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Chronic Anti-Stress Properties of a Polyherbal-Formulated Tea (*Citrus limon*, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, *and Moringa oleifera*) Using a Non-Social Stressor Animal Model

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ABSTRACT

Polyherbal remedies used for stress relief are considered a cost-effective and safe choice. This warranted a study to evaluate the chronic anti-stress activity of a polyherbal-formulated tea (*Citrus limon*, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, *and Moringa oleifera*) using a non-social stressor animal model. Chronic stress was induced using a non-social stressor mouse model. Sixteen mice were allotted into 4 groups of 4 mice each: Group one received 10 mL/kg of distilled water, while groups 2-4 received 5 mg/kg and 10 mg/kg extracts of polyherbal-formulated tea and 20 mg/kg of fluoxetine, respectively. The tea was administered orally, along with the various stressors, for 21 days. The mice were sacrificed, and blood was collected from the abdominal aorta for cortisol analysis, and the brain was homogenised for antioxidant analysis of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and malondialdehyde. In the forced swimming test, the polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and fluoxetine increased the swimming time when compared with chronic stress control ($p < 0.01$). In the tail suspension test, 10 mg/kg of polyherbal-formulated tea and fluoxetine decreased the time of immobility when compared with chronic stress control ($p < 0.05$). Tea at 10 mg/kg and fluoxetine decreased the level of cortisol when compared with chronic stress control ($p < 0.05$). The tea at 10 mg/kg increased the level of catalase and glutathione reductase when compared with chronic stress control ($p < 0.01$). The tea had no effect on glutathione peroxidase, superoxide dismutase, or malondialdehyde. Conclusively, the polyherbal-formulated tea has stress-relieving properties.

Keywords

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INTRODUCTION

Plants that have medicinal properties continue to play an important role in healthcare, particularly in less developed countries that have a long history of using herbal medicine (Boy *et al*., 2018). The importance of these plants, both as a source of medical treatment and economic assistance, is growing on a global scale, spanning both industrialised and developing countries (WHO, 1998). Traditional medical practices have their roots in plant-based therapies, which have stood the test of time for millennia (Gurib-Fakim, 2006). In different regions and cultures, herbal products are used as single herbs, combinations of herbs, or combinations of herb(s) and drug(s) in the treatment of different diseases, including stress and depression (Chen *et al*., 2012; Che *et al*., 2013; Khan and Khan, 2017). Numerous plants, including lemon, tumeric, ginger, garlic, and moringa, have been used in traditional medicine either singly or in combinations as polyherbal formulations (Clement *et al*., 2015; Badal *et al*., 2019; Tung *et al*., 2019; Dubey and Dixit, 2023). Polyherbal formulations are used across various cultures to combat chronic stress, leveraging their synergistic effects (Sen and Chakraborty, 2015). Stress is a complex physiological and psychological re-

sponse to external or internal stimuli, known as stressors that disrupt the body's equilibrium (Chrousos, 2009; Singh *et al*., 2022). Stressors can be acute or chronic (Steptoe and Kivimäki, 2012). Chronic stress occurs when an individual is exposed to prolonged or repeated stressors over an extended period, leading to persistent activation of the stress response system (Agorastos and Chrousos, 2022). *Citrus limon* (L.), or lemon, is a member of the Rutaceae family and is characterised by permanent green leaves and tasty yellow fruits (Mabberley, 2004; Goetz, 2014). Lemon juice is traditionally used to treat diseases such as high blood pressure, the common cold, and menstrual irregularities. In addition, the essential oil of the fruit is effective in the treatment of coughs (Papp *et al*., 2011; Bhatia *et al*., 2015; Clement *et al*., 2015). *Curcuma longa,* or turmeric, belongs to the family Zingiberaceae. It is traditionally used in the treatment, prevention, and control of a wide variety of diseases and conditions, such as cancer, diabetes, arthritis, diarrhoea, inflammation, and gastrointestinal ulcers (Ayman *et al*., 2019; Tung *et al*., 2019). *Zingiber officinale*, or ginger, belongs to the family Zingiberaceae. It is used to alleviate conditions such as headaches, coughs, colds, and inflammation (Baliga *et al*., 2011; Dehghani *et al*., 2011; Khaki and Fathiazad, 2012; Semwal *et al*., 2015). Ginger has potential as an anti-inflammatory, carminative, antispasmodic, diaphoretic, and stimulant for peripheral circulation (Vasala, 2004; Ali *et al*., 2008). *Allium sativum L*., or garlic, is a member of the family Alliaceae (Gomes *et al*., 2021). Garlic and its constituents possess a variety of biological functions, including anti-cancer, antioxidant (Rahman and Lowe, 2006), anti-diabetic, kidney-protective, anti-atherosclerotic, antibacterial, and anti-hypertensive effects (Davis, 2005; Badal *et al*., 2019). Garlic has been shown to be effective as a carminative, a fever-reducing agent, a sedative, an aphrodisiac, and a diuretic (Souza *et al*., 2011; El-Saber *et al*., 2020). *Moringa oleifera,* which belongs to the family Moringaceae, acts as a cardiac and circulatory stimulant and possesses a wide range of beneficial properties, such as antitumor, antipyretic, anti-epileptic, anti-inflammatory, antiulcer, antihypertensive, hypoglycaemic, cholesterol-lowering, antioxidant, antibacterial, antifungal, and hepatoprotective properties (Stohs and Hartman, 2015; Chen *et al*., 2017; Razis *et al*., 2014; Agrawal and Mehta, 2008).

The aim of this study was to evaluate the chronic anti-stress activity of a polyherbal-formulated tea (*Citrus limon, Curcuma longa, Zingiber officinale, Allium sativum, and Moringa oleifera*) using a non-stressor mouse model.

MATERIALS AND METHODS

Plant Collection*:* Garlic, turmeric, and ginger were obtained from Oregbeni Market in Ikpoba Okha Local Government Area. Lemons were obtained at the New Benin Market in the Oredo Local Government Area. *Moringa oleifera* leaves were gotten from the Faculty of Agricultural Sciences farm land at the University of Benin City in the Ovia North East Local Government Area, all in Edo State.

The plants were identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

Plant Preparation: Plant preparation was done according to the modified method of Builders *et al*. (2020). Garlic, turmeric, ginger, and lemon were washed and chopped into smaller bits. The *Moringa oleifera* leaf was removed from the stalk and washed. Each of the lemon, garlic, turmeric, ginger, and moringa leaves was dehydrated using a sonifer dehydrator (Model: SF-4006 China). After dehydration, they were separately ground to powder using an impact mill. Their powder was weighed and mixed in an equal proportion (1:1:1:1:1) to formulate the herbal tea, which contained 200 mg of each powdered plant material.

Experimental Animals: *S*ixteen adult Swiss mice of either sex weighing 20–30 g were obtained from the College of Medicine, Ambrose Ali University, Ekpoma, Edo State. The mice were housed within the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, of the University of Benin and allowed two weeks of acclimatisation 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). This research was approved by the ethical committee of the Faculty of Life Sciences, University of Benin, with registration number LS23011.

Chronic Stress Experiment

Chronic stress was induced using a non-social stressor mouse model (Uwaya *et al*., 2022; Inès and Anne-Kathrin, 2023; Uwaya *et al*., 2023) for 21 days. The sixteen mice were allotted into four groups of four mice each, as follows: Group one received 10 mL/kg of distilled water; groups two and three received 5 mg/kg and 10 mg/kg extracts of polyherbal-formulated tea, respectively. Group four received 20 mg/kg of fluoxetine.

For each of the 21 days, mice underwent specific stressors an hour after oral administration of polyherbal-formulated tea or fluoxetine, as follows:

Wet Beddings: The home cage beddings were soaked with water and left for 24 h. This stressor was done on the first, eighth, and 15th days.

Food Deprivation: The animal feeds were removed for 24 h. This stressor was done on the second, seventh, and 17th days.

Water Deprivation: Drinking water was removed from the animal cages for 24 h. This was done on the third, sixth, and ninth days.

Introduction of Mice in Rat Bedding: Each mouse from each group was placed in a rat cage containing rat bedding for 30 min. It was done on the fourth day.

Introducing Predators (Rats) in Mouse Cages: A rat was placed in the cages of the mice for 30 min. It was done on the fifth day.

Tail Suspension Test: On the 10th day, each mouse was suspended from the edge of a shelf 60 cm above a tabletop for two hours. The mice were suspended by adhesive tape placed approximately 1 cm from the tip of the tail. The time of immobility was also observed and recorded.

Cold Restraint Stress Test: On the 11th and 20th days, a cold restraint stress test was given to all the mice by tying the limbs and placing the mice in the refrigerator at 4°C for two hours.

Heat Exposure: On the 12th and 18th days, each mouse was placed on a hot plate at 50 °C for 30 min.

Forced Swimming Test: On the 13th day, each mouse was placed in a transparent cylindrical container (45 x 40 x 30 cm) filled with water to a level of 20 cm and a temperature of 25 °C, and the swimming time was recorded for a period of 30 min.

Animal Isolation: On the 14th day, mice were individually isolated in different cages for two hours.

Acetic Acid-Induced Pain: On the 17th day, mice were administered 0.1 mL of 6% glacial acetic acid intraperitoneally, and the writhing time was recorded.

Physical Restraint Stress Test: On the 19th and 21st days, mice were individually restrained by placing them in a restraint tube (internal diameter 26 mm) for two hours.

Sucrose Preference Test: On the 20th day, mice were allowed a free choice between two bottles containing 1% sucrose solution and distilled water for 24 h.

Blood and Brain Sample Collection and Analyses
At the end of the administrations, mice were placed in a container containing chloroform (anaesthesia), and their abdominal region was opened up. Blood was collected via the abdominal aorta into plain containers for cortisol analysis, and their brains were harvested and homogenised using 5 mL of cold normal saline. The blood and the homogenised tissues were centrifuged at 600 revolutions per minute (rpm) for 10 min. The sera and supernatants, or tissue homogenates, were collected and stored in different plain containers in the refrigerator at -20 °C (Idu *et al.*, 2016).

Estimation of Cortisol Level
Prewashed and sterilised test tubes were labelled as blank or test. Plasma (1,000 uL) was pipetted into test tubes labelled test, and distilled water (1,000 uL) was pipetted into test tubes labelled blank. A freshly prepared methanol (2:1 v/v) mixture, 200 uL, was added to all the test tubes, followed by 3,000 uL of chloroform. Following that, 300 uL of 0.1N sodium hydroxide and 3,000 uL of sulphuric acid were added to all the test tubes. The test tubes were incubated for 45 min in the dark room at room temperature. Absorbance was read at 533 nm using a UV spectrophotometer (Model: T80 + UV/VIS). A standard curve of hydrocortisone (g<pH) absorbent against concentration was plotted, from which the concentrations of cortisol were extrapolated. r = 0.999 (Iniaghe *et al.*, 2018).

Determination of Superoxide Dismutase (SOD)
The assay relies on the reaction by Misra and Fridovich (1980), but modified by Idu *et al.* (2016). Two steps were required for the assay of SOD. The first was the reference tube, which was prepared by mixing 0.2 mL of distilled water with 2.5 mL of carbonate buffer. This was quickly followed by the addition of 0.3 mL of freshly prepared adrenaline solution, which was rapidly mixed.

The sample tubes were prepared by introducing 2.5 mL of carbonate buffer into the test tubes. This was followed by the addition of 80 uL of the test samples and 120 uL of adrenaline solution. This was very rapidly mixed and read at 420 nm absorbance every 30 to 120 s with a UV-visible spectrophotometer (Model: T80 + UV/VIS), and distilled water was used to zero the machine.

Determination of Catalase Activity
Distilled water was measured into the blank test tubes, while 0.5 mL of sample was measured into the labelled test tubes. 2.5 mL of 30 M hydrogen peroxide was added into the labelled sample and blank test tubes. After 3 min, 1 mL of 6 M H_2SO_4 and 3.5 mL of 0.01 M potassium permanganate were added to the test and the blank. Absorbance was read within 30–60 s. A spectrophotometric standard was prepared by adding 3.4 mL of 0.01 M potassium permanganate to a mixture of 5.5 mL of 0.05 M phosphate buffer pH 7.0 and 1.0 mL of sulphuric acid solution. The spectrophotometer (Model: T80 + UV/VIS) was zeroed with distilled water (Idu *et al.*, 2016).

Determination of Glutathione Peroxidase Activity
Test tubes were filled with sodium phosphate buffer in the following amounts: 1,500 uL for the labelled sample, 1,900 uL for the labelled control, 1,900 uL for the labelled standards, and 2,000 uL for test tubes labelled blank. 100 uL of tissue homogenates were added to the sample and control, whereas 200 uL of reduced glutathione was added to the sample and standard. 200 uL of H_2O_2 were added to the sample and standard to start the reaction. The test tubes were combined and heated at 37 °C for 10 min. The addition of 0.5 mL of 8% TCA stopped the process. The test tubes were centrifuged at 3,000 rpm for 15 min. In brand-new test tubes marked sample, standard, control, and blank, 1 mL of the supernatant was added to 3 mL of the working reagent. For 30 min, the absorbance was measured at 450 nm against a blank. It was determined how much GPx was present by using: (A test-A control) ÷ (A standard) × concentration of standard (Iniaghe *et al.*, 2018).

Determination of Glutathione Reductase Activity
A 0.5 mL of TCA (5%) was added to the test tubes, and 1.5 mL of blood sample was added to each test tube. A precipitate was formed. Each test tube was then centrifuged for 10 min, and after 0.5 mL of Ellman's reagent was added to another set of glass test tubes. 1.5 mL of glutathione phosphate buffer was added to the new set of glass test tubes. 2.5 mL of the already centrifuged blood sample was then added to the new set of glass test tubes. This was then read at 416 nm wavelength in comparison with the standard (which did not have a blood sample included in it) using a spectrophotometer (Model: T80 + UV/VIS) (Iniaghe *et al.*, 2018).

Determination of Malondialdehyde

Malondialdehyde was estimated by the method modified by Idu *et al*. (2016). A volume (0.6 mL) of the tissue homogenate was added to 3 mL of (1:1 v/v) TA-TBA-HCl reagent thiobarbituric acid (0.375% w/v, 15% TAC w/v, and 0.25 HCl) and mixed. Solutions were heated for 15 min in a boiling water bath. The solution was cooled and centrifuged at 1,000 rpm for 10 min. The absorbance of the supernatant was measured against a reference blank at 535 nm using a UV-visible spectrophotometer (Model: T80 + UV/VIS) (Iniaghe *et al*., 2018).

Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted, followed by the Newman-Keuls post hoc test. GraphPad Prism software version 6 from the UK (Graphpad Software, UK) was used for all data analysis. A significance level of $P<0.05$ indicated notable differences between the compared data. The data were presented as mean ± standard error of the mean (SEM).

RESULTS

Effects of a Polyherbal-Formulated Tea on a Swimming Endurance Test and Tail Suspension

Figures 1a and b show the effect of polyherbal-formulated tea on the swimming time and time of immobility on the forced swimming endurance test and tail suspension on mice induced with depression-like stress. The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and fluoxetine at 20 mg/kg increased the swimming test when compared with chronic stress control ($p<0.01$). The polyherbal-formulated tea at 10 mg/kg and the standard (fluoxetine) at 20 mg/kg decreased the time of immobility when compared with chronic stress control ($p<0.01$; $p<0.05$).

Effects of a Polyherbal-Formulated Tea On Cortisol Level

Figure 2 shows the effect of a polyherbal-formulated tea on cortisol levels in mice induced by chronic stress. The polyherbal-formulated tea of 10 mg/kg and fluoxetine of 20 mg/kg decreased the level of cortisol when compared with chronic stress control ($p<0.05$).

Effects of Polyherbal-Formulated Tea on Malondialdehyde Level

Figure 3 shows the effect of polyherbal-formulated tea on malondialdehyde levels in mice induced by chronic stress. There was no significant difference in malondialdehyde level when compared to chronic stress control ($p > 0.05$).

Effects of a Polyherbal-Formulated Tea on Levels of Catalase, Glutathione Peroxidase, Glutathione Reductase, and Superoxide Dismutase

Figures 4a, b, and c show the effect of a polyherbal-formulated tea on levels of catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase in mice induced by chronic stress. The polyherbal-formulated
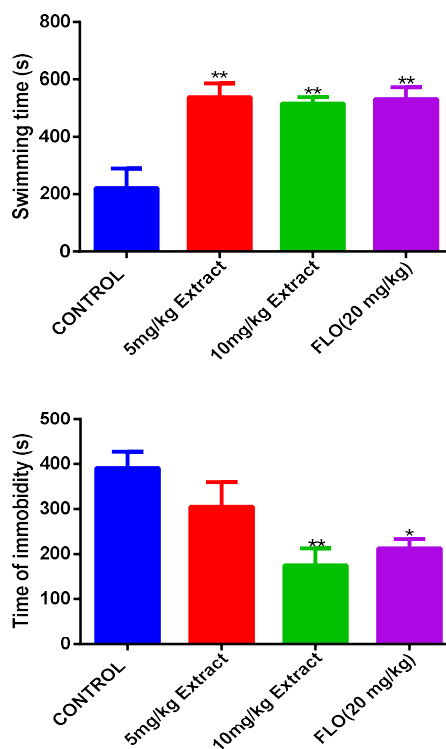

Fig. 1: The effect of a polyherbal-formulated tea on the time of immobility of the forced swimming endurance test and time of immobility of tail Suspension. A: The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg and fluoxetine at 20 mg/kg increased the swimming time when compared with chronic stress control (**$p<0.01$). B: The polyherbal-formulated tea at 10 mg/kg and fluoxetine at 20 mg/kg decreased the time of immobility when compared with chronic stress control (**$p<0.01$; *$p<0.05$). FLO: Fluoxetine. Values were presented as mean ± S.E.M n=4.
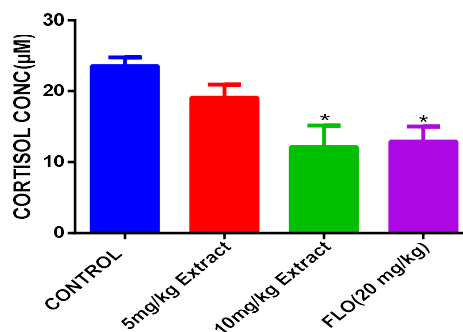

Fig. 2: The effect of polyherbal-formulated tea on cortisol level in mice induced with chronic stress. The polyherbal-formulated tea at 10 mg/kg and fluoxetine at 20 mg/kg decreased the level of cortisol when compared with chronic stress control (*$p<0.05$). FLO: Fluoxetine. Values were presented as mean ± S.E.M, n=4.

tea at 10 mg/kg increased catalase levels when compared with chronic stress control ($p < 0.01$) (Fig. 4a). There was no significant difference in glutathione peroxidase level when compared to chronic stress control ($p > 0.05$) (Fig. 4b). The polyherbal-formulated tea at 10 mg/kg increased

the level of glutathione reductase when compared with chronic stress control (p<0.01) (Fig. 4c). There was no significant difference in the superoxide dismutase level when compared to chronic stress control (p > 0.05) (Fig. 4d).
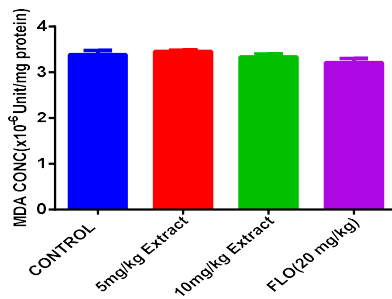

Fig. 3: The effect of polyherbal-formulated tea on malondialdehyde level in mice induced with chronic stress. There was no significant difference on malondialdehyde level when compared to chronic stress control (P> 0.05). FLO: Fluoxetine. Values were presented as mean ± S.E.M, n=4.

DISCUSSION

In evaluating herbs for long-term stress, researchers in the laboratory conduct tests on mice to observe behaviours similar to depression. These tests include the forced swimming endurance test and the tail suspension test (Tran and Gellner, 2023). The forced swimming test assesses a rodent's susceptibility to negative emotions and reflects an animal's perception of being unable to control its situation. This is a test that examines how a mouse reacts to the fear of drowning (Petit-Demouliere *et al*., 2005; Millstei and Holmes, 2007; Can *et al*., 2012). Forced swimming is the most extensively used approach for assessing the anti-stress ability of a new chemical. This paradigm is based on the discovery that animals trained to swim in water gradually developed a typical motionless position devoid of any activity. As a result, the appearance of immobility indicates a condition of tiredness, fatigue, and reduced stamina, with the end point being the moment when the mouse could not swim farther and started drowning. However, greater swimming time has been recorded in mice pre-treated with antistress and adaptogenic drugs (Ishola *et al*., 2008). In this study, we found that the aqueous extract of the polyherbal-formulated tea, which contains *Citrus limon, Curcuma longa, Zingiber officinale, Allium sativum, and Moringa oleifera* at different doses (5 mg/kg and 10 mg/kg), along with the standard drug (fluoxetine 20 mg/kg), enhanced the swimming time in the forced swimming endurance test compared to the stress control. The polyherbal-formulated tea's capacity to extend swimming time indicates that the tea has anti-stress properties, as mentioned by Ishola *et al*. (2019) and Ansari and Shashikantsorte (2018).

The tail suspension test is a widely used method in animal research to evaluate possible antidepressant and anti-stress effects. It involves inducing a condition of immobility
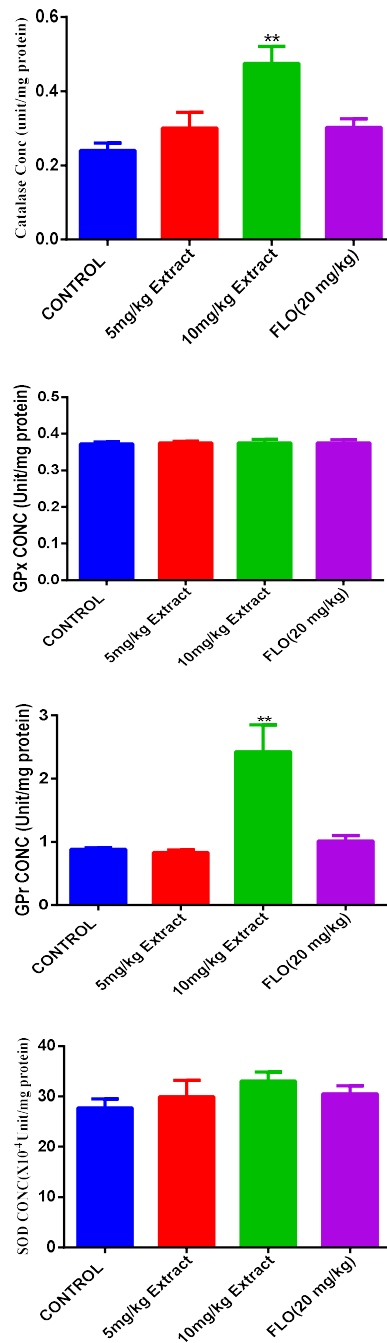

Fig. 4: Effects of polyherbal-formulated tea on levels of catalase, glutathione peroxidase, glutathione reductase and sodium dismutase in chronic stressed mice. A: The polyherbal-formulated tea at 10 mg/kg increased catalase level when compared with chronic stress control (**p<0.01). B: There was no significant difference on glutathione peroxidase level when compared to chronic stress control (P> 0.05). C: The polyherbal-formulated tea at 10 mg/kg increased glutathione reductase level when compared with chronic stress control (**p<0.01). D: There was no significant difference on superoxide dismutase level when compared to chronic stress control (P> 0.05). FLO: Fluoxetine. Values were presented as mean ± S.E.M n=4.

in animals when they are faced with an inescapable scenario (Inès and Anne-Kathrin, 2023; Ansari and Shashikantsorte, 2018). Researchers have suggested that such a lack of movement activity may indicate a state of hopelessness, potentially indicative of depressive disorders in humans (Maier and Seligman, 2016). Researchers most commonly use the tail suspension test, similar to the forced swim test, to evaluate the effectiveness of antidepressant and anti-stress medicines. However, researchers also use it to examine the effects of environmental, neurobiological, and genetic changes. This study demonstrates that both the conventional (fluoxetine 20 mg/kg) and the polyherbal-formulated tea at 10 mg/kg were found to decrease the duration of immobility in the tail suspension test. The polyherbal tea's ability to reduce times of immobility suggests that the tea contains properties that can help with depression and stress, as stated by Maier and Seligman (2016) and Ishola *et al.* (2008). Fluoxetine, which served as the benchmark, belongs to a class of medications known as selective serotonin reuptake inhibitors (SSRIs), commonly prescribed for the treatment of depression. By blocking a protein known as the reuptake transporter in certain nerve cells, fluoxetine stops the reabsorption of serotonin into those cells (Cao *et al.*, 2019; Robertson *et al.*, 2019). The polyherbal-formulated tea may have an antistress effect due to the presence of Zingiber officinale, Curcuma longa, and Allium sativum. Studies have indicated that *Zingiber officinale, Curcuma longa, and Allium sativum* have antistress properties (Singh *et al.*, 2012; Ittiyavirah and Paul, 2013; Khushboo, 2017; Moragrega and Ríos, 2021).

Elevated cortisol levels indicate the presence of stress in mice (Sentari *et al.*, 2019). In this study, the stress control mice showed a notable increase in cortisol levels and a notable reduction in cortisol levels following the administration of the normal medication (fluoxetine 20 mg/kg) and the polyherbal tea formulation at 10 mg/kg. The outer part of the adrenal gland cortex releases cortisol, a hormone with a steroid structure that has glucocorticoid effects (Gatti *et al.*, 2009; Cay *et al.*, 2018). This hormone has a regular release rhythm, reaching its highest level in the morning to promote alertness and progressively decreasing afterwards (Ehlert *et al.*, 2001; Tsigos and Chrousos, 2002; Hannibal and Bishop, 2014). In addition to its important role in daily functioning, cortisol also plays a significant part in the stress response. When faced with a physical or mental hazard, cortisol levels increase to supply the required energy and resources to deal with stress-causing triggers or avoid harm (Blackburn-Munro and Blackburn-Munro, 2003; Jankord and Herman, 2018). It is commonly believed that long-term stress causes an increase in cortisol release, which may result in depression, digestive issues, headaches, muscle tension and pain, heart disease, heart attack, high blood pressure and stroke, sleep problems, weight gain, difficulties with memory and concentration, and anxiety (Stephens and Wand, 2012; Qin *et al.*, 2016). The polyherbal tea's capacity to decrease cortisol levels after exposure to various stressors over a period of 21 days suggests that it possesses antistress properties and may be beneficial in managing conditions such as high

blood pressure, stroke, heart attack, headache, muscle tension, sleep disturbances, weight gain, cognitive difficulties, anxiety, and depression.

Catalase is an important antioxidant enzyme that is present in almost all organisms that require oxygen. Catalase acts as a treatment option for different disorders linked to oxidative stress (Nandi *et al.*, 2019). This study has shown that polyherbal-formulated tea at a dosage of 10 mg/kg elevated catalase levels. The polyherbal-formulated tea's capacity to enhance catalase levels demonstrates the tea's antioxidant potential. Catalase deficiency or dysfunction is linked to various diseases, including diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anaemia, certain dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia (Nandi *et al.*, 2019; Habib *et al.*, 2010). Catalase has a key function in controlling the amount of hydrogen peroxide in cells, and its breakdown of hydrogen peroxide helps protect cells from oxidative damage. For instance, it protects pancreatic cells from hydrogen peroxide-induced injury (Nandi *et al.*, 2019). Nandi *et al.* (2019) observed reduced catalase activities in individuals diagnosed with schizophrenia and those with atherosclerosis.

In this study, a polyherbal-formulated tea at a dosage of 10 mg/kg increased the activity of glutathione reductase, as shown in Figure 4c. The polyherbal-formulated tea's capacity to enhance catalase levels has demonstrated the plant's antioxidant potential. Glutathione reductase's main job is to keep the balance of thiol redox in cells by helping to change glutathione disulphide (GSSG) into glutathione (GSH) with NADPH as a cofactor (Tutic *et al.*, 1990; Qiao *et al.*, 2007). The process that is catalysed by glutathione reducatase helps keep the balance between reduced glutathione and oxidised glutathione (GSH/GSSG) inside cells. Furthermore, clinical settings employ the glutathione reducatase enzyme to diagnose liver ailments, evaluate nutritional status, detect riboflavin insufficiency, and diagnose genetic problems (Bakirezer *et al.*, 2019). In this study, the polyherbal-formulated tea did not have any impact on malondialdehyde (MDA), glutathione peroxidase (GPx), or SOD.

The polyherbal-formulated tea has the potential to raise the levels of catalase and glutathione reductase, suggesting that it has antioxidant properties. This combination of herbs may be beneficial in fighting diseases associated with oxidative stress and stress-induced disorders in the brain, such as Alzheimer's disease and Parkinson's disease (Pizzino *et al.*, 2017). Research has revealed that *Citrus limon*, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, and *Moringa oleifera* possess antidepressant, cardioprotective, antioxidant, anti-inflammatory, anticough, anti-asthmatic, and antidiabetic properties. The phytochemical constituents present in each of these plants are responsible for these properties. Uwaya and Effiong (2024) reported the amount of alkaloids, flavonoids, phenolic compounds, vitamins, carotenoid, and lycopene in the polyherbal-formulated tea, which comprises *Curcuma longa, Citrus limon, Zingiber officinale, Allium sativum,* and *Moringa oleifera.* Thus, the phytochemicals and antioxi-

dants found in the tea's constituent plants may be responsible for the polyherbal tea's anti-stress capability.

Conclusion
The polyherbal-formulated tea has stress-relieving qualities. The polyherbal-formulated tea's antioxidant potential may be the cause of its antistress effects.

Conflict of Interest
None declared.

Authors' Contribution
DOU - conception and designed of research; DOU and SOO - Data collection, analysis, and interpretation of the results; DOU and JCA - Statistical analysis; SOO and DUO - Draft manuscript preparation; DUO, SOO and JCA - reviewed the results and approved the final version of the manuscript.

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