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ACUTE ANTI-STRESS PROPERTIES OF POLYHERBAL FORMULATED TEA (*Citrus limon, Curcuma longa, Zingiber officinale, Allium sativum, and Moringa oleifera*)

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ABSTRACT

Polyherbal formulation involves combining multiple herbs in specific ratios to create potent mixtures for the treatment of illnesses. The aim of this study is to evaluate the acute anti-stress activity of poly-herbal-formulated teas (Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum, and Syzygium aromaticum). This study was evaluated using a tail suspension, a force swimming test, and a cold restraint stress test. The stress control group had more mobility during the tail suspension test than the groups that were given the polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and Standard drug group, fluoxetine, 20 mg/kg ($p < 0.05$). In the forced swimming test, the polyherbal-formulated tea at 10 mg/kg and fluoxetine at 20 mg/kg increased swimming time when compared with stress control ($p < 0.05$). In the cold restraint test, the polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and fluoxetine at 20 mg/kg reduced the cortisol level in mice when compared to stress control ($p < 0.05$). When compared to the stress control, the polyherbal tea at 5 mg/kg and 10 mg/kg significantly raised the activity of superoxide dismutase in mice ($p < 0.001$). The activity of catalase went up when polyherbal-formulated tea at 10 mg/kg and fluoxetine (20 mg/kg) were given compared to stress control. The levels of malondialdehyde (MDA)decreasewhen the animals were administered5 mg/kg and 10 mg/kg, compared to the stress control group ($p<0.05$). In conclusion, the polyherbal-formulated tea possesses antistress properties.

Keywords: Anti-stress, Polyherbal-formulated Tea, Antioxidant, Cortisol.

INTRODUCTION

Plants have been utilized for medical purposes since the beginning of time and are still used now (Grover *et al.*, 2002). Today, thousands of higher plants are grown in many parts of the world for their medicinal and pharmaceutical benefits (Kinghorn *et al.*, 2020). Different kinds of seeds, roots, leaves, fruits, skins, flowers, and even the entire plant can be utilized as a source of medicine. Herbal teas are more properly referred to as "tisanes," which are combinations of several herbs. Tisanes are created from mixtures of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical materials that give them their flavour and offer the advantages of herbal teas. The majority of herbal teas may have a single primary herbal component or a combination of herbal compounds, each of which is designed to achieve a particular goal, such as relaxation, rejuvenation, or alleviation

from a particular condition (Aoshima *et al.*, 2007). Some of these plants used in combination or singly in the formulation of herbal teas include moringa, lemon, garlic, ginger, turmeric, and cloves (Uwaya *et al*., 2024).

Turmeric, or *Curcuma longa*, is an herb that belongs to the Zingiberaceae family and the genus Curcuma. Turmeric has historically been used as a natural treatment for wound healing. Additionally, it aids in the treatment of bacterial infections, cancer, atherosclerosis, osteoarthritis, liver illness, eye conditions, and women's menstruation problems(Fuloria *et al*., 2022). It is considered a good source of blood purification, brain and heart tonics, and manages conditions like asthma, leucoderma, piles, bronchitis, spleen enlargement, tumors, biliary disorders, anorexia, cough, rheumatism, sinusitis, tuberculous glands in the neck, diabetic wounds, hepatic disorders, leucorrhea, and gonorrheal secretion. It helps to lower blood clotting and blood sugar levels (Zhang et al., 2013; Reddy and Rao, 2002). According to Kiso *et al.* (1983). The plant species *Moringa oleifera*, often known as the miracle tree, tree of life, or drumstick, is a member of the Moringa genus and Moringaceae family. *Moringa oleifera* and its active components have been found to have a wide range of pharmacological effects, including anti-inflammatory, antioxidant, anti-diabetic, anti-microbal, anti-bacterial, anti-cancer, anti-fertility, and wound healing (Mishra *et al.,* 2011). According to Dixit *et al.* (2016), it is used to treat poisonous bites, pneumonia, and ascites in Africa.

Zingiber officinalis is a tropical plant; Roscoe, often known as ginger, is a member of the Zingiberaceae family and thrives in hot, humid regions. It is used to treat a variety of conditions in traditional medicine, including cough, asthma, indigestion, nausea, vomiting, allergic reactions, the common cold, fever, allergic rhinitis, acute chronic bronchitis, respiratory issues, pain, headaches, backaches, and any type of muscular ache, as well as painful teeth and swollen gums (Kumar *et al.*, 2011).

The genus *Citrus limon* is a hybrid plant belonging to the Rutaceae family. Before vitamin C was discovered, citrus fruit juice, namely lemon juice, had long been used as a treatment for scurvy. In traditional medicine, lemon juice has been used to treat a variety of conditions, including high blood pressure, the common cold, irregular menstruation, cough, sore throats, fever, rheumatism, and chest discomfort (Papp *et al*., 2011; Balogun and Ashafa, 2019). *Citrus limon* fruit extract, juice, and essential oil exhibit a variety of pharmacological effects, including antibacterial, antifungal, anti-inflammatory, anti-cancer, hepato-regenerating, and cardio-protective functions (Otang and Afolayan, 2015).

Garlic is a member of the Alliaceae or Liliaceae family and is referred to botanically as *Allium sativum*. *Allium sativum* has been used as a medicine to treat a wide range of illnesses and conditions related to the heart and blood system, including high blood pressure, high cholesterol, coronary heart disease, heart attacks, and hardening of the arteries known as atherosclerosis, by the biological active component allicin and its derivatives (Mikaili *et al.,* 2013). Additionally, it promotes effects against asthma, cough, arthritis, backache, bronchitis, persistent fever, tuberculosis, and rhinitis while preserving healthy liver function (Pendbhaje *et al.,* 2000; Jung *et al.,* 2000).

Clove, also known as *Syzygium aromaricum*, is a dried flower bud from the Myrtaceae family (Cortès *et al.,* 2014). The warming and energizing properties of clove (*Syzygium aromaticum*) are employed in traditional Chinese and Indian medicine (Batiha *et al.,* 2019). Clove has traditionally been used to cure nausea, liver, intestine, and stomach diseases, as well as to stimulate the nerves and treat vomiting, flatulence, and vomiting-related symptoms. Cloves are known to treat a variety of disase condition, including scabies, cholera, malaria, and tuberculosis, in tropical

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Asia. Clove has been traditionally used in America to inhibit growth of viruses, worms, andand therefore find application in the treatment of several food-borne diseases

, by preventing their growth (Bhowmik *et al.*, 2012). Clove has been shown to have aphrodisiac, antipyretic, appetizer, hypnotic, anxiolytic, antiemetic, analgesic, decongestant, antimicrobial, antiepileptic, myorelaxant, anti-inflammatory, and expectorant properties, as well as a therapeutic effect on trophic disorder (Han and Parke, 2017; Elkwakeel *et al.*, 2007).

Aim of the Study

The aim of this study is to evaluate the acute anti-stress activity of poly-herbal-formulated teas composed of *Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*.

MATERIALS AND METHODS

Plant collection: *Citrus limon* was bought from the New Benin Market in Oredo Local Government Area. *Curcuma longa, Zingiber officinale, Allium sativum, and Syzygium* aromatic were purchased at Oriegbeni Market in Ikpoba Okha Local Government Area. *Moringa oleifera* leaves were gotten from farm land of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, all in Edo State.

Plant preparation: *Citrus limon, Curcuma longa, Zingiber officinale, and Allium sativum* were washed and chopped into smaller bits. *Syzygium aromatica* was bought in its dried form. *Moringa oleifera* leaves were washed. The chopped lemon was dehydrated using a dehydrator, while *Curcuma longa, Zingiber officinale, Allium sativum,* and *Moringa oleifera* leaves were first air dried in a shade before using the dehydrator for proper drying. The dried leaves of *Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*were each ground into a powder using an impact mill. To make the herbal tea, equal amounts of powdered *Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*were weighed out and mixed together (1:1:1:1:1:0.5). The herbal tea was formulated in such a way that 1.1g in a tea bag contained 200 g each of *Citrus limon, Curcuma longa, Zingiber officinale, Allium sativum,* and 100 g of *Syzygium aromatic* plant material(Uwaya and Effiong, 2024).

Experimental animals: Healthy adult Swiss albino mice of either sex weighing 20–30 g were purchased from the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. The mice and Guinea pigs were housed within the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, and were acclimatize for 2 weeks under normal laboratory conditions with a 12-hour light/dark cycle. They were fed normal animal pellets ad libitum. The animals were handled in accordance with normal protocols for laboratory animals (National Institute of Health, USA, Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Experimental design

Antidepressants

The antidepressant activity of the formulated polyherbal tea was carried out using the Forces Swimming Test and Tail Suspension Test as described by Nikunj *et al*. (2011) and Porsolt *et al*. (1977), with a slight modification.

Forced Swimming Test

Sixteen mice were randomly distributed into four groups of four mice per group. Group one received distilled water (10 ml/kg, orally), group two and group three received 5 mg/kg and 10 mg/kg of the polyherbal-formulated tea orally, respectively, and group four received the aqueous solution of fluoxetine (20 mg/kg) orally. All the groups were treated for seven days. One hour after the drug administration on the seventh day, each mouse was placed in a transparent cylindrical container (45 x 40 x

30 cm) filled with water to a depth of 20 cm at a temperature of 25 °C, and the time of immobidity was recorded for a period of 10 minutes.

Tail Suspension Test

Sixteen mice were randomly allotted into four groups of four mice per group. Group one received the vehicle (10 ml/kg distilled water, p. o.), group two received 5 mg/kg, and group three received 10 mg/kg of the polyherbal-formulated tea orally, respectively, while group four received the standard treatment (20 mg/kg of fluoxetine). All treatments were done for seven days. On the seventh day, one hour following the administration, each mouse was suspended from the edge of a shelf 60 cm above a tabletop. At about 1 cm from the tail tip, adhesive tape held the mice in place. The time of immobility was then observed and recorded for duration of 10 minutes.

Acute Antistress.

The acute antistress activity of the polyherbal-formulated tea was studied using the cold restraint stress test according to the method of Nikunj *et al*. (2011), with a slight modification on the doses and standard drug. Sixteen Swiss albino mice were allotted into four groups of four animalsin each group, as follows:

Group 1 received distilled water (10 ml/kg).

Group 2 received a 5 mg/kg extract of a polyherbal-formulated tea.

Group 3 received a 10 mg/kg extract of polyherbal-formulated tea.

Group 4 received 20 mg/kg of fluoxetine (the standard drug).

The animals were administered polyherbal-formulated tea and standard drugs orally for 7 days. On day seven, one hour after oral administration of polyherbal-formulated tea, all the mice in all groups received cold restraint stress by tying their limbs and placing them in the refrigerator for two hours at 4 °C. The animals were sacrificed under chloroform anesthesia. Blood was collectedvia abdominal aorta and placed in a plain container for cortisol analysis. The brain was harvested and homogenized for antioxidant analysis.

Brain Homogenate and Serum Separation

The extracted mice's brains were ground using a mortar and pestle. The ground brain tissue was then homogenized using 5 ml of cold normal saline and transferred to plain containers. These containers holding the blood and homogenized brain samples were placed in a centrifuge and spun for 10 minutes at 3000 revolutions per minute (rpm). After centrifugation, the serum was separated from the whole blood, and the supernatant from the homogenized brain samples was transferred to new regular containers and stored in a refrigerator.

Procedure for Analysis

Estimation of malondialdehyde (MDA)

Malondialdehyde (MDA) was evaluated as describe in Idu et al. (2016) with slight modifiication. Using a micropipette, equal amounts of malondialdehyde (MDA) reagent (15% TCA-0.375% TBA-0.25NHCl) were pipetted into different test tubes. Then, 600 µl of a homogenized sample of brain tissue was added and mixed well. The solution was then heated for 15 minutes in a boiling water bath at 100°C. Thereafter, the reacted mixture was allowed to cool and then centrifuged for 10 minutes at 3000 rpm (revolutions per minute) in order to remove the flocculants precipitates. A spectrophotometer was used to compare the clear supernatant's absorbance to that of a reference tube containing 3 ml of MDA reagent and 600 µl of distilled water at a wavelength of 535 nm.

Determination of Superoxide Dismutase (SOD) Activity

The assay uses a modified version of the Misra and Fridouich (1972) reaction that Idu et al. (2016) proposed. Two steps were required for the assay of SOD. The first was the reference tube, which was prepared by mixing together 0.2 ml of distilled water and 2.5 ml of

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carbonate buffer. The addition of 0.3 ml of freshly made adrenaline solution was immediately after that, and it was quickly mixed. After adding 2.5 ml of carbonate buffer to the test tubes, the sample tubes were ready for the addition of 0.2 ml of adrenaline solution. This was rapidly mixed and read at 420 nm absorbance every 30 to 120 seconds with a UV-visible spectrophotometer (model T80 + UV spectrometer, PG Instruments Ltd.). Distilled water was used to zero the machine.

Determination of Catalase Activity

Catalase activity was evaluated as describe in Idu et al. (2016) .

The sample (50 µl) was introduced into a test tube, and the reaction was initiated by adding 500 µl of H_2O_2 and thorough mixing by inversion. Following the addition of 1000 µl of 6M H_2SO4, mixing by inversion stopped the reaction 3 minutes later. The addition of 700 µl of 0.01 M $KMnO_4$ came next, and it was then mixed once more using inversion. Absorbance was then read within 30–60 s against a blank using a spectrophotometer with a visible wavelength of 480 nm. The spectrophotometer was zero with distilled water. To make the spectrophotometric standard, 700 µl of 0.01 M $KMnO_4$ was added to a test that already had 550 µl of phosphate buffer at pH 7.4 and 1000 µl of 6 M H_2SO4. The blank was prepared by adding 500 µl of H_2O_2 to a test tube containing 50 µl of distilled water, 1000 µl of 6M H_2SO_4, and 700 µl of 0.01M $KMnO_4$.

Estimation of Cortisol Level

Test tubes that had been cleaned and sterilized were marked "blank" and "test." 1000 µl of serum and 1000 µl of distilled water were pipetted into test tubes with the labels "test" and "blank," respectively. Following the addition of 200 µl of a freshly made chloroform:methanol (2:1 v/v) combination, 3000 µl of chloroform was added to each test tube. Then, 300 µl of sodium hydroxide solution (0.1 N) and 3000 µl of sulfuric acid solution were added to each test tube, respectively. The test tube blank and sample were incubated for 45 minutes at room temperature in the dark. A UV spectrophotometer was used to measure absorbance at 533 nm. The concentration was determined from a standard curve of hydrocortisone of 10 – 100 µM absorbent against the concentration of cortisol extrapolated. r^2 =0.999

Statistical Analysis

The data were presented as mean ± standard error of the mean (SEM), with "n" indicating the number of mice in each experimental group. A one-way analysis of variance (ANOVA) and the Newman-Keuls post hoc test were both performed. GraphPad Prism software version 6 from the UK was used for all data analysis. A significance level of $P < 0.05$ indicated notable differences between the compared data.

RESULTS

The effect of polyherbal formulated tea (***Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,*** **and** ***Syzygium aromaticum*****) on tail suspension and forced swimming test**

Figure 1 and 2 shows the effect of a polyherbal formulated tea (*Citrus limon, Curcuma longa, Zingiber officinale, Allium sativum, Moringa oleifera, and Syzygium aromaticum*) onforced swimming test and tail suspension test. The formulated polyherbal tea at 5 mg/kg, 10 mg/kg, and fluoxetine(20 mg/kg) significantly reduced the time of immobility when compared to the stress control (***p <0.001; **p <0.01; *p <0.05)in tail suspension test and the formulated tea at 10 mg/kg and fluoxetine(20 mg/kg) increase swimming time when compared with stress control (**p <0.01; * p<0.05) in forced swimming test.

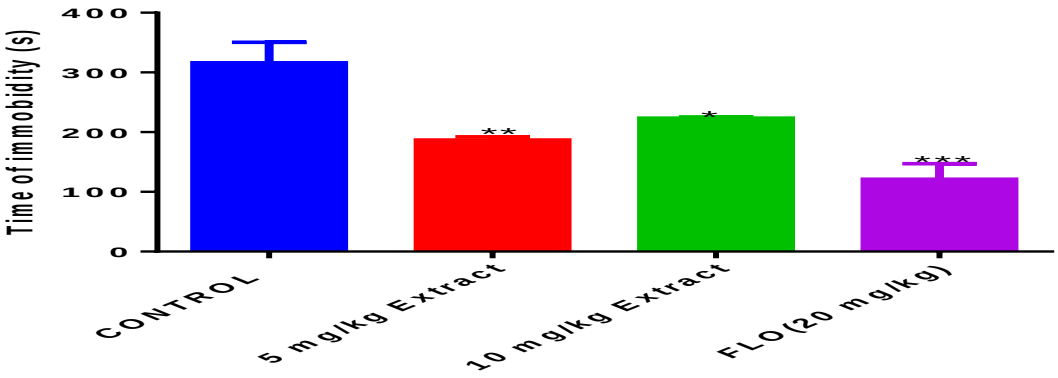

Figure 1: The effect of a polyherbal formulated tea (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on the time of immobility of the tail suspension test in mice induced. depression. The polyherbal formulated tea at 5 mg/kg, 10 mg/kg and the standard (fluoxetine) at 20 mg/kg significantly reduced the time of immobility when compared to the stress control. (***p <0.001; **p <0.01; *p <0.05). FLO: fluoxetine. SC: stress control. The data were presented as mean S.E.M. n = 4.

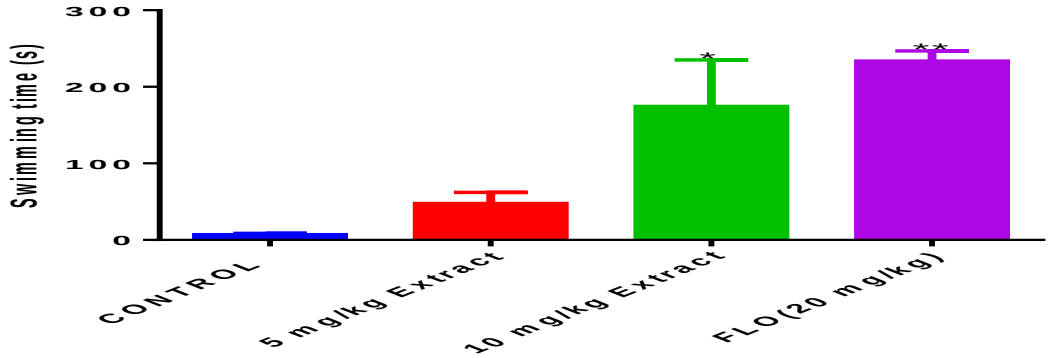

Figure 2: The effect of polyherbal formulated tea (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on swimming time of forced swimming test in mice induced depression**.** The polyherbal formulated tea at 10 mg/kg and the standard (fluoxetine) at 20 mg/kg increase swimming time when compared with stress control. (**p <0.01; *p <0.05). FLO**:** Fluoxetin. SC**:** Stress control. The data were presented as Mean ± S.E.M n=4.

The effect of polyherbal tea formulation on cortisol and malondialdehyde levels.

Figure 3 and **Figure 4** show the effect of the polyherbal tea formulations (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum)* on cortisol and malondialdehyde levels in mice induced by stress. The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and the standard (fluoxetine) at 20 mg/kg reduce the cortisol level in mice when compared to stress control. The stress control group (p <0.05) had higher MDA levels than the animals that were given polyherbal tea formulations at 5 mg/kg, 10 mg/kg, or 20 mg/kg of fluoxetine. The stress support group (p <0.05;) had lower MDA levels.

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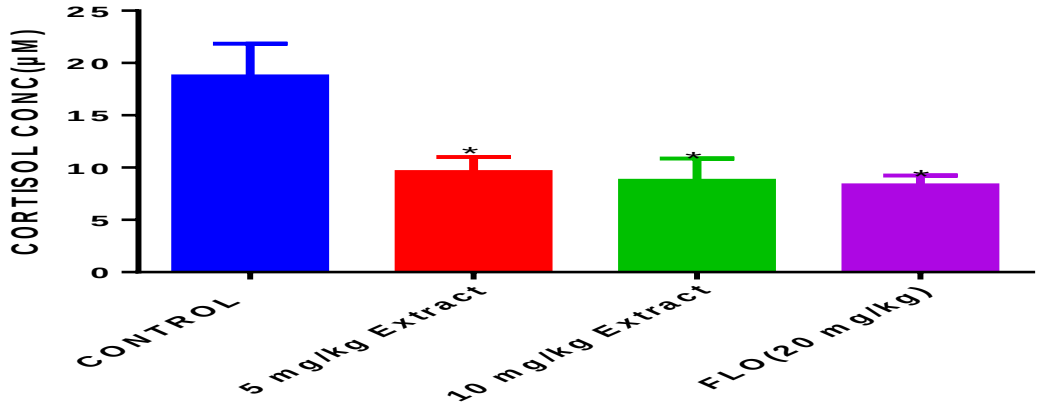

Figure 3: The effect of the polyherbal tea formulation (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on cortisol levels in mice under stress**.** The polyherbal formulated tea at 5 mg/kg, 10 mg/kg, and the standard (fluoxetine) at 20 mg/kg reduce the cortisol level in mice when compared to stress control. ($p < 0.05$).FLO**:** Fluoxetine. SC**:** Stress control. The data were presented as Mean ± S.E.M n=4.

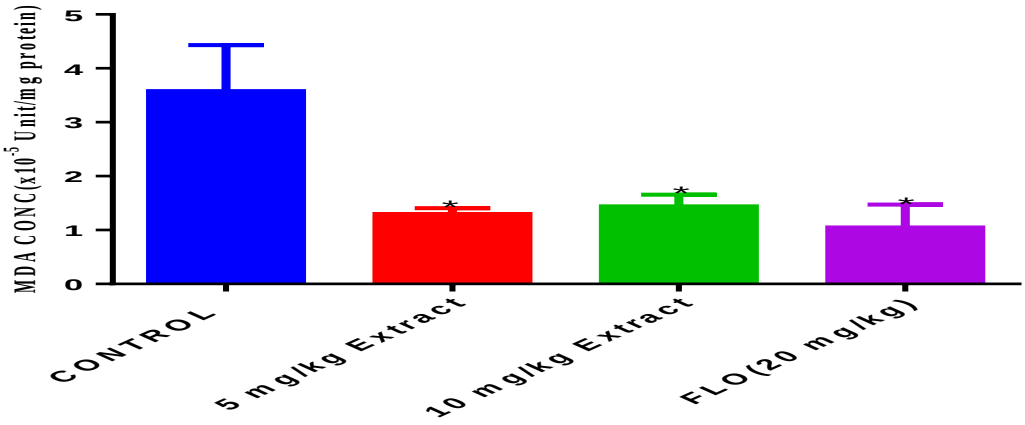

Figure 4: The effect of the polyherbal formulated tea (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on MDA levels in mice induced stress**.** The polyherbal formulated tea at 5 mg/kg, 10 mg/kg, and standard (fluoxetine at 20 mg/kg) significantly reduced the MDA level in the animals when compared to stress control ($p < 0.05$). FLO: Fluoxetine. SC: Stress control. The data were presented as Mean ± S.E.M n=4.

The effect of polyherbal formulated tea on superoxide dismutase (SOD) and catalase levels.

Figures 5 and 6 show the effect of the polyherbal tea formulations (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on SOD and catalase activity in mice induced by stress. The polyherbal-formulated tea at 5 mg/kg and 10 mg/kg significantly increased SODactivity in mice when compared to the stress control (****$p < 0.0001$; ***$p < 0.001$) and the polyherbal tea formulation at 10 mg/kg and the standard (fluoxetine 20 mg/kg) increase catalase activity when compared to stress control($p < 0.05$).

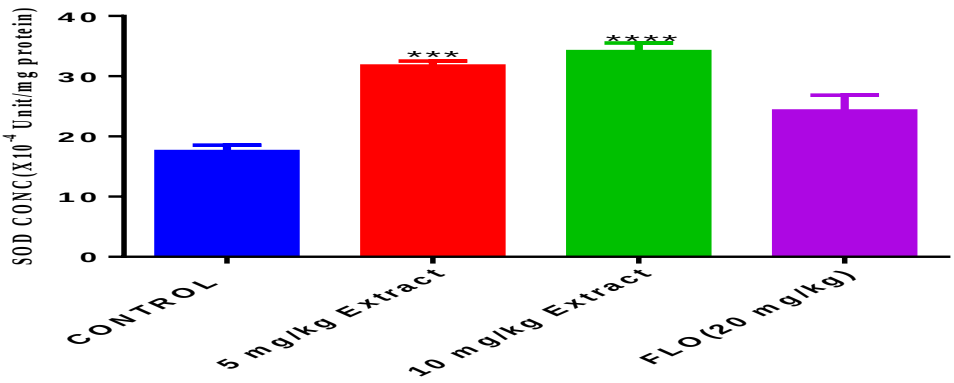

Figure 5:The effect of the polyherbal formulated tea (*Curcuma longa, Moringa oleifera, Zingiber officinale Roscoe, Citrus limon, Allium sativum,* and *Syzygium aromaticum*) on SOD level on mice induced stress**.** The polyherbal formulated tea at 5 mg/kg and 10 mg/kg significantly increased SOD levels in mice when compared to the stress control. (****p <0.0001; ***p <0.001). FLO**:** Fluoxetine. SC**:** Stress control. The data were presented as Mean ± S.E.M n=4.

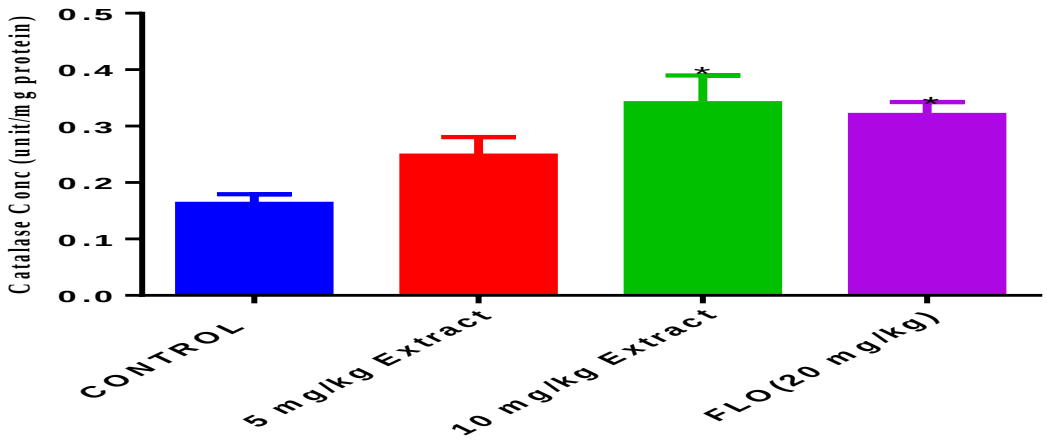

Figure 6: The effect of the polyherbal formulated tea (*Cuitrus limon, Curcuma longa, Zingiber officinale, Allium sativum, Moringa oleifera, and Syzygium aromaticum*) on catalase activity under mouse-induced stress. The polyherbal formulated tea at 10 mg/kg and the standard (fluoxetine at 20 mg/kg) increase catalase activity when compared to stress control (*p <0.05; *p <0.05). FLO: Fluoxetine. SC: Stress control. The data were presented as Mean ± S.E.M n = 4.

DISCUSSION

Stress is a psychological and physiological condition that causes the adrenal gland to generate cortisol and, if left untreated, is linked to a number of issues (Solati *et al*., 2017; Khan and Khan, 2017). Depression and anxiety are two factors that can affect stress (Solati et al., 2017). The forced swimming test and the tail suspension test are antidepressant models used in evaluating stress. In this present study, the antistress and antidepressant activity of the formulated polyherbal teas (*Cuitrus limon, Curcuma longa, Zingiber officinale, Allium sativum, Moringa sleifera, and Syzygium aromaticum*) at doses of 5 mg/kg, 10 mg/kg, and fluoxetine reduced the immobility time in tail suspension-induced depression (**Figure 1**). The tail suspension test is a proven procedure for assessing the effectiveness of antidepressant medications. It is also employed to assess the results of environmental, neurobiological, and genetic alterations (Crowley *et al.*, 2005; Lad *et al.*,

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2007). The tail suspension test is the most common animal model used for screening potential antidepressant agents, which induce a state of immobility in animals facing an inescapable situation. Such immobility behavior has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans (Nizamudee *et al*., 2015). The ability of the polyherbal-formulated tea to reduce the time of immobility suggests that the herb combination possesses antidepressant activity. In a force swimming test, 10 mg/kg of the polyherbal-formulated tea and fluoxetine (20 mg/kg) increased swimming time. Research has shown that an increase in swimming time in force swimming tests suggests antidepressant activity (Inès and Anne-Kathrin, 2023). The ability of the polyherbal-formulated tea to increase swimming time suggests that the polyherbal-formulated tea has antidepressant and antistress activity (**Figure 2**). Fluoxetine is a well-known antidepressant drug that belongs to the Selective Serotonin Reuptake Inhibitor (SSRI) class. Fluoxetine acts by inhibiting the reuptake transporter protein found in the presynaptic terminal to prevent serotonin from being reabsorbed into presynaptic serotonin neurons (Robertson *et al*., 2019; Cao *et al*., 2019). The antidepressant and antistress activity of the polyherbal-formulated tea could be a result of *Zingiber officinale, Curcuma longa,* and *Allium sativum*. It has been reported that *Zingiber officinale, Curcuma longa,* and *Allium sativum* possess antistress and antidepressant activity (Ittiyavirah and Paul, 2013; Singh *et al*., 2012; Moragrega and Ríos, 2021; Khushboo and Sharma, 2017). In this study, the polyherbal formulation (*Citrus limon, Moringa oleifera, Zingiber officinale, Curcuma longa, and Allium sativum*) at a dose of 5 mg/kg and 10 mg/kg reduced the cortisol level in the cold restraint test (**Figure 3**). The cold restraint test is an animal model used to induce stress in animals (Nikunj *et al*., 2011). The kidney's adrenal cortex produces the essential catabolic hormone cortisol (Blackburn-Munro et al., 2003). It is released in a diurnal fashion, with blood levels peaking in the morning to facilitate arousal and steadily declining thereafter (Ehlert *et al*., 2001; Tsigos and Chrousos, 2002). Throughout the day, cortisol maintains blood glucose and suppresses nonvital organ systems to provide energy to an actively functioning brain and neuromuscular system (Heim *et al*., 2000). Stress increases the cortisol level. An increase in cortisol level can lead to anxiety, depression, digestive problems, headaches, muscle tension and pain, heart disease, heart attack, high blood pressure and stroke, sleeping problems, weight gain, and problems with memory and focus (Qin *et al*., 2016; Stephens and Wand, 2012). The ability of the formulated polyherbal tea to reduce cortisol level indicates that the polyherbal formulated tea has antistress properties and can also be used to manage high blood pressure, stroke, heart attack, headache, muscle tension, sleeping problems, weight gain, problems with memory and focus, anxiety, and depression. Superoxide dismutase is a crucial antioxidant that the body uses to protect itself from oxidative stress (Landis and Tower, 2005). Boosting SOD levels can help stop a number of diseases connected to oxidative stress, such as cancer, inflammation, rheumatoid arthritis, atherosclerosis, heart disease, diabetes, Parkinson's and Alzheimer's disease, liver damage, and immune system problems (Yonus, 2018). In this study, the polyherbal tea raised superoxide dismutase levels in mice that were under a lot of stress at doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg of standard fluoxetine (Figure 5). The polyherbal-formulated tea's capacity to boost superoxide dismutase activity may demonstrates the tea's potential ability to lower the risk of oxidative stress. This research has shown that polyherbal-formulated tea at 10 mg/kg and fluoxetine at 20 mg/kg increase catalase activity (**Figure 6**). The ability of the polyherbal-formulated tea to increase catalase levels has proven that the plant possesses antioxidant activity. Catalase deficiency or malfunctioning is associated with many diseases, such as diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, some

dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia (Nandi *et al*., 2019; Habib *et al*., 2010). Catalase is very important for controlling the level hydrogen peroxide in cells, and breaking down hydrogen peroxide protects cells from oxidative damage. For example, it protects pancreatic β cells from hydrogen peroxide damage (Nandi et al., 2019). Low catalase activities have been reported in schizophrenic patients as well as in patients with atherosclerosis (Nandi *et al*., 2019). Malondialdehyde is a lipid peroxidation product with a low molecular weight that results from the breakdown of very reactive lipid hydroperoxides (Cherubini *et al.*, 2005; Spickett *et al.*, 2010). It is well known that the end result of lipid peroxidation is malondialdehyde (MDA). MDA levels in the serum or plasma can therefore be used as a sign of lipid peroxidation. Increased levels of free oxygen radicals can result in excessive lipid peroxidation, which harms tissues by oxygenating them (Herken *et al*., 2001). According to Mahadik *et al*. (2001), elevated MDA levels in the blood and cerebrospinal fluid can harm the brain through oxidative stress. Cytoprotective enzymes and antioxidants can prevent this kind of damage. In this study, the polyherbal tea formulation at doses of 5 mg/kg, 10 mg/kg, and the standard (fluoxetine) at 20 mg/kg all reduced malondialdehyde levels in mice under acute stress (Figure 4). The polyherbal formulation's capacity to lower malondialdehyde levels demonstrates that it has the capacity to stop oxygen damage to tissues and oxidative damage to the brain. The antistress ability of tea maybe acts by inhibiting the reuptake transporter protein found in the presynaptic terminal to prevent serotonin from being reabsorbed into presynaptic serotonin neurons fluoxetine.

CONCLUSION

According to this study, the tea made from *citrus limon, curcuma longa, ginger, allium sativum, moringa sleifera, and syzygium aromaticum*have properties that help protect against stress. The mechanism of the antistress property of this polyherbal-formulated tea could be due toanti-oxidant potential.

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Conflict of interest

According to the authors, there is no conflict of interest.

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Author contribution statement

The authors affirm their involvement in the paper as follows: examine development and planning: Dr. Dickson O. Uwaya. Data collection, analysis, and interpretation of results: Dr. Dickson O. Uwaya,EmoghwareHope Oghenenyore and Osagie Osakpolor Success. Draft manuscript preparation: EmoghwareHope Oghenenyore, Osagie Osakpolor Successand Dr. Dickson O. Uwaya. Statistical analysis: The final draft of the manuscript was approved by the authors after they had evaluated the findings.

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