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Antioxidant Activities of *Desmodiumadscendens*;a Trial on WistarRats

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Abstract:*Antioxidants are substances that inhibit oxidation, especially one used to counteract the deterioration of stored food products. They are substances that remove potentially damaging oxidizing agents in the body of human and other living organisms. Desmodiumadscendens is a medicinal herb commonly used to manage several disease conditions. The current study determines the antioxidant activities of the herb, relating them to the phytochemistry of the herb.Twenty-four (24) rats were grouped into four (n=6): A, B, C, and D. While group A served as control, groups B, C, and D were administered low, median, and high doses of aqueous leave extract of the herb. The antioxidant effect of the herb was then determined across the various doses based on the determination of serum concentration of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Malondialdehyde (MDA). There was no significant (p>0.05) effect of Desmodiumadscendenson SOD in the groups treated with low, median and high doses of the extract when compared with the control group. There was however a significant (p<0.05) increase in serum concentration of catalase (CAT) in the groups treated with medium and high doses when compared with the control (32.30 ±2.16 nmmol/mL) and low dose (25.97 ±2.20 nmol/mL) groups. Glutathione peroxidase (GPx) concentration also increased significantly in the groups treated with median and high doses of extract when compared at p<0.05 with control (78.38 ±4.94 nmol/mL) and low dose (72.52 ±3.18 nmol/mL) groups. To determine lipid peroxidation, Malondialdehyde (MDA) concentration decreased significantly in all the test groups when compared with control (0.57 ±0.07 nmol/L).Extract may improve health condition as it possesses the body's ability to mop-up free radicals by increasing anti-oxidant enzymes concentration, and preventing lipid peroxidation with decreased MDA.*

Keywords:*Glutathione peroxidase, Superoxide dismutase, Catalase, Malondialdehyde, Freeradicals, Lipid peroxidation*

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I. Introduction

Free radicals

During normal metabolic functions, highly reactive compounds called free radicals are generated in the body, they could also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence becoming highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them, causing vital cellular structures and functions to be lost and ultimately resulting in various pathological conditions **[1].**

Antioxidant enzymes

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals.

It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of food rich in antioxidant enzymes or antioxidant enzyme supplements, the body's potential in reducing the risk of free radicals and related health problems is improved. Antioxidant enzymes are, therefore critical for maintaining optimal cellular and systemic health and well-being **[1].**

Activities of free radicals

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the

initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. The ability of the cell to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS). About 5% or more of the inhaled O_2 is converted to ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals by univalent reduction of O_2 **[1].**Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect**[1].**

This antioxidant system includes, antioxidant enzymes (e.g. Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods. Whenever the balance between ROS production and antioxidant defence is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions**[2].**

1. Superoxide dismutase (SOD)

In 1967 biochemist Irwin Fridovitch of Duke University and Joe McCord discovered the antioxidant enzyme SOD, which provides an important means of cellular defence against free radical damage. This breakthrough caused medical scientists to begin to look seriously at free radicals. In most cases the process is automatically controlled and the number of free radicals does not become dangerously high. Fortunately, the body has, throughout the course of millions of years of evaluation become accustomed to coping with free radicals and has evolved various schemes for doing this **[2,3].**

SOD (EC 1.15.1.1) is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions **[4,5,6,].**

$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ (catalysed by SOD)

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) **[7,]**SOD destroys O_2- by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates **(Donovan *et al*.,1998) [8].** All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe-, Mn- or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N3- , CN-**,** and by F-**[9].**Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit those cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide**[10].**The respiratory chain in mitochondria is a major source of oxygen radicals.Mn-SOD has been shown to be greatly induced and depressed by cytokines, but is only moderately influenced by oxidants **[10].**Inactivation of recombinant human mitochondrial Mn- SOD by peroxynitrite is caused by nitration of a specific tyrosine residue **[11].**The biological importance of Mn-SOD is demonstrated among others by the following observations: (a) inactivation of Mn-SOD genes in *Escherichia coli* increases mutation frequency when grown under aerobic conditions**;** (b) elimination of the gene in *Saccharomyces cerevisiae*increases its sensitivity to oxygen, (c) lack of expression in Mn- SOD knockout mice results in dilated cardiomyopathy and neonatal lethality**;** (d) tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu/Zn- SOD, CAT or GPX mRNA in various mouse tissues and cultured cells**;** (e) transection of Mn- SOD cDNA into cultured cells rendered the cells resistant to**;** f) expression of human Mn-SOD genes in transgenic mice protects against oxygen induced pulmonary injury and Adriamycin-induced cardiac toxicity**[12, 13,14].**

This enzyme has been known to promote the rejuvenation and repair of cells, while reducing the damages caused by free radicals. SOD is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts. Among the common natural sources of SOD are cabbage, Brussels sprouts, wheat grass, barley grass and broccoli. SOD plays a significant role in preventing the development of the Lou Gehrig's disease, also known as Amyotrophic Lateral Sclerosis (ALS). This kind of illness can lead to death because it affects the nerve cells in the spinal cord and the brain.Apart from that, this enzyme is also used for treatment of inflammatory diseases, burn injuries, prostate problems, arthritis, corneal ulcer, and reversing the long term effects of radiation and smoke exposure. Additionally, if superoxide dismutase is made into a lotion and applied to the skin, it will prevent the formation of wrinkles. It will also heal wounds, reduce the appearance of scars, and lighten skin pigmentation that has been caused by UV rays **[15].**

SOD is also known to help carry nitric oxide into our hair follicles. This is beneficial for people who are experiencing premature hair loss due to a genetic predisposition or free radicals. Because this enzyme is a very potent antioxidant, SOD combats the effects of free radicals that are causing hair follicles to die. Since nitric oxide relaxes the blood vessels and allows more blood to circulate to the hair follicles and SOD helps to

remove the free radicals, hair loss can be prevented and even reversed. Taking dietary supplements that provide an adequate supply of Superoxide dismutase will be helpful in maintaining overall well-being and health because it protects our entire body from the harmful effects of free radicals **[15].**

2. Catalase

Catalase (CAT) is an enzyme responsible for the degradation of hydrogen peroxide. It is a protective enzyme present in nearly all animal cells.

Specificity

The reaction of CAT occurs in two steps. A molecule of hydrogen peroxide oxidizes the heme to an oxyferryl species. A porphyrincation radical is generated when one oxidation equivalent is removed from iron and one from the porphyrin ring. A second hydrogen peroxide molecule acts as a reducing agent to regenerate the resting state enzyme, producing a molecule of oxygen and water.

CAT (EC 1.11.1.6) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa**[16].** CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity.

In animals, hydrogen peroxide is detoxified by CAT and by GPX. CAT protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Survival of rats exposed to 100% oxygen was increased when liposome's containing SOD and CAT were injected intravenously before and during the exposure **[17].** The increased sensitivity of transfected CAT-enriched cells to some drugs and oxidants is attributed to the property of CAT in cells to prevent the drug-induced consumption of O_2 either for destroying H_2O_2 to oxygen or for direct interaction with the drug **[18].**

Application:

CAT is used in the food industry for removing hydrogen peroxide from milk prior to cheese production. Another use is in food wrappers where it prevents food from oxidizing CAT is also used in the textile industry, removing hydrogen peroxide from fabrics to make sure the material is peroxide-free. A minor use is in contact lens hygiene - a few lens-cleaning products disinfect the lens using a hydrogen peroxide solution; a solution containing CAT is then used to decompose the hydrogen peroxide before the lens is used again. Recently, CAT has also begun to be used in the aesthetics industry. Several mask treatments combine the enzyme with hydrogen peroxide on the face with the intent of increasing cellular oxygenation in the upper layers of the epidermis**[18]**.

3. Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The GPx (EC 1.11.1.19) contains a single selenocysteineselenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity **[17]** GPX (80 kDa) catalyses the reduction of hydro peroxides using GSH, thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential antioxidative defence mechanisms.

There are five GPxisoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type. Cytosolic and mitochondrial glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione. GPX1 and the phospholipid hydroperoxide glutathione peroxidase (PHGPX or GPX4) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. PHGPX can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins **[19].** GPX1 is predominantly present in erythrocytes, kidney, and liver, and GPX4 is highly expressed in renal epithelial cells and testes. Cytosolic GPX2 or GPX-G1, and extracellular GPX3 or GPX-P is poorly detected in most tissues except for the gastrointestinal tract and kidney, respectively. Recently, a new member, GPX5, expressed specifically in mouse epididymis, is interestingly selenium-independent **[20].** Although GPX shares the substrate, H_2O_2, with CAT, it alone can react effectively with lipid and other organic hydroperoxides, being the major source of protection against low levels of oxidant stress.

This is one of the most important enzymes in the body with antioxidant properties. Levels of GPx in the body are closely linked with that of glutathione, the master antioxidant. Glutathione (GSH for short) is a tripeptide that not only protects the cells against ill effects of pollution; it is also acts as your body's immune system boosters. It is present in high concentrations in the cells and plays a pivotal role in maintaining them in reduced state lest they suffer damage by oxidation (from free radicals). The role as antioxidant is particularly

important for brain as it is very sensitive to presence of free radicals. Combination of certain antioxidants like glutathione, vitamin C and E, selenium and glutathione peroxidase are very powerful in helping the body fight against the free radicals. GSH ensures that the red blood cells remain intact and protect the white blood cells (which are responsible for immunity). Glutathione is found in vegetables and fruit, but cooking will significantly reduce its potency. Taking it as a supplement is a good idea.

Clinical applications of antioxidant enzymes

1. Chronic Inflammation: Chronic inflammatory diseases such as rheumatoid arthritis are self-perpetuated by the free radicals released by neutrophils. Both corticosteroids and non-steroids anti-inflammatory drugs interfere with formation of free radicals and interrupt the disease process.
2. Acute Inflammation:At the inflammatory site, activated macrophages produce free radicals. Respiratory burst and increased activity of NADPH oxidase are seen in macrophages and neutrophils.
3. Respiratory Diseases: Breathing of 100 % oxygen for more than 24 hr produces destruction of endothelium and lung edema. This is due to the release of free radicals by activated neutrophils **[21].** In premature newborn infants, prolonged exposure to high oxygen concentration is responsible for bronchopulmonary dysplasia. Adult respiratory distress syndrome (ARDS) is characterized by pulmonary edema. ARDS is produced when neutrophils are recruited to lungs which subsequently release free radicals. Cigarette smoking enhances the emphysema in alpha-1 protease inhibitor deficiency. Cigarette smoke contains free radicals. Soot attracts neutrophils to the site which releases more free radicals. Thus, there is more elastase and less protease inhibitor, leading to lung damage.
4. Diseases of the Eye:Retrolental fibroplasia or retinopathy of prematurity is a condition seen in premature infants treated with pure oxygen for a long time. It is caused by free radicals, causing thromboxane release, sustained vascular contracture and cellular injury. Cataract formation is related with ageing process. Cataract is partly due to photochemical generation of free radicals. Tissues of the eye, including the lens, have high concentration of free radical scavenging enzymes.
5. Shock Related Injury: Release of free radicals from phagocytes damage membranes by lipid peroxidation. They release leucotrienes from platelets and proteases from macrophages. All these factors cause increased vascular permeability, resulting in tissue edema. Anti-oxidants have a protective effect.
6. Arthrosclerosis and Myocardial Infraction: Low density lipoproteins (LDL) promote atherosclerosis. They are deposited under the endothelial cells, which undergo oxidation by free radicals released from endothelial cells. This attracts macrophages. Macrophages are them converted into foam cells. This initiates the atherosclerotic plaque formation. Alpha tocopherol offers some protective effect.
7. Peptic Ulcer:Peptic ulcer is produced by erosion of gastric mucosa by hydrochloric acid. It is shown that superoxide anions are involved in the formation of ulcer. Helicobacter pylori infection perpetuates the disease. This infection potentiates the macrophage oxidative burst leading to tissue destruction.
8. Skin Diseases: due to inborn defects, porphyrins accumulate in the skin. Exposure of sunlight will lead to erythema and eruptions in the patients. Sunlight acting on porphyrins produces singlet oxygen, which trigger inflammatory reaction, leading to the above symptoms. Certain plant products, called psoralens are administered in the treatment of psoriasis and leukoderma. When the drugs is applied over the affected skin and then irradiated by UV light, singlet oxygen produced with clinical benefit.
9. Cancer Treatment: Free radicals contribute to cancer development because of their mutagenic property. Free radicals produce DNA damage, and accumulated damages lead to somatic mutations and malignancy. Cancer is treated by radiotherapy. Irrational produces reactive oxygen species in the cells which trigger the cell death. To increase the therapeutic effect of radiation, radio-sensitisers are administered, which increase the production of ROS **[21].**

Dietary antioxidants

Vitamin C, vitamin E, and beta-carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E. Beta-carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta-carotene may work synergistically with vitamin E. A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E **[22].**

Antioxidant Phytonutrients

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plant kingdom: approximately 3,000 flavonoid substances have been described. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers". Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages **[22].**

Generally, on Antioxidant enzymes, Oxidative stress plays a major role in the pathogenic of many disorders including aging, cancer, diabetes, Alzheimer's, strokes, viral infections (that cause airway epithelial inflammation), neurodegenerative processes (including cell death, motor neuron diseases and axonal injury) and infraction, and brain edema. Antioxidant enzyme plays an important role in protecting oxidative injury to the body. One of the therapeutic approach by which these disorders can be prevented is to increase the levels of these enzymes (SOD, CAT, GPx etc.) in the body by interventions which may include increases intake of dietary supplements rich in antioxidants/antioxidant enzymes and regular exercise.

Lipid Peroxidation

Currently, lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. First, lipid peroxidation was studied for food scientists as a mechanism for the damage to alimentary oils and fats, nevertheless other researchers considered that lipid peroxidation was the consequence of toxic metabolites (e.g. CCl4) that produced highly reactive species, disruption of the intracellular membranes and cellular damage **[23].** Lipid peroxidation is a complex process known to occur in both plants and animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers **[24].** In pathological situations the reactive oxygen and nitrogen species are generated at higher than normal rates, and as a consequence, lipid peroxidation occurs with α-tocopherol deficiency. In addition to containing high concentrations of polyunsaturated fatty acids and transition metals, biological membranes of cells and organelles are constantly being subjected to various types of damage **[25,26].** The mechanism of biological damage and the toxicity of these reactive species on biological systems are currently explained by the sequential stages of reversible oxidative stress and irreversible oxidative damage. Oxidative stress is understood as an imbalance situation with increased oxidants or decreased antioxidants **[23].** The concept implies the recognition of the physiological production of oxidants (oxidizing free-radicals and related species) and the existence of operative antioxidant defenses. The imbalance concept recognizes the physiological effectiveness of the antioxidant defenses in maintaining both oxidative stress and cellular damage at a minimum level in physiological conditions **[24].**

Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA). Since polyunsaturated fatty acids are more sensitive than saturated ones, it is obvious that the activated methylene (RH) bridge represents a critical target site. The presence of a double bond adjacent to a methylene group makes the methylene C-H bond weaker and therefore the hydrogen in more susceptible to abstraction. This leaves an unpaired electron on the carbon, forming a carbon-centered radical, which is stabilized by a molecular rearrangement of the double bonds to form a conjugated diene which then combines with oxygen to form a peroxyl radical. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid and so of starting a chain reaction **[10].**

Molecular oxygen rapidly adds to the carbon-centered radicals (R.) formed in this process, yielding lipid peroxyl radicals (ROO.). Decomposition of lipid peroxides is catalyzed by transition metal complexes yielding alcoxyl (RO.) or hydroxyl (HO.) radicals. These participate in chain reaction initiation that in turn abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The formation of peroxyl radicals leads to the production of organic hydroperoxides, which, in turn, can subtract hydrogen from another PUFA. This reaction is termed propagation, implying that one initiating hit can result in the conversion of numerous PUFA to lipid hydroperoxides. In sequence of their appearance, alkyl, peroxyl and alkoxyl radicals are involved. The resulting fatty acid radical is stabilized by rearrangement into a conjugated diene that retains the more stable products including hydroperoxides, alcohols, aldehydes and alkanes. Lipid hydroperoxide (ROOH) is the first, comparatively stable, product of the lipid peroxidation reaction **[10].**

Reduced iron complexes (Fe^{2+}) react with lipid peroxides (ROOH) to give alkoxy radicals, whereas oxidized iron complexes (Fe^{3+}) react more slowly to produce peroxyl radicals. Both radicals can take part in the propagation of the chain reaction. The end products of these complex metal ion-catalyzed breakdowns of lipid hydroperoxides include the cytotoxic aldehydes and hydrocarbon gases such as ethane. The free radical chain reaction propagates until two free radicals conjugate each other to terminate the chain. The reaction can also terminate in the presence of a chain-breaking antioxidant such as vitamin E (α-tocopherol). In conditions in which lipid peroxidation is continuously initiated it gives non-radical products destroying two radicals at a time. In the presence of transition metal ions, ROOH can give rise to the generation of radicals capable of re-initiating lipid peroxidation by redox-cycling of these metal ions **[10].**

Lipid peroxidation causes a decrease in membrane fluidity and in the barrier functions of the membranes. The many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter chemotactic signals and enzyme activity **[25].**

Implications of Lipid Peroxidation

The biological production of reactive oxygen species primarily superoxide anion (O_2-) and hydrogen peroxide (H_2O_2) is capable of damaging molecules of biochemical classes including nucleic acids and amino acids. Exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen **[26].** However, the most damaging effect is the induction of lipid peroxidation. The cell membrane which is composed of poly-unsaturated fatty acids is a primary target for reactive oxygen attack leading to cell membrane damage. The lipid peroxidation of polyunsaturated fatty acids may be enzymatic and non-enzymatic. Enzymatic lipid peroxidation is catalyzed by the lipoxygenases family, a family of lipid peroxidation enzymes that oxygenates free and esterified PUFA generating as a consequence, peroxy radicals. Non-enzymatic lipid peroxidation and formation of lipidperoxides are initiated by the presence of molecular oxygen and is facilitated by Fe^{2+} ions **[27].** Oxidative breakdown of biological phospholipids occurs in most cellular membranes including mitochondria, microsomes, peroxisomes and plasma membrane. The toxicity of lipid peroxidation products in mammals generally involves neurotoxicity, hepatotoxicity and nephrotoxicity **[24].** The principal mechanism involves detoxification process in liver. Toxicity from lipid peroxidation affect the liver lipid metabolism where cytochrome P-450s is an efficient catalyst in the oxidative transformation of lipid derived aldehydes to carboxylic acids adding a new facet to the biological activity of lipid oxidation metabolites. Cytochrome P-450-mediated metabolism operates in parallel with other metabolic transformations of aldehydes; hence, the P450s could serve as reserve or compensatory mechanisms when other high capacity pathways of aldehyde elimination are compromised due to disease or toxicity. Finally, 4-hydroxynonenal (HNE), unsaturated aldehydes, such as acrolein, trans-2-hexenal, and crotonaldehyde, are also food constituents or environmental pollutants, P-450s may be significant in favoring lipid peroxidation that has significant downstream effects and possibly play a major role in cell signaling pathways. Oxidized lipids appear to have a signalling function in pathological situations, are proinflammatory agonists and contribute to neuronal death under conditions in which membrane lipid peroxidation occurs. For example, mitochondrial lipid cardiolipin makes up to 18% of the total phospholipids and 90% of the fatty acyl chains are unsaturated. Oxidation of cardiolipin may be one of the critical factors initiating apoptosis by liberating cytochrome c from the mitochondrial inner membrane and facilitating permeabilization of the outer membrane. The release of cytochrome c activates a proteolytic cascade that culminates in apoptotic cell death **[27].** Previous results indicate that lipid peroxidation has a role in the pathogenesis of several pathologies as neurodegenerative, inflammatory, infectious, gastric and nutritional diseases. Oxidative damage in liver is associated with hepatic lipid metabolism, and may be affecting the absorption and transport mechanisms of α-tocopherol in this organ. In the liver, the morphological damage is previous to the lipid peroxidation and the consumption of endogenous antioxidants. In kidney and heart, indeed, lipid peroxidation and oxidative damage preceded necrosis **[27,28].**

Lipid peroxidation is a chain reaction process characterized by repetitive hydrogen abstraction by HO. and RO., and addition of O_2 to alkyl radicals resulting in the generation of ROO. And in the oxidative destruction of polyunsaturated fatty acids, in which the methylene group (=RH-) is the main target. The association between increased phospholipid oxidation, free-radical mediated reactions and pathological states was early recognized. The contribution by Sies of the concept of oxidative stress followed with the implication that increased free-radical mediated reactions, basically by HO. and RO. would produce phospholipid, protein, lipid, DNA, RNA or carbohydrate oxidation, whatever is close. The increased oxidation of the cell biochemical constituents is associated with ultra-structural changes in mitochondrial morphology with mitochondrial swelling and increased matrix volume. In human liver, the morphological changes can affect the organ structure and function as it is the case for the bile canaliculi that are damaged in liver transplanted patients; a fact that is interpreted as consequence of the oxidative damage that is associated to ischemia reperfusion. Interestingly,

there are reports in rat liver experimental models, of increased peroxidation secondary to increased mitochondrial production of O_2- and H_2O_2**[25].**

Lipid Peroxidation and Human Pathologies

The organism must confront and control the balance of both pro-oxidants and antioxidants continuously. The balance between these is tightly regulated and extremely important for maintaining vital cellular and biochemical functions. This balance often referred to as the redox potential, is specific for each organelle and biological site, and any interference of the balance in any direction might be deleterious for the cell and organism. Changing the balance towards an increase in the pro-oxidant over the capacity of the antioxidant is defined as oxidative stress and might lead to oxidative damage. Changing the balance towards an increase in the reducing power, or the antioxidant, might also cause damage and can be defined as reductive stress. Oxidative stress and damage have been implicated in numerous disease processes, including inflammation, degenerative diseases, and tumor formation and involved in physiological phenomena, such as aging and embryonic development. The dual nature of these species with their beneficial and deleterious characteristics implies the complexities of their effects at a biological site. Lipid peroxidation has been pointed out as a key chemical event in the oxidative stress associated with several inborn and acquired pathologies. Disruption of organelle and cell membranes together with calcium homeostasis alterations are the main supramolecular events linked to lipid peroxidation. However, it is not clear if lipid peroxidation process is a cause, triggering step of the clinical manifestations of the disease, or a consequence of toxic effects of lipid peroxidation products. In pathological situations the reactive oxygen species are generated and as a consequence lipid peroxidation occurs with α-tocopherol deficiency. In addition to containing high concentrations of polyunsaturated fatty acids and transitional metals, red blood cells are constantly being subjected to various types of oxidative stress. Red blood cells however are protected by a variety of antioxidant systems which are capable of preventing most of the adverse effects under normal conditions. Among the antioxidant systems in the red cells, α-tocopherol possesses an important and unique role. α- tocopherol may protect the red cells from oxidative damage via a free radical scavenging mechanism and as a structural component of the cell membrane (Sies, 1997).Levels of Met-Hb. are regarded as an index of intracellular damage to the red cell and it is increased when α-tocopherol is consumed and the rate of lipid peroxidation is increased. Scavenging of free radicals by α-tocopherol is the first and the most critical step in defending against oxidative damage to the red cells. When α-tocopherol is adequate, GSH and ascorbic acid may complement the antioxidant functions of α-tocopherol by providing reducing equivalents necessary for its recycling/regeneration. On the other hand, when α -tocopherol is absent, GSH and ascorbic acid release transitional metals from the bound forms and/or maintain metal ions in a catalytic state. Free radical generation catalysed by transition metal ions in turn initiates oxidative damage to cell membranes. Membrane damage can lead to release of heme compounds from erythrocytes. The heme compounds released may further promote oxidative damage especially when reducing compounds are present **[24].**

Analytical Determination of Lipid Peroxidation

Since the acceptation of the oxidative stress concept, scientists and physicians have been searching for a simple assay or a small group of determination that would result useful for the assessment of oxidative stress and lipid peroxidation in clinical situations. The determinations of marker metabolites are usually performed in blood, red blood cells or plasma. The markers for systemic oxidative stress are normally present in healthy humans and the assays for systemic oxidative stress are comparative, which makes necessary to have reference values from normal individuals. At present, the plasma levels of oxidation products derived from free-radical mediated reactions and of antioxidants are used as indicators of systemic oxidative stress in humans and experimental animals. The more utilized determination of an oxidation product is MDA, which is determined with low specificity but with great efficiency by the simple and useful assay of TBARS with measurements made by spectrophotometry or spectrofluorometry. The normal plasma levels of TBARS are 2-3 μM. Oxidative damage is characterized by increases in the levels of the oxidation products of macromolecules, such as thiobarbituric acid reactive substances (TBARS), and protein carbonyls. Many of these products can be found in biological fluids, as well as addition derivatives of these reactive end-products. As a result of lipid peroxidation a great variety of aldehydes can be produced, including hexanal, malondialdehyde (MDA) and 4-hydroxynonenal. Oxidation of an endogenous antioxidant reflects an oxidative stress that is evaluated by measuring the decrease in the total level of the antioxidant or the increase in the oxidative form. The only way not to be influenced by nutritional status is to measure the ratio between oxidized and reduced antioxidants present in blood. The published literature provides compelling evidence that (a) MDA represents a side product of enzymatic PUFA oxygenation and a secondary end product of no enzymatic (autoxidative) fatty peroxide formation and decomposition and (b) sensitive analytical methods exist for the unambiguous isolation and direct quantification of MDA **[27].**

Conceptually, these two facts indicate that MDA is an excellent index of lipid peroxidation. However, this conclusion is limited in practice by several important considerations:
a) MDA yield as a result of lipid peroxidation varies with the nature of the PUFA peroxidised (specially its degree of instauration) and the peroxidation stimulus
b) Only certain lipid oxidation products decompose yield MDA
c) MDA is only one of several end products of fatty peroxide formation and decomposition,
d) The peroxidation environment influences both the formation of lipid-derived precursors and their decomposition to MDA
e) MDA itself is a reactive substance which can be oxidative and metabolically degraded
f) Oxidative injury to no lipid biomolecules has the potential to generate MDA.

With biological materials, it appears prudent to consider the TBARS test more than an empirical indicator of the potential occurrence of peroxidative lipid damage and not as a measure of lipid peroxidation **[27].** The thiobarbituric acid test (TBARS) has been employed to a uniquely great degree over the last five decades to detect and quantify lipid peroxidation in a variety of chemical as well as biological material. Two underlying assumptions are implicit from the widespread use of the TBARS test to assess lipid peroxidation:
a) An operative and quantitative relationship exists between lipid peroxidation and MDA
b) Product formation during the TBARS test is diagnostic of the presence and amount of fatty peroxides.

Lipid peroxidation proceeds by a free-radical mediated chain reaction that includes *initiation*, *propagation* and *termination* reactions. The chain reaction is initiated by the abstraction of a hydrogen atom from a methylene group of an unsaturated fatty acid. Propagation is cycled through rounds of lipid peroxyl radical abstraction of the bismethylene hydrogen atoms of a polyunsaturated fatty acyl chain to generate new radicals, after O_2 addition, resulting in the conversion of alkyl radical in hydroperoxyl radical. Termination involves the reaction of two hydroperoxyl radicals to form non-radical products. This reaction is particularly interesting since it is accompanied, although at low yield, by emission of light or chemiluminiscence. Some lipid peroxidation products are light-emitting species and their luminescence is used as an internal marker of oxidative stress **[24,26,27]**. The measurement of light emission derived from $^1O_2$ and excited triplet carbonyl compounds, which are the most important chemiluminiscent species in the lipid peroxidation of biological systems, is directly related to the rate of lipid peroxidation and allows an indirect assay of the content of lipophilic antioxidants in the sample. Lipophilic antioxidants react with lipid peroxyl radicals and lower antioxidant content is associated with higher chemiluminescence**[27].** The low-level chemiluminescence which accompanies the peroxidation of polyunsaturated fatty acids has been used as a tool in kinetic and mechanistic studies of biological samples to estimate the extent of the reactions and even to indicate tissue damage promoted by oxidants. Triplet carbonyls and singlet oxygen formed in the annihilation of intermediate peroxyl radicals (ROO) have been identified as the chemiluminescence emitters. Chemiluminescence is a very interesting way to evaluate an oxidative stress and lipid peroxidation in biological samples and living systems. The emission of light has been observed during stress in different experimental models. Chemiluminescence is very sensitive and thus can be applied to measure free radical production in human tissues. Chemiluminescent systems may be classified in two classes based on the origin of the emitting molecule. In the first class, the emitter is a product of the chemical reaction (direct chemiluminescence). In the second class, there is energy transfer between an electronically excited product molecule and a second substance which then becomes the emitter (sensitized chemiluminescence) **[24,27].** The chemical mechanism responsible for spontaneous organ light emission is provided by the Russell's reaction in which two secondary or tertiary peroxyl radicals (ROO•) yield $^1O_2$ and excited carbonyl groups (=CO*) as products. In turn, two 1O2, through dimol emission, lead to photoemission at 640 and 670 nm, whereas =CO* yields photons at the 460-470 nm band. The main sources of the chemiluminescence detected in the direct and sensitized chemiluminescence is the dimol emission of $^1O_2$ (reaction 27) and the photon emission from excited carbonyl groups (reaction 28) **[23].**

$2\ ^1O_2 \rightarrow 2\ O_2 + h\cup(634\text{-}703\ nm)$ (27)

$RO^* \rightarrow RO + h\cup(380\text{-}460\ nm)$ (28)

These reactions are accompanied by chemiluminescence whose intensity may serve as an indirect measure of peroxide free radical and α-tocopherol concentration in the sample. Lipid peroxidation has been recognized as free radical-mediated and physiologically occurring **[23,27]** with the supporting evidence of *in situ* organ chemiluminescence Spontaneous chemiluminescence of *in situ* organs directly reports the intracellular formation of singlet oxygen ($^1O_2$) and represents an issue of direct chemiluminescence. The generation of $_1O_2$ implies the collision of two peroxyl radicals (ROO·) with formation of excited species, 1O2 itself and excited carbonyls, followed by photoemission. Light emission from *in situ* organs is a physiological phenomenon that provides a determination of the steady state concentration of singlet oxygen and indirectly of the rate of oxidative free radical reactions **[24].** *In situ* liver chemiluminescence has been recognized as a reliable indicator of oxidative stress and damage in rat liver upon hydroperoxide infusion ischemia-reperfusion

and chronic and acute alcohol intoxication The increases in photoemission observed were parallel to increased contents of indicators of lipid peroxidation (malonaldehyde and 4-HO-nonenal) but with a higher experimental/control ratio in organ chemiluminescence. Tert-butyl hydroperoxide initiated chemiluminescence is an example of sensitized chemiluminescence, and it has been used to enhance the chemiluminescence accompanying lipid peroxidation and the α-tocopherol content of tissues. This method has been successfully utilized to detect the existence of oxidative damage associated to experimental or pathological situations in tissue homogenates, subcellular fractions, and in human heart, liver and muscle biopsies. Tissue homogenates or blood samples are subjected to *in vitro* oxidative damage by supplementation with tert-butyl hydroperoxide. It reacts with haemoproteins and Fe^{2+} producing peroxyl and alcoxyl free radicals, which enter to the propagation phase of the lipid peroxidation radical chain reaction. The termination steps of the chain reaction generate compounds in an excited state: singlet oxygen and carbonyl groups. This assay is useful to evaluate the integral level of the non-enzymatic antioxidant defenses of a tissue. The increase of tert-butyl hydroperoxide-initiated chemiluminescence is indicative that α-tocopherol is the antioxidant consumed in erythrocytes and suggest that reactive oxygen species and lipid peroxidation catalyzed by reduced transition metals may be responsible for the onset of oxidative damage and the occurrence of systemic oxidative stress in patients suffering oxidative damage associated to neurological pathologies as Parkinson , Alzheimer disease and vascular dementia; immunological diseases as HIV infection and AIDS, hyperthyroidism and hypothyroidism. These methods were used to evaluate lipid peroxidation and oxidative damage in experimental models of oxidative stress in rats. A common question of the researchers in the field is which the method of choice is. The answer is: none of them, and all of them. Each assay measures something different. Diene conjugation tells one about the early stages of peroxidation, as a direct measurement of lipid peroxides. In the absence of metal ions to decompose lipid peroxides there will be little formation of hydrocarbon gases, carbonyl compounds, or their fluorescent complexes, which does not necessarily mean therefore that nothing is happening. Even if peroxides do not decompose, the TBARS test can still detect them because of decomposition of peroxides. Changes in the mechanism of peroxide decomposition might alter the amount generated without any change in the overall rate of lipid peroxidation. Whatever method is chosen, one should think clearly what is being measured and how it relates to the overall lipid peroxidation process. Whatever possible, two or more different assay methods should be used **[24, 29].**

Desmodiumadscendens

*Desmodiumadscendens*is of the Family - "*Fabaceae*"; and genus - *Desmodium*. Common names of *Desmodiumadscendens* are Beggar- lice, Beggar weed, Tick Clover, Tick trefoil **[30]**.

It is a rainforest herb which has been traditionally used by the natives for a wide variety of medical conditions including: muscle cramp, tendon, spinal pain, bronchitis, epilepsy and some central nervous system disorders. Other uses include rheumatism, jaundice, hepatitis, protection of liver from cirrhosis, asthma (*owing to its bronchial-dilating effects*), allergic symptoms and eczema. It is also a very potent natural antispasmodic agent **[30].**

It could also be used in women to manage leucorrhoea (*a thick yellowish vaginal discharge usually caused by oestrogen imbalance*), vaginal infections, and ovarian inflammations **[31].** Also, disorders of the ovary and reproductive tract infections have been treated using extracts from the herb **[32].** It has been known to also promote lactation in women. *Desmodiumadscendens* has also been used in treating wounds and sores **[33].**

Aim of Study is to determine the antioxidant activities of *Desmodiumadscendens.*

Note that *Ethical approval was given by Faculty of Basic Medical Science Animal Research Ethics Committee of University of Calabar for the research. Approval number is FAREC/PA/011PY31016.*

II. Method

Phytochemical analysis

The aqueous leaf extract of *Desmodiumadscendens*was subjected to phytochemical analysis. 2g of the crude extract was weighed and dissolved in 20 ml of distilled. The solution was screened for the presence and absence of alkaloids, flavonoids, tannins, saponins, glycosides, reducing agents, polyphenols, anthraquinones, and phlobatanins following standard methods **[34].**

Test for alkaloids:

Mayer's test: 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of Mayer's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for the presence of precipitate and colour change.

Wagner's test: 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of Wagner's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for the presence of precipitate and colour change.
Dragendorrf's test: 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of Dragendorff's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for presence of precipitate and colour change.

Test for tannins:
i. 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops of 10 % ferric chloride. The mixtures were observed for presence of precipitate and colour change.
ii. 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops of 10 % lead acetate. The mixtures were observed for presence of precipitate and colour change.

Test for flavonoids:
i. 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of NaOH. The mixtures were allowed to stand for 2 min and then observed for presence of precipitate and colour change.
ii. 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of NaOH and 3 drops of 0.5 N HCl. The mixtures were observed for presence of precipitate and colour change.

Test for saponnins:
Emulsifying test: 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops olive oil and the mixture shaken vigorously. The mixtures were observed for presence of brown emulsion.

Frothing test: 1 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 4 ml distilled water. The mixture was shaken vigorously and then observed for presence of frothing.

Test for Anthraquinone:
0.1 g of the crude extract was dissolved in 10 ml concentrated chloroform. The solution was filtered and used for this test. To 5 ml of filtrate and control solution in separate test tubes was added 5 ml ammonia solution. The mixtures were shaken vigorously. The mixtures were observed for presence of precipitate and colour change.

Test for glycoside:
To 2 ml of filtrate and control solutions in separate test tubes were added 2 ml of Fehling I and Fehling's II solutions. The solutions were mixed thoroughly and boiled in a water bath for 2 min. The mixture was observed for the presence of precipitate and colour change**.**

Test for terpenes:
0.1 g of the crude extract was dissolved in 10 ml concentrated chloroform. The solution was filtered and used for this test. To 1 ml of filtrate and control solutions in separate test tubes were added 1 ml acetic anhydride. The solutions were mixed thoroughly with a glass rod. The test tubes were then placed in slanting positions and 1 ml H_2SO_4 was added to the side of each test tube into the mixture. The junction of the two liquid layers was observed for the presence of colour change.

Experimental animals
A total of 24 adult male albino Wistar rats weighing between 120-160g were used for this experiment.The animals were obtained from the faculty of Basic medical science animal house, University of Calabar. The experimental animals were handled in accordance with the principles guiding the use and handling of experimental animals as stipulated by faculty animal research ethics committee of the faculty of Basic medical sciences. The rats were maintained on standard rat feed (growers feed) and tap water available all through the period of experiment. The animals were maintained at an ambient temperature between 28 - 30ºC, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 hours of light (06:30 hour – 18:30

hour) alternating with approximately 12 hours of darkness (18:30 hour - 06:30 hour). The rats were allowed to get familiarized with the environment for a period of 7 days before treatments commenced.

Preparation of extract

Two (2) grams of the aqueous leaves extract of *Desmodiumadscendens*was dissolved in 10ml of distilled water as follows; 2 g = 10 ml of water (200 mg = 1 ml). If 200 mg = 1 ml, therefore, 300 mg = 1.5 ml, 450mg = 2.25 ml, and 600 mg = 3 ml of water. Volume per animal was determined as follows; for the low dose treated group, 300mg of extract was dissolved in 1.5 ml of water. A rat in the low dose group with a body weight 120g received 36mg of the extract. If 300 mg of extract was dissolved in 1.5 ml of water, 36 mg of the extract will be dissolved in 0.18 ml of water. Therefore, an animal in the low dose group with a body weight of 120g will receive 0.18 ml of the extract daily all through the treatment period. Same was applicable to all the experimental animals all the extract treated groups.

Extract administration was done orally with the aid of an orogastric cannula and treatment lasted for four (4) weeks.

Experimental design

At the end of the acclimatization period, the animals were randomly assigned into four (4) groups, n=6, as follows;

i. Control (Received normal rat chow and tap water)
ii. Low dose treated group (Received low dose of extract (300mg/kg)
iii. Median dose treated group (Received middle dose of extract (450mg/kg)
iv. High dose treated group (Received high dose of extract (600mg/kg)

Treatments lasted for a period of four (4) weeks, all animals had free access to fed and water *ad libitum.*

Collection of blood samples At the end of treatment period, animals from all the experimental groups were sedated and made unconscious using chloroform anesthesia. Blood samples from each rat was collected via cardiac puncture **[35]** into plain sample bottles for the estimation of haematological and biochemical parameters.

Analysis of serum

Serum from the different groups was analyzed for the following Antioxidant Enzymes; Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and marker of Lipid peroxidation (MDA).

Determination of antioxidant enzymes

1. Superoxide Dismutase (SOD) activity:

Principle: Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, also produces a water-solubleformazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XD) activity and is inhibited by SOD. Therefore, the inhibition of the activity of SOD can be determined by a colorimetric method.

Procedure: Serum was mixed with 150mm KCL to remove any red blood cells. 1ml of serum was homogenized in ice-cold 0.1m tris, pH 7.4. The crude serum homogenate was centrifuged at 14000xg for 5minutes at 4^0C and the cell debris discarded.

Plates were incubated at 37^0C for 20mintes and absorbance read at 450nm using a microplate reader.

Calculation:

SOD Activity = $\frac{(A_{blank\ 1}-A_{blank\ 3})-(A_{blank\ 1}-A_{blank\ 2})}{(A_{blank\ 1}-A_{blank\ 3})}$ x 100

(Inhibition rate %).

2. Glutathione Peroxidase (GPx) activity:

Principle: Glutathione peroxidase (GPX) converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water.

GPX reduces cumenehydroperoxide while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease of NADPH (easily measured at 34onm) is proportional to GPx activity.

Procedure: 0.5ml of tissue was homogenized on ice in 0.2ml cold assay buffer and centrifuged at 10,000x9 for 15 minutes at 4^0C. The supernatant was collected for assay.

40µL of the samples was added into a 96 well plate and the volume made up to 50µL with assay buffer. The standard curve was prepared by diluting 25µL of the 40nm NADPH solution into 975µL distilled water to generate 1mm NADPH standard. 0,20,40,60, 80 and 10µL of the 1mm NADPH standard was added into 96 well plate in duplicate to generate 0, 20, 40, 60, 80, 100nmol /well standard. The final volume is brought to 100µL with assay buffer. The optical density at 340nm was measured and the NADPH standard curve was plotted.

The positive control was prepared to put 5-10µL of the GPx positive control into the desired wells and adjusted to 50µL with assay buffer. 50µL of assay buffer was added to the wells as a reagent control (Rc). 40µL of the reaction mix was added to each test samples, positive controls, and reagent controls were mixed well and incubated for 15minutes to deplete all GSSG in the sample. 10µL cumenehydroperoxide solution was added to start GPx reaction and then mixed well.

The OD at 340nm at T was measured to read A, OD at 340nm was measured again at T_2 after incubating the reaction of 25^0C for 5minutes to read A_2

A_{340nm} = [(sample A_1 - sample A_2) – (RCA_1 – RCA_2)]

Calculation:

GPx activity (nM/min/mg protein)

$$=\frac{B}{T_1-T_2 XV}\text{x sample dilution}$$

3. Catalase (CAT) activity:

Principle: Catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. The unconverted reacts with oxiRed^{TM} probe to produce a product which can be measured colourimetrically at 570nm. The light produced is inversely proportional to the amount of catalase activity.

Procedure: 0.5ml serum was homogenized in 0.2ml cold assay buffer and centrifuged at 10,000xg for 15mintues at 4^0C. The supernatant was collected for assay. 40µL of samples solution was added into each well and volume adjusted to total 78µL with assay buffer. High control (Hc) was prepared with the same amount of sample in separate wells then made up to 78µL with assay buffer. 10µL of stop solution was added into the sample Hc, mixed and incubated for 5minutes at 25^0C. 12µL fresh 1mmol H_2O_2 is added to each well of both samples and sample HC to start the reaction, then incubated at 25^0C for 30minutes. 10µL stop solution was then added into each sample vial to stop the reaction. 50µL of the developer mix was added to each test sample, controls and standard. It was mixed well and incubated at 25^0C for 10minutees. The OD at 570nm is read in a Mindray Chemistry Analyzer BS-120

Calculation:

Catalase activity (U/ml) $= \frac{B}{30xv}$

B = decomposed H_2O_2 amount (nmol) from H_2O_2 standard curve

V = pre-treated sample volume (ml) added into the reaction well

30 = reaction time in minutes

4. <u>Malondialdehyde (MDA) level:</u>

Principle: Lipid peroxidation forms malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural by-products. MDA reacts with thiobarbituric acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically at 532nm.

Procedure: 1ml serum was homogenized on ice in 300µl of the MDA lysis buffer, then centrifuged at 13,600xg for 10min. 200µl of the supernatant from the homogenized sample was placed into a microcentrifuge tube. 0,2,4,6,8,10µL of the 2mmol MDA standard was added into separate microcentrifuge tubes and the final volume adjusted to 200µL with distilled water to generate 0,4,8,12,16 and 20nmol standard per well. 600µL to TBA solution was added into each vial containing standard and sample and incubated at 95^0C for 60 minutes. It was then cooled to room temperature in an ice bath for 40 minute and 200µL (from each 800µL reaction mixture) was pipette into a 96-well microplate for analysis. The absorbance was read at 532nm.

Calculation:

The MDA standard curve was plotted and MDA amount in the test sample was calculated as follows: concentration (nmol/mg) = [(A/mg)] X 4

Where;

A = sample MDA amount from standard curve in nmol

mg = original tissue amount used

3 = correction for using 200µL of the 800µL reaction mix.

III. Results

Table 1: Phytochemical constituents of aqueous leaves extracts of *Desmodiumadscendens*

Phytochemicals	Aqueous extract
Alkaloids (%)	2.56 ± 0.02
Glycosides (%)	2.45 ±0.01
Saponins (%)	2.10 ±0.01
Tannins (%)	0.42 ±0.01
Flavonoids (%)	8.23 ±0.02*
Polyphenols (%)	13.71 ±0.02*
Reducing sugars (mg%)	5.70 ±0.01*

Each value represents the mean of 3 determinants ±SEM
* = high percentage

Superoxide dismutase (SOD) concentration

Result showed no significant ($p>0.05$) effect on SOD in the groups treated with low, median and high doses of extract when compared with the control group at $p<0.05$. (Fig 1)

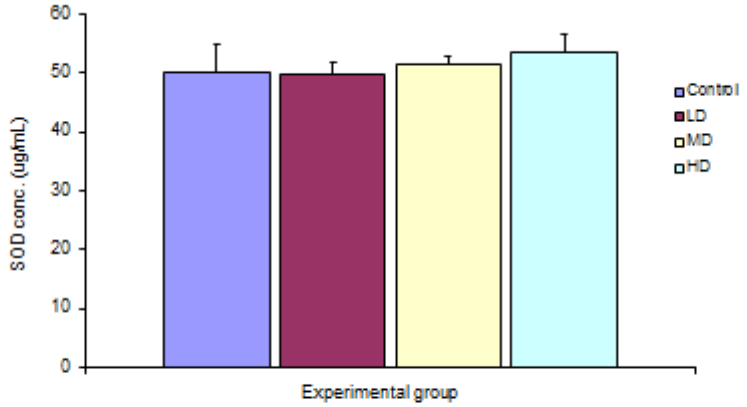

FIG. 1: Comparison of superoxide dismutase concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
No significant differences among groups

Catalase (CAT) concentration

There was a significant ($p<0.05$) increase in serum concentration of catalase (CAT) in the groups treated with medium and high doses when compared with the control (32.30 ±2.16 nmmol/mL) and low (25.97 ±2.20 nmol/mL) dose groups at $p<0.05$. (Fig 2)

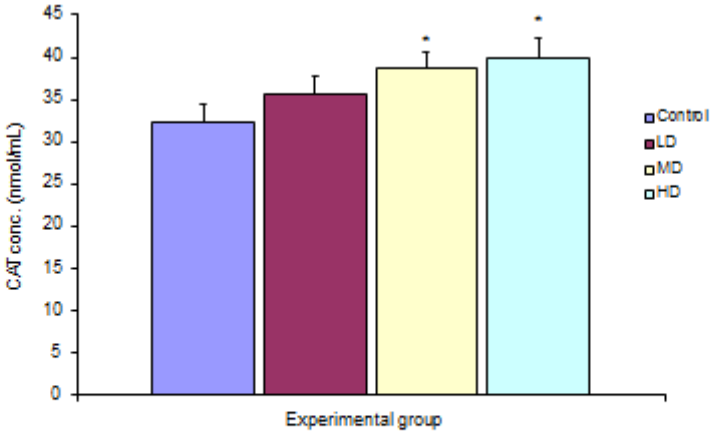

FIG. 2: Comparison of catalase concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = significantly different from control at p<0.05

Glutathion peroxidase (GPx) concentration

There was a significant increase in GPx concentration in the groups treated with median and high doses of extract when compared at $p<0.05$ with control (78.38 ±4.94 umol/mL) and low (72.52 ±3.18 umol/mL) dose groups. (Fig 3)

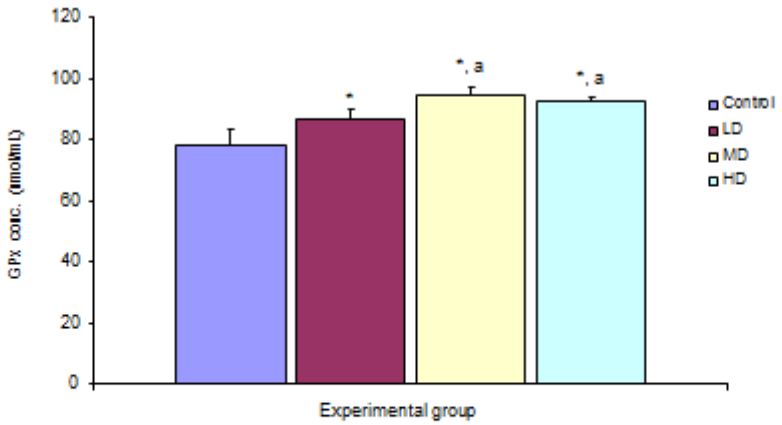

FIG. 3: Comparison of glutathion peroxidase concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = significantly different from control at p<0.05
a = significantly different from low dose at p<0.05

Malondialdehyde (MDA) concentration

Comparison of Malondialdehyde (MDA) concentration in the different experimental groups showed significant decrease in MDA in all the test groups when compared with control (0.57 ±0.07 nmol/L).(Fig 4).

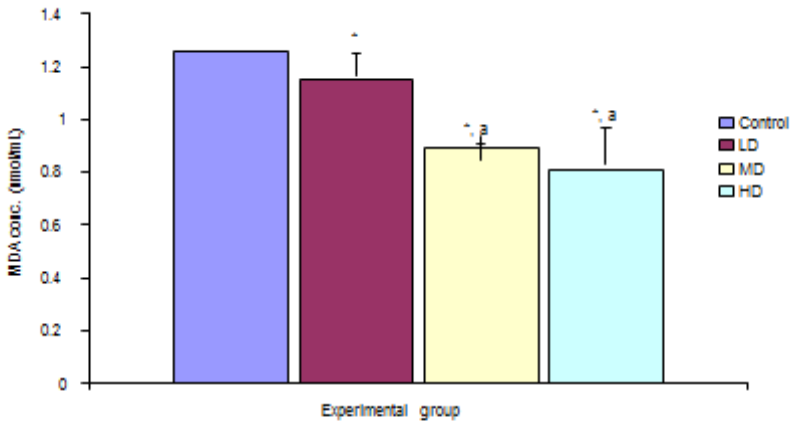

FIG. 4: Comparison of malondialdehyde concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = significantly different from control at p<0.05

IV. Discussion

Antioxidant enzymes; Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) represent the first line of defence against oxidative tissue injury (or stress). SOD catalyzes the dismutation of highly reactive and potentially toxic superoxide radicals to H_2O_2, while GPx and CAT metabolize H_2O_2 to non-toxic products **[10].** The results of this study revealed that treatment of rats with *Desmodiumadscendens* extract caused a significant ($p<0.05$) increase in the tissue activities of GPx and CAT. This implies that extract may improve the body's ability to mop-up free radicals.

The extract has high concentrations of polyphenols, flavonoids, and reducing sugar(*which are strong antioxidants*) compared to other phytochemical constituents like tannins, saponins, glycosides and alkaloids.

The flavonoids, apart from mopping up free radicals from the body system, serve to inhibit the pro-inflammatory activities of enzymes involved in free radical production, such as cyclo-oxygenase, lipoxygenase or inducible nitric oxide synthase.

Most hepatotoxic chemicals or herbal products damage liver by inducing directly or indirectly lipid peroxidation. The malondialdehyde (MDA) is one of the end products of the lipid peroxidation process **[10].** Lipid peroxidation can reduce membrane fluidity and inactivate membrane-bound proteins and thus inhibition of lipid peroxidation is a crucial effect of antioxidant compounds. In this study, there was significant ($p<0.05$) decrease in the liver tissue level of MDA in experimental groups. Therefore, with decreased MDA, the extract may have reduced lipid peroxidation.

The phytochemical constituents of *D. adscendens* have been attributed to its pharmacological activities. For instance, Alkaloids, glycosides and saponinshave antimalarial, antiasthma, anticancer, vasodilatory, antiarrhythmic, analgesic, antibacterial and antihyperglycemic activities.

Alkaloidsare nitrogen-containing natural compounds having a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, antibacterial and antihyperglycemic activities. Alkaloids also possess psychotropic and stimulant activities

Saponins are naturally occurring plant glycosides that offer tremendous health benefits. Studies have shown they may support the immune system, promote normal cholesterol levels, and support overall wellness. Its foam-like product with water helps it bind with water as well as fats and oils. This means that, in the digestive tract, saponins produce an emulsification of fat-soluble molecules. Specifically, saponins bind to bile acids and help eliminate them from the body, preventing cholesterol from being reabsorbed. You might even say saponins "wash away" various toxins.

V. Conclusion

Extract may improve body's ability to mop-up free radicals and prevent oxidative stress as it increased anti-oxidant enzymes concentration. It may also prevent lipid peroxidation which is evident with decreased MDA.

References

[1]. Yen G.C., Chang Y.C., Su S.W. Antioxidant activity and active compounds of rice Koji fermented with *Aspegilluscandidus*. *Food Chemistry*,2003. 83: 49-54.

[2]. Khal R. and danHilderbrantA.G.Methodology for studying antioxidant activity and mechanism of action of antioxidant. *Food Chemistry Toxicology, 1986.* 24: 1007-1014.

[3]. Frankel E.N. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Science Technology, 1993.*4: 220-225.

[4]. Zhang, S., Cui Y.L, DiaoM.Y., Chen D.C., Lin Z.F. Use of platelet indices for determining illness severity and predicting prognosis in critically ill patients. *Chinese Medical Journal*, 2015. 128: pg2012-2018.

[5]. Gazzani G, Papetti A, Massolini G, Daglia M. Antioxidant and prooxidant activity of soluble components of some common diet vegetables and effect of thermal treatment. *Journal of Agricultural Food Chemistry*, 1998. 46: 4118-4122.

[6]. Arena E, Fallico B, danMaccarone E. Evaluation of antioxidant capacity of blood orange juices as influences by constituents, concentration process and storage. *Food Chemistry*, 2001. 74(4): 423-427.

[7]. Miller N.J., DiplockA.T.,dan Rice-Evans C.A. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *Journal of Agricultural Food Chemistry*, 1995. 43: 1794-1801.

[8]. Donovan J.L., Meyer A.S., Waterhouse A.L. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunusdomestica).* Journal of Agricultural Food and Chemistry, 1998.

[9]. Halliwell B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Res. 1996. 25: 57-74.

[10]. Halliwell B. and Gutteridge J.M.C. *Free Radicals in Biology and Medicine*, 2nd ed., Clarendon Press, Oxford, 1999.

[11]. Desmarchelier C., Ciccia G., Cussio J. Recent advances in the search for antioxidant activity in South American plants. In: Atta-ur-Rahman editor. *Studies in Natural ProductsChemistry*, 2000. 22:343-367.

[12]. Gordon,T.,Castelli,W.P.,Hjortland,M.C.High density lipoprotein as a protective factor against coronary health disease.*The Framingham study American Journal of Medicine*,1977. 62(5):707-714

[13]. Jacob RA. The integrated antioxidant system. *Nutritional Research, 1995*. 15: 755-766.

[14]. Gutteridge J.M.C and Halliwell B. Antioxidants in nutrition, health and diseases. Oxford University Press, 1994: New York.

[15]. Velioglu Y.S, Mazza G, Gao L, Oomah B.D. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Agricultural Food Chemistry*,1998. 46: 4113-4117.

[16]. Kahkonen M.P, HopiaA.I, Vuorela H.J, Rauha J. Antioxidant activity of plant extracts containing phenolic compounds. *Agricultural Food Chemistry, 1999*. 47: 3954-3962.

[17]. McDonald S., Prenzler P.D., Antolovich M., Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 2001. 73: 73-84.

[18]. Huang D., Ou B., Prior R.L. The chemistry behind antioxidant capacity assays. *Agricultural Food Chemistry*2005. 53: 1841-1856.

[19]. Wolfe K., Wu X, Liu R.H. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 2003. 51: 609-614.

[20]. Yildirim A., Oktay M, BilaogluV.The antioxidant activity of the leaves of *Cydonia vulgaris*. *Turkey Journal of Medical Science*, 2001. 31: 23-27.

[21]. Benavente-Garcia. Uses and properties of Citrus flavonoids. *Journal of Agricultural. Food and Chemistry. 1997* 45: 4505-4515.

[22]. Koleva II, Van Beek T.A., Linssen J.P.H., de Groot A., danEvstatievaL.N.Screening of plant extract for antioxidant activity: a comparative study on three testing methods. *Phytochemistry Analysis 2002.* 13: 8-17.

[23]. Boveris, A.; Cadenas, E.; Reiter, R.; Filipkowski, M.; Nakase, Y., Chance, B. Organ chemiluminescence: noninvasive assay for oxidative radical reactions *Proceeding of theNational Academy of Sciences of the United States,1980*.177, 347-351
[24]. Boveris, A.; Repetto, M.G.; Bustamante, J.; Boveris, A.D. and Valdez, L.B. The concept of oxidative stress in pathology. In: Álvarez, S.; Evelson, P. (ed.), *Free Radical Pathophysiology*,008. 2 1-17, Transworld Research Network: Kerala, India
[25]. Fridovich, I. Superoxide radicals, superoxide dismutases and the aerobic lifestyle. *Photochemistry and Photobiology*, 1978. 28: 733-741
[26]. Chance, B., Sies, H., Boveris, A.Hydroperoxide metabolism in mammalian organs. *Physiological Reviews 1979*.59:527-605.
[27]. Repetto, M.G.; Reides, C.; Evelson, P.; Kohan, S.; Lustig, E.S. de and Llesuy, S. Peripheral markers of oxidative stress in probable Alzheimer patients. *European Journalof Clinical Investigation, 1999*.29:643-649
[28]. Fiszman, M.; D´Eigidio, M.; Ricart, K.; Repetto, M.G.; Llesuy, S.; Borodinsky, L.; Trigo, R.; Riedstra, S.; Costa, P.; Saizar, R.; Villa, A. &Sica, R. Evidences of oxidative stress in Familial Amyloidotic Polyneuropathy Type 1. *Archives of Neurology*,60:593-597. 2003
[29]. Famulari, A.; Marschoff, E; Llesuy, S.; Kohan, S.; Serra, J.; Domínguez, R.; Repetto, M.G.; Reides, C. and Lustig, E.S. de. Antioxidant enzymatic blood profiles associated with risk factors in Alzheimer's and vascular diseases. A predictive assay to differentiate demented subjects and controls. *Journal of the Neurological Sciences*, 141: 69-78. 1996
[30]. Taylor, L. The healing power of rainforest herbs. Square One Publishers: USA; 528. 2005
[31]. Guarin, N.G. *Plantasmedicinais do Estado do MatoGrosso*. AssociaçãoBrasileiradeEducaçãoAgrícola Superior, p.31. 1996
[32]. Barreto, G.S. "Effect of butanolic fraction of *Desmodiumadscendens* on the anococcygeus of the rat." *Braz. J. Biol*; 62(2): 223–30. 2002
[33]. Addy, M.E. Some Secondary Plant Metabolites in *Desmodium Adscendens* and their effects on Anaihidonic Acid Metabolism. Prostaglandins LeukotrienesEssent. *Fatty Acids*, 47(1): 85-91. 1997
[34]. Trease, G.E and Evans W.C. .Pharmacognosy 12th Ed. Bailliere Tindal, London: 1984. 622.
[35]. Ohwada, K. Improvement of cardiac puncture in mice: JikkenDodutsu.35 (3):353-35. 1986

Seriki SA. "Antioxidant Activities of Desmodiumadscendens; A Trial On Wistar Rats." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 10, 2019, pp51-66.