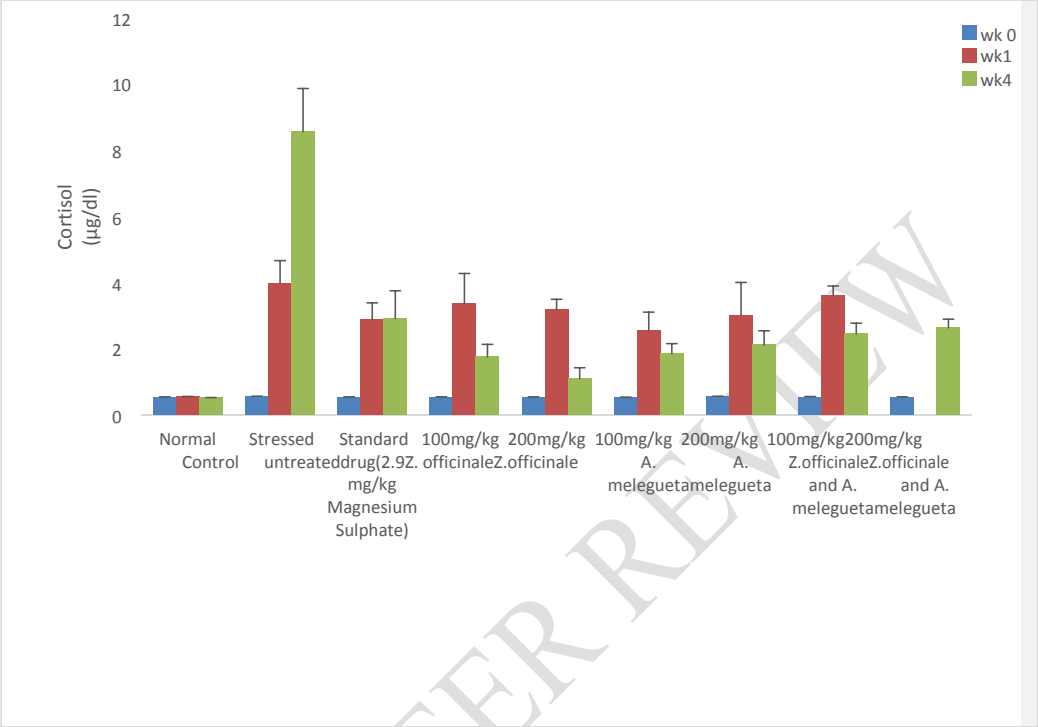

Figure2: Showstheeffectofethanolextractof*Z.officinale*and*A.melegueta*onCortisollevelof electric-shock stressed-induced rats before, during and after treatment period

Comment [a15]: Please identify the significance above the bars.

~~**Result for Kidney function test**~~

Theresultofthekidneyfunctiontestispresentedin Table1, itshowedasignificantdecreaseinthe urea and creatinine levels of the extracts treated groups compared with the untreated control.

Comment [a16]: Please determine the significance as put the letters or symbols of significance beside the means

Table1:Effectsofethanolextractof*Z.officinale*and*A.melegueta*onUreaandCreatinineconcentration of Electric-shock stress-induced rats.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Group A: Normal Control	0.48 ± 0.06	0.17 ± 0.01
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

Comment [a17]: Please adjust the format of table

Magnesium Sulphate)		
Group D: 100mg/kg *Z. officinale*	0.61 ± 0.07	0.17 ± 0.01
Group E: 200mg/kg *Z. officinale*	0.63 ± 0.13	0.18 ± 0.02
Group F: 100mg/kg *A. melegueta*	0.41 ± 0.07	0.18 ± 0.01
Group G: 200mg/kg *A. melegueta*	0.60 ± 0.11	0.17 ± 0.01
GroupH:100mg/kg*Z.officinalemelegue* and *A. ta*	0.51 ± 0.10	0.19 ± 0.05
Group I: 200mg/kg *Z. officinalemelegueta* **and *A.***	0.49 ±0.11	0.18 ± 0.01

Comment [a18]: Please adjust the format of table

Comment [a19]: Please adjust the format of table

~~**Result for Liver Function Test**~~

Table 2 shows the effect of ethanol extract of *Z. officinale* and *A. melegueta* on liver functiontestsinelectric-shockstress-inducedrats.Theresultshowedasignificantdecreaseintheactivities of ALT, AST, ALP, direct bilirubin, and total bilirubin in the extract-treated groupscompared with the untreated control.

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

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

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

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

Comment [a20]: Please insert the abbreviations and number of animals used and level of significant (p value) in all the tables footnotes.

~~**Result for White Blood Cell Differentials**~~

Figure3presentstheeffectofethanolextractof*Z.officinale*and*A.melegueta*onthedifferentials of white blood cells (neutrophil, eosinophil and basophil) in electric shock stressedinduced 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 theuntreated control while there was a significant decrease in the eosinophil level of the extract-treated group compared to the untreated control.

Comment [a21]: Please identify the significance above the bars.

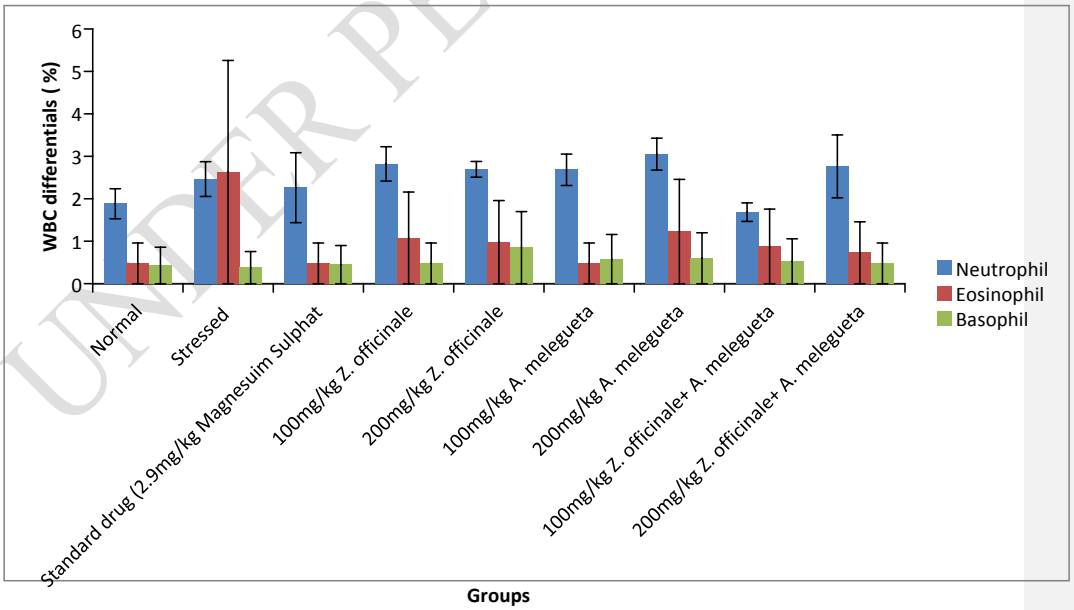

Figure 3: Effect of ethanol extract of *Z. officinale and A. melegueta* on white blood cellsdifferentials of electric-shock stressed-induced rats before, during and after treatment period

Comment [a22]: Please unite the format for all figures.

~~Result for White Blood Cell Count~~

Figure 4 showed the effects of ethanol extract of *Z. officinale* and *A. melegueta* on white bloodcell 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 showedan increase in the white blood cell compared with the untreated group.

Comment [a23]: Please identify the significance above the bars.

Comment [a24]: Please check

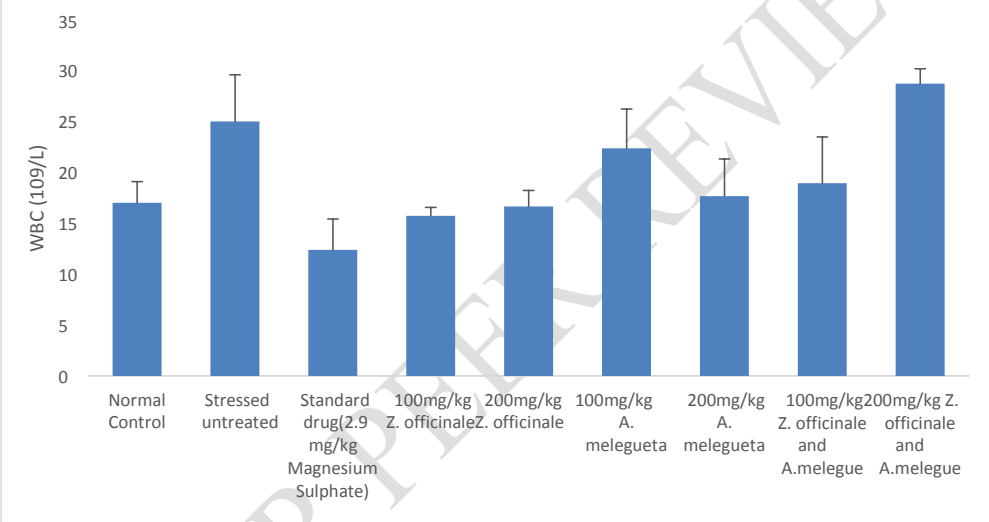

Figure 4: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cellconcentration of Electric-shock stress-induced rats.

~~Results for Platelet~~

Resultsontheeffectsofethanolextractof*Z.officinale*and*A.melegueta*onPlateletsconcentration of electric-shock stress-induced rats are presented in Figure 5. Oral administrationof100mg/kgbodyweightof*Z.officinale*andco-administrationof*Z.officinale*and*A.melegueta* at 200 mg/kg significantly increases the platelet level of the treated groups whencompared with the control group.

Comment [a25]: Please identify the significance above the bars.

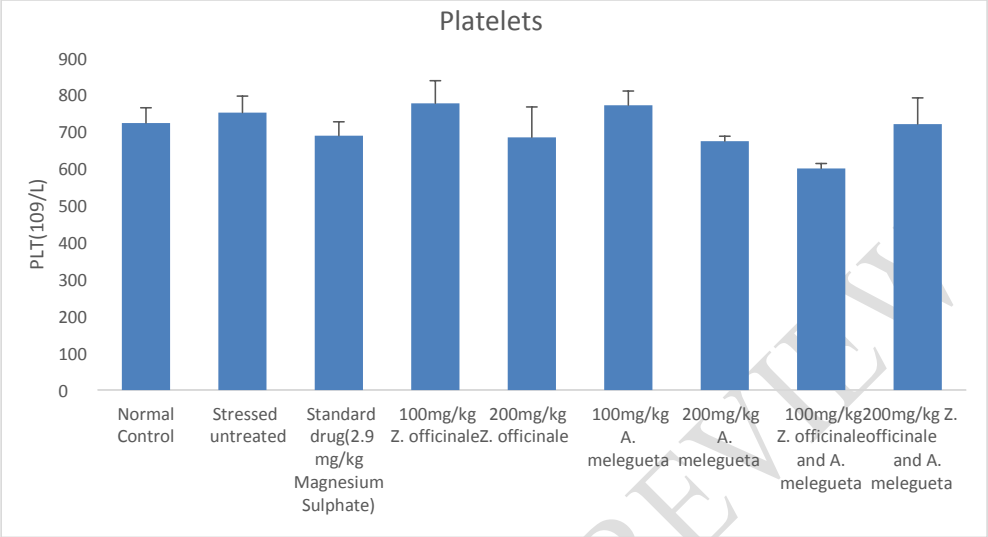

Figure5: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Platelets concentration of electric-shock stress-induced rats.

~~Result for C – reactive Protein~~

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

Comment [a26]: Please check and correct

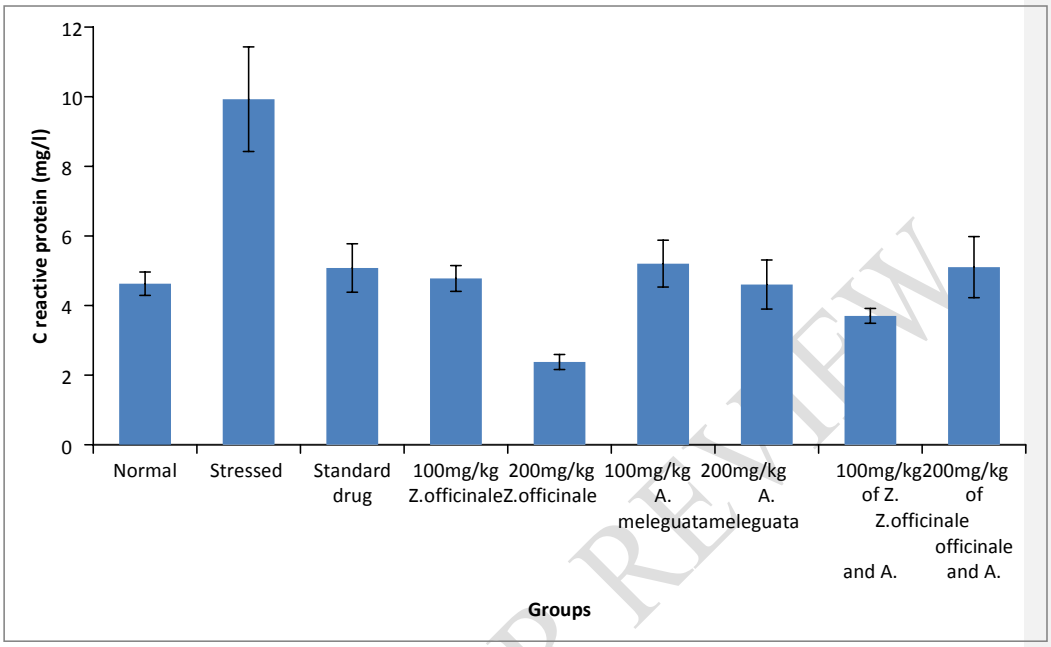

Figure6: Effectofethanolextractof*Z.officinale*and*A.melegueta*onc-reactiveproteinconcentration of electric-shock stress-induced rats

Comment [a27]: Please retitle the subtitles of result section as (The effect of ethanol extract of *Z. officinale*and *A. melegueta*on (variable)).

Discussion

Comment [a28]: Please discussion need to be rewrite and explain the causes and attributions of improvements of measured parameters by extract treatments.

Stress has been defined to be a feeling of emotional or physical tension which could arise fromany event or thought that makes one feel frustrated, angry, or nervous. It is often a normalreaction the body has when changes occur, resulting in physical, emotional, and intellectualresponses. As depicted in this study (Figure 2), there was a significant decrease ($p>0.05$) in thecortisol levels of the extracts treated groups compared to the untreated control. It is worth notingthat co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight decreases thecortisol levels to nearly zero at week 1 of administration. This is an important observation sincecortisol has been implicated in stressful conditions. Cortisol is a steroid hormone that is producedby the adrenal glands, which sit on top of each kidney and it is released into the bloodstream in astressedstate.Thisresultisconsistentwiththefindingsof O'Connoretal.[1],whoexplainedthat when the homeostasis of the body is altered, the body releases hormones such as cortisolswitches on the autonomic nervous system (ANS) which allows the body to adapt and respond today-to-dayactivities.Sincetheinductionofelectricfootshockhasbeenimplicatedinthepathogenesis of inflammatory response, the ability of these extracts to cause a decrease in thecortisol level supports its anti-inflammatory potentials Brody et al. made a similar assertion [22].

Comment [a29]: correct please

Assessment of renal function is vital in the management of patients with kidney disease. Test ofrenal function has utility in identifying the presence of renal disease monitoring the response ofkidneys to treatment and determining the progression of renal disease [23]. The result showingtheeffectofethanolextractof*Z.officinale*and*A.melegueta*onureaandcreatinineconcentration in electric-shock stress-induced rats is presented in Table 1, it showed a significantdecrease in the urea and creatinine levels of the extracts treated groups compared with theuntreated control, which is consistent with the findings of Okeke et al [24]. Urea is a naturallyoccurring molecule that is produced by protein metabolism and found abundantly in mammalianurine. The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl. Accumulation ofurea above this reference range has been implicated in several health conditions, especiallyuremia. The levels of urea concentrations in the extract-treated groups, as observed in this studyimplies that the administration of the extract does not pose any threat to the proper physiologicalfunctions of the kidney. Similarly, the normal reference range of creatinine has been reported torange between 0.7 to 1.3mg/dl for adult males and 0.5 to 1.1 mg/dl for adult females. Theobserved creatinine level in this study falls within the reference range of apparently healthypatients. 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, anyimpairment in the proper physiological functions of the kidneys could plausibly lead to theaccumulation of creatinine in the blood and consequently impair health.

Liverfunctiontestsarebloodteststhatmeasuredifferentenzymes,proteins,andothersubstances made by the liver. As shown in Table 2 above, there was a significant decrease in theserum activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in theextract-treatedgroupscomparedwiththeuntreatedstressedanimals.ASTandALTareimportant 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 plantextracts 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 theeffect of foreign substances including plant extracts in vivo [10]. They are used to ascertain anypossiblechangesinthelevelsofbiomoleculessuchasenzymes,metabolicproducts,hematology, normal functioning, and histopathology of the organs. In this study, the effect of theextract of *Z. officinale* and *A. melegueta* on white blood cells and its differentials showed thatthere was a significant increase ($p \geq 0.05$) in the levels of the WBC in Figure 4 in the grouptreated with 200mg/kg of the combination of *Z. officinale* and *A. melegueta* gave a value of28.72±1.46 which is almost double of the value of stressed untreated 25.00±4.59 and that of thenormal group 17.01±2.08, although the dose-dependent value of *A. melegueta* at 100mg/kg gavea high value of 22.36±3.86 but was suppressed at 200mg/kg, the combination of both extract at200mg/kgprovedtobemoreeffectiveinproducingmoreWBCintheprocessoftreatingstressed related inflammation. In Figure 3 there was a significant increase of neutrophils ingroups treated with 200mg/kg of both extracts it gave a value of 27.65±7.41 which was higherthan the stressed untreated group and those treated with standard drug, neutrophils are the firstrespondersastheyareswiftlyrecruited,constitutingabout50%ofallcellattheinflammationsite [26]. The primary function of neutrophils at the site of inflammation includes compactinginvadingpathogensviavariousantimicrobialresponsesinvolvingphagocytosis,thereforet he

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 thatof 200mg/kg of the combination of the extracts. While Eosinophil levels were significantlyreduced as seen in Figure 3, Eosinophils less commonly called acidophils are one of the immunesystem components responsible for combating multicellular parasites and certain infections invertebrates [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 notsignificantly increased, but rather there was a decrease as compared to the stressed untreatedgroup, therefore this depicts that there was no presence of allergy or tissue damage, this isconsistent with the findings of Oladejo and Osukoya [27]. Neutrophils and monocytes as well asother hematological parameters are measurable indices of the blood, which can be used toevaluatehematopoieticfunction[10].Neutrophilsareimportantphagocyticcellsnormallyelevatedat earlystagesofstressedconditions[28].Monocytesareknowntobethelargesttypeof 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 theprocess of adaptive immunity [27].

The platelets level is known to aggregate where there is wound healing or inflammation it alsohas diagnostic and prognostic roles in certain diseases [29], from the results the groups treatedwith 200mg/kg of ginger and Alligator pepper combinations suppressed the platelets aggregationwhile 100mg/kg of ginger and Alligator pepper enhanced platelets aggregation comparing it tothe groups treated with the standard drug, thereby enhancing the immune system. Thus themaximum efficacy of platelets is at 100mg/kg of both extracts than the groups treated with200mg/kg of both extracts and the standard drug.

C-reactive protein (CRP) is a pentameric protein synthesized by the liver, whose level rises inresponse to 9 of 11 inflammation. CRP is an acute-phase reactant protein that is primarilyinduced by the IL-6 action on the gene responsible for the transcription of CRP during the acutephaseofaninflammatory/infectiousprocess.AhighlevelofCRPinthebloodhasbeenimplicated in the pathogenesis of inflammation and inflammatory diseases. As depicted in thisstudy (Figure 6), induction of stress in the experimental animals significantly caused an increasein their serum CRP in the stressed untreated group, the CRP levels rose from 4.63± 0.33 to twiceits normal level of 9.93±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. Thiscould 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-inflammatoryeffect by inhibiting the production of inflammatory mediators, such as prostaglandin E2, NO,inflammatory cytokines (TNF-α), interleukin-1β (IL-1β)), and pro-inflammatory transcriptionfactor (NF-κB) [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 animalsinduced with acute inflammation.

Conclusions

The abundance of these nutritional fruits *Z. officinale* and *A. melegueta* makes it easily accessibleandaffordablebyall;theseplantshaveshownanti-inflammatoryabilitiesandhaveproventobe

useful in the management and treatment of the silent pathway to many severe illnesses which areStress. However, from the studies, a combination of the two extracts showed some favorableincrease in some biochemical parameters such as the neutrophils and white blood cells, but thetreatment with the extracts separately at 100mg/kg bw and 200mg/kg bw dose was also highlybeneficialinreducingimmunologicalindicessuchastheCRPcomparingtothestresseduntreated group,fromtimeimmemorial,medicinalplantsespecially*Z.officinale*and*A.melegueta*havebeenusedi nthetreatmentofdifferentdiseases.Fromtheresultsofthisstudy,the administration of extract of *Z. officinale* and *A. melegueta* showed that the plants could beused to relieve stressed conditions, tackle inflammation and consequently help to fight variouspathological conditions.

Abbreviations

PTSD, Post-traumatic stress disorder; CFSS, Chronic foot shock stress; ROS, Reactive oxygenspecies;IACUC,InstitutionalAnimalCareandUseCommittee;ELISA,Enzyme-linkedimmunosorbent assay; CRP, C-reactive protein; ALT, Alanine transaminase; AST, Aspartatetransaminase; ALP, Alkaline phosphatase; ANS, Autonomic nervous system; WBC, White bloodcells; Z. officinalle, Zingiberofficinalle; A. melegueta, Aframomummelegueta.

References

Comment [a30]: Please revise the formats of references to follow the instruction of journal.

1. O'Connor DB, Thayer JF, Vedhara K: Stress and Health: A Review of PsychobiologicalProcesses.Annu.Rev.Psychol..2021,72:663-688.https://dx.doi.org/10.1146/annurev-psych-062520-122331
2. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A.: The impact of stress on bodyfunction: A review.. EXCLI J.. 2017 , 16:1057-1072.https://dx.doi.org/10.17179/excli2017-480.
3. FellerL,KhammissaRAG,BallyramR,ChandranR,LemmerJ:ChronicPsychosocialStressinRelationtoCancer.MiddleEastJournalofCancer.2019,10:1-8.https://dx.doi.org/10.30476/mejc.2019.44680
4. Lin TK, Zhong L, Santiago JL : Association between Stress and the HPA Axis in the AtopicDermatitis . Int J Mol Sci.. 2017, 18:2131.https://dx.doi.org/10.3390/ijms18102131
5. CattaneoA,RivaMA.:Stress-inducedmechanismsinmentalillness:Aroleforglucocorticoidsignaling.JSteroidBiochemMolBiol..2016,160:169-174.https://dx.doi.org/10.1016/j.jsbmb.2015.07.021.
6. Bali A, Jaggi AS.: Electric foot shock stress: a useful tool in neuropsychiatric studies . RevNeurosci.. 2015,26:655-677.https://dx.doi.org/10.1515/revneuro-2015-0015.
7. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB.: Bioactive Compounds andBioactivitiesofGinger(ZingiberofficinaleRoscoe)..Foods.2019,8:185.https://dx.doi.org/10.3390/foods8060185.
8. Thirumalai T, Kelumalai E, Senthilkumar B, David E: Ethnobotanical Study of MedicinalPlantsUsedbytheLocalPeopleinVelloreDistrict,Tamilnadu,India.EthnobotanicalLeaflets. 2009, 13:1302-1311.
9. Ezekwesili- Ofili J, Josephine O, Antoinette N : Herbal Medicines in African TraditionalMedicine.HerbalMed.PhilipF.Builders(ed):IntechOpen,Online;2019.10:314.https:/

/dx.doi.org/10.5772/intechopen.80348
10. ChibuikeID,NwobodoE,AnyaehieBU,UmegboluEI:Hematologicalandhistologicaleffect of IRACARP® (fractionated neem leaf extract) in healthy Wistar rats model. PhysiolPharmacol.. 2020, 24:314-321.https://dx.doi.org/10.32598/ppj.24.4.60
11. Tohma H, Gülçin İ, Bursal E, Goren AC, Alwasel SH, Koksal E: Antioxidant activity andphenolic compounds of ginger (Zingiber officinale Rosc.) determined by HPLC-MS/MS.Food Measure. 2017, 11:556-566.https://dx.doi.org/10.1007/s11694-016-9423-z
12. Kazeem MI, Akanji MA, Yakubu MT: Amelioration of pancreatic and renal derangements instreptozotocin-induced diabetic rats by polyphenol extracts of Ginger (Zingiber officinale)rhizome. Pathophysiology. 2015,22:203-209.https://dx.doi.org/10.1016/j.pathophys.2015.08.004.
13. Azeez AA, Akeredolu OA, Igata DF, Akomolede LA, Ojokunle AM, Ogundoyin AA: Areview on the phytochemistryand medicinal values of ten common herbs used in Nigeria. J.Res. For. Wildl. Environ. 2020, 12:115-121.
14. Ernst M, Durbin K. (2019). Accessed: 25-05-2023: https://www.uky.edu/ccd/sites/www.uky.edu.ccd/files/ginger_turmeric.pdf.
15. BamideleHO:AntifungalPotencyofAframomummelegueta(AlligatorPepper)SeedExtractsonPostharvestRotFungiofTwoCitrusSpecies.Sustain.FoodProd.2019,6:1-11. https://dx.doi.org/10.18052/www.scipress.com/SFP.6.1
16. TijjaniH,LukaCD:EffectsofAframomummelegueta,ZingiberofficinaleandPipernigrumon Some Biochemical and Hematological Parameters in Rats Fed with HighLipid Diet. Int. J. Pure Appl. Biosci. 2013,1:61-67.
17. Juliani HR, Simon JE, Ho C-T: African Natural Plant Products Volume II: Discoveries andChallenges in Chemistry, Health, and Nutrition. Juliani HR, Simon JE, Ho C-T (ed): OxfordUniversityPress,AmericanChemicalSociety,Washington,DC;2013. https://dx.doi.org/10.1021/bk-2013-1127.fw001
18. Osuntokun OT: Aframomum Melegueta (Grains of Paradise). Ann. Microbiol Infect. Dis.2020, 3:1-6.
19. Barros L, Soraia F, Baptista P, Cristina F, Miguel VB, Isabel CF, Ferreira R: Antioxidantactivity of Agaricus specie mushrooms by chemical, biochemical and electrochemical assays.Food Chemistry. 2008, 111:61-66.https://dx.doi.org/10.1016/j.foodchem.2008.03.033
20. Limdi JK, Hyde GM. : Evaluation of abnormal liver function tests. . Postgrad Med J.. 2003,79:307-312.https://dx.doi.org/10.1136/pmj.79.932.307
21. KarabağY,ÇağdaşM,RencuzogullariI,etal.:UsefulnessofTheC-ReactiveProtein/AlbuminRatioforPredictingNo-ReflowinST-elevationmyocardialinfarctiontreated with primary percutaneous coronary intervention. Eur J Clin Invest. 2018, 48:e12928.https://dx.doi.org/10.1111/eci.12928.
22. Brody GH, Yu T, Chen E, Miller G E, Barton AW, Kogan S : Family-centered preventioneffectsontheassociationbetweenracialdiscriminationandmentalhealthinBlackadolescents: Secondary analysis of 2 randomized clinical trials.. JAMA Netw Open.. 2021,4:e211964.https://dx.doi.org/10.1001/jamanetworkopen.2021.1964
23. IgbokweGE,IkwukaDC,NworguCC,etal.:EffectofVernoniaamygdalinaonhematologicalindicesandkidneyfunctioninratsexposedtostress..TeikyoMedicalJ..2021, 44:1135-1142.
24. OkekeCB,OladejoAA,MetuMD,NdubuezeIK,AyoEI:TheEffectofInhalationofFumesfromEsbi

othrinBasedMosquitoCoilonSomeRenalFunctionMarkersandHematological Parameters in Male Wistar Rats. Int. J. Adv. Nephrol. Res.2023, 6:46-53.
25. Onwubuya EI, Oladejo AA: Cardio-Protective Effect of the Leaf Extract of Andrographispaniculata in Isoproterenol-Induced Myocardial Infarction. Cardiol. Angiol.: Int. J.. 2022,11:467-478.https://dx.doi.org/10.9734/ca/2022/v11i4302
26. IgbokweGE,EzeigweOC,NwobodoVOG:C-reactiveProteinandImmunologicalIndicesofLectinExtractfromEdibleFungus(Pleurotustuber-regium)onWoundedrats..J.Sci.Res. Rep.. 2023, 29:10-22.
27. Oladejo AA, Osukoya O : Hematological Profiles of Naturally Infected Pigs Treated withBridelia ferruginea Leaf Extracts. Asian Hematology Research Journal. 2021, 4:40-49.
28. Ballester-FerrerJA,Bonete-LópezB,RoldanA,CervellóE,PastorD:Effectofacuteexerciseintensityoncognitiveinhibitionand well-being:RoleoflactateandBDNFpolymorphisminthedose-responserelationship.FrontPsychol..2022,9:1057475.https://dx.doi.org/10.3389/fpsyg.2022.1057475.
29. IkwukaDC,AnyaehieBU,IyareEE,etal.:Assessmentofhematologicalandserumelectrolytes effects of intermittent fasting on mice. J. Pharm. Res. Int. . 2021, 33:207-216.https://dx.doi.org/10.9734/JPRI/2021/v33i62B35188
30. BoarescuI,PopRM,BoarescuPM,etal.:Ginger(Zingiberofficinale)RootCapsulesEnhance Analgesic and Antioxidant Efficacy of Diclofenac Sodium in Experimental AcuteInflammation. . Antioxidants (Basel). 2023, 12:745.https://dx.doi.org/10.3390/antiox12030745.