Sub-Acute Effects of Hot Aqueous *Arachis hypogaea* Pericarp Extract on Serum Antioxidant Markers Following Doxorubicin Administration in Male Wistar Rats

Ruth Asonye [1], **Lucky Legbosi Nwidu** [1], **Muskan** [2], **Miracle Chidinma Amaefule** [3], **Isaac Excellent** [4]

[1] *University of Port Harcourt*
P. M. B. 5323, Choba, Rivers State, Nigeria

[2] *Birmingham City University*
Curzon Building, 4 Cardigan Street, Birmingham, B4 7BD, UK

[3] *Imo State University*
Samek Road off Okigwe Owerri Rd, Owerri, Imo 23401, Nigeria

[4] *Michael Okpara University of Agriculture*
P. M. B. 7267 Umuahia Umudike, Abia State, Nigeria

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Corresponding Author:
Ruth Asonye

Abstract. The toxicity of Doxorubicin, an anthracyclic antibiotic, a valuable drug as the drug of choice for treatment of various cancer types, has been an issue of great concern. The drug generates reactive oxygen species (ROS), which induce oxidative stress and inflammation in body tissues; hence, there is a need for adjuvant therapy while monitoring the concentration of Doxorubicin in serum. This study aims to evaluate the antioxidant properties of *Arachis hypogaea* L. (Fabaceae family) pericarp, commonly known as groundnut peel, and its subacute effect on serum antioxidant markers following doxorubicin administration. Researchers have studied several antioxidant markers, including superoxide dismutase, lipid peroxidation, glutathione peroxidase, and catalase. These markers indicate the balance between the body's antioxidant defence and oxidative damage. Results showed a significant decline in oxidative stress and a reduction in the prospects of tissue injury induced by Doxorubicin administration. Hence, *Arachis hypogaea* pericarp extract can play a protective role against Doxorubicin-induced oxidative stress and toxicity in serum, thereby enhancing antioxidant defence.

Keywords: *Arachis hypogaea* L. pericarp extract (AH-HAPE); antioxidant properties; serum antioxidant markers; doxorubicin toxicity; sub-acute effect; oxidative stress; lipid peroxidation; superoxide dismutase; glutathione peroxidase; catalase.

INTRODUCTION

Background to the Study. *Arachis hypogaea* L. (Fabaceae), commonly known as peanut, is a widely cultivated and economically important crop. Its geographical source, however, can be traced back to Brazil and Peru in South America, where it has been cultivated for thousands of years and is now grown in various regions worldwide, including Nigeria. Although called nuts, it is a legume known for its edible seeds and oil. The pericarp of *Arachis hypogaea* L. refers to the outer covering or shell of the peanut. It is commonly known as groundnut peel. The pericarp serves as a protective layer for the peanut seed inside. It is composed of multiple layers, including the exocarp, mesocarp, and endocarp. The exocarp is the outermost layer, followed by the mesocarp, which provides cushioning and moisture regulation. The endocarp is the innermost layer, forming a tight seal around the seed [1].

In Nigeria, peanuts are referred to by various local names, such as "groundnut" or "earth nut," reflecting their significant importance in daily life. They are often used for cooking oil production, animal feed, and as a snack due to their high nutritional value. Additionally, peanut butter made from *Arachis hypogaea* L. is gaining popularity among health-conscious individuals.

1) Pericarp Uses of *Arachis hypogaea* L. The pericarp of *Arachis hypogaea* L. is vital for various reasons. Firstly, it plays a crucial role in the overall quality and marketability of the peanut. Secondly, the peanut's pericarp contributes to its nutritional value. It contains a significant amount of fibre, vitamins, minerals, and antioxidant properties, which are essential for human health. Additionally, the pericarp serves as a barrier against external factors, including pests, diseases, and environmental stresses, thereby protecting the seed from damage.

In recent years, there has been growing interest in studying the pericarp of *Arachis hypogaea* L. to gain a deeper understanding of its structure and composition. Researchers have conducted extensive research to uncover the genetic mechanisms responsible for the development and characteristics of the pericarp [1].

This knowledge can aid in the development of improved peanut varieties with desirable pericarp traits, including enhanced protection and improved nutritional content. Understanding the pericarp of *Arachis hypogaea* L. can also lead to advancements in post-harvest processing techniques. For example, knowledge of the pericarp composition and structure can guide efforts to improve methods for shelling and processing peanuts, resulting in increased efficiency and reduced waste.

Knowledge of the composition and structure of the pericarp of *Arachis hypogaea* L. can provide insights into its potential uses beyond the peanut seed itself. For instance, researchers have discovered that the pericarp of peanuts is known to accumulate various bioactive compounds, including anti-fungal and antibacterial metabolic by-products and flavonoids. These compounds play a crucial role in protecting the seed from microbial infections. By studying the pericarp of *Arachis hypogaea* L., researchers can potentially identify and isolate specific genes responsible for the synthesis of these protective compounds, and develop strategies to enhance their production in peanut cultivars with improved resistance against fungal and bacterial pathogens. This approach reduces the need for chemical pesticides and improves overall crop yield [2]. Studying the pericarp of *Arachis hypogaea* L. can provide valuable insights into the plant's defence mechanisms against biotic and abiotic stresses, serving as a bio-mimicry for anti-oxidative stress studies [1]. These compounds also hold potential for various applications in the pharmaceutical, cosmetic, and food industries. The pericarp extracts have found applications in traditional medicine for treating ailments such as diarrhoea and respiratory disorders. Further investigations are being conducted to explore their potential use in skincare products, due to their ability to promote wound healing and protect against UV-induced damage [3].

2) Ameliorative Properties on Cardio Toxicity. Cardio toxicity refers to the damaging effects exerted on the heart by certain medications or toxins. It is a significant concern since it can lead to severe cardiac dysfunction or even heart failure. Biomarkers such as electrocardiographic changes, as well as levels of troponin T and Troponin I, are commonly used to assess the presence and extent of cardiac toxicity.

Doxorubicin is an anticancer drug widely used due to its potent cytotoxic effects against cancer cells; however, it also exhibits cardiotoxic side effects, which limit its long-term use. Studies have shown that *Arachis hypogaea* L. pericarp extracts possess ameliorative properties against cardiotoxicity induced by Doxorubicin. These extracts can reduce oxidative stress, protect against mitochondrial damage, and preserve cardiac function [4].

Statement of Problem. In the year 2020, the number of new cases of cancer in Nigeria rose to an estimated value of 124,815, with breast cancer accounting for 22.7% of the cases; this is followed by prostate cancer (12.3%), cervical cancer (9.7%), colorectal cancer (6%), and non-Hodgkin lymphoma, accounting for about 5.9% of the cases. Every Nigerian has an 11.7% risk of developing cancer before the age of 75. The number of deaths resulting from cancer-related cases in Nigeria was estimated to be 78,899 in 2020 [5]. According to the Global Cancer Observatory, there were 115,950 cases of cancer in Nigeria recorded in 2018. The most common cases of cancer seen in Nigeria are breast cancer, cervical cancer, prostate cancer, colorectal cancer, and non-Hodgkin lymphoma [6].

Treatment of various types of cancer, including Hodgkin's and non-Hodgkin's lymphomas, has become possible with Doxorubicin as an effective chemotherapy agent. However, it is known to cause damage to the muscles of the heart, resulting in a condition known as Congestive heart failure (CHF). The early damage occurs within 1 to 2 days following the administration of the drug, while the late stage of damage gradually becomes serious and begins about a year or more after the initiation of doxorubicin chemotherapy [7].

The heart muscles are mostly affected. They become weakened, making it more difficult for the heart to pump blood. The risk of CHF is dose-dependent, ranging from approximately 4% to 36%. Ideally, the total dose of Doxorubicin a person should receive throughout their lifetime should not exceed 450 mg per square metre of the body surface [7].

A recent study shows that 9% of patients treated with anthracyclines such as Doxorubicin experienced cardiotoxicity [8], which is indicated by a decline in left ventricular ejection fraction by greater than 10% and an absolute value of <50% within the first year following completion of anthracycline-based chemotherapy [9].

Aim and Objective of the Study

The study aims to evaluate the sub-acute effect of *Arachis hypogaea* pericarp extract on serum antioxidant markers following the administration of Doxorubicin. The study will also assess the impact on cardiac biomarkers.

To determine the sub-acute effect of hot aqueous *Arachis hypogaea* L. pericarp extract (AH-HAPE) on serum antioxidant markers following Doxorubicin administration.

To evaluate the antioxidant changes in the serum of rats following administration of AH-HAPE and Doxorubicin.

The significance of this study is to evaluate the sub-acute effect of AH-HAPE on serum antioxidant markers following the administration of Doxorubicin.

Literature Review

Doxorubicin. In the early 1950s, a group of Italian researchers from Farmitalia Research Laboratories discovered a new strain of Streptomyces peucetius var. caesius, which produced a red pigment. This bacterium produced an antibiotic that showed significant activity against murine tumours. The research team named the compound Daunorubicin, combining the names "daun" and "rubicin." This pre-Roman tribe occupied the area of Italy where the compound was isolated at the time, with the French word for ruby, rubis, which describes the colour. Researchers discovered in the 1960s that Daunorubicin produced fatal cardiac toxicity [10].

Minor adjustments to the compound's structure enhanced its biological activity. Researchers mutated a strain of Streptomyces, which then produced a different, red-coloured antibiotic. Scientists then changed the name to Doxorubicin to conform to the established naming convention. Doxorubicin showed greater activity than Daunorubicin against solid tumours. It also showed a higher therapeutic index, but cardiotoxicity was still found to be present. Doxorubicin and Daunorubicin, together, can be considered prototype compounds for the anthracyclines [10].

Doctors use Doxorubicin as a chemotherapy drug to treat various forms of cancer. They administer it intravenously, and the body rapidly distributes it to different tissues. The liver primarily metabolises the drug, and the bile excretes most of it, while the kidneys eliminate a small percentage. Its pharmacokinetics show a high volume of distribution, extensive tissue binding, and a relatively slow elimination rate.

1) *Mechanism of Action*. The mechanism of action of Doxorubicin involves intercalation into DNA and the disruption of topoisomerase II-mediated repair activities, thereby inhibiting replication and transcription processes and inducing apoptosis, which results in DNA damage. This process leads to the generation of reactive oxygen species (ROS), which causes cellular damage. Doxorubicin is effective against a variety of cancers- breast cancers, gastric cancers, lung cancer, cancer of the thyroid gland, ovarian cancer, non-Hodgkin's and Hodgkin's lymphoma, Sarcoma, multiple myeloma and paediatric cancer. One of the known significant side effects of Doxorubicin is cardiotoxicity; this does not exclude toxicity to other organs such as the liver, kidney and brain.

2) *Pharmacokinetics of Doxorubicin*. Understanding the pharmacokinetics of Doxorubicin is crucial for maximising its dosage, achieving the intended therapeutic effects, and reducing side effects. Here is an overview of Doxorubicin's

pharmacokinetics, with particular attention to its absorption, distribution, metabolism, and elimination.

2.1. Absorption. Following an intravenous infusion administration, Doxorubicin is almost entirely absorbed [11]. It permeates the cell via passive diffusion and is found to accumulate at higher concentrations within intracellular compartments than in extracellular ones.

2.2. Distribution. Due to its broad absorption into tissues and significant binding to plasma proteins (up to 75%), it has a vast volume of distribution. The steady-state distribution volume of Doxorubicin ranges from 809 L/m^2 to 1214 L/m^2 [12]. It is worth noting that Doxorubicin does not cross the blood-brain barrier.

2.3. Metabolism. Doxorubicin is rapidly and extensively metabolised in the liver, mainly via the cytochrome P450 3A4 enzyme. Doxorubicinol, an active metabolite produced by this metabolism, enhances the overall therapeutic efficacy of Doxorubicin [13]. This metabolic process contributes to Doxorubicin's effectiveness against cancer cells but also explains its tendency to cause cardiotoxicity [12].

2.4. Elimination. Doxorubicin has a long half-life of 20–48 hours. The primary excretion routes for drugs from the body are the faeces and urine. Nevertheless, a considerable amount of the medication is also removed by biliary excretion, which plays a role in its enterohepatic circulation. Approximately half of the dose is eliminated from the body unchanged [12].

3) *Clinical Indication*. Physicians prescribe Doxorubicin to treat neoplastic conditions such as: a) Acute myeloblastic leukaemia and Acute lymphoblastic leukaemia; b) Hodgkin and non-Hodgkin lymphoma; c) Metastatic bronchogenic carcinoma, metastatic breast cancer, metastatic soft tissue, metastatic Wilms' tumour, metastatic ovarian carcinoma, metastatic neuroblastoma, metastatic thyroid carcinoma, metastatic gastric carcinoma and metastatic transitional cell bladder carcinoma; d) Bone sarcomas.

Doctors also recommend Doxorubicin as part of adjuvant therapy for women who have had primary breast cancer resection and show evidence of axillary lymph node involvement.

It is also prescribed for AIDS-related Kaposi's Sarcoma following the failure of previous systemic chemotherapy or intolerance to such therapy and cases of ovarian cancer that have progressed or recurred after platinum-based chemotherapy in the liposomal formulation [12].

Silymarin

1) *Origin*. The dried fruits and seeds of the plant Silybum marianum, commonly referred to as milk thistle, are the source of Silymarin. It belongs to the class of polyphenolic flavonoid complexes. Chemically, it consists mainly of silybin, which accounts for approximately 50-70% of its extract [14]. It also contains small amounts of taxifolin and other polyphenolic compounds, as well as various flavonolignan compounds such as isosilybin, silychristin, and silydianin [15].

2) *Clinical indications*. Researchers have extensively studied Silymarin's hepatoprotective properties. Although they initially identified it as an antioxidant for the liver, more recent studies suggest that it may also be effective in treating other neurological conditions. These include neurological illnesses, mental disorders, cognitive deficits, metabolic diseases, and other brain-related disorders [15].

3) *Mechanism of Action*. Silymarin protects the liver by stabilising cell membranes, thereby preventing the penetration of toxins into liver cells. Additionally, it could mitigate oxidation and inflammation in the liver while promoting the development of hepatocytes [16].

4) *Pharmacokinetics*

4.1. Absorption. Following oral administration, the liver extensively metabolises Silymarin during first-pass metabolism. The primary components that reach systemic circulation are silybin A and silybin B. Due to its poor solubility in water, the oral absorption of this Silymarin is only approximately 23–47%. Rapid phase II conjugation further limits its bioavailability. Furthermore, Silymarin shows improved absorption in patients with hepatitis C and non-alcoholic fatty liver disease [14].

4.2. Distribution. Silybin A and silybin B, the diastereomers of Silymarin, are highly lipophilic and are extensively bound to plasma proteins, mainly albumin. This high protein binding results in a large volume of distribution. It is widely distributed to various tissue organs, including the liver, stomach, colon, and pancreas [14].

Experiments on mice have shown the following:

- Liver: Approximately 8.8 μg of free silibinin/g/g of tissue was found at peak values 0.5 hours after administration;

- Lung: At 0.5 hours, peak levels of 4.3 μg silibinin/g/g tissue were observed;

- Stomach: At 0.5 hours, peak levels were noted, constituting about 123 μg of silibinin/g/g of tissue;

- Pancreas: 5.8 μg of silibinin/g/g of tissue was present at peak levels, which happened at 0.5 hours;

- Prostate: The tissue reached peak silibinin levels of approximately 2.5 μg/g within an hour;

- Skin: At one hour, peak levels of 1.4 μg silibinin/g/g tissue were reached [17].

4.3. Metabolism. Phase II reactions rapidly metabolise Silymarin after absorption.. Silymarin undergoes both phase I and phase II biotransformation, primarily the latter. The CYP2C8 isoenzyme predominantly mediates the phase I metabolism of silybin, resulting in the production of O-demethylated metabolites. Four minor metabolites are also detected, three of which are monohydroxy and one of which is dihydroxy. After oral administration, the body rapidly metabolises Silymarin flavonolignans into their conjugates. These conjugates essentially produce glucuronides, which are the primary constituents of human plasma, comprising roughly 28% sulfated and 55% glucuronidated compounds [14].

4.4. Excretion. Hepatobiliary excretion is extensive. Like most flavonoids, silybin undergoes enterohepatic circulation in vivo. The secondary peak in the plasma concentration curve indicates that bacteria enzymatically break the β-glucosidic linkages of excreted glucuronidated silybin, allowing the body to reabsorb it. The body primarily excretes silybin via urine and bile [14].

4.5. Adverse effects. Silymarin is generally well-tolerated, with minimal toxicity. However, some adverse effects have been found associated with the use of Silymarin in a few populations: Hypersensitivity reactions are significant; Disorders of the skin and subcutaneous tissue, such as pruritus; Anorexia; Disorders of the digestive system, which include flatulence, diarrhoea, nausea, dyspepsia, and bloating or pain in the abdomen; Headaches.

Cardiac Biomarkers. Cardiac biomarkers are substances measured in the blood that help assess heart health. They may help with the early diagnosis or prognosis of cardiac problems. In other words, they are predictors of likely cardiotoxicity or coronary heart disease.

Creatine kinase (CK-MB) and cardiac troponins are the two most widely used cardiac biomarkers. Cardiac troponins (TnT, TnI & TnC) are the most sensitive and specific tests for myocardial damage, while CK-MB is relatively specific when skeletal muscle damage is not present [18]. Cardiac biomarker measurements, which are less complex and costly than cardiac imaging, can help doctors determine whether more involved or invasive procedures are necessary. It is, however, essential to note that elevated cardiac biomarker levels do not always signify heart disease [19].

The pharmacokinetics of cardiac biomarkers are characterised by their rapid release into the bloodstream following myocardial injury, followed by a gradual decline over time. One limitation is that some cardiac biomarkers, such as Troponin, can remain elevated for several days following a heart attack, leading to false-positive results and making it difficult to determine the timing of the event [19].

There are various cardiac biomarkers, and they include the following:

1) *Cardiac Troponins*. Troponin is a complex of three globular proteins (Troponin T, Troponin I & Troponin C) involved in muscle contraction that appears in the blood only when damage occurs to the heart. They are the most commonly used biomarkers in evaluating cardiac toxicity. It has the highest known sensitivity.

Although there are several other causes of elevated Troponin during (such as acute infarction, severe pulmonary embolism causing acute right heart overload, heart failure, myocarditis), heart attack is, however, the most common [20].

During a myocardial infarction, myocytes release Troponin from their cytosolic pool. The degradation of actin and myosin filaments prolongs this release. Troponin I (TnI) occurs only in the myocardium, whereas other isoforms (TnT and TnC) also appear in different muscles.

Troponin is released 2–4 hours after myocyte injury and persists for up to 7 days, with a maximum peak at 12 hours. Following myocardial injury, the heart secretes Troponin I as the first cardiac biomarker. Its high affinity for damaged myocardial tissue results from the prolonged degradation of actin and myosin filaments.

TnI level will remain elevated for 4-7 days after the damage to the heart. TnT level, however, remains elevated for at least several days, in some cases as long as 3 weeks. Troponin I is the most sensitive and specific test for myocardial injury because it is found only in the heart muscle and has increased specificity compared to CK-MB.

Typical values for Troponin I are <0.3 ng/ml and <0.2 ng/ml for Troponin T.

Clinicians can calculate infarct size using Troponins, but they must measure the peak on the third day [20].

2) *Creatine Kinase (CK-MB) Test*. The CK-MB test is a blood test that detects the enzyme creatine kinase-myocardial band (CK-MB) in blood to help diagnose or rule out a heart attack as a possible diagnosis. Doctors no longer use it as a first-choice blood test for diagnosing heart attacks because its short duration (2–3 days) prevents late diagnosis of acute myocardial infarction. There are better test methods currently available, such as the cardiac troponin tests [21].

The heart muscle expresses the CK-MB isoform of creatine kinase, which typically peaks between 10 and 24 hours after the onset of symptoms. Its standard range is 2 to 6 ng/ml [21].

3) *Lactate Dehydrogenase (LDH)*. The LDH enzyme is found in nearly all living organisms. It is involved in cellular respiration, the processes of which include:

- Conversion of Pyruvate: The LDH enzyme catalyses the conversion of pyruvate (the end product of glycolysis) into lactic acid. During this process, LDH also oxidises a unit of the energy-transferring molecule NADH to NAD+, allowing for the continuity of glycolysis [22];

- Tissue Damage Indicator: LDH is released into the bloodstream or other body fluids in the event of tissue injury. Therefore, a test indicating elevated LDH levels suggests chronic or acute diseases or injuries affecting specific tissues/organs.

The heart muscle typically contains the LDH-1 isozyme, while blood serum predominantly contains LDH-2. A high LDH-1 to LDH-2 ratio indicates myocardial infarction. Tissues also release high LDH levels during breakdown or haemolysis [20].

4) *Myoglobin*. Myoglobin (MB) is an iron & oxygen-binding protein present in the cardiac and skeletal muscle tissues of most mammals. It is the primary oxygen-carrying pigment of muscle tissues released in response to muscle tissue damage. Although it responds very rapidly, giving rise to a rise and fall earlier than Troponin or CK-MB, myoglobin is used less frequently than other markers due to its low specificity for myocardial infarction. Doctors recommend carrying out the test within the first 2 hours, as the approximate peak occurs at that time [20].

5) *D-Dimer*. This is a sensitive yet nonspecific marker produced as a result of fibrin degradation following the formation of blood clots. An elevated D-dimer level is indicative of a blood-clotting condition, especially in cases of Deep Vein Thrombosis (DVT) [23]. Monitoring D-dimer levels is essential to assess the presence of D-dimer in acute myocardial infarction, as a coronary thrombus may rupture, which is a prerequisite for its pathophysiology [23].

Oxidative Stress. The standard cancer treatment drug doxorubicin exerts its anti-tumour activity by inhibiting DNA replication and transcription. Dose-dependent cardiotoxicity, however, restricts its clinical utility. The generation of ROS causes oxidative stress, which is a significant factor in the cardiac damage caused by Doxorubicin. Reactive oxygen species (ROS) are oxygen-containing molecules with high chemical activity. These molecules can take many different forms, but they all share a common characteristic: the presence of an electron with only one bond [24].

1) *Antioxidants*. Antioxidants play a crucial role in slowing down lipid peroxidation. They neutralise free radicals by terminating radical chain reactions. Examples of antioxidants include vitamin C, vitamin E, superoxide dismutase, catalase, and peroxidase.

2) *Serum Antioxidants*. When evaluating oxidative stress and general health, serum antioxidant biomarkers are essential. These indicators show how the body's antioxidant defence systems interact with the generation of reactive oxygen species (ROS). Some frequently employed antioxidant labels include:

2.1. Antioxidants with enzymes: they consist of:

- Malondialdehyde (MDA): The malondialdehyde (MDA) test measures the amount of MDA, a by-product of lipid peroxidation that can potentially damage tissues. The presence of catalase, an enzyme that converts hydrogen peroxide into water and oxygen, can be detected with the catalase test [25].

- Superoxide Dismutase (SOD): Converts superoxide radicals to hydrogen peroxide. The activity of the enzyme superoxide dismutase (SOD), which breaks down superoxide radicals into oxygen and hydrogen peroxide, is measured using the SOD test [26].

- Catalase (CAT): Breaks down hydrogen peroxide into water and oxygen. Catalase is one of the enzymes that can prevent or reduce oxidative stress by breaking down hydrogen peroxide, a potent ROS, into harmless products. Its activity is quantified based on the decomposition of hydrogen peroxide [25].

- Glutathione Peroxidase (GPx): GPx reduces lipid hydroperoxides by utilising glutathione. The GPx family consists of eight members: GPX1 to GPX8. The reduction of hazardous hydro-/organo peroxides, like hydrogen peroxide ($H2O_2$), into water (H2O) or related alcohols (ROH) is catalysed by these selenium compounds. To counteract oxidative stress and maintain redox balance, members of the GPx family are crucial [27].

2.2. New Indicators of Serum Antioxidants.

F2-Isoprostanes: Arachidonic acid is oxidised by ROS, leading to the formation of F2-isoprostanes. Researchers can use these markers to accurately detect lipid peroxidation. Elevated levels are indicative of oxidative stress and can be linked to various disease conditions, including myocardial infarction [28].

Lipid Peroxidation Mechanism:

- Initiation: A free radical attacks the fatty acid chain, and reactive oxygen species (ROS), such as hydrogen peroxide (HOO·) and hydroxyl radicals (OH·), participate in this step.

- Transmission: When the fatty acid radical combines with molecular oxygen, it produces a peroxyl-fatty acid radical. This radical can react in several ways, such as adding to another unsaturated fatty acid or removing hydrogen atoms from other fatty acids. New radicals are formed through these processes, initiating a "radical chain reaction."

- Termination: Radical reactions are terminated when two radicals combine.

The process of oxidising lipids to produce lipid peroxides as the main product is known as "lipid peroxidation". The primary byproduct of lipid peroxidation, lipid hydroperoxides (LOOH), is created during the propagation stage. One common aldehyde byproduct of lipid peroxidation is Malondialdehyde (MDA). Elevated levels of MDA in blood, urine and other tissues have been linked to several pathological conditions and are a potential biomarker. It is essential, however, to consider other factors and use MDA in combination with other biomarkers for a precise and comprehensive evaluation.

4-Hydroxynonenal (4-HNE) is a byproduct of aldehydes. It is highly toxic and contributes significantly to damage caused by oxidative stress. Their potential therapeutic effects are still under study by researchers [28].

Peanut (Arachis hypogaea L.) Pericarp Extract. Grown primarily for its edible seeds, the peanut (*Arachis hypogaea*), also called the groundnut, is a legume crop. It is widely grown in the tropics and subtropics, important to both small and large commercial producers. Its high oil content classifies it as both an oil crop and a grain legume. It is unusual for legume crop plants to develop peanut pods underground (geocarpy) as opposed to above ground. The botanist Carl Linnaeus named peanuts Hypogaea, which translates to "under the earth," in recognition of this trait [29].

The pericarp is the soft outer tissue covering the peanut. Despite having a significant number of bioactive phytochemicals, peanut pericarps are a byproduct of peanuts and have little socioeconomic value. Research is ongoing to enhance the nutritional value of peanut pericarp and eliminate some of its astringent qualities.

In one study, powdered peanut pericarps, both raw and roasted, underwent a 48-hour extraction process in 70% methanol. The results of the phytochemical screening included the determination of the total phenolic and flavonoid contents (TPC, TFC). The assays used to determine and compare the antioxidant activity with standard antioxidants included Fe2+ chelating, ferric reducing antioxidant power (FRAP), OH radical scavenging, and reducing power.

In comparison to the raw peanut pericarp extract, the roasted peanut pericarp extract exhibited significantly higher ($p < 0.05$) DPPH radical, OH- radical scavenging capacity, Fe2+-chelating, and ferric reducing antioxidant property. The concentration-dependent percentage inhibitions produced by both extracts were significantly lower than those of the applied antioxidant standards. The results, however, showed that roasting improved the in vitro antioxidant properties of the peanut pericarp compared to the

raw peanut pericarp; this may enhance its utility as an antioxidant, a functional food ingredient, and in the animal feed sector [30].

1) *Arachis hypogaea Pharmacological activities. Arachis hypogaea*, commonly known as groundnuts or peanuts, exhibits good antioxidant properties [29]. Researchers have reported that the pericarp of groundnuts is rich in phenolic compounds, which confer excellent antioxidant properties [29, 31]. The class of phenolic compounds found in ground pericarp is known as procyanidins, which have been shown to possess potent antioxidant, anti-inflammatory, anti-ageing, and anticancer activities, as well as immunity-regulating properties [32].

2) *Arachis hypogaea pericarp Chemical constituents*. The pericarp (skin) of *Arachis hypogaea* is rich in several compounds with diverse biological activities, most notably polyphenolic compounds [32, 33]. Researchers divide the phenolic compounds into four classes: phenolic acids, flavonoids, tannins, and stilbenes [33]. Coumaric acid, ferulic acid, and caffeic acid comprise most of the phenolic acids found in A. hypogaea pericarp [32, 33]. Resveratrol is the main stilbene found in A. hypogaea pericarp [32, 33]. Much research has shown that procyanidins, the primary phenolic compound in A. hypogaea pericarp, have antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-ageing effects [32].

3) *Arachis hypogaea* scientific classification.

Table 1 – Classification of *Arachis hypogaea* according to the United States Department of Agriculture [34]

Rank	Scientific Name and Common Name
Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae* Lindl. - Pea family
Genus	*Arachis* L. – peanut
Species	Arachis hypogaea L.

METHODS

Drugs, Chemicals & Reagents. Doxorubicin, Silymarin (Micro LABS LTD), Hot aqueous *Arachis hypogaea* L. pericarp extract, Distilled water, Tween 80, Normal saline, Phosphate buffer, Thiobarbituric acid (TBA reagent), Adrenaline, Carbonate buffer, Hydrogen peroxide, 10% Formalin, Ethyl alcohol, Alcohol (70% and 95%), Absolute Alcohol, Xylene, Paraffin wax, Haematoxylin, Glycerine gel.

Equipment and Materials. Wire mesh cages (locally made), Hot plate, Measuring cylinder, Hand gloves (Latex), Teabags, Syringes 1 ml (Danagel, Nigeria), Oral cannula (16G size), Electronic Digital Scale (Xin Yuan, China), Dissecting set, Surgical blade, Centrifuge (Sorvall Instruments, Germany), Micro pipette, Cuvette, Vis Spectrophotometer (Searchtech Instruments, England), Test tubes, EDTA bottles, Thermostatic water bath (HH-W420, Jinotech instruments), Test tubes rack, Blood collection tube (Non vacuum plain tube), Tea bag, Hot air oven, Slides, Spatula, Glass mortar and pestle, Stop watch, Cylindrical Beakers, Cover slips, Microscope.

Collection of the Arachis hypogaea L. Pericarp. Researchers obtained the pericarp of *Arachis hypogaea* L. from local groundnut vendors. They carefully deseeded it and removed unwanted particles. Using a manual grinder, they ground the deseeded pericarp. Afterwards, they transferred 5 g of the pericarp into each of 24 tea bags.

1) Preparation of *Arachis hypogaea* L. Pericarp Extract (AH-HAPE). A tea bag containing 5 g of ground *Arachis hypogaea* L. pericarp was inserted into a beaker containing 100 *ml* of boiled distilled water. Researchers shook the mixture intermittently for 10 minutes and then gently squeezed the teabag to extract more liquid. They administered the respective doses of the hot aqueous *Arachis hypogaea* L. pericarp extract to the experimental animals according to their weight.

2) Preparation of 1% Tween 80. Researchers transferred 1 *ml* of Tween 80 into a beaker and diluted the preparation to 100 *ml* by mixing it with 99 *ml* of distilled water. They then used 5 *ml* of this 1% Tween 80 solution to dissolve the Silymarin tablet (70 mg). Animals in group 6 each received 1 *ml* of the prepared Silymarin suspension.

3) Preparation of Doxorubicin. Researchers dissolved a 50 mg dose of Doxorubicin in 10 *ml* of normal saline and administered the corresponding doses to each experimental animal in its respective group.

4) Experimental Animal Care and Handling. Researchers procured twenty-four male Wistar rats (weighing 100–150 g) from the Basic Medical

Sciences Animal House at the University of Port Harcourt, Rivers State. They acclimatised the animals for one week (7 days) under ambient temperature and standard humid conditions. They were also placed on a top feed diet (grower's mash) and provided with suitable drinking water obtained from the Faculty of Basic Medical Sciences at the University of Port Harcourt. Researchers cleaned the animal cages daily and replaced their feed and water as often as necessary. The University of Port Harcourt's Ethics Committee approved the experimental design and protocol with approval number UPH/CEREMAD/REC/MM95/009 on February 29 2024. See the original copy of the approval letter in the appendix.

Acute Toxicity Study (Modified Lorke's Method). A total of 15 healthy male mice were rando*mly* selected and allowed to acclimatise for seven days. They were divided into five groups, each containing three mice.

For group 1, 100 ml of boiled distilled water was used to extract 1g of powdered *Arachis hypogaea* L. pericarp, and 5 ml/kg was administered to the mice.

For group 2, 100 ml of boiled distilled water was used to extract 2 g of powdered *Arachis hypogaea* L. pericarp, and 5 ml/kg was administered to the mice.

For group 3, 100 ml of boiled distilled water was used to extract 3 g of powdered *Arachis hypogaea* L. pericarp, and 5 ml/kg was administered to the mice.

For group 4, 100 ml of the boiled distilled water was used to extract 4 g of the powdered *Arachis hypogaea* L. pericarp, and 5ml/kg was administered to the mice.

For group 5, 100 ml of boiled distilled water was used to extract 5 g of powdered *Arachis hypogaea* L. pericarp, and 5 ml/kg was administered to the mice.

The researchers observed the animals for 24 hours.

Experimental Study Protocols. Researchers divided a total of 36 male Wistar rats into six groups, ensuring that each group contained at least four rats. The researchers fed and weighed the rats daily for 10 days. A fresh, hot aqueous extract of *Arachis hypogaea* L. pericarp was prepared daily by saturating a 5 g tea bag of pericarp that had been soaked in 100 *ml* of boiled water, shaking intermittently for 10 minutes, and then extracting.

Group 1 (Negative Control): The animals in this group received 5 ml/kg of distilled water daily for 10 days.

Group 2 (positive group): The animals in this group received 2.5 mg/kg of Doxorubicin on alternate days per week.

Group 3: The animals in this group received 0.625 ml/kg of the hot aqueous *Arachis hypogaea* L. pericarp extract (AH-HAPE) followed by 2.5 mg/kg doxorubicin one hour later.

Group 4: The animals in this group received 1.25 ml/kg of the hot aqueous AH-HAPE, followed by 2.5 mg/kg doxorubicin one hour later.

Group 5: The animals in this group received 2.5 ml/kg of the hot aqueous AH-HAPE, followed by 2.5 mg/kg doxorubicin one hour later.

Group 6: The animals in this group received 100 mg/kg silymarin followed by 2.5 mg/kg doxorubicin one hour later.

The Doxorubicin was administered on alternate days per week.

Determination of Oxidative Stress Parameters

a) Catalase Enzyme Activity (According to AEBI, 1984). The researchers added 4 ml of H_2O_2 reagent (0.036%) to a test tube and pipetted 100 µL of the sample into the same tube. The researchers measured the absorbance at 0, 30-second, and 1-minute intervals and recorded the change in absorbance at 240 nm.

b) Super Oxide Dismutase (SOD), Method According to authors [35]. The researchers measured 4 ml of carbonate buffer into a test tube, pipetted 100 µL of the sample mix, and incubated it at 37 °C. They initiated the reaction by pipetting 100 µL of adrenaline and took readings at 0, 30 seconds, and 1 minute at 480 nm. They repeated the same procedure for a blank without the sample.

c) Lipid Peroxidation According to authors [36]. The researchers prepared a 1% TBA solution in 100 ml of 0.05 M sodium hydroxide and glacial acetic acid, labelling it as the working reagent.

Procedure. The researchers added 100 µL of the sample to 3 ml of working reagent, boiled it for 15–20 minutes, and then centrifuged it for 15 minutes to obtain a clear supernatant. They measured the sample at a wavelength of 532 nm.

1) Nitric Oxide Assay. Procedure:

Sample Preparation: Preparation of samples, or any other biological or chemical samples that may contain nitrite and nitrate.

Standard Curve Preparation (Optional): A standard solution containing known concentrations of nitrite or nitrate. (0 to 10 mg/ml) was prepared.

Assay Setup: Each test tube for the samples, standards, and blank was labelled.

Add Griess Reagent I: Griess Reagent I was added to each test tube containing the samples, standards, and blank. The Griess Reagent was added in equal volume to the samples and mixed properly.

Incubation: The researchers incubated the cuvettes at room temperature for 10 minutes, allowing the Griess Reagent I to react with nitrite to form a diazo compound.

Add Griess Reagent II: After incubation, the researchers added an equal volume of Griess Reagent II to each test tube. Griess Reagent II contains sulphanilic acid and N-1-naphthylethylenediamine dihydrochloride. The researchers thoroughly mixed the solution to ensure the reaction mixture was homogeneous.

Incubation (Part 2): The researchers incubated the cuvettes for an additional 10–15 minutes at room temperature. This incubation allows the diazo compound to react with Griess Reagent II to form a coloured azo dye.

Read Absorbance: The researchers measured the absorbance of the samples at 542 nm using a spectrophotometer. The absorbance is directly proportional to the concentration of nitric oxide. The concentration of Nitric oxide is calculated from the standard.

2) Glutathione Peroxidase. Pipette 50 µL of the sample into a test tube and add 1,000 µL of buffered hydrogen peroxide solution (0.74 mM or 0.25%). Add 300 µL of pyrogallol solution (20 mM). Mix and read the absorbance at 430 nm. Allow to stand at room temperature for 5 minutes. Stop the reaction by the addition of 100 µL sulphuric acid (2.5 M). Reread the absorbance.

GPx activity (U/mg or µmol/min/mg) = (Change in Absorbance/Reaction time × Sample volume) × Dilution Factor.

RESULTS AND DISCUSSION

The biochemical results of this study, as presented in Table 2, show no mortality in all animals following the administration of respective doses of Doxorubicin and AH-HPE extract in the respective groups.

Table 2 – Mortality Rate from Acute Toxicity

Group	Drugs Given	Number of deaths
Group 1 (Negative control)	5 ml/kg of distilled water	0
Group 2 (Positive control)	2.5 mg/kg of Doxorubicin	0
Group 3	0.625 ml/kg of AH-HAPE* + 2.5 mg/kg Doxorubicin	0
Group 4	1.25 ml/kg of AH-HAPE + 2.5 mg/kg Doxorubicin	0
Group 5	2.5 ml/kg of AH-HAPE + 2.5 mg/kg Doxorubicin	0
Group 6 (Reference group)	100 mg/kg of Silymarin + 2.5 mg/kg Doxorubicin	0

Notes: AH-HAPE – Hot aqueous *Arachis hypogaea* pericarp extract.

The results of this study, as presented in Table 3, indicate a significant increase in body weight among animals in groups 3 and 5, compared to group 2, following the administration of AH-HAPE to doxorubicin-induced oxidative stress in the serum of male Wistar rats over 10 days.

Table 3 – Effect of supplementation of AH-HAPE on body weight following Doxorubicin-induced toxicity in rats for 10 days

Groups	Initial body weight, g	Final weight, g
Group 1 (negative control)	115.25 ±4.65	131 ±21.69
Group 2 (positive control)	124.75 ±15.15	129 ±29.14
Group 3	118.5 ±14.84	143.5 ±22.48
Group 4	115 ±12.14	131 ±15.96
Group 5	132.66 ±16.19	162.67 ±28.57
Group 6 (reference group)	95.25 ±7.93	117±14.49

Notes: Group 1: Negative control received 5 ml/kg of distilled water; Group 2: Positive control received 2.5 ml/kg of AH-HAPE, received 2.5 mg/kg and Doxorubicin; Groups 3-5: 0.625 ml/kg, 1.25 ml/kg, 2.5 mg/kg, respectively and 2.5 mg/kg Doxorubicin; Values represented as mean ± standard deviation from the mean (n=4); Values are compared with initial weights and control groups using a one-way ANOVA test.

Groups 1 and 4, respectively, showed no significant increase in body weight compared to the toxic group. Only group 6 showed a decrease in body weight compared to group 2.

The biochemical results, as shown in Table 4, demonstrated that AH-HAPE had a significant effect on serum antioxidant markers following Doxorubicin (DOX)-induced oxidative stress in the rats. MDA levels were significantly decreased in groups 3-5 compared with the negative control group 1 & positive control group 2.

Table 4 – Sub-Acute exposure effect of AH-HAPE on antioxidant markers in Doxorubicin (DOX) - induced oxidative stress in serum of Wistar male rats

Group	MDA (nmol/ml)	SOD (units/ml)	CAT (mmol/min/ml)	Gpx activity (μmol/min/mg tissue)
Group 1 (negative control)	0.1125 ±0.0005774	0.2045 ±0.00238	5.143 ±0.03202	12.83 ±0.9708
Group 2 (positive control)	0.1343 ±0.003304****	0.1053 ±0.003304****	3.268 ±0.06898****	9.39 ±0.2689****
Group 3	0.1225 ±0.001732***	0.2225 ±0.00238****####	4.24 ±0.09764***###	11.97 ±0.559####
Group 4	0.1143 ±0.003202###	0.2353 ±0.0035****####	3.593 ±0.3378****	13.4 ±0.3575####
Group 5	0.1153 ±0.003304###	0.2448 ±0.002986****####	4.498 ±0.3568*####	12.81 ±0.4506####
Group 6 (reference group)	0.1098 ±0.002217###	0.2053 ±0.002872####	4.588 ±0.2654*####	13.26 ±0.1187####

Notes: Group 1: Negative control received 5 ml/kg of distilled water; Group 2: Positive control received 2.5 ml/kg of AH-HAPE, received 2.5 mg/kg of Doxorubicin; Groups 3-5: 0.625 ml/kg, 1.25 ml/kg, 2.5 mg/kg, respectively and 2.5 mg/kg doxorubicin; AH-HAPE – Arachis hypogaea hot aqueous pericarp extract; MDA: Malondialdehyde; sod: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; Values represented as mean ± standard deviation from the mean (n=4); **** – P<0.0001; *** – P 0.0004; * – P 0.0002 compared to group 1; #### – P<0.0002; ### – P<0.0001 compared to group 2.

Table 4 further shows a significant decrease in levels of Superoxide dismutase (SOD), an enzyme that scavenges superoxide radicals, in groups 3-5, especially in comparison to groups 1 and 2, respectively. The treatment exhibited substantially more antioxidant activity compared to Silymarin. From Table 4, the biochemical results also demonstrate that (toxic) group 2 presented with decreased CAT activity. Groups 3-5, however, showed significantly increased levels of CAT, with Group 5 having the most significant elevation ($p < 0.01$).

Glutathione peroxidase (GPx) enzyme activities were significantly increased in groups 3-5 compared to groups 1 and 2, respectively (Table 4). As the dose of AH-HAPE increased, so did its antioxidant activity, even more effective than Silymarin at protecting against oxidative stress.

It is well known that Doxorubicin, a cytotoxic, cell-cycle-nonspecific anthracycline antibiotic, causes DNA strand breakages and damage by intercalating into DNA structures to destabilise them and induce damage. The compound not only alters the transcriptomes of cells but also initiates apoptotic pathways when it is unable to repair DNA damage. Furthermore, essential enzyme activities, such as those of DNA polymerase, RNA polymerase, and topoisomerase II, can be disrupted by doxorubicin intercalation, leading to cell cycle arrest. Lastly, Doxorubicin can potentially cause cellular damage by producing cytotoxic reactive oxygen species [37].

A particular type of flavonoid that is derived from milk thistle plant seeds is called Silymarin. It has demonstrated hepatoprotective, anti-inflammatory and antioxidant properties. Silymarin has been shown to mitigate oxidative stress and inflammation, hence protecting against doxorubicin-induced cardiotoxicity [16]. It exerts its effects by scavenging free radicals, preventing lipid peroxidation, and enhancing the activity of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione. It also improves liver regeneration by stimulating DNA and protein synthesis. RNA synthesis within the hepatocytes [16].

Results from the research provide information on the extent to which AH-HAPE effectively reduced Doxorubicin-induced oxidative stress in these rats.

ROS are crucial for the body's ability to adjust to stress. They can result in lipid peroxidation and are quite reactive, yet they are also essential to many physiological functions [38]. Malondialdehyde showed a significant increase in the blood serum of animals in group 2, indicating possible tissue injury and increased oxidative stress. When compared to groups 3-6, however, there was a substantial decline in MDA levels, with group 6 reaching nearly the same level as the negative control (Table 4); this suggests that the AH-HAPE was able to reduce lipid peroxidation.

Superoxide dismutase enzyme is abundantly produced in the body in three different isoforms-SOD1 distributed in the cytosol, SOD2 distributed in the mitochondria, and SOD3 distributed in the extracellular compartment [39, 40]. The activity of the enzyme superoxide dismutase (SOD) showed a significant dose-dependent increase across Groups 3-5, especially in comparison to the positive and negative groups 1 & 2, respectively. (Table 3). The action of superoxide may have been restricted by intracellular SODs, which are responsible for protecting iron-sulfur (Fe-S)-containing enzymes and mitigating their primary cause of tissue damage. This suggests that AH-HAPE enhances SOD function, scavenging reactive oxygen species, as effectively demonstrated in Maurya's research paper [40].

Catalase aids in tissue healing and serves as the first line of defence against oxidative stress. Despite its moderate reactivity, catalase can traverse biological membranes and create hydroxyl radicals (·OH). These radicals have the potential to harm tissues [41]. As demonstrated, AH-HAPE enhances CAT-mediated hydrogen peroxide breakdown, supporting Ransy's observations on the action of cellular catalase [42].

As protectors of the cell against oxidative stress, GPx enzymes maintain the delicate equilibrium between oxidation and reduction. Their numerous mechanisms of action underscore their importance in maintaining cellular health and preventing damage. The reduced GPX activity in the toxic group was indicative of oxidative stress induced by Doxorubicin. Groups 3-5 showed a dose-dependent increase in GPx levels, with Group 5 exhibiting the highest GPx activity. GPx1-GPx4 and GPx6 utilise selenocysteine as their active site. They catalyse the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols, thus reducing their toxicity. While its primary purpose is to reduce complex lipid compounds (small molecule hydroperoxides), GPx4 has the unique capability to destroy complicated lipid hydroperoxides directly. It also prevents the accumulation of lipid peroxides, which causes damage to cellular membranes. This dual enzymatic activity emphasises GPx4's importance in cellular defence against oxidative stress [27]. This study suggests that AH-HAPE likely enhances GPx expression, counteracting doxorubicin-induced oxidative stress.

CONCLUSIONS

The study reveals that *Arachis hypogaea* hot aqueous pericarp extract (AH-HAPE) possesses potent antioxidant properties against Doxorubicin-Induced Toxicity in the serum of male Wistar rats. Further study is required to exploit its effectiveness in the management of doxorubicin-induced toxicity effects in patients receiving chemotherapy treatment.

This study shows that AH-HAPE protects serum against oxidative stress. Researchers can develop it as an agent for the associated condition and as an antioxidant for adjuvant therapy in patients receiving Doxorubicin. They should also conduct further studies to determine the effects of prolonged or long-term use.

This study sheds light on the toxicity to blood serum with the administration of Doxorubicin as an anticancer agent.

This study assesses the antioxidant properties of *Arachis hypogaea* pericarp, a byproduct of a well-known legume crop (peanut), which is often considered waste.

This report also provides insight, as the first of its kind, into in vivo studies on the antioxidant effect of *Arachis hypogaea* on Doxorubicin-induced oxidative stress in rats.

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