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EFFECT OF LEAF AND STEM EXTRACTS OF *MUCUNA PRURIENS* (L) ON OXIDATIVE STRESS MARKERS IN LEAD ACETATE-INDUCED TESTICULAR TOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

The purpose of this study was to evaluate the effect of extracts of Mucuna pruriens leaves and stems on hormone levels in lead acetate-induced testicular toxicity in wistar albino rats. A total of fifty-four (54) adult male albino rats were randomly assigned into nine (9) groups of six (6) rats in each group. Then, toxicity was induced by administration of 60mg/kg of lead acetate (LA) twenty-four hours before administration of Mucuna pruriens extracts. All the administration was by oral intubation for 28 days as follows: Group A (Normal control) received only food and water. Group B was induced with 60mg/kg of LA and Untreated (Negative Control). Group C (Positive control) was induced with 60mg/kg of LA but was treated with a Standard drug (Clomid-1.0mg/kg). Group D was induced with 60mg/kg of LA and was treated with 200mg/kgBW Mucuna pruriens leaves ethanol extract (MPLE). Group E was induced with 60mg/kg of LA and was treated with 400mg/kgBW of MPLE. Group F was induced with 60mg/kg of LA and was treated with 600mg/kgBW of MPLE. Group G was induced with 60mg/kg of LA and was treated with 200mg/kgBW Mucuna prurien sstem ethanol extract (MPSE). Group H was induced with 60mg/kg of LA and was treated with 400mg/kgBW of MPSE. Group I was induced with 60mg/kg of LA and was treated with 600mg/kgBW of MPSE. Lead acetate (LA) administration resulted in a significant ($P<0.05$) reduction in antioxidant markers such as superoxide dismutase, catalase, and glutathione reductase but increased the malondialdehyde levels. Treatment with the extracts significantly ($P<0.05$) reversed the effects of the lead acetate. These findings suggest that Mucuna pruriens ethanol extracts have potential therapeutic effects against lead acetate toxicity. The presence of bioactive compounds with reported antioxidant effects suggests antioxidant mechanisms of Mucuna pruriens ethanol extracts against lead toxicity.

Keywords: Catalase, Superoxide dismutase, Glutathione reductase, Malondialdehyde, Lead Acetate.

Introduction

Medicinal plants are defined as those capable of alleviating or curing diseases and they have a traditional use as a remedy in a population or community (Dar *et al*., 2017). They have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries (Okujagu *et al*., 2008). The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics (Edeoga *et al*., 2005).

The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes, including treatment of infectious diseases (Igoli *et al*.,2003). Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials (Dar *et al*., 2017). Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials (Edeoga *et al*., 2005).

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Okujagu *et al*., 2008). These plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world (Edeoga *et al*., 2005). The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17.000 species of higher plants, of which approximately 8.000 species, are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely accepted (Igoli *et al*.,2003).

Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Dar *et al*., 2017). During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons (Lawal *et al*., 2010).

Background of the Study

Medicinal plants are an integral component of research developments in the pharmaceutical industry (Edeoga *et al*., 2005). Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds (Kantor, 2009). The development and commercialization of medicinal plant-based bioindustries in the developing countries is dependent upon the availability of facilities and information concerning upstream and downstream bioprocessing, extraction, purification, and marketing of the industrial potential of medicinal plants (Okujagu *et al*., 2008). Furthermore, the absence of modernized

socio-economic and public healthcare systems reinforces reliance of rural and lower-income urban populations on the use of traditional medicinal herbs and plants as complementary aids to routine pharmaceutical market products. Recent estimates suggest the over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Kantor, 2009). In the male reproductive system, the scrotum houses the testicles or testes, including providing passage for blood vessels, nerves, and muscles related to testicular function (Kefer *et al*., 2009). The testes are a pair of male reproductive organs that produce sperm and some reproductive hormones (Fisher *et al*.,1999). Each testis is approximately 2.5 by 3.8 cm (1.5 by 1 in) in size and divided into wedge-shaped lobules by connective tissue called septa. Coiled in each wedge are seminiferous tubules that produce sperm (Kumbhare *et al*.,2023).

Sperm are immobile at body temperature; therefore, the scrotum and penis are external to the body, so that a proper temperature is maintained for motility. In land mammals, the pair of testes must be suspended outside the body at about 2° C lower than body temperature to produce viable sperm. Infertility can occur in land mammals when the testes do not descend through the abdominal cavity during fetal development (Kesari *et al*.,2018).

Sperm mature in seminiferous tubules that are coiled inside the testes. The walls of the seminiferous tubules are made up of the developing sperm cells, with the least developed sperm at the periphery of the tubule and the fully developed sperm in the lumen (Kefer *et al*., 2009). The sperm cells are mixed with "nursemaid" cells called Sertoli cells which protect the germ cells and promote their development. Other cells mixed in the wall of the tubules are the interstitial cells of Leydig. These cells produce high levels of testosterone once the male reaches adolescence (Kesari *et al*.,2018).

When the sperm have developed flagella and are nearly mature, they leave the testicles and enter the epididymis. This structure resembles a comma and lies along the top and posterior portion of the testes; it is the site of sperm maturation (Kefer *et al*., 2009). The sperm leave the epididymis and enter the vas deferens (or ductus deferens), which carries the sperm, behind the bladder, and forms the ejaculatory duct with the duct from the seminal vesicles. During a vasectomy, a section of the vas deferens is removed, preventing sperm from being passed out of the body during ejaculation and preventing fertilization (Kesari *et al*.,2018).

Semen is a mixture of sperm and spermatic duct secretions (about 10 percent of the total) and fluids from accessory glands that contribute most of the semen's volume. Sperm are haploid cells, consisting of a flagellum as a tail, a neck that contains the cell's energy-producing mitochondria, and a head that contains the genetic material. An acrosome is found at the top of the head of the sperm. This structure contains lysosomal enzymes that can digest the protective coverings that surround the egg to help the sperm penetrate and fertilize the egg. An ejaculate will contain from two to five milliliters of fluid with from 50–120 million sperm per milliliter (Fahim *et al*.,2013).

The penis is an organ that drains urine from the renal bladder and functions as a copulatory organ during intercourse (Arafa and Agarwal, 2020). The penis contains three tubes of erectile tissue

running through the length of the organ. These consist of a pair of tubes on the dorsal side, called the corpus cavernosum, and a single tube of tissue on the ventral side, called the corpus spongiosum (Fahim *et al*.,2013). This tissue will become engorged with blood, becoming erect and hard, in preparation for intercourse. The organ is inserted into the vagina culminating with an ejaculation. During intercourse, the smooth muscle sphincters at the opening to the renal bladder close and prevent urine from entering the penis (Fisher *et al*.,1999). An orgasm is a two-stage process: first, glands and accessory organs connected to the testes contract, then semen (containing sperm) is expelled through the urethra during ejaculation. After intercourse, the blood drains from the erectile tissue and the penis becomes flaccid (Ekeh *et al*, 2015).

The walnut-shaped prostate gland surrounds the urethra, the connection to the urinary bladder. It has a series of short ducts that directly connect to the urethra. The gland is a mixture of smooth muscle and glandular tissue (Fahim *et al*.,2013). The muscle provides much of the force needed for ejaculation to occur. The glandular tissue makes a thin, milky fluid that contains citrate (a nutrient), enzymes, and prostate specific antigen (PSA). PSA is a proteolytic enzyme that helps to liquefy the ejaculate several minutes after release from the male. Prostate gland secretions account for about 30 percent of the bulk of semen (Ekeh *et al*, 2015).

The bulbourethral gland, or Cowper's gland, releases its secretion prior to the release of the bulk of the semen. It neutralizes any acid residue in the urethra left over from urine. This usually accounts for a couple of drops of fluid in the total ejaculate and may contain a few sperm. Withdrawal of the penis from the vagina before ejaculation to prevent pregnancy may not work if sperm are present in the bulbourethral gland secretions (Arafa, and Agarwal, 2020).

Statement of Problem

Despite the traditional use, the effects of *Mucuna pruriens* leaf and stem extracts on testicular function, particularly on sperm quality, hormone regulation, remain poorly understood, necessitating systemic investigation to elucidate its potential benefits or risks for male reproductive health. There has been a trend of decreasing male fertility in terms of sperm count, quality, and other changes in male reproductive health (Ekeh *et al.*, 2015). Lately, due to high cost of orthodox drugs and its attendant toxicity, research has been moving worldwide towards the use of drugs of phyto-therapeutics and nutraceuticals origin for the management of male infertility and other diseases because of their little or no side effect (Bhaskar *et al.*, 2011). The leaves of *Mucuna pruriens* have tremendous medicinal values such as a remedy for various diseases like diabetes, arthritis, dysentery, and cardiovascular diseases (Agbafor and Nwachukwu, 2011), hence the need to evaluate its effect on male reproductive health.

Aim/Objectives of the Study

The aim of this study is to determine effect of leaf and stem extracts of *mucuna pruriens* (l) on oxidative stress markers in lead acetate induced testicular toxicity in wistar albino rats.
The specific objectives of the study will be to determine the:

1. Proximate and vitamin composition of the leaf extract
2. Mineral and phytochemical compositions of the leaf extract.

3. Effects of the extracts on oxidative stress markers Superoxide Dismutase (SOD) activity in the rats.
4. Effects of the extracts on Glutathione Reductase (GR) activity in the rats.
5. Effects of the extracts on Malondialdehyde (MDA) levels in the rats
6. Effects of the extracts on Catalase (CAT) activity in the rats.

Review of Related Literature

Description of *Mucuna pruriens*

The genus *Mucuna*, belonging to the Fabaceae family, sub family Papilionaceous, includes approximately 150 species of annual and perennial legumes. Among the various under-utilized wild legumes, the velvet bean *Mucuna pruriens* is widespread in tropical and sub-tropical regions of the world (Lee *et al*., 2005). It is considered a viable source of dietary proteins due to its high protein concentration (23–35%) in addition to its digestibility, which is comparable to that of other pulses such as soybean, rice bean, and lima bean. It is therefore regarded as a good source of food (Bhaskar *et al.*, 2011). The dozen or so cultivated *Mucuna* spp. found in the tropics probably result from fragmentation deriving from the Asian cultigen, and there are numerous crosses and hybrids. The main differences among cultivated species are in the characteristics of the pubescence on the pod, the seed color, and the number of days to harvest the pod. “Cowitch” and “cowhage” are the common English names of *Mucuna* types with abundant, long stinging hairs on the pod (Lee *et al*., 2005).

Nutritional aspects of *Mucuna pruriens*

This legume has a high content of lipids, minerals, carbohydrates, fiber as well as amino acids. To improve the nutritional status of this bean, several types of *Mucuna* have been developed as nourishment in numerous parts of the world according to the nutritional thickness of the beans (Adebowale and Lawal, 2003). The *Mucuna* composition ranges between 42.79 and 64.88% crude carbohydrate, 4.1 and 14.39% crude lipid, 5.3 and 11.5% crude fiber, and 2.9 and 5.5% ash content. *Mucuna* beans are rich in minerals, particularly potassium (806–2790 mg/100 g), magnesium (85–477 mg/100 g), calcium (104–900 mg/100 g), iron (1.3–15 mg/100 g) sodium (4–70 mg/100 g), phosphorus (98–498 mg/100 g), copper (0.33–4.34 mg/100 g), zinc (1–15 mg/100 g) and manganese (0.56–9.26 mg/100 g) (Adebowale and Lawal, 2003). Different proteins and amino acids are also found in *M. pruriens*, such as threonine, proline, tyrosine, phenylalanine, tryptophan, glutamic corrosive, aspartic corrosive, serine, lysine, histidine, and arginine. The crude protein concentration of natural *Mucuna* bean ranges between 21 and 38%. The protein digestibility of this bean resembles that of another grain legume. Therefore, these beans have a reasonable protein digestibility extending from 69 to 82% in rats (Chauhan *et al*., 2014). The protein digestibility studies conducted in human subjects showed that digestibility is a problem in grain legumes due to enzyme-inhibiting polyphenols and the concentration of dietary fiber has increased during the cooking of beans. Such polyphenols clarify the difference in the shade of Mucuna beans. Colors are white to dull, reflecting differences in amino acid absorbability. In this manner, white beans have the most effective absorbability (62.1%) as compared to dull and red beans (49.6 and 55.7%). This is because greater concentrations of the digestibility-inhibiting polyphenolic compounds in the seed coat are present in black or red than in white beans (Champatisingh *et al*., 2011).

Male Fertility

Male fertility requires the cooperation of the different organs of the male urogenital system, each carrying out its assigned function. Through their interactions, the testes (which contain germ cells, Sertoli cells and Leydig cells), the epididymis, and the male accessory glands (prostate, seminal vesicles and bulbourethral glands) simultaneously contribute to the production of the human seminal plasma (Boe-Hansen *et al*., 2005). The testes contribute by producing germ cells (spermatozoa), whereas the main contributions of the accessory glands include the secretion of proteins (for example, kallikreins (KLKs) and semenogelins by the prostate and seminal vesicles, respectively), growth factors (for example, testosterone and insulin-like 3 protein by Leydig cells), trace elements (for example, Zn2+ by prostate epithelium) and other factors (for example, the metabolites citrate and spermine by the prostate epithelium and the glycoprotein mucin MG by the bulbourethral glands). Semen is composed of spermatozoa, which make up about 2–5% of the volume of the whole ejaculate, and seminal plasma, which mostly consists of various fluids secreted by the seminal vesicles, prostate epithelium and bulbourethral glands. Ejaculation, liquefaction and clotting make up a synchronized cascade that enables sperm to perform all the biological processes necessary in order to reach and fertilize the egg. Human seminal plasma is a very complex bodily fluid, the composition of which is strictly regulated by the hypothalamic–pituitary–adrenal (HPA) axis via the gonadotrophic or hypothalamic–pituitary–gonadal (HPG) axis (Hazra *et al*., 2013).

Oxidative stress

Lead acetate promotes oxidative stress primarily by inducing the generation of reactive oxygen species (ROS) within cells and simultaneously inhibiting antioxidant defense mechanisms. ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are highly reactive molecules that can cause oxidative damage to cellular components such as lipids, proteins, and DNA (Pearce, 2007; Famurewa *et al.*, 2023). This oxidative damage disrupts normal cellular function and can lead to inflammation, tissue injury, and various health problems. Lead acetate contributes to ROS generation through multiple pathways, including the activation of oxidative enzymes and the disruption of mitochondrial function. Additionally, lead acetate interferes with antioxidant defense systems by depleting antioxidant molecules like glutathione and impairing the activity of antioxidant enzymes such as superoxide dismutase and catalase (Flora, 2011). As a result, the balance between ROS production and antioxidant defense mechanisms is disrupted, tipping the scale towards oxidative stress (Qader *et al.*, 2021). Oxidative stress induced by lead acetate exposure has been implicated in the pathogenesis of various diseases and health conditions such as neurotoxicity, cardiovascular diseases, kidney damage, and reproductive disorders (Haouas *et al.*, 2015; Abu-Khudir *et al.*, 2023).

Superoxide Dismutase

Superoxide dismutase (SOD) is an antioxidant that catalyzes dismutation of superoxide into oxygen and hydrogen peroxide. It scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane (Garrido *et al*., 2004). SOD spontaneously dismutase anion to form O_2 and H_2O_2. SOD also prevents premature hyperactivation and capacitation induced by superoxide radicals before ejaculating. Oxidative

stress has been implicated in the pathophysiology of damage to human spermatozoa. It is a consequence of an imbalance between Reactive Oxygen Species (ROS) production and degradation (Aitken *et al*.,2014).

Catalase

Catalase (CT) catalyzes the decomposition of hydrogen peroxide to molecular oxygen and water. A characteristic feature of its structure is a heme system with a centrally–located iron atom (Habib *et al*.,2010). Its activity has been demonstrated in peroxisomes, mitochondria, endoplasmic reticulum and the cytosol in many types of cells. In semen, it was found in the sperm cells of humans and rats, as well as seminal plasma, where its source is the prostate. Catalase activates the sperm cell capacitation induced by nitric oxide, which is a complicated mechanism using hydrogen peroxide (Aitken *et al*.,2014).

Another enzyme of the antioxidant system in the semen is glutathione peroxidase (GPX), which catalyzes the reduction of hydrogen peroxide and organic peroxides, including the peroxides of phospholipids (Habib *et al*.,2010). In its active site, it contains selenium in the form of selenocysteine. In sperm it is located mainly in the mitochondrial matrix, but a nuclear form that protects sperm DNA from oxidative damage and participates in the process of chromatin condensation has also been found. The presence of GPX has also been demonstrated in the seminal plasma, suggesting its origin from the prostate (Aitken *et al*.,2014).

Materials and Methods

Collection and authentication of plant materials

Mucuna pruriens leaves and stems will be collected from Awka in Awka South Local Government Area, Anambra state. The plant sample will be classified and authenticated by a plant taxonomist.

Preparation of *Mucuna pruriens* extracts

Mucuna pruriens leaves and stems will be washed repeatedly with clean water to reduce dust and microbial contamination before it will be air-dried at room temperature for two weeks and then ground to powdered form with an automated grinder. The pulverized leaves and stem samples of 400 g each will be soaked in 90% ethanol for 48 hours with intermittent stirring every 8 hours. The extraction mixture will be filtered into a flat bottom flask using a separatory funnel and Whatman No 1 filter paper. The filtrate will be evaporated to dryness using a rotary evaporator at 30^0C for 10 minutes. The sample will be stored in an air-tight container in a refrigerator before the experiment (Hamed and Bahareh, 2012).

Chemical analysis

The proximate, mineral, vitamin, and phytochemical contents will be determined using the method of A.O.A.C. (1997).

Acute Toxicity Test

The acute toxicity (LD_{50}) of *Mucuna pruriens* leaves in male wistar albino rats will be determined by a modified method of Lorke (1983). Twenty-four (24) rats will be randomized into six (6)

groups of four rats each, Group A- Control, Group B- 1000mg/kg, Group C- 2000mg/kg, Group D- 3000mg/kg, Group E- 4000mg/kg and Group F- 5000mg/kg.

Animal collection and handling

The experimental animals used in this study will be adult male wistar albino rats purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State Nigeria. The rats will be kept in stainless steel rat cages in a well-ventilated animal house of the Biochemistry Department, Ebonyi State University, Abakaliki. They will be acclimatized for seven days under good laboratory conditions (12 hours light/dark cycle; room temperature) and allowed access to standard rodent chow and water. The procedures for experimental studies will be performed consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Experimental Design

A total of fifty-four (54) male albino rats will be randomly assigned into nine (9) groups (A-I) of six (6) rats in each group. Testicular toxicity will be induced by oral administration of 60mg/kg of Lead acetate (LA) twenty-four hours before administration of *Mucuna pruriens* extracts (Offor *et al*., 2019). All the administrations will be by oral intubation for 28 days as follows; Group A: Normal control, Group B: Induced with 60mg/kg of LA and Untreated (Negative Control), Group C: Induced with 60mg/kg of LA but will be treated with Standard drug (Clomid- 1.0mg/kg) (Positive control), Group D: Induced with 60mg/kg of LA and treated with 200mg/kgBW *Mucuna pruriens* leaves ethanol extract (MPLE), Group E: Induced with 60mg/kg of LA and treated with 400mg/kgBW of MPLE, Group F: Induced with 60mg/kg of LA and treated with 600mg/kgBW of MPLE, Group G: Induced with 60mg/kg of LA and treated with 200mg/kgBW *Mucuna pruriens* stem ethanol extract (MPSE), Group H: Induced with 60mg/kg of LA and treated with 400mg/kgBW of MPSE, Group I: Induced with 60mg/kg of LA and treated with 600mg/kgBW of MPSE.

Testes Sample collection

The rats will be sacrificed under mild anesthesia. Blood samples will be collected by femoral cut into an Ethylenediaminetetraacetic acid (EDTA) container and the testicles of each member of the group will be harvested and placed in specimen bottles stored at very low temperature and transferred to the laboratory within 3 hours after sacrificing for sample analysis (Römisch-Margl *et al*, 2011).

Determination of Glutathione Reductase (GR) activity rat testicles

Glutathione reductase activity in the testicular homogenate will be quantified by the method of Benke *et al.* (1974).

(a) Principles: The assay involves a carefully optimized enzymatic recycling method using glutathione reductase and 5-5'- dithiobis [2-nitrobenzoic acid] (DTNB) where Glutathione reductase reduces GSSG to GSH and DTNB reacts with GSH to form yellow color chromophore, 5- thionitrobenzoic acid (TNB) and glutathione-5- thionitrobenzoic acid complex (GS-TNB) with an absorbance maximum at 412 nm. Thus, GS-TNB is further

reduced to GSH and TNB by glutathione reductase hence, this enzymatic recycling of GSH enhances the sensitivity of the assay.

(b) Procedure: One ml of testicular homogenate will be incubated with 1 ml of 20% TCA and 1 ml of EDTA for 5 min and then centrifuged at 1000 rpm at 4°C for 30 min. Next, 200 µl of supernatant was mixed with 1.8 ml of DTNB. GSH reacts with DTNB and forms a yellow compound. The absorbance will be read at a wavelength of 412 nm. The GSH values are expressed as nmol/mg mitochondria protein.

Determination of catalase activity in rat testicles

Catalase (CAT) (EC. 1.11.1.6) activity in the testicular homogenate will be assayed by monitoring the decomposition of H_2O_2 at 240 nm as described by Hadwan *et al.* (2024).

(a) Principles: Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen. Its activity in a sample is determined by measuring the decrease in H_2O_2 concentration observed following incubation of the analyte sample with an H_2O_2 standard solution.

(b) Procedure: In brief, testicular homogenates will be added to Tris-HCl (0.05 mM) and H_2O_2 (0.01 M), mixed, and incubated for 10 min. Subsequently, ammonium molybdate (4 %) will be added, and absorbance will be measured at 410 nm.

Determination of Malondialdehyde level in rat testicles

Lipid peroxidation in the testicular homogenate will be determined by measuring thiobarbituric acid reactive substances (TBARS) expressed in terms of malondialdehyde (MDA) content using the method of Tsikas (2017).

(a) Principles: In lipid peroxidation assay, the malondialdehyde (MDA) in the sample reacts with thiobarbituric acid (TBA) to generate a pink MDA-TBA adduct. The colored complex was extracted into organic solvents, and butanol and measured by spectrophotometry using a wavelength of 532 nm. In this process free radicals take electrons from the lipids (generally in cell membranes), resulting in cell damage. Quantification of lipid peroxidation is essential to assess oxidative stress. Lipid peroxidation forms reactive aldehydes such as malondialdehyde (MDA) as natural by-products. MDA is often used as a marker of lipid peroxidation, and to assay for oxidative damage.

(b) Procedure: Testicular tissue preparation (4 mg/ml) will be mixed with an equal volume of Buege and Aust reagent (TCA 15 % w/v in 0.25 M HCl and thiobarbituric acid (TBA) 0.37 % w/v in 0.25 M HCl) and will be heated for 15 min in boiling water. After cooling, the precipitate will be removed by centrifugation at 1000g for 10 min at room temperature. Absorbance will be measured at 532 nm. TBARS will be quantified using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol of TBARS/mg protein.

Determination of Superoxide dismutase Activity in rat testicles

Superoxide dismutase (SOD) (EC. 1.15.1.1) in the testicular homogenate will be determined by the method of Flohe and Otting (1984).

(a) Principles: Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide anion produced enzymatically by the xanthine and xanthine oxidase system

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into hydrogen peroxide and molecular oxygen, thus, the SOD activity is measured by its ability to inhibit 50 % reduction of ferricytochrome c.

(b) Procedure: Briefly, testicular homogenate (1 mg) will be suspended in 50 mM phosphate buffer and 0.1 mM EDTA, (pH 7.8) and disrupted by sonication. The supernatant will be collected after centrifugation and assayed for MnSOD activity. The assay mixture consisted of sample, reaction buffer (0.5 M xanthine (Sigma), 0.1 mM NaOH (EMerck), and 2 lM cytochrome c (SRL) in 50 mM phosphate buffer and 0.1mM EDTA, at pH 7.8. The reaction will be initiated by adding xanthine oxidase (Sigma) (0.2 U/ml in 0.1 mM EDTA) and the change in absorbance will be monitored for 3 min at 438 nm. The SOD activity will be calculated as its ability to inhibit 50 % reduction of ferricytochrome c and expressed as U/mg protein.

Results

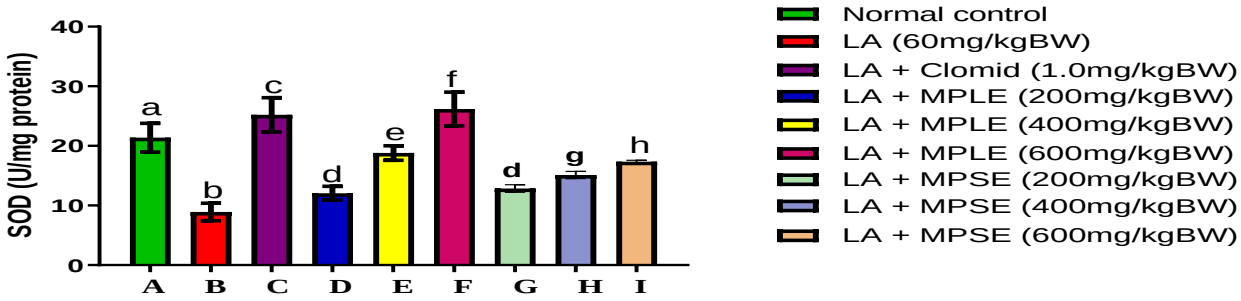

Figure 1: Superoxide Dismutase (SOD) activity after treatment with *Mucuna pruriens* leaf and stem extracts. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. LA (Lead acetate), MPLE (*Mucuna pruriens* ethanol leaf extract), MPSE (*Mucuna pruriens* ethanol stem extract)

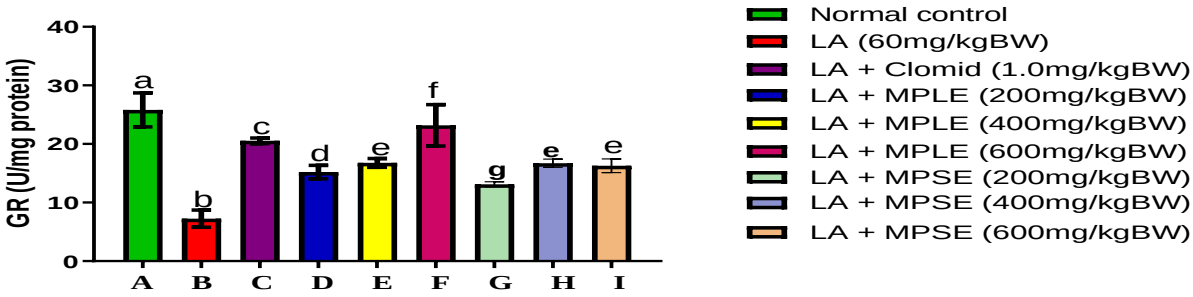

Figure 2: Glutathione Reductase (GR) activity after treatment with *Mucuna pruriens* leaf and stem extracts. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. LA (Lead acetate), MPLE (*Mucuna pruriens* ethanol leaf extract), MPSE (*Mucuna pruriens* ethanol stem extract).

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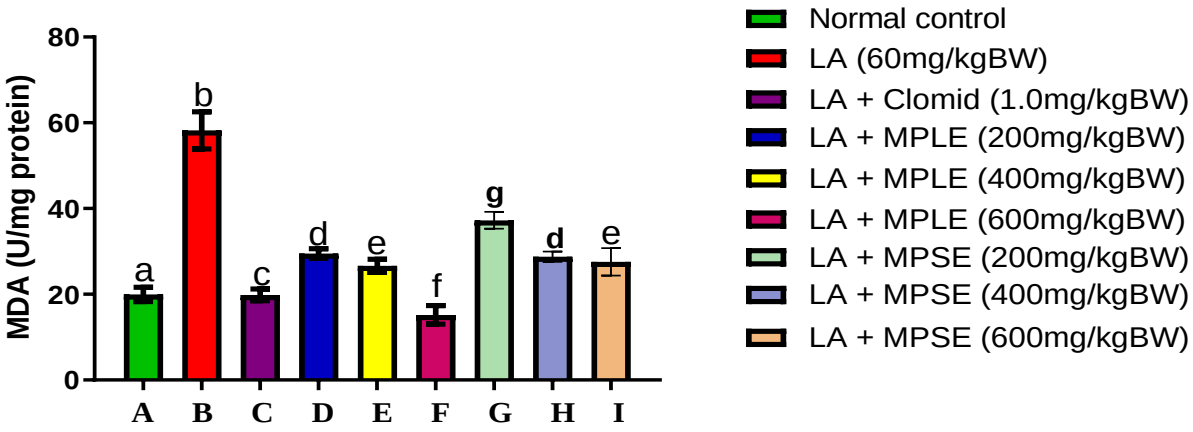

Figure 3: Malondialdehyde (MDA) after treatment with *Mucuna pruriens* leaf and stem extracts. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. LA (Lead acetate), MPLE (*Mucuna pruriens* ethanol leaf extract), MPSE (*Mucuna pruriens* ethanol stem extract)

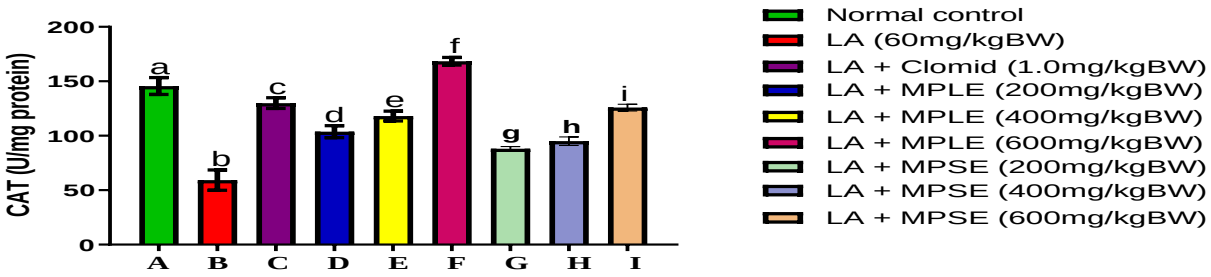

Figure 4: Catalase (CAT) activity after treatment with *Mucuna pruriens* leaf and stem extracts. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. LA (Lead acetate), MPLE (*Mucuna pruriens* ethanol leaf extract), MPSE (*Mucuna pruriens* ethanol stem extract).

Discussion

Dysregulated oxidative stress status has been established with detrimental effects on testicular functions (Aja *et al.*, 2023). In this study, administration of LA significantly decreased the activities of superoxide dismutase (SOD), glutathione reductase (GR), and catalase but increased the levels of malondialdehyde (MDA), a marker of lipid peroxidation, in rats' testicular homogenates. However, co-administration of LA with MPLE and MPSE significantly increased the activities of SOD, GR, and catalase but significantly lowered the levels of MDA in a dose-dependent manner. These effects are comparable to those observed with the standard drug Clomid, indicating the potential efficacy of MPLE and MPSE in mitigating oxidative stress induced by LA. Previous studies have established that lead has a detrimental impact on oxidative stress markers in testicular tissue, primarily through the generation of reactive oxygen species (ROS) and subsequent oxidative damage. For example, research by El-Magd *et al.* (2016), Ekeh *et al.* (2015),

and Famurewa *et al.* (2023) has demonstrated that lead exposure significantly reduces antioxidant enzyme activities and increases lipid peroxidation, consistent with the current findings. Consistently, this study proposed that the protective effects of MPLE can be attributed to its rich antioxidant content, including vitamins and phytochemicals, which help neutralize ROS and enhance the antioxidant defense system (Valko *et al.*, 2006).

Biochemically, the observed improvements in oxidative stress markers suggest that MPLE and MPSE mitigates oxidative damage through several possible mechanisms. Specifically, the antioxidants present in MPLE, such as vitamins A, C, and E, as well as flavonoids and alkaloids, play a crucial role in scavenging free radicals, thereby reducing oxidative stress as robustly reported in existing pieces of literature (Samuel *et al.*, 2011; Mene-Saffrane and DellaPenna, 2010; Interdonato *et al.*, 2015). Our findings in this current investigation upheld that this antioxidant properties led to the restoration of antioxidant enzyme activities such as SOD, GR, and catalase, which are essential for detoxifying ROS and maintaining cellular redox balance. Additionally, the reduction in MDA levels indicates decreased lipid peroxidation, suggesting that MPLE and MPSE effectively protects cell membranes from oxidative damage (Aja *et al.*, 2023). Therefore, this study suggests that Mucuna pruriens ethanol extracts have significant protective effects against lead acetate-induced oxidative stress in rats' testicular homogenates perhaps due to its antioxidant properties. Hence, *Mucuna pruriens* is an emerging natural remedy against male infertility and reproductive challenges.

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