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The Effect of Methanolic Extract of *Costus afer* Leaves on the Lipid Profile of Chronically Stressed and Non-Stressed Male Wistar Rats

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Abstract

The present study was conducted to investigate the effects of the methanolic extract of *Costus afer* leaves on the lipid profile of chronically stressed and non-stressed Wistar rats. A total of 30 male Wistar rats, weighing 180–220 g, were used for the study. The rats were divided into six (6) groups: Control group (CT), Stress group (STR), *Costus afer* 200 mg/kg body weight (CA1), *Costus afer* 400 mg/kg body weight (CA2), Stress + *Costus afer* 200 mg/kg body weight (STR + CA1), and Stress + *Costus afer* 400 mg/kg body weight (STR + CA2). The animals were maintained under standard laboratory conditions. The stress groups (STR, STR + CA1, STR + CA2) were subjected to a chronic unpredictable stress protocol (CUS) for 21 days to induce stress. After the initial 21 days of stress administration, the STR + CA1 and STR + CA2 groups were treated with *Costus afer* alongside another sequence of stress for an additional 21 days. The CT group received distilled water throughout the 42-day period, while the CA1 and CA2 groups were administered *Costus afer* extract. The STR group was exclusively stressed for 42 days. The lipid profile of all the animals was measured at the end of the experiment. The total cholesterol level in the STR group increased significantly ($p < 0.01$) compared with the control group. Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglyceride levels increased significantly in the STR group ($p < 0.0001$, $p < 0.05$, and $p < 0.05$, respectively). The level of high-density lipoprotein (HDL) increased significantly in the CA2 ($p < 0.05$) and STR + CA1 ($p < 0.05$) groups, while it decreased significantly in the STR group ($p < 0.05$). The results of this study revealed that the methanolic extract of *Costus afer* leaves has an antilipidemic effect. The extract contains agents capable of depleting low-density lipoprotein (bad cholesterol) levels in the body systems of rats.

***Keywords*:** Methanolic extract, Chronically stressed, Non-stressed, Lipid profile, *Costus afer*.

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BACKGROUND OF STUDY

Lipid profile or lipid panel is a panel of blood tests that serves as an initial screening tool for abnormalities of lipids such as cholesterol and triglycerides. It is used to measure the amount of good and bad cholesterol and triglycerides, a type of fat in the blood (Onwe*et al*., 2015). Their studies have shown that the lipid profile level of humans changes in different conditions. When there is a decrease or increase in any lipid profile level, it can affect the body systems positively or negatively depending on the specific lipid profile parameter.

Stress can be defined as a feeling of emotional or physical tension. It can come from any event or thought that makes a person feel frustrated, angry or nervous (Ahmed *et al.*, 2016). It can also be defined as any external or internal condition that challenges the homeostasis of a cell or an organism (Kagias*et al.,* 2012).

Chronically unpredictable stress (CUS) protocol has been widely used to study the impact of stress exposure in several animal models. It consists of random intermittent and unpredictable exposure to a variety of stressors for several weeks (Monteiro *et al.,* 2015). The type and timing of stress exposure varies at each instance of administration. According to American Psychological Association (APA), chronic stress is the most harmful type of stress. If stress is left untreated over a long period of time, it can significantly and often reversibly damage physical health and deteriorate mental health. Chronic stress is grinding stress. It wears people away day after day, year after year. Chronic stress destroys lives, bodies and minds (Freshwater, 2018). Stress has been reported to have an effect on the lipid profile in humans (Negar, 2017).

The use of herbal medicine to manage or cure diseases dates back to the Stone Age. There has been an advancement in pharmacological discoveries over the years that has resulted in the production of many synthetic drugs. This therefore reiterates the worth of ethno medicinal plants for drug discovery. A number of research studies have gone into finding bioactive compounds of plant origin with pharmacological properties to be used in the design of new drugs with lesser side effects. The several medicinal importance of *Costusafer* makes it serve as one of the plants to attract this kind of research. *Costusafer Ker-Gawl (Costusafer*) is a rhizomatous herb commonly known as ginger lily or “bush cane” (Anyasor*et al.,* 2014). Almost every part of this plant is endowed with medicinal potential in diseases such as malaria, measles, diabetes mellitus, arthritis, and stomach disorders. In West Africa for instance, the succulent stem is chewed to quench thirst and also to treat cough and its accompanying sore throat (Taiwo *et al*., 2003). The stem and leaves of *Costusafer*contain substantial amounts of micro and macronutrients that serve beneficial health features. Various solvent extracts of the plant leaves, stem, rhizomes, and roots have been studied and reported to contain chemical compounds that could be useful in the alleviation of oxidative stress-related conditions (Anyasor*et al.,* 2010).

Costusafer has also been found to have antilipidemic effect on the body (Boison*et al.,* 2019).

Statement of problem

Stress affects lipid profile specifically the level of High density lipoprotein (good cholesterol) and Low density lipoprotein (bad cholesterol) (Neger, 2017). There is a significant reduction of high density lipoprotein and an increased level of low density lipoprotein which results to higher risk of death from cardiovascular causes (cardiovascular diseases). Although available drugs such as naloxone have been utilized to treat lipid profile disorders associated with stress, the negative effects such as restlessness, weakness, headache, nausea etc., limits their usage (Koenigsberg*et al.,* 1987)

Justification

Recent studies show that *Costusafer*possess antioxidant and antilipidemic properties (Boison*et al.,* 2019). However, its potency to improve or ameliorate lipid disorders associated with chronic stress has not been reported, thus necessitating this work.

Aim

The aim of the study is to determine the effect of *Costusafer*on the lipid profile of chronically unpredictable stressed and non-stressed-induced male Wistar rats.

Objectives

To determine the effect of *Costusafer* on:

1. Low density lipoprotein Cholesterol (LDL-C) levels of stressed rats.
2. High density lipoprotein cholesterol (HDL-C) levels of stressed rats.
3. Triglyceride (TG) levels of stressed rats.
4. Total cholesterol (TC) levels of stressed rats.
5. Very low density lipoprotein (VLDL) levels of stressed rats.

LITERATURE REVIEW

Stress

Stress can be defined as any type of change that causes physical, emotional, or psychological strain. Stress is the body's response to anything that requires attention or action (Scott, 2020). Stress management can be complicated and confusing because there are different types of stress, namely; acute stress, episodic acute stress, and chronic stress each with its own characteristics, symptoms, duration and treatment approaches (Scott, 2020).

Mechanism of stress

Stress is a multidimensional phenomenon which involves both nervous and endocrine system. The first step in stress response is the perception of the threat (stressor). Whenever there is some stressor (real or imagined), it acts at the level of brain. In the brain, it is the

hypothalamus which perceives the stressor. When the hypothalamus encounters a threat it performs some specific functions:

1. Activates autonomic nervous system (ANS)
2. Stimulates Hypothalamic Pituitary Adrenal (HPA) axis by releasing Corticotrophin Releasing Hormone (CRH)
3. Secretes vasopressin (Antidiuretic Hormone, ADH).

Autonomic nervous system consists of sympathetic (arousal) and parasympathetic (relaxed) nervous system. The ANS regulates visceral activities like circulation, digestion, respiration, temperature regulation and some vital organs (Sharma, 2018).

The sympathetic system accounts for the flight-or-flight response. In response to a stressor, catecholamines (epinephrine and norepinephrine) are released at various neural synapses. The release of these catecholamines causes several changes like increase in the heart rate, force of myocardial contraction, vasodilatation of arteries throughout working muscles, dilation of pupil and reduction of digestive activities in the body (Sharma, 2018). All these changes are required to prepare the body for fight-or-flight response. The effects of these hormones last for few seconds. The functions of parasympathetic nervous system are opposite to that of sympathetic nervous system and help in energy conservation and relaxation (Sharma, 2018).

CRH acts at the anterior pituitary gland an endocrine gland located in the brain. Pituitary gland is also called 'master gland', as it controls the secretion of other endocrine glands in the body. On stimulation by CRH, anterior pituitary secretes Adrenocorticotropin Hormone (ACTH) (Sharma, 2018; Scatamburlo*et al*., 2001). ACTH released from anterior pituitary gland in response to CRH stimulates adrenal glands located in the kidneys. There are two parts of adrenal - the outer part called cortex and the inner part known as medulla. ACTH stimulates adrenal cortex to release corticoids (glucocorticoids and mineralocorticoids).

The major function of glucocorticoids is to release energy, which is required to cope with the ill effects of stressors. The energy is released by conversion of glycogen into glucose (glycogenolysis) and also by breakdown of fats into fatty acids and glycerol (lipolysis). In addition to these corticoids have several other functions such as increased urea production, appetite suppression, suppression of immune system, exacerbation of gastric irritation, associated feeling of depression and loss of control. These are the symptoms generally seen in a person under stress. Mineralocorticoid (aldosterone) promotes Na^+ retention and elimination of K^+. It increases blood pressure by increasing blood volume. The medulla part of the adrenal gland secretes epinephrine and norepinephrine. The functions of these hormones are the same as that of those secreted from nerve endings of sympathetic nervous system. These hormones secreted by adrenal medulla, reinforce the functions of sympathetic nervous system. The release of these hormones from adrenal medulla acts as a backup system to ensure the most efficient means of physical survival (Sharma, 2018). The effects brought out by epinephrine and nor-epinephrine from the sympathetic nervous system may be termed as immediate effects and the effects brought out by those of adrenal medulla are intermediate effects (Sharma, 2018).

The basic function of vasopressin or ADH synthesized by hypothalamus and released by posterior pituitary is to regulate fluid loss through urinary tract (Sharma, 2018). This is achieved by reabsorption of water. In addition, ADH also has a prominent role on regulation of blood pressure during stress when the homeostasis of the body is disturbed in addition to

release of energy second major change occurring during stress is distribution of energy to a particular organ that needs it most. This is achieved by increasing blood pressure. This occurs either through enhanced cardiac output or through constriction of blood vessel (Sharma, 2018).

In addition to the hypothalamo-pituitary adrenal axis some other hormones such as Growth Hormone (GH) and thyroid hormones also play significant role in stress. Growth hormone is a peptide hormone, released from anterior pituitary gland. GH is a stress hormone that raises the concentration of glucose and free fatty acids. It has been observed that, in human beings' psychological stimuli increase the concentration of thyroid hormones. Thyroid releases thyroxin and triiodothyronine. These hormones also have some significant function in stress (Sharma, 2018). The main function of thyroid hormones is to increase overall metabolic rate or Basal Metabolic Rate (BMR). Thyroxin also increases heart rate and also the sensitivity of some tissues to catecholamines (Sharma, 2018).

Acute stress

Acute stress is one of the least damaging types of stress. It is also one of the most common types of stress (Scott, 2020). Acute stress is thrilling and exciting in small doses, but too much is exhausting. A fast run down a challenging ski slope, for example, is exhilarating early in the day. That same ski run late in the day is taxing and wearing. Skiing beyond your limits can lead to falls and broken bones. By the same token, overdoing on short-term stress can lead to psychological distress, tension headaches, upset stomach and other symptom (APA, 2018). Acute stress is short-termed, hence doesn't have enough time to do the extensive damage associated with long-term stress. Such stress can crop up in anyone's life, and it is highly treatable and manageable (APA, 2018).

Episodic acute stress

This is the term used when someone experiences acute stress with some regularity or frequency. They are several reasons why it may happen, example taking too much on oneself, being in an unusually demanding job, being responsible for a loved one with frequent or significant difficulties, bad luck, having interpersonal difficulties, having the tendency to interpret situations in a catastrophic way (Greene, 2020).

It is common for people with acute stress reactions to be over aroused, short-tempered, irritable, anxious and tense. Often, they describe themselves as having "a lot of nervous energy." Always in a hurry, they tend to be abrupt, and sometimes their irritability comes across as hostility (APA, 2018). Interpersonal relationships deteriorate rapidly when others respond with real hostility. The workplace becomes a very stressful place for them (APA, 2018).

The symptoms of episodic acute stress are the symptoms of extended over arousal: persistent tension headaches, migraines, hypertension, chest pain and heart disease. Treating episodic acute stress requires intervention on a number of levels, generally requiring professional help, which may take many months (APA, 2018).Only the promise of relief from pain and

discomfort of their symptoms can keep them in treatment and on track in their recovery program (APA,2018).

Chronic stress

While acute stress can be thrilling and exciting, chronic stress is not. This is the grinding stress that wears people away day after day, year after year. Chronic stress destroys bodies, minds and lives (Shawna, 2018). It wreaks havoc through long-term attrition. It's the stress of poverty, dysfunctional families, being trapped in an unhappy marriage or in a despised job or career (APA, 2018).

Chronic stress comes when a person never sees a way out of a miserable situation. It's the stress of unrelenting demands and pressures for seemingly interminable periods of time. With no hope, the individual gives up searching for solutions (APA, 2018).

It may also stem from traumatic, early childhood experiences that become internalized and remain forever painful and present. Some experiences profoundly affect personality (APA, 2018).

The worst aspect of chronic stress is that people get used to it. They forget it's there. People are immediately aware of acute stress because it is new; they ignore chronic stress because it is old, familiar, and sometimes, almost comfortable (APA, 2018).

Chronic stress kills through suicide, violence, heart attack, stroke and, perhaps, even cancer. People wear down to a final, fatal breakdown. Because physical and mental resources are depleted through long-term attrition, the symptoms of chronic stress are difficult to treat and may require extended medical as well as behavioral treatment and stress management (APA, 2018).

Chronic unpredictable stress (CUS)

This is a protocol that has been widely used to study the impact of stress exposure in several animal models and consists in the random, intermittent, and unpredictable exposure to a variety of stressors during several weeks (Monterio *et al.,* 2015). Exposure to chronic stress can have broad effects on health ranging from increased predisposition for neuropsychiatric disorders to deregulation of immune responses. CUS has consistently been shown to induce behavioral and immunological alterations typical of the chronic stress-response (Monterio*et al.,* 2015).

Effect of stress on the Body function

Any intrinsic or extrinsic stimulus that evokes a biological response is known as stress. The compensatory responses to these stresses are known as stress responses. Based on the type, timing and severity of the applied stimulus, stress can exert various actions on the body ranging from alterations in homeostasis to life-threatening effects and death (Yaribeygi*et al.,* 2017).

Effect of stress on Brain function

McEwen *et al.* (1968) formulated a hypothesis that stress can cause functional changes in the central nervous system (CNS). This hypothesis was accepted and from that time on, two types of corticotropic receptors (glucocorticosteroids and mineralocorticoids) were recognized (Kloet*et al*., 1999).The effects of stress on the nervous system have been investigated for 50 years (Thierry *et al*., 1968). Chronic stress can lead to atrophy of the brain mass and decrease its weight (Sarahian*et al.,* 2014). These structural changes bring about differences in the response to stress, cognition and memory (Lupien*et al.,* 2009). Of course, the amount and intensity of the changes are different according to the stress level and the duration of stress.

Effect of stress on Immune system functions

The prevailing attitude between the association of stress and immune system response has been that people under stress are more likely to have an impaired immune system and, as a result, suffer from more frequent illness (Khansari*et al*., 1990). Stress can affect the function of the immune system by modulating processes in the Central Nervous System (CNS) and neuroendocrine system (Khansari*et al*., 1990; Glaser and Glaser, 1991). Following stress, some neuroendocrine and neural responses result in the release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and other stress mediators (Carrasco and Kar, 2003).

However, evidence suggests that the lymphatic system, which is a part of the immune system, also plays a role in releasing these mediators (Khansari*et al.,* 1990). For instance, thymus peptides, such as thymopentine, thymopoietin, and thymosin fraction-5, cause an increase in ACTH production (Goya *et al*., 1993; Yaribeygi, 2017). Severe stress can lead to malignancy by suppressing the immune system (Reiche*et al.,* 2004). In fact, stress can decrease the activity of cytotoxic T lymphocytes and natural killer cells and lead to growth of malignant cells, genetic instability, and tumor expansion. Studies have shown that the plasma concentration of norepinephrine, which increases after the induction stress, has an inverse relationship with the immune function of phagocytes and lymphocytes. Catecholamines and opioids which have immune-suppressing properties are released following stress (Reiche*et al*., 2004; Yaribeygi, 2017).

Effect of stress on Musculoskeletal system

When the body is stressed, muscles tense up. Muscle tension is almost a reflex reaction to stress (the body's way of guarding against injury and pain). With sudden onset of stress, the muscles tense up all at once, and then release their tension when the stress passes (APA, 2018). Chronic stress causes the muscles in the body to be in a more or less constant state of guardedness. When muscles are taut and tense for long periods of time, this may trigger other reactions of the body and even promote stress-related disorders (APA, 2018). For example, both tension-type headache and migraine headache are associated with chronic

muscle tension in the area of the shoulders, neck and head. Musculoskeletal pain in the low back and upper extremities has also been linked to stress, especially job stress (APA, 2018).

Effect of stress on the Respiratory system

The respiratory system supplies oxygen to cells and removes carbon dioxide waste from the body. Air comes in through the nose and goes through the larynx in the throat, down through the trachea, and into the lungs through the bronchi. The bronchioles then transfer oxygen to red blood cells for circulation (APA, 2018). Stress and strong emotions can present with respiratory symptoms, such as shortness of breath and rapid breathing, as the airway between the nose and the lungs constricts (APA, 2018). For people without respiratory disease, this is generally not a problem as the body can manage the additional work to breathe comfortably, but psychological stressors can exacerbate breathing problems for people with pre-existing respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD; includes emphysema and chronic bronchitis) (APA, 2018).Some studies show that an acute stress such as the death of a loved one can actually trigger asthma attacks. In addition, the rapid breathing or hyperventilation caused by stress can bring on a panic attack in someone prone to panic attacks (APA, 2018).

Effect of stress on the Blood (blood pressure)

The body produces a surge of hormones during stressful situations. These hormones temporarily increase blood pressure by causing the heart to beat faster which leads to narrowing of blood vessels (American Heart Association, 2021). There is no proof that stress by itself causes long-term high blood pressure. But reacting to stress in unhealthy ways can increase the risk of high blood pressure, heart attacks and strokes. Certain behaviors are linked to higher blood pressure, such as: Smoking, drinking too much alcohol and eating unhealthy foods (American Heart Association, 2021). Also, heart disease may be linked to certain health conditions related to stress, such as: Anxiety, depression, isolation from friends and family. But there is no evidence these conditions are directly linked to high blood pressure. Instead, the hormones produced during emotional stress may damage your arteries, leading to heart disease (American Heart Association, 2021). Increase in blood pressure related to stress can be dramatic. But when stress goes away, the blood pressure returns to normal. However, even frequent, temporary spikes in blood pressure can damage blood vessels, heart and kidneys in a way similar to long-term high blood pressure (American Heart Association, 2021).

Effect of stress on the Digestive system (GIT)

Stress shows both short and long-term effects on the functions of the gastrointestinal tract (Konturek*et al.,* 2011).Exposure to stress results in alterations of the brain-gut interactions ("brain-gut axis") ultimately leading to the development of a broad array of gastrointestinal disorders including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and other functional gastrointestinal diseases, food antigen-related adverse

responses, peptic ulcer and gastroesophageal reflux disease (GERD). The major effects of stress on gut physiology include: alterations in gastrointestinal motility, increase in visceral perception, changes in gastrointestinal secretion, increase in intestinal permeability, negative effects on regenerative capacity of gastrointestinal mucosa and mucosal blood flow (Konturek*et al.*, 2011).

Effect of stress on the Reproductive system (Male)

The male reproductive system is influenced by the nervous system. The parasympathetic part of the nervous system causes relaxation whereas the sympathetic part causes arousal. In the male anatomy, the autonomic nervous system, also known as the fight or flight response, produces testosterone and activates the sympathetic nervous system which creates arousal (APA, 2018). Stress causes the body to release the hormone cortisol, which is produced by the adrenal glands. Cortisol is important to blood pressure regulation and the normal functioning of several body systems including cardiovascular, circulatory, and male reproduction. Excess amounts of cortisol can affect the normal biochemical functioning of the male reproductive system (APA, 2018).

Sexual desire

Chronic stress, ongoing stress over an extended period of time, can affect testosterone production resulting in a decline in sex drive or libido, and can even cause erectile dysfunction or impotence (APA, 2018).

Reproduction

Chronic stress can also negatively impact sperm production and maturation, causing difficulties in couples who are trying to conceive (APA, 2018). Researchers have found that men who experienced two or more stressful life events in the past year had a lower percentage of sperm motility (ability to swim) and a lower percentage of sperm of normal morphology (size and shape), compared with men who did not experience any stressful life events (APA, 2018).

Effect of stress on the Excretory system (Liver)

In vertebrates, stress negatively affects body homeostasis and triggers a battery of metabolic responses, with liver playing a key role. This organ responds with altered metabolism, leading the animal to cope with the stress situation, which involves carbohydrate and lipid mobilization. However, metabolism among other physiological functions is under circadian control within the liver (Juan *et al.*, 2019). During chronic stress, cortisol maintains the liver metabolic response by modulating carbohydrate- and lipid-related metabolism (Juan *et al*., 2019)

Effect of stress on the Endocrine system

During a challenging, threatening, or uncontrollable situation, the brain initiates a cascade of events involving the hypothalamic-pituitary-adrenal (HPA) axis, which is the primary driver of the endocrine stress response (APA, 2018). This ultimately results in an increase in the production of steroid hormones called glucocorticoids, which include cortisol, often referred to as the “stress hormone" (APA, 2018).

The Hypothalamo-pituitary adrenal axis

During times of stress, the hypothalamus, a collection of nuclei that connects the brain and the endocrine system, signals the pituitary gland to produce a hormone, which in turn signals the adrenal glands, located above the kidneys, to increase the production of cortisol (APA, 2018). Cortisol increases the level of energy fuel available by mobilizing glucose and fatty acids from the liver. Cortisol is normally produced in varying levels throughout the day, typically increasing in concentration upon awakening and slowly declining throughout the day, providing a daily cycle of energy (APA, 2018). During a stressful event, an increase in cortisol can provide the energy required to deal with prolonged or extreme challenge (APA, 2018).

Effect of stress on the Cardiovascular system

The effects of stress on the cardiovascular system are not only stimulatory, but also inhibitory in nature. It can be postulated that stress causes autonomic nervous system activation and indirectly affects the function of the cardiovascular system (Vrijkotte*et al.*, 2000; Yaribeygi, 2017). Upon activation of the sympathetic nervous system, there is an increase in heart rate (tachycardia), strength of contraction, vasodilatation in the arteries of skeletal muscles, a narrowing of the veins, contraction of the arteries in the spleen and kidneys, and decreased sodium excretion by the kidneys (Yaribeygi, 2017). Sometimes, stress activates the parasympathetic nervous system. Specifically, if it leads to stimulation of the limbic system, it results in a decrease, or even a total stopping of the heart-beat, decreased contractility, reduction in the guidance of impulses by the heart stimulus-transmission network, peripheral vasodilatation, and a decline in blood pressure.

The initial effect of stress on heart function is usually on the heart rate (Vrijkotte*et al.*, 2000). Depending upon the direction of the shift in the sympatho-vagal response, the heart beat will either increase or decrease (Hall *et al.*, 2004). Stress can stimulate the autonomic sympathetic nervous system to increase vasoconstriction, which can mediate an increase in blood pressure, an increase in blood lipids, disorders in blood clotting, vascular changes, atherogenesis; all of which can cause cardiac arrhythmias and subsequent myocardial infarction (Rozanski *et al.*, 1999; Yaribeygi*et al.*, 2017).

***Costusafer* (Nomenclature and Classification)**

Costusafer Ker-Gawl belongs to the domain Eukaryota, kingdom Plantae, and the family Zingiberaceae now known as Costaceae I (Edeoga and Boison, 2019). It is of the

genus *Costus* and species *afer*. The pharmacological significance that is attached to the use of the plant has led to numerous scientific research publications. *Costusafer Ker-Gawl* is usually an unbranched tropical plant often seen as a herb with a creeping rhizome. It is a relatively small monocot shrub which is commonly found in humid and monstrous forests and riverside. It is a perennial plant which can grow as tall as 4 m and bears white and yellow flowers (Ekpo*et al.,* 2008). *Costus* is pan tropical with about seventy species, of which forty are found in tropical America, twenty-five in West tropical Africa, and five in South-East Asia. In Africa, the plant is found in the forest belt from Senegal to Ethiopia and in the East to Tanzania. In tropical West Africa, it is found in the rain forest and riverbanks of countries including Ghana, Sierra Leone, Senegal, Guinea, Togo, Cameroon, and Nigeria (Atere*et al.,* 2018).

Nutritional and Phytochemical composition of *Costusafer*

*Costusafer*is used by the local folks due to its nutritional and medicinal properties. This involves the use of the plant parts such as leaf, stem, and the rhizome in preparation of food (Aweke, 2007). The proximate analysis of different parts of *Costusafer*shows the presence of both macro and micronutrients (Anyasor*et al.,* 2014). Both the leaves and stem are rich in macronutrients such as carbohydrate, crude protein, fat, ash, moisture, and a good source of fiber. There are also reports of the presence of certain vital nutrients such as vitamins B (1, 2, 3, 6, and 12), E, and C in the leaves (Ekpe, 2018).

The oil extracted from the plant is made up of 78% saturated fatty acids and 22% unsaturated fatty acids. The phytochemical analysis of the leaves, stem, and the rhizome of this plant in different solvents shows the presence of;

1. Alkaloids
2. Phenols
3. Saponins
4. Triterpenes
5. Tannins
6. Glycosides (Martins *et al.,* 2016).

These phytochemicals and nutrients may justify the nutraceutical use of the plant. Research on the chemical identification and isolation of bioactive compounds from *Costusafer*has been carried out, and this has led to the elucidation of structures from different parts of the plant. For instance, the rhizome is reported to contain steroidal saponins such as diocin, paryphyllin C, aferoside B, and aferoside C. Kaempferol-3-O-R-L-rhamnopyranoside, which is a flavonoid glycoside, has also been identified from the aerial part of the plant. Additional aferoside A and aferosides B and C have been isolated from the roots of *Costusafer*(Anyasor*et al.,* 2014).

Effect of *Costusafer* on the Cardiovascular system

Diosgenin (DSG) is a plant sterol saponin found in *costusafer*. More and more studies have reported that diosgenin has significant pharmacological activities such as anticancer, cardiovascular protection, hypolipidemic, anti-inflammatory, and neuroprotection (Jiang,

2019). Numerous preclinical studies have shown that DSG has great potential in the treatment of various cardiovascular diseases in vivo and in vitro, especially in atherosclerosis (Jiang, 2019).

Effect of *Costusafer* on the Reproductive system

Diosgenin is the material for the synthesis of hormonal products in the manufactory such as dehydroepiandrosterone (DHEA), the precursor of testosterone (Ching *et al.,* 2011). Studies also showed that diosgenin enhanced sperm motility and also increased the weight of accessory sex organs (Ching *et al.*, 2011).

The protective effect of aqueous leaf extract of *Costusafer*on lead-induced testicular damage was evaluated in a study reported by Ezejiofor and Orisakwe in 2019. The result showed insignificant changes in the weights of epididymis and testes when extract treated lead (Pb) group was compared with the normal control. Marked rise was noted in the sperm analysis, blood-Pb level, and luteinizing hormone (LH) and a decrease was observed in follicle-stimulating hormone (FSH) with non-significant changes in testosterone in the extract treated Pb group compared to the normal control. The outcome according to the researchers depicts the fact that aqueous leaf extract of*Costusafer*may be protective against lead-induced testicular damage (Ezejiofor and Orisakwe, 2017).

Effect of *Costusafer* on the Nervous system

Numerous studies have reported that Diosgenin a plant saponin found in *Costusafer*is useful in the prevention and treatment of neurological diseases (Bangrong*et al.,* 2020). Its therapeutic mechanisms are based on the mediation of different signaling pathways, and targeting these pathways might lead to the development of effective therapeutic agents for neurological diseases (Bangrong*et al.,* 2020).

Numerous studies have also demonstrated that Diosgenin and its derivatives have preventive and therapeutic effects against various neurological disorders. Animal experiments have shown that Diosgenin is active in the treatment of nervous system diseases such as Parkinson's disease and Alzheimer's disease (Bangrong*et al.,* 2020).

Effect of *Costusafer* on the Excretory system (Kidney)

In a disease state of the kidney, its detoxifying capacity is impaired. Toxicity of the kidney results in elevated concentrations of sodium and potassium in the serum and enlarged kidney. In cyclosporin-a- (Csa-) induced nephrotoxicity animal model, there is generally a significant elevation of serum K^+, Na^+, Blood Urea Nitrogen (BUN), and creatinine in negative control animals compared to the normal rats. A report published by Ezejiofor*et al.* (2017) indicates that oral administration of aqueous extract of *Costusafer*leaf showed a significant dose-dependent reduction of serum BUN and K^+. This depicts the fact that *Costusafer*leaves have nephroprotective property (Ezejiofor*et al.*, 2017).

Antidiabetic property and Hypolipidemic effect of *Costusafer*

Diabetes mellitus is a chronic hormonal and metabolic disorder that is characterized by a persistent increase in blood glucose levels. In an alloxan-induced rat model, there was a significant reduction in blood glucose level when *Costusafer*leaf extract at concentrations of 375, 750, and 1125 mg/kg and the control drug (glibenclamide 5 mg/kg) were orally given (Uwah, 2015). A study by Ezejiofor and colleagues reported in 2014, 2015, and 2017 showed that *Costusafer*leaf and stem extracts are able to reverse histopathological damage of pancreatic β-cells in alloxan-induced diabetes mellitus (Ezejiofor *et al.,* 2015).

According to a report by Ezejiofor and colleagues published in 2015, oral administration of 750 and 1125 mg/kg of *Costusafer*leaf extract produced a more prominent regeneration and repopulation of islet cells. The same research group in 2017 in a histopathological study of *Costusafer*stem extract on alloxan-induced damaged pancreatic cells noticed an organ protective effect. This therefore indicates that *Costusafer*leaf and stem extracts have pancreatic islet cell protective and regenerative effect that could be explored in managing type I diabetes mellitus (Ezejiofor*et al.*, 2017).

The concentration of lipids such as triacylglyceride (TAG), total cholesterol (TC), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) is highly regulated to avoid certain clinical conditions such as steatosis. This condition occurs when there is abnormal retention of lipids within a cell as a result of impairment in the normal synthesis and degradation of fats. Accumulation of these fats is often associated with disorders and diseases such as diabetes mellitus, obesity, and hepatitis C. When the body is unable to control fat regulation, there is the need for extracellular regulation, which includes the use of a natural product such as *Costusafer*. In both carbon tetrachloride-induced model (Njoku *et al.,* 2017) and streptozotocin-induced diabetic rat model (Ekpe *et al*., 2018); there was a significant rise in the TAG, TC, and LDL levels in negative control animals. On the administration of *Costusafer*extract, there was a significant improvement in the lipid profile as indicated by lowering serum TAG, TC, and LDL levels to near normal. Results from these studies indicate that *Costusafer*plant leaves could be explored in the management of diabetes mellitus and its complications such as dyslipidemia (Ekpe *et al*., 2018).

Lipids

Lipids are chemically defined as substances that are insoluble in water but soluble in alcohol, ether, and chloroform. Lipids are an important component of living cells (Charles, 2021). Together with carbohydrates and proteins, lipids are the main constituents of plant and animal cells. Cholesterol and triglycerides are lipids. Lipids are easily stored in the body. They serve as a source of fuel and are an important constituent of the structure of cells. Lipids include fatty acids, neutral fats, waxes and steroids (like cortisone). Compound lipids (lipids complexed with another type of chemical compound) comprise the lipoproteins, glycolipids and phospholipids (Charles, 2021).

Lipoprotein

Lipoproteins are special particles made up of droplets of fats surrounded by a single layer of phospholipid molecules. Phospholipids are molecules of fats which are attached to a phosphorus-containing group. They are distinctive in being amphipathic, which means they have both polar and non-polar ends (Thomas, 2018).

In a lipoprotein, the polar ends of all the phospholipid molecules face outwards, so as to interact with water, itself a polar molecule. This enables the lipoprotein to be carried in the blood rather than rising to the top, like cream on milk. The non-polar fat balled up inside the phospholipid layer, at the center of the lipoprotein, is thus transported to the place where it must be stored or metabolized, through the bloodstream, despite being insoluble in blood. Thus lipoproteins are molecular level trucks to carry fats wherever they are required or stored (Thomas, 2018).

Types of Lipoproteins

Different lipoproteins are differentiated based on specific proteins attached to the phospholipid outer layer, called the apo-lipoproteinn (Thomas, 2018). This also helps to make the fatty molecule more stable, and also binds to cell surface receptors in some cases, to enable the cell to take up the lipoprotein by receptor-mediated endocytosis. The types of lipoproteins with their function are as follows:

Chylomicrons

These are the largest and least dense of the lipoproteins, with the highest triglyceride content. They consist of a protein component synthesized in the liver, which wraps around diet-derived cholesterol and fats (Thomas, 2018). It travels from the intestinal lymphatics to the large veins, and sticks to the inner surface of the tiny capillary blood vessels inside the muscles and the fat storage cells in various parts of the body. There the fat is digested, while the cholesterol remains. This is now called the chylomicron remnant. It travels to the liver, where the cholesterol is metabolized. Thus chylomicrons deliver fats and cholesterol from the intestines to the muscles, fat cells and the liver (Thomas, 2018.)

VLDL (Very low Density Lipoprotein)

This is composed of protein, fats and cholesterol synthesized in the liver. It is associated with 5 different apoproteins, namely, B-100, C-I, C-II, C-III and E. It is converted to Intermediate Density Lipoprotein (IDL) and low density Lipoprotein (LDL) by removal of the apoproteins, except for one called apoprotein B100, along with esterification of the cholesterol. They are second only to chylomicrons in the percentage triglyceride content (Thomas, 2018).

IDL – Intermediate density lipoprotein, is created by the metabolism of VLDL.

LDL (Low Density Lipoprotein)

This is the last VLDL remnant, and contains chiefly cholesterol. The only apoprotein associated with it is apoB-100. Thus all these forms carry fats and cholesterol produced in the liver to the tissues (Thomas, 2018).

HDL (High Density Lipoprotein)

This has the highest protein: lipid ratio, and so is the densest. It has the apoprotein A-1. This is also called 'good cholesterol', because it carries cholesterol away from the tissues to the liver, lowering blood cholesterol levels. High HDL levels are associated with lowered risk of cardiovascular disease. HDL levels are higher with exercise, higher estrogen levels, with alcohol consumption, and weight loss (Thomas, 2018). Stress and medications such as progestins, anabolic steroids and benzodiazepines depress HDL (Rosa, 1988; Neger, 2017).

The Importance of Lipoproteins

Lipoproteins show varying patterns that correlate with the risk of having a fatal cardiovascular event. High LDL, VLDL and triglyceride levels are associated with a high risk of atherosclerosis and heart disease. High HDL is correlated with reduced cholesterol levels, and a lower cardiovascular risk. Thus high measurements of apo-A-1 correlates with a low atherosclerosis risk (Thomas, 2018). HDL levels drop with cigarette smoking, and rise with regular exercise, alcohol use, estrogen levels and weight loss (Thomas, 2018).

Lipid Profile

This is a panel of blood tests that serves as an initial screening tool for abnormalities of lipids such as cholesterol and triglycerides. An important part of the health evaluation is the lipid profile. This consists of measuring the total plasma cholesterol, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and High density lipoprotein (HDL) levels, as well as the triglyceride level. High cholesterol does not produce any signs or symptoms, so a blood test is essential to evaluate the risk of atherosclerosis (Onwe*et al.*, 2015).

Effect of stress on Lipid metabolism

A study carried out by Zahaĭko*et al.* (2008) on the effect of chronic social stress on lipid metabolism in golden Syrian hamsters showed that the increase of cholesteryl ester transfers protein (CETP) activity in HDL, which is observed at stress, can be accompanied by atherogenic LDL accumulation in the blood plasma. The chronic social stress is proatherogenic owing to lipid and lipoprotein metabolism changes, which lead to the shift of balance during lipids transport and their use by tissues (Zahaĭko*et al.*, 2008).

Effect of stress on Lipid profile

A research carried out by Negar also suggests that psychological stress was a risk factor for increasing triglycerides, LDL and decreasing HDL (Negar, 2017). He tested the abnormalities of lipids after stress exposure and his result showed the significance of HDL (good cholesterol) during or immediately after stress exposure. HDL plays an interesting role in that it removes LDL cholesterol from the walls of arteries. Low levels of HDL are linked to an increased risk of developing cardiovascular disease (Negar, 2017). During stressful conditions the body releases adrenaline and cortisol (stimulates fat metabolism for fast energy). Constant stress increases the mobilization of cholesterol in form of LDL from the liver to the cells. Cells use cholesterol but too much can build up in the arteries. This build up in the arteries (formation of plaque) can cause health problems (such as atherosclerosis) so that some scientists refer to LDL as "bad cholesterol". Atherosclerosis is a disease with metabolic, inflammatory and autoimmune elements (Ekaterina *et al*., 2016). Immune cells accumulate in the wall of arteries and in plaque (formed by the accumulation of LDL). This inflammatory reaction reduces the level of HDL and reduces the ability of HDL to participate in reverse cholesterol transport. HDL plays a role in mopping away of cholesterol back to the liver where it can be excreted. This is why HDL is termed as "good cholesterol". This high level of stress will also increase the level of triglycerides and VLDL. During constant stress, VLDL (which carries triglycerides) is produced from the liver and released into the bloodstream. It also contributes to the buildup of plaque in the arteries. VLDL along with LDL is sometimes called "bad cholesterol" (Feingold *et al.*, 2016).

MATERIALS AND METHODS

Materials

Materials used include: Animal Cages, weighing scale, dissecting kits, Dissecting boards and kits, flasks and beaker (50ml), cotton wool, syringes (1ml,5ml, 10ml) needles, canula, plain sample bottles, distilled water, chikun broiler pellet (crown flour mall manufacturers), soxhlet extractor(chemistry department UNICAL), Digital and manual weighing balance, rat beddings(saw dust from timber market), electric blender, rotary evaporator(chemistry department UNICAL), sample bottles containing gel, disposable gloves, etc.

Chemicals

Ketamine (Bez pharmacy), Dettol (Bez pharmacy), Methanol and Methylated spirit.

Plant

Costusafer leaves

Methods

Collection and Preparation of plant extract

The extract was prepared according to the methods employed by Sutharson*et al.* (2007). Fresh *Costusafer* leaves were gotten from a farm land in Calabar, Cross River state and authenticated by Mr. Damian Anthony Okachi of the department of Plant and Ecological studies, University of Calabar. The fresh leaf samples were washed with clean water to remove any dirt or sand present. The leaves were dried under room temperature for 28 days, after which they were grinded into coarse powder using an electric blender. The pulverized *Costusafer*were weighed to determine the initial weight thereafter, introduced into the extraction chamber of Soxhlet extractor and extracted with 80% methanol solvent. At the end of the extraction, the methanol was evaporated from the filtrate using hot plate under 40˚C. The extract was further stored at 4˚C in a refrigerator from which a stock solution was produced and used for the administration (Sutharson*et al.*, 2007)

Experimental animals

Thirty (30) male rats (weighing between 180-220g) were used for this study. They were purchased from the Genetics and Biotechnology departmental animal house, University of Calabar, and housed in the Faculty of Basic medical science animal house. Animals were maintained under standard laboratory conditions and housed in well ventilated plastic cages at room temperature (28-30°C) under controlled light cycles (12-hr light/12-hr dark).The animals were acclimatized for 2 weeks, during which they were fed with standard rat pellet and given free access to clean water *ad libitum*.

Ethical approval

Ethical approval was obtained from the animal ethical committee of the Faculty of Basic Medical Sciences, University of Calabar. All procedures used for the experimental animals were in accordance with the guidelines of the Faculty of Basic Medical Sciences, University of Calabar, Ethical committee.

Experimental Design

The animals were divided into six (6) groups of five (5) animals each.

1 Control group (CT)
2 Stress group (STR)
3 *Costusafer*200mg/kg body weight (CA1)
4 *Costusafer*400mg/kg body weight (CA2)
5 Stress + *Costusafer*200mg/kg body weight (STR +CA1)
6 Stress + *Costusafer*400mg/kg body weight (STR +CA2)

From Day 1 to Day 21*:*
Group 1, 3 and 4 = no treatment
Group 2, 5 and 6 = stress-induced

Day 22 to Day 42*:*
Group 1 - received only distilled water (orally)
Group 3 and 4 - received *C. afer*only (orally)
Group 2 - stress-induced + distilled water (orally)
Group 5 and 6 - stress-induced + *C.afer* administration (orally)
The experiment lasted for 42 days

Stress Induction

The Chronic unpredictable stress carried out was a modification of Katz *et al*. (1981); Lu *et al*. (2006) and Wilner*et al.* (1987).
The stress induction was as follows:
Day 1: Rats were placed in sealed cages containing water and without food for 3 hours.
Day 2: Stress was induced by poking their tails alongside food deprivation for 2 hours.
Day3: Stress was induced by shaking their cages for 3 hours.
Day 4: Animals were deprived of food and water 4 hours
Day 5: Stress was induced by noise exposure for 3 hours.
Day 6: Stress was induced by clustering rats in their cages overcrowding (without food) for 3 hours.
Day 7: Rats were deprived of sleep and food by continuous shaking of their cages for 2 hours.
Day8: Rats were placed in sealed cages containing water and without food for 3 hours.
Day 9: Rats were starved for 4 hours.
Day 10: Deprivation of sleep by poking their tails for 2 hours.
Day 11: Animals were overcrowded for 2 hours.
Day 12: Stress was induced by leaving them under intense sun rays for 1hour 30 minutes.
Day 13: Stress was induced by clustering rats for 3 hours.
Day 14: Stress induced by overcrowding 3 hours.
Day 15: Stress induced by noise exposure for 3 hours.
Day 16: Rats were subjected to stress by putting them in plastic cages containing water for 2 hours.
Day 17: Stress induced by clustering rats for 2 hours.
Day 18: Stress induced by starving rats for 4 hours.
Day 19: Stress was induced by exposure to noise for 2 hours.
Day 20: Stress was induced by exposure to sun rays for 4 hours.
Day21: Stress induced by shaking rat's cages for 3 hours.

The stress administered from day 1 to day 21 was same as that administered from day 22 to 42.

Dosage

The doses used in this study were considered following LD_{50} test carried out according Lorke's method (Lorke, 1983), in which zero mortality was recorded and *C. afer* was safe at 5000 mg/kg body weight.

Sacrifice (collection of blood samples)

At the end of the 42 days of stress and *Costusafer* administration, the animals were anesthetized using Ketamine. The abdomen was dissected up to the level of the mediastinum in order to locate the heart. Blood was further collected via cardiac puncture using a 5ml syringe into sample bottles containing gel. The blood was later centrifuged at 3000rpm for 15min. The serum was separated and used for the evaluation of biochemical parameters.

Lipid profile Analysis

Lipid analysis was performed using a spectrophotometric method with TECO diagnostic kits on blood samples obtained using heparinized bottles. The following parameters will be analyzed; Total cholesterol, High density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglyceride (TG). LDL and VLDL will be calculated using formula described by Friedewald (1972) as stated below;
LDL (mg/dl) = (Total cholesterol) – (High density lipoprotein) -(TG/5)
VLDL (mg/dl) =TG/5

Statistical Analysis

All values were presented as mean ± Standard error of mean (SEM). Statistical analysis was done using one-way analysis of variance (ANOVA), followed by a post hoc comparison test. All analysis was done using Graph Pad Prism (version 7). The statistical differences at level $p<0.05$ was considered significant.

RESULTS

Comparison of Serum Total cholesterol concentration of chronically unpredictable stressed Wistar rats treated with *Costusafer*

The mean serum total cholesterol (TC) concentration of stressed Wistar rats treated with *Costusafer*compared with control group were as follows; 0.37 ± 0.02, 0.58 ± 0.05, 0.34 ± 0.03, 0.40 ±0.04 and 0.41 ± 0.02 for control (CT), stress (STR), *Costus afer*200mg/kg (CA1), *Costusafer*400mg/kg (CA2), Stress + CA1 (STR+CA1) and Stress + CA2 (STR+CA2) respectively. The serum TC level significantly increased in STR ($p<0.01$) compared with control. However, TC significantly decreased in CA1 ($p<0.001$) and CA2 ($p<0.01$) as well as in STR+CA1 ($p<0.01$) and STR+CA2 ($p<0.05$) compared with STR.

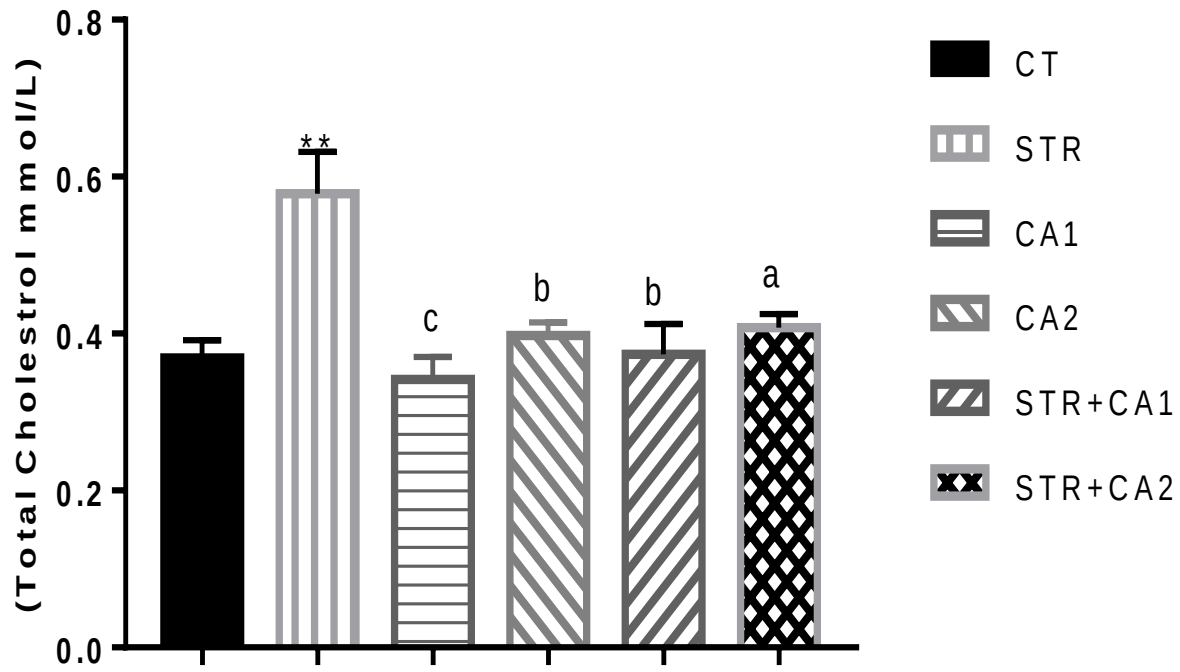

Fig. 1: comparison of serum total cholesterol levels in different experimental groups. Values are expressed as mean ± SEM, n = 5 ** p<0.01 vs CT, [a]p<0.05 vs STR, [b]p< 0.01 vs STR, [c]p<0.001 vs STR

Comparison of Serum High density lipoprotein (HDL) concentration of chronically unpredictable stressed Wistar rats treated with *Costus afer*

The mean serum High density lipoprotein (HDL) concentration of stressed Wistar rats treated with *Costus afer* compared with control group were as follows; 0.29 ± 0.04, 0.17 ± 0.01, 0.25 ± 0.03, 0.29 ± 0.01, 0.30 ± 0.03, 0.28 ± 0.02 for CT, STR, CA1, CA2, STR+CA1, STR+CA2 respectively. The serum HDL level significantly decreased in STR ($p<0.05$) compared with CT. However, serum HDL levels significantly increased in CA2 ($p<0.05$) and STR+CA1 ($p<0.05$) compared with STR.

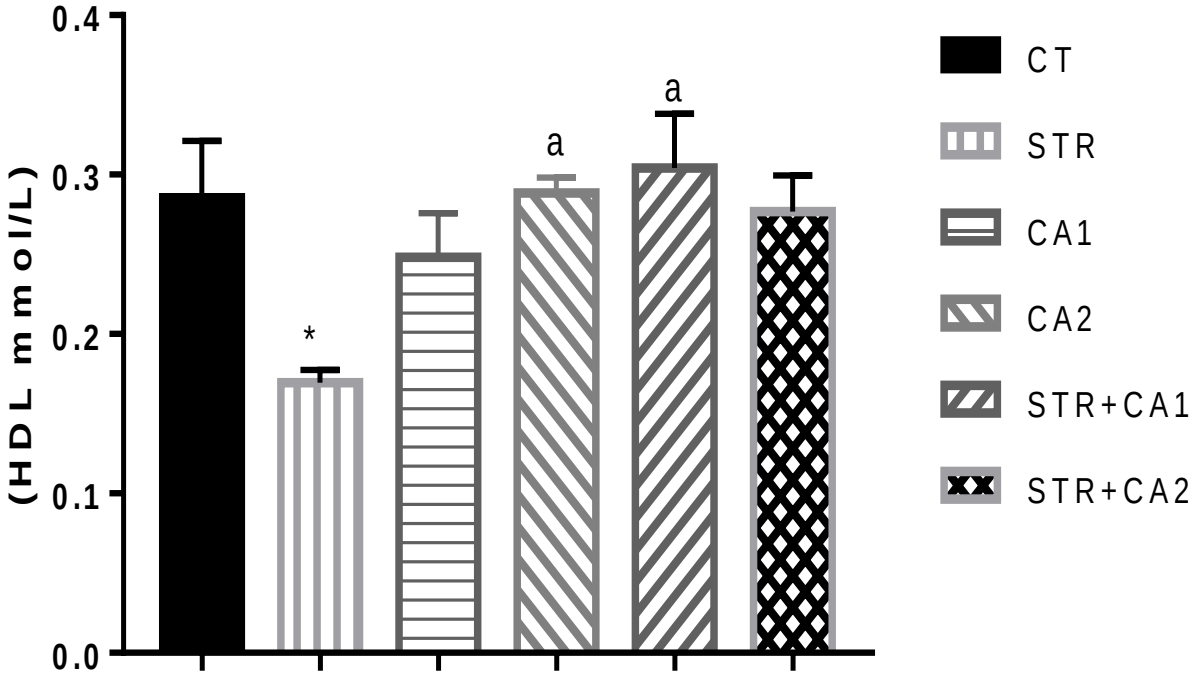

Fig. 2: Comparison of serum High density lipoprotein levels in different experimental groups. Values are expressed as mean ± SEM, n = 5

***p<0.05 vs CT, [a]p< 0.05 vs STR**

Comparison of Serum Triglyceride (TG) concentration of chronically unpredictable stressed Wistar rats treated with *Costus afer*

The mean serum Triglyceride (TG) concentration of stressed Wistar rats treated with *Costus afer* compared with control group are as follows; 0.15 ± 0.02, 0.26 ± 0.02, 0.14 ± 0.02, 0.15 ± 0.01, 0.18 ± 0.03, 0.14 ± 0.03 for CT, STR, CA1, CA2, STR+CA1, STR+CA2 respectively. The serum TG level significantly increased in STR ($p<0.05$) compared with CT. However, serum TG levels significantly decreased in CA1 ($p<0.05$), CA2 ($p<0.05$) and STR+CA2 ($p<0.05$) compared with STR.

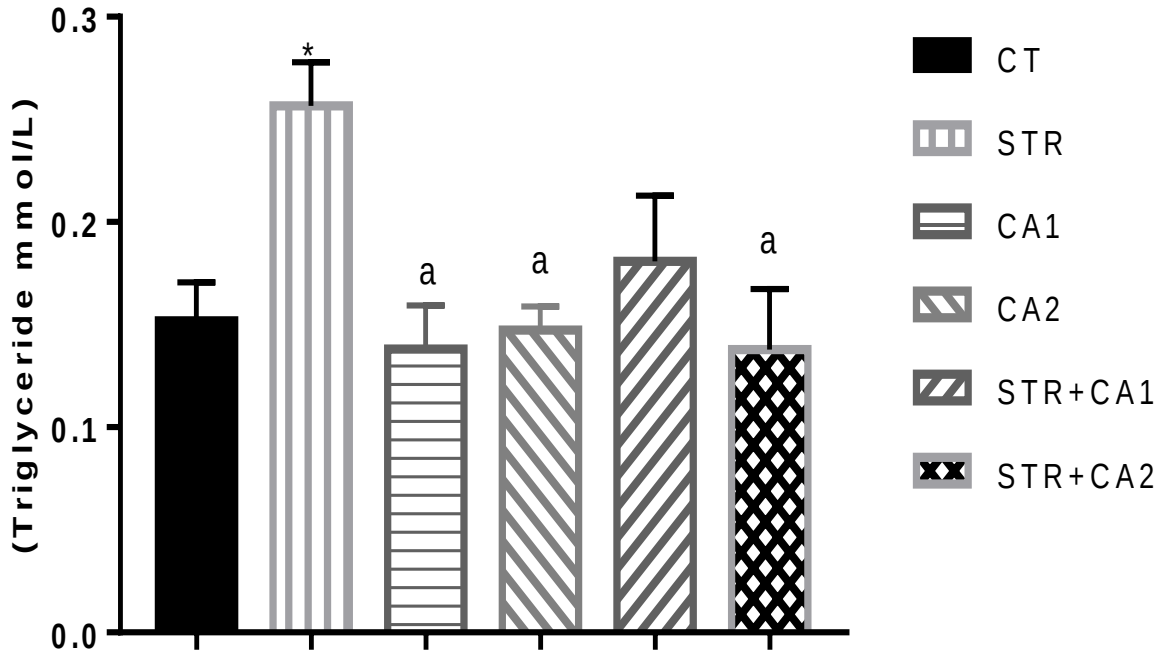

Fig. 3: Comparison of serum Triglyceride (TG) levels in different experimental groups.
Values are expressed as mean ± SEM, n = 5
***p<0.05 vs CT, [a]p<0.05 vs STR**

Comparison of Serum Very low density lipoprotein (VLDL) concentration of chronically unpredictable stressed Wistar rats treated with *Costus afer*

The mean serum Very low density lipoprotein (VLDL) concentration of stressed Wistar rats treated with *Costus afer* compared with control group were as follows; 0.69 ± 0.01, 0.12 ± 0.01, 0.06 ± 0.01, 0.07 ± 0.01, 0.08 ± 0.01, 0.06 ± 0.01 for CT, STR, CA1, CA2, STR+CA1, STR+CA2 respectively. The serum VLDL level significantly increased in STR ($p<0.05$) compared with CT. However, serum VLDL levels significantly decreased in CA1 ($p<0.05$), CA2 ($p<0.05$) and STR+CA2 ($p<0.05$) compared with STR.

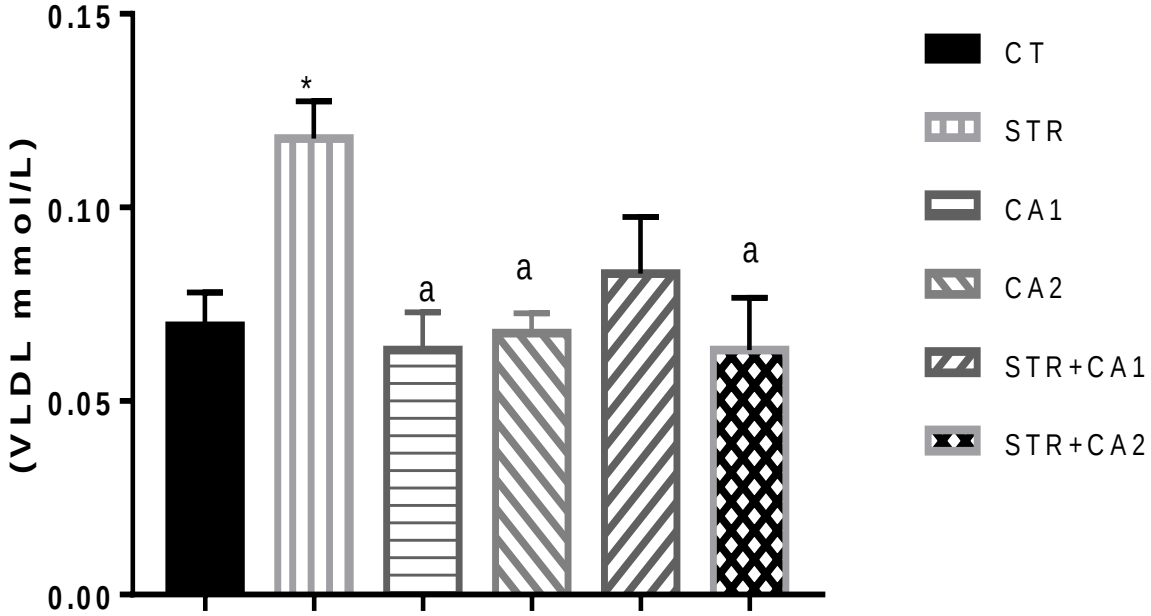

Fig. 3: Comparison of serum Very low density lipoprotein (VLDL) levels in different experimental groups. Values are expressed as mean ± SEM, n = 5

***p <0.05 vs CT, [a]p<0.05 vs STR**

Comparison of Serum low density lipoprotein (LDL) concentration of chronically unpredictable stressed Wistar rats treated with *Costus afer*

The mean serum low density lipoprotein (LDL) concentration of stressed Wistar rats treated with *Costus afer* compared with control group were as follows; 0.04 ± 0.01, 0.29 ± 0.05, 0.03 ± 0.01, 0.04 ± 0.01, 0.05 ± 0.01, 0.07 ± 0.02 for CT, STR, CA1, CA2, STR+CA1, STR+CA2 respectively. The serum LDL level significantly increased in STR ($p<0.0001$) compared with CT. However, serum LDL levels significantly decreased in CA1 ($p<0.0001$), CA2 ($p<0.0001$), STR+CA1 ($p<0.0001$) and STR+CA2 ($p<0.0001$) compared with STR.

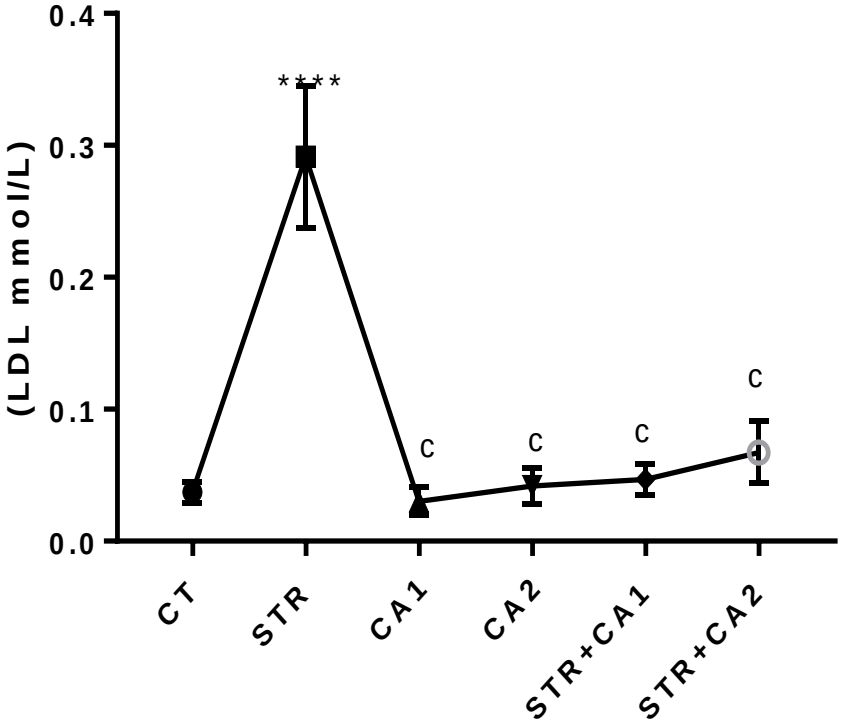

Fig. 4: Comparison of serum low density lipoprotein (LDL) levels in different experimental groups.
Values are expressed as mean ± SEM, n = 5
******p<0.0001 vs CT; ^{d}p<0.0001 vs STR**

DISCUSSION AND CONCLUSION

Discussion

The aim of the study was to determine the effects of *Costus afer* on the lipid profile of chronically stressed and non-stressed male Wistar rats. Chronic unpredictable stress was used to induce stress on animals. It has to do with the random, intermittent, and unpredictable exposure to a variety of stressors during several weeks (Monterio *et al.*, 2015). Lipid profile, a panel of blood test consisting of Total cholesterol (TC), Triglyceride (TG), High density Lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) was assessed after the serum was collected through cardiac puncture.

Negar (2017) suggested that stress affects lipid profile, specifically the level of HDL (good cholesterol), LDL (bad cholesterol) and TG. The significant reduction of HDL, an increased level of LDL, TG and TC, results in a higher risk of death from cardiovascular cause (cardiovascular diseases).

The results of the current study showed that the different parameters of the lipid profile of animals except HDL (which decreased significantly) increased significantly

following the exposure to chronic stress. The group of animals that were administered *Costus afer* after subjection to stress showed a significant decrease in LDL, TG, VLDL and TC. This revealed the negative effects of stress on the lipid profile parameters on animals, and the antilipidemic property of *Costus afer*. This is in line with a research carried out by Ekpe *et al*. (2018) which showed significant improvement in the lipid profile of animals as indicated by lowering serum TG, TC, and LDL levels to near normal after administration of *Costus afer* (Ekpe *et al*., 2018).

HDL plays an important role in removing LDL cholesterol from the walls of arteries (Feingold *et al*., 2016). Low levels of HDL are linked to an increased risk of developing cardiovascular diseases (Negar, 2017). During stressful conditions, the body releases adrenaline and cortisol (which stimulates fat metabolism for fast energy). Constant stress increases the mobilization of cholesterol in form of LDL from the liver to the cells. Cells use cholesterol but too much can build up in the arteries. This build up in the arteries (formation of plaque) can cause health problems (such as atherosclerosis) so that some scientists refer to LDL as "bad cholesterol" (Ekaterina *et al.*, 2016).

The results of this study showed that HDL level significantly decreased in STR group when compared to the CT group. This was reversed in the STR+CA1 and STR+CA2 stressed groups that were treated with *Costus afer*. HDL levels also increased in the non-stressed groups.

Studies have shown that high level of stress will also increase the level of triglycerides and VLDL (Ekaterina *et al.*, 2016). During constant stress, VLDL (which carries triglycerides) is produced from the liver and released into the bloodstream. It also contributes to the buildup of plaque in the arteries. VLDL along with LDL is sometimes called "bad cholesterol" (Feingold *et al.,* 2016). The results of the present study showed that the STR group level of TG, TC and VLDL increased significantly when compared to the CT group. Although other groups in which *Costus afer* was administered showed a significant reduction of TG, TC and VLDL near normal thereby reducing the risk of cardiovascular diseases.

Conclusion

The study has shown that exposure to chronic unpredictable stress causes significant increase in bad cholesterol (VLDL and LDL) and decrease in good cholesterol (HDL) thus increasing the chances of developing cardiovascular diseases. This abnormality in lipid profile reported in the study was however reversed following *Costus afer* treatment, indicating that *Costus afer* exhibit antilipidemic effect against lipid profile alterations associated with chronic stress.

REFERENCES

Ahmed, S. M., Hershberger, P. J., Lemkau, J. P., (2016). Psychosocial influences on health. *Textbook of Family Medicine* Elsevier 9(3), 25 -33.

American Heart Association (2021). Managing stress to control high blood pressure. Retrieved on the 10th September, 2021 from http://www.heart.org/en/health-topics/high-blood-pressure.

American Psychological Association., APA (2018). Stress effects on the body. Retrieved on 7th September, 2021 from http://www.apa.org/topics/stress/body.

Anyasor, G. N., Onajobi, F. D., Osilesi, O., & Adebawo, O., (2014). Hexane fraction of *Costus afer ker-Grawl* leaves inhibited mitochondrial membrane permeability transition, FIFO ATPase and scavenged nitric oxide and hydrogen peroxide. *American Journal of Physiology, Biochemitry and phamarcology*, 3(2), 79-85.

Atere, T. G., Akinloye, O. A., Ugbaja, R. N., Ojo, D. A., & Dealtry, G. (2018). In vitro antioxidant capacity and free radical scavenging evaluation of standardized extract of *Costus afer* leaf. Food Science and Human Wellness, 7(4), 266-272.

Aweke, G. (2007). *Costus afer* ker gawl. Record from PROTA4U. PROTA (Plant Resources of Tropical Africa/Ressourcesvégétales del'Afrique tropicale). PROTA Foundation, Wageningen, Netherlands.

Bobryshev, Y. V., Ivanova, E. A., Chistiakov, D. A., Nikiforov, N. G., & Orekhov, A. N. (2016). Macrophages and Their Role in Atherosclerosis: Pathophysiology and Transcriptome Analysis. BioMed research international, 2016, 9582430. https://doi.org/10.1155/2016/9582430

Cai, B., Zhang, Y., Wang, Z., Xu, D., Jia, Y., Guan, Y., ... & Li, J. (2020). Therapeutic potential of diosgenin and its major derivatives against neurological diseases: recent advances. Oxidative medicine and cellular longevity, 2020.

Carrasco, G. A., & Van de Kar, L. D. (2003). Neuroendocrine pharmacology of stress. European journal of pharmacology, 463(1-3), 235-272.

Charles, patrick Davies., (2021). Retrieved on 5th August, 2021 from https://www.rxlist.com/lipid/definition.htm

Daniel, Boison., Cynthia, A. A., Godwin, K. B., Olga, Q., Rosemary, A., Wiabo-Asabil, G. K., Adinotey, M. B., (2019). *Costus Afer*: A systematic review of evidence-based data in support of its medicinal relevance. *Scientifica, 2019.*

De kloet, E. R., Oitzl, M. S., & Joels, M. (1999). "Stress and cognition : are corticosteroids good or bad guys?". *Trends in neurosciences,* 22(10), 422-426. doi :10.3389/fgene.2012.00222.

Edeoga, H. O., & Okoli, B. E. (1998). Anatomy and systematics in the Costus afer-C. lucanusianus complex (Costaceae). *Acta phytotaxonomica et geobotanica*, 48(2), 151-158.

Ekpe, I. P., Udosen, E. O., & Amaechi, D. (2018). Evaluation of some vitamins and macro-nutrients composition of ethanolic extract of tecoma stans and *Costus afer* leaves. *International Journal of Biochemistry Research & Review*, 23(4), 1-5.

Ekpe, I., Udosen, E. O., Amaechi, D., & Yisa, B. (2018). Impact of ethanolic extract of Tecoma stans and *Costus afer* leaves on lipid profile status of streptozotocin induced diabetic Wistar rats. *International Journal of Sciences*, 4(8), 16-20.

Ekpo, B. A., Bala, D. N., Essien, E. E., & Adesanya, S. A. (2008). Ethnobotanical survey of Akwa Ibom state of Nigeria. *Journal of Ethnopharmacology*, 115(3), 387-408.

Ezejiofor, A. N., & Orisakwe, O. E. (2019). The protective effect of *Costus afer Ker Gawl* aqueous leaf extract on lead-induced reproductive changes in male albino Wistar rats. JBRA assisted reproduction, 23(3), 215.

Ezejiofor, A. N., Igweze, Z. N., Udowelle, N. A., & Orisakwe, O. E. (2017). Histopathological and biochemical assessments of *Costus afer* stem on alloxan-induced diabetic rats. *Journal of basic and clinical physiology and pharmacology*, 28(4), 383-391.

Ezejiofor, A. N., Orish, C. N., & Orisakwe, O. E. (2015). Morphological changes in the pancreas and glucose reduction of the aqueous extract of *Costus afer* leaf on alloxan-induced diabetic rats. *Journal of basic and clinical physiology and pharmacology*, 26(6), 595-601.

Ezejiofor, A. N., Udowelle, N. A., & Orisakwe, O. E. (2017). Nephroprotective and antioxidant effect of aqueous leaf extract of *Costus afer* Ker gawl on cyclosporin-a (Csa) induced nephrotoxicity. *Clinical Phytoscience*, 2(1), 1-7.

Feingold, K. R., & Grunfeld, C. (2016). Effect of inflammation on HDL structure and function. *Current opinion in lipidology*, 27(5), 521-530.

Goya, R. G., Castro, M. G., Hannah, M. J., Sosa, Y. E., & Lowry, P. J. (1993). Thymosin peptides stimulate corticotropin release by a calcium-dependent mechanism. Neuroendocrinology, 57(2), 230-235.

Hall, M., Vasko, R., Buysse, D., Ombao, H., Chen, Q., Cashmere, J. D., ... & Thayer, J. F. (2004). Acute stress affects heart rate variability during sleep. Psychosomatic medicine, 66(1), 56-62.

Hernández-Pérez, J., Naderi, F., Chivite, M., Soengas, J. L., Míguez, J. M., & López-Patiño, M. A. (2019). Influence of stress on liver circadian physiology. A study in rainbow trout, Oncorhynchus mykiss, as fish model. *Frontiers in physiology*, 10, 611.

Jesus, M., Martins, A. P., Gallardo, E., & Silvestre, S. (2016). Diosgenin: recent highlights on pharmacology and analytical methodology. *Journal of analytical methods in chemistry*.

Kaigas, K., Camilla, N., Pocock, R., (2012). Neuronal response to physiological stress.

Katz RJ, Roth KA, Carroll BJ (1981). Acute and chronic effects onopen field activity in the rat: implications for a model ofdepression. *NeurosciBiobehav Revs* 5: 247–251

Khansari, D. N., Murgo, A. J., & Faith, R. E. (1990). Effects of stress on the immune system. Immunology today, 11, 170-175.

Kiecolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Psychoneuroimmunology: psychological influences on immune function and health. *Journal of consulting and clinical psychology*, 70(3), 537.

Koenisberg, M. D., & Schwartz, J. A., (1987). Naloxone-induced pulmonary edema. *Annals of Emergency Medicine*, 16(11),1294-1296.

Konturek, P. C., Brzozowski, T., & Konturek, S. J. (2011). Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*, 62(6), 591–599.

La Rosa J. C. (1988). The varying effects of progestins on lipid levels and cardiovascular disease. *American journal of obstetrics and gynecology*, 158(6 Pt 2), 1621–1629. https://doi.org/10.1016/0002-9378(88)90200-1

Lu X-Y, Kim CS, Frazer A, Zhang W (2006).Leptin: a potentialnovel antidepressant. *ProcNatlAcadSci* USA 103: 1593–1598.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature reviews neuroscience, 10(6), 434-445.

McEwen, B. S., Weis, J. M., & Schwartz , L. S., (1986). Selective retension of corticosterone by limbic structures in rat brain. *Nature*, 220(5170), 911-912.

Monterio, S., Roque, S., de Sa-calcada, D., Sousa, N., Correia-Neves, M., & Cerqueira, J. J., (2015). An efficient chtonic unpredicatable stress protocol to induce stress-related responses in C57BL/6 mice. *Frontiers in psychiatry*, 6, 6.

Njoku, U. O., Nwodo, O. F. C., & Ogugofor, M. O. (2017). Cardioprotective potential of methanol extract of *Costus afer* leaf on carbon tetrachloride-induced cardiotoxicity in albino rats. *Asian Journal of Pharmaceutical Research and Health Care*, 9(2), 51-58.

Onwe, P., Folawiyo, A. M., Okike, P. (2015). Lipid profile and growing concern on lipid related disease. *IOSR Journal of pharmacy and biological sciences* 10(5), 22-27.

Paul, Greene., (2020). What is Episodic stress?. Manhattan centre for cognitive Behavioural Therapy.

Reiche, E. M. V., Nunes, S. O. V., & Morimoto, H. K. (2004). Stress, depression, the immune system, and cancer. The lancet oncology, 5(10), 617-625.

Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation, 99(16), 2192-2217.

Sarahian, N., Sahraei, H., Zardooz, H., Alibeik, H., & Sadeghi, B. (2014). Effect of memantine administration within the nucleus accumbens on changes in weight and volume of the brain and adrenal gland during chronic stress in female mice. Pathobiology Research, 17(2), 71-82.

Scantamburlo, G., Ansseau, M., Legros, J. J., (2001). Role of the neurohypophysis in psychological stress. Encephale 27 : 245-259.

Scott, Elizabeth., (2020). Stress management and its effect on health. Retrieved on 10th September, 2021 from https://www.veryellmind.com/stress-and-health-3145086.

Seyedeh, Negar (2017). Effects of psychological stress and physical work on blood lipid profiles. *Journal of medicine* doi:1097/MD.0000000000006816.

Sharma, Dushyant., (2018). Physiology of stress and its management. *Journal of Medicine* 1:1-5.

Shawna, Freshwater., (2018) 3 types of stress and health hazards. *Spacious therapy* 17

Sutharson, L., Lila, K. N., Prasanna, K. K., Shila E. B. &Rajan, V. J. (2007). Anti-inflammatory and anti-nociceptive activities of methanolic extract of the leaves of *Fraxinus floribunda* Wallic.*African Journal of Traditional, Complementary and Alternative Medicines,* 4(4):411-416.

Taiwo, A.O., & Bolanle, A. A (2003). The leaf essential oil of *Costus afer ker-Grawl* from Nigeria. *Flavour and fragrance Journal*, 18(4), 309-311.

Thierry, A. M., Javoy, F., Glowinski, J., & KETY, S. S. (1968). Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. *Journal of Pharmacology and Experimental Therapeutics*, 163(1), 163-171.

Thomas, Liji. (2018). What are Lipoproteins?.Retreived on 9th September, 2021 from https://www.news-medical.net/amo/life-sciences/what-are-Lipoproteins.aspx.

Uwah, A. F., Ewere, E. G., & Ndem, J. I. (2015). Hypoglycemic and haematologic effects of crude stem juice of *Costus afer* on alloxaninduced diabetic Wistar rats. *American Journal of Ethnomedicine*, 2(4), 2348-9502.

Vrijkotte, T. G., Van Doornen, L. J., & De Geus, E. J. (2000). Effects of work stress on ambulatory blood pressure, heart rate, and heart rate variability. *Hypertension*, 35(4), 880-886.

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987).Reduction of sucrose preference by chronic unpredictable mildstress, and its restoration by a tricyclic antidepressant.*Psychopharmacology*93: 358–364.

Wu, F. C., & Jiang, J. G. (2019). Effects of diosgenin and its derivatives on atherosclerosis. Food & function, 10(11), 7022–7036.

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function : A review. *EXCLI journal*, 16, 1057.

Yu, C. H., Wang, K. L., Hsu, R. L., Ho, Y. J., & Wang, P. S. (2011). Effects of Diosgenin on the Reproductive Function of D-galactose-induced Aging Model of Male Rats.

Zahaĭko, A. L., Voronina, L. M., Kaliman, P. A., & Strel'chenko, K. V. (2008). *Ukrains'kyi biokhimichnyi zhurnal* (1999), 80(4), 120–129.

Treatment/Groups	**Mean**	**SEM**	**SD**
HDL			
CT	0.2854	0.03566	0.07975
STR	0.1696	0.007884	0.01763
CA1	0.2842	0.02776	0.06207
CA2	0.2884	0.009621	0.02151
STR+CA1	0.3042	0.03395	0.07592
STR+CA2	0.2769	0.2246	0.05022
Total cholesterol			
CT	0.3688	0.02286	0.05112
STR	0.5786	0.05302	0.1186
CA1	0.3417	0.02856	0.06387
CA2	0.3978	0.01632	0.03649
STR+CA1	0.3738	0.03882	0.03928
STR+CA2	0.4074	0.01757	0.03928
Triglyceride			
CT	0.1519	0.01868	0.04178
STR	0.2566	0.02116	0.04731
CA1	0.1382	0.02131	0.04766
CA2	0.1474	0.0114	0.0255
STR+CA1	0.181	0.03188	0.07129

STR+CA2	0.1379	0.02962	0.6623
VLDL			
CT	0.06944	0.01913	0.008557
STR	0.1178	0.009661	0.0216
CA1	0.06323	0.009732	0.02176
CA2	0.06752	0.005224	0.01168
STR+CA1	0.08292	0.01461	0.03266
STR+CA2	0.06316	0.01357	0.03034
LDL			
CT	0.03707	0.008062	0.01803
STR	0.2914	0.05379	0.1203
CA1	0.03023	0.01058	0.02367
CA2	0.004187	0.01344	0.03006
STR+CA1	0.04668	0.01163	0.02601
STR+CA2	0.06732	0.02336	0.05224