THE IMPACT OF *MIMOSA PUDICA* ON THE HISTOARCHITECTURE OF HYPOTHALAMIC-PITUITARY-TESTICULAR AXIS IN CADMIUM TREATED RATS

*Edem Edem Ekpenyong¹, Enye Linus Anderson¹, Towobola Adewale Abdullateef¹,
  Akingbade Adebaji Modupe¹, Kunlere Oladunni Eunice¹

¹Department of Anatomy, College of Medicine and Health Sciences,
  Afe Babalola University, Ado Ekiti, Nigeria.

ABSTRACT

**Background:** Cadmium is a known environmental and industrial pollutant with an enormous neuroendocrine disrupting potential. *Mimosa pudica* Linn is a creeping annual or perennial herb known to possess antiasthmatic, antiepileptic, antitumour, aphrodisiac, analgesic, antidepressant properties and a strong radical scavenging activity. This research was aimed at investigating the impact of *Mimosa pudica* on the histoarchitectural integrity of the hypothalamic-pituitary-testicular axis in cadmium-treated rats. **Materials and Methods:** Twenty five mature wistar rats (Rattus rattus norvegicus) were employed in the study. These animals were divided into five groups - 5 Rats/Group; Control, Cadmium Toxicity, *Mimosa pudica* Extract, Protection and Therapeutic Groups. The Control Group was orally administrated with distilled water. **Result:** Toxicity was achieved with 1.2mg/kg body weight for forty days with apparent histological abnormalities and alterations to the axis components. Administration of *Mimosa pudica* (200mg/kg) body weight with cadmium in both the Protection and Therapeutic Groups showed remarkable histological improvements and markedly reduced tissue damage when compared with Cadmium Toxicity Group. **Conclusion:** Results of this study demonstrate that *Mimosa pudica* possesses protective, therapeutic as well as restorative capacity on the histoarchitecture of hypothalamic-pituitary-testicular axis components in cadmium-treated rats.
KEYWORDS: Cadmium, Mimosa pudica, histoarchitecture, hypothalamic-pituitary-testicular axis, toxicity.

INTRODUCTION

*Mimosa pudica* Linn is a creeping annual or perennial herb found to possess anti-asthmatic, aphrodisiac, analgesic, and antidepressant properties. It is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and neurological disorders like epilepsy, convulsions, and various urogenital infections. Phytochemical studies on *M. pudica* have revealed the presence of alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids (Genest et al., 2008).

The hypothalamus is that portion of the brain that maintains the body’s internal balance (homeostasis). The hypothalamus is the link between the endocrine and nervous systems. The hypothalamus produces releasing and inhibiting hormones, which stop and start the production of other hormones throughout the body (Dindyal et al., 2007).

Pituitary gland is a pea-sized gland that sits in a protective bony enclosure called the sella turcica. It is composed of three lobes: anterior, intermediate, and posterior. This endocrine structure consists of ductless glands, distinct clusters of cells within certain organs of the body, and diffuse neuro-endocrine cells, regulates metabolic activities in certain organs and tissues of the body, thereby helping to bring about homeostasis brought about by chemical substances called hormones, which are released into the bloodstream to influence target cells at remote sites. Cadmium is recognized as an endocrine disruptor that modifies, among other secretions, prolactin (PRL) secretion in a number of species including humans. Cd is readily absorbed and retained in the pituitary gland of rats and affects lactotroph cell activity causing biochemical, genomic and morphological changes (Jiménez et al., 2012).

The testes (or testicles) are a pair of sperm-producing organs that maintain the health of the male reproductive system. In addition to their role in the male reproductive system, the testes also have the distinction of being an endocrine gland because they secrete testosterone—a hormone that is vital to the normal development of male physical characteristics (Shackelford et al., 2007).
Cadmium (Cd) is ranked eighth in the top 20 hazardous substances; it is released into water as a by-product of smelting, into air by combustion of coal and oil, and into soils as impurities. In human populations, cadmium exposure occurs primarily through dietary sources and drinking water as well as cigarette smoking. Its main uses are for nickel–cadmium battery manufacture, pigments and plastic stabilizers (Jarup & Akesson, 2009). Cadmium is recognized as an endocrine disruptor that modifies, among other endocrine secretions, prolactin (PRL) secretion in a number of species including humans. Cd is readily absorbed and retained in the pituitary gland of rats and affects lactotroph cell activity causing biochemical, genomic and morphological changes, which invariably affects the different endocrine structures influenced by the pituitary gland in other parts of the body, example, testis, thyroid, etc. (Jiménez et al., 2012). Based on its nervine and reproductive benefits, *mimosa pudica* is being investigated in the present study to determine its protective as well as its therapeutic role on the histoarchitecture of hypothalamic-pituitary-testicular axis in cadmium-treated adult rats.

**MATERIALS AND METHODS**

**Animals**

All experiments were designed in strict adherence to the guidelines of the Afe Babalola University’s Animal Ethics Committee. Twenty-five male healthy Wistar rats weighing between 98-132g of the same specie *Rattus norvegicus* was purchased from the Department of Biochemistry, Afe Babalola University, were used for the research Work. They were housed in well ventilated plastic cages, kept and maintained under laboratory condition of temperature, humidity and light. At the time of purchase, they weighed between 77 - 117g. They were allowed to acclimatize for a period of two weeks and fed with growers Mash. The rats were also given tap water at pleasure using water bottles. At the end of two weeks, the rats were weighed and were randomly assigned to five different groups A as the control group B, C, D and E as the experimental groups.

**Collection and Preparation of aqueous extract from the leaves of *Mimosa pudica***

Fresh, healthy leaves of *Mimosa pudica* after identification were harvested and properly washed in tap water and then rinsed in sterile distilled water to remove dirt and possible mycotoxins. The leaves were air dried for three days and then pulverized into fine powder using an electric blender. Sixty (60) grams of the powder was extracted in 300ml of distilled water and left to stand for 48 hours at room temperature. The extract was filtered through
cheese cloth and the resulting filtrate was concentrated on steam bath until a semisolid residue (green black paste) which weighed 4.5g was obtained. The percentage yield was calculated and equivalent amount of the residue was separately reconstituted in 100ml of normal saline to give the required doses of 200mg/kg body weight.

**Induction of Toxicity**
Induction of toxicity was achieved by the oral administration of cadmium at 1.2kg/kg body weight. The induction was done orally using syringe and canula.

**Treatment**
The aqueous extract of leaves of *Mimosa pudica* was given orally at the dose of 200mg/kg for 40 consecutive days.

**Experimental Design**
The rats were grouped into 5 of five animals per group according to the weight of the rats. Group A weighing 114g served as the Control group; Group A - Control Group; Group B - Cadmium Toxicity Group; Group C - Mimosa Pudica Extract Group; Group D - Protective Group; and Group E-Therapeutic Group. The Control Group animals received distilled water only through the 40 days duration of the experiment. Rats in the Cadmium Toxicity Group were given 1.2mg/kg body weight for 40 days of the experiment. *Mimosa pudica Extract Group* rats were administered with 200mg/kg body weight of aqueous extract of *Mimosa pudica* for 40 days. Protection Group rats were given 1.2mg/kg body weight of cadmium and 200mg/kg body weight of aqueous extract of *Mimosa pudica* simultaneously for 40 days. Wistar rats in the Therapeutic Group received 1.2mg/kg body weight of cadmium for the first 20 days and 200mg/kg body weight of aqueous extract of *Mimosa pudica* for the remaining 20 days of the experiment.

**Sacrification**
At the end of the experiment, the rats were sacrificed. This was done by cervical dislocation. The whole brain was collected and fixed in 10% formal saline and the same was done for the pituitary gland. The testes were collected and fixed in Bouin’s fluid.

**RESULTS**
At the end of the histological analysis, marked morphological alterations were observed in cadmium-treated rats; and varying degrees of regenerations and cellular restorations were
noticeable in animals treated with *Mimosa pudica* after toxicity induction with cadmium, as presented below.

**Plate 1: Control Group (Hypothalamus)**

Micrograph (x400) of hypothalamus given distilled water shows section cluster of hypothalamic nuclei (arrow head), ependymal layer (arrow); lining the third ventricle (3VT). The parenchyma appears compact and the neurons have a spherical nucleus with scattered heterochromatin, the glia (star) cells appear essentially unremarkable.

**Plate 2: Cadmium Toxicity Group (Hypothalamus)**

Micrograph (x400) of hypothalamus treated with 1.2mg/kg body weight of Cadmium for 40 days reveals ghost neurons characterized by oval empty spaces (vacuolizations) indicative of neurodegeneration.

**Plate 3: Extract Group (Hypothalamus)**

Micrograph (x400) of hypothalamus treated with 200mg/kg body weight of aqueous extract Mimosa pudica for 40 days shows cluster of hypothalamic nuclei with scattered heterochromatin, the neuroglia (arrow head) appear normal, no congestion or inflammatory cells seen.

**Plate 4: Protection Group (Hypothalamus)**

Photomicrograph (x1000) of hypothalamus treated with 1.2mg/kg body weight of cadmium and 200mg/kg body weight of Mimosa pudica simultaneously for 40 days shows the nuclei of ependymal cell (arrow) lining the third ventricle (3rd vt), and a congested capillary (CAP). Neuronal recovery remarkable.

**Plate 5: Therapeutic Group (Hypothalamus)**

Micrograph (x400) treated with 1.2mg/kg body weight of Cadmium for 20 days and 200mg/kg body weight of Mimosa pudica for the remaining 20 days reveals eosinophilic collections (evidence of regeneration after degeneration). No inflammatory cells seen. Cellular restoration evident.

**Plate 6: Controls Group (Nesitary Gland)**

Photomicrograph (x400) given distilled water shows section of the posterior pituitary (pars nervosa) showing axons of nerve fibers, the Schwann cells and pituicytes. Micromorphology appears remarkable.
Photomicrograph (x400) of Pituitary Gland treated with 1.2mg/kg body weight of Cadmium for 40 days. Section shows perivascular inflammatory cells (arrow) and vacuolation (V) within the stroma.

Micrograph (x400) of Pituitary Gland treated with 200mg/kg body weight of Mimosa pudica for 40 days shows section of the posterior pituitary (pars nervosa) showing axons of nerve fibers, the Schwann cells (SC) and pituicytes. Micromorphology appears remarkable.

Micrograph (x400) of Pituitary Gland treated with 1.2mg/kg body weight of Cadmium and 200mg/kg body weight of Mimosa pudica simultaneously for 40 days. Section shows perivascular inflammatory cells (arrow) and vacuolation (V) within the stroma. Mild restorative activity seen with reduced vacuolations.

Micrograph (x400) of Pituitary Gland treated with 1.2mg/kg body weight of Cadmium for 20 days followed by 200mg/kg body weight of Mimosa pudica for the remaining 20 days. Section of the posterior pituitary (pars nervosa) shows axons of nerve fibers, the Schwann cells (SC) and pituicytes. Remarkable restored micromorphology.

Micrograph (x400) of Testis given distilled water only, shows seminiferous tubules (ST) with defined lumen (L), composed spermatocytes (SZ). Tubules are composed of germ cells (S2) at different levels of maturation, the sertoli cells (STC) and interstitial cells (IC). Normal testicular cytoarchitecture intact.

Micrograph (x400) of Testis treated with 1.2mg/kg body weight of Cadmium for 40 days shows outstanding testicular alteration; seminiferous tubules with reduced spermatogenic cells (line) within the lumen (L), also seen is marked congestion of the Interstitium (ICG).
DISCUSSION
The current investigation showed the efficacy of *mimosa pudica* in preventing/ameliorating the toxic effects of cadmium on the histoarchitecture of the hypothalamic-pituitary-testicular axis in adult Wistar rats. At the moment, this is the first report showing these effects.

Histopathology is considered the most reliable parameter for the detection of toxic effects on male reproduction (Creasy, 2001; Lanning *et al.*, 2002); and as such histological investigation was carried out to ascertain the extent of cellular and tissue disruption induced by cadmium on the different components of the hypothalamic-pituitary-testicular axis.

Cadmium exposure has been reported to be a risk factor for infertility and it is very dangerous on testicular function (Sarah *et al.*, 2012; Sokol, 1997). The study of the hypothalamic pituitary-gonadal axis in animals exposed to the metal is of great interest since the levels of...
Cadmium in air, water, soil, and foods have increased several-fold in many parts of the world as a result of emissions from industrial activities (Sebahat et al., 2005).

Cd-induced neurotoxicity might be caused by impaired neurogenesis, resulting in markedly reduced neuronal differentiation and axonogenesis, leading to neuronal cell death (Chen et al., 2008). The present study evaluated the histomorphological damage of cadmium on the hypothalamus, the result revealed ‘ghost neurons’ characterized by oval empty spaces (vacuolizations) which are indicative of neurodegeneration. Though the scope of this study did not cover hormonal analysis, the impaired cytoarchitecture is an evidence of altered plasma gonadotropin levels as shown in different studies of the hypothalamus (Lafuente & Esquifino, 1999).

Cadmium toxicity changes the general morphology of the pituitary gland. In the present study, the tissue appeared slightly atrophied in a few areas with perivascular inflammatory cells and vacuolations within the stroma. This is in conformity with the work of Favorito et al., (2010). This evidence has been reported also in other glandular tissues as in the thyroid of the catfish Clarias batrachus (Jadhao et al., 1994), in the testis of the cyprinid Puntius sarana (Kumari et al., 1991) and of the monkey Presbytis entellus entellus (David & Ramaswami, 1971). Underlying these histological disorganizations are cadmium’s inhibitory effect on the hormonal secretion of many adenohypophyseal cells, which was reported in mammals: the levels of LH, PRL and GH in serum of the rat exposed to cadmium decrease (Lafuente et al., 2001; Lafuente et al., 1997) as well as the levels of GTH in pig (Han et al., 2006) and female rats (Pillai et al., 2003). Recently it has been also reported that Cd modifies the lactotrophs activity of pituitary gland through biochemical, genomic and morphological changes, contributing directly or indirectly to the levels of serum prolactin in rat (Calderoni et al., 2010).

Pituitary secretion activity has been shown to be affected by metals and this endocrine gland is a particularly sensitive target to cadmium toxicity (Lafuente et al., 2001; Poliandri et al., 2006; Cano et al., 2007), but not very much has been reported regarding the mechanism of action of the Cd like endocrine disruptor. It is just reported that divalent cations, as Cd, inhibit in vitro release of GH and PRL from bovine adenohypophyseal secretory granules (Lorenson et al., 1983). Calderoni et al., (2005) report that cadmium modifies the lipid contents of pituitary gland and directly or indirectly the levels of prolactin and growth hormone in serum. Lafuente et al., (2005) have reported that the inhibitory effect of cadmium...
on PRL and LH secretion may be partially explained by a decrease in the content of glutamate and aspartate in anterior hypothalamus. Several studies have shown that cadmium could compete with calcium at the pituitary level (Waalkes & Poirier, 1984; Milos et al., 1989), which results in altered calcium regulation. Thus it either interferes with calcium influx through the membrane channel (Kasprzak & Poirier, 1985; Cooper et al., 1987) and disrupts intracellular calcium mobilization and subsequently alters the cytoarchitectural integrity of the gland. Further researches are however certainly required to define the mechanism of cytotoxic action of cadmium on the pituitary cells.

Cadmium accumulates in male reproductive organs, in both humans and animals Numerous studies have confirmed that the testis is more sensitive to cadmium than other important organs (Souza et al., 2010). Histopathological observations also showed remarkable destruction of the testis in the rats poisoned with cadmium in the present study. This is consistent with the earlier works of Habeebu et al., (1998); Jones et al., (1988) in which they showed that cadmium caused liver, kidney and testicular damage. Similarly, other agents like lansoprazole, oxolinic acid and procynidone appear to induce leydig cell damage and tumors of the testis in rats by perturbation of testosterone production and overstimulation of testicular interstitia via increase serum luteinising hormone (Fort et al., 1995; Murakani et al., 1995; Yamada, 1994). This indicates a reduction in normal feedback inhibition mechanism in rats by cadmium at the level of hypothalamus and/or pituitary resulting from reduced testosterone production by the testes which is critical in the formation of testicular interstitial cell tumors (Waalkes et al., 1997b). Furthermore the loss of testosterone feedback can result in pituitary cell hypertrophy, hyperplasia and eventually pituitary neoplasia (Nyska et al., 1998). Thus the disruption of the hypothalamic-pituitary-testicular axis may contribute to the causation of both testicular and pituitary destructions in the present study.

Exposure of animals to cadmium induced oxidative stress, stimulates the synthesis of cadmium binding proteins metallothioneins (MT) and heat proteins (Klassen et al., 1999). Cadmium-induced oxidative stress has been associated with production of reactive oxygen species (ROS) comprising mainly superoxide radical anion (O2 - ), hydrogen peroxide and hydroxyl radical (OH) which lead to lipid peroxidation, membrane protein and DNA damage which can also result in carcinogenesis (Bagchi et al., 1996). This had been reported to cause apoptosis, necrosis and cell proliferation (Habeebu et al., 2000; Stoh et al., 2001). From this study, cadmium stimulated the histopathological damages which include seminiferous tubules
with reduced spermatogenic cells within the lumen, with marked congestion of the Interstitium (ICG). This disorganization could be positively correlated with increase in phosphatase levels.

In drug discovery, random screening as a tool in identifying new biologically active molecules has been the most productive. Free radicals are generated by both internal (cellular respiration, etc.) and external (alcohol, pollution, smoking, etc.) sources. These free radicals can damage all cellular macromolecules (proteins, carbohydrates, lipids and nucleic acids) and attribute to many disorders (Thirumalai et al., 2011). However, these radicals are controlled by antioxidants, which can safely interact and terminate the chain reaction before vital molecules are damaged. There are several enzyme systems (catalase, superoxide dismutase etc.) within our body that scavenge free radicals. In addition micronutrients such as vitamin E, beta-carotene and vitamin C form dietary sources and can act as antioxidants (Sharma et al., 2010; Nonita & Mylene, 2010). The present study explored the scavenging antioxidant property of *Mimosa pudica* on the cytostructural integrity of the hypothalamic-pituitary-testicular axis in cadmium treated rats.

Anti-oxidants are agents that significantly inhibit the rate of oxidative activity (Murray et al., 2000). They can be generally categorized into preventive and non preventive antioxidants (Parker, 1986). Vitamin C, E and selenium are the best known preventive antioxidants (Parker, 1986). Vitamins C, E and selenium inhibited oxidation by an effect on calcium metabolism, (Stoh et al., 2001), protein kinase C (Das & King, 2007) inhibition and catalysis of the reduction of hydrogen peroxide which protect biological membranes from oxidative degradation (Murray et al., 2000).

The *Mimosa pudica* invites attention of the researchers worldwide for its pharmacological activities such as antiepileptic, anticonvulsant, antidiabetic, antitoxin, antihepatotoxin, antioxidant, aphrodisiac and wound healing activities. It is reported to contain alkaloid, glycoside, flavonoid and tannis. All parts of the plant are considered to possess medicinal properties and used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, blood diseases (Chauhan et al., 2009). *Mimosa pudica* leaves, which were used in this investigation is reported to be the richest in total flavonoid and total phenolic contents compared to other parts of the plants (Jing et al., 2011). Flavanoids and other phenolic compounds of plant
origin have been reported as scavengers and inhibitors of lipid peroxidation (Meenakshi et al., 2012).

Toxicity in the hypothalamic tissue induced by cadmium resulted in cell death with apparent ‘ghost neurons’ following inflammatory mechanisms. Treatment with *Mimosa pudica* in both the Protection and Therapeutic Groups showed neuronal recovery (Plate 4 and 5). This was due to its antioxidant properties which protect DNA against damage induced by the reactive oxygen species. Perivascular inflammatory cells and vacuolation within the stroma (Plate 7) observed in the pituitary gland, of the cadmium group would compromise the endocrine function of the hypothalamic-pituitary axis (Massanyi et al., 2007). These changes were attenuated by 200mg/kg body weight of *Mimosa pudica* (Plate 10).

The testes are endocrine organs hence damage to the tissue will result in abnormal endocrine responses. Because the integrity of the testes is compromised histologically following cadmium toxicity, furthermore Cd is known to also directly cause destruction to the testicular organs and hypothalamus-pituitary gonadal axis thus destroying the secretory organs of hormones (Massanyi et al., 2007) and compromising hormonal release. In this study, 1.2mg/kg body weight of cadmium produced cytoarchitectural alterations including seminiferous tubules with reduced spermatogenic cells [line] within the lumen and marked congestion of the Interstitium (Plate 12). This damage is believed to be brought about by the inhibition of the serum hormonal levels of FSH and LH which induce the signals for testosterone synthesis (Cooke et al., 1981). An inhibition of these signals results in the time-dependent monophasic serum inhibition in testosterone levels showing reproductive dysfunction, cell death and apoptosis by cadmium (Habeebu et al. 1998; Massanyi et al., 2007; Stoh et al., 2000) resulting in liver and accessory sex tissues atrophy such as the prostate (Waalkes et al., 1997a). This will result in reproductive dysfunction.

Testis treated with 200mg/kg body weight of *Mimosa pudica* aqueous extract for 40 days showed seminiferous tubules filled with germinal cells, the germinal epithelium is composed of germ cells (GC) and varying degree of maturation, within the Interstitium lay blood vessels (BV) and interstitial cells with no apparent toxic histological evidence. At this dosage, aqueous extract is safe, and this is in agreement with Pradeep’s (2012) toxicity studies on *Mimosa pudica*.

Cadmium-induced testicular toxicity is caused by the interactions between complex networks, involving the inhibition of oxidative stress, which leads to an increase in germ cell
apoptosis (Siu et al., 2009; Turner & Lysiak, 2008) and/or distortion of the blood-testis barrier with subsequent germ cell loss, testicular edema, and hemorrhage (Cheng & Mruk, 2012). After prolonged exposure, damage inflicted by cadmium can be found at interstitial and tubular level (Mathur et al., 2011). The present study revealed a distorted testicular histoarchitecture (Plate 12) induced by cadmium toxicity, and it confirms the above assertions. The present research revealed treatment with aqueous leaf extract of *mimosa pudica* showed reactive testicular germ cells undergoing recovery from cellular damage (Plate 15). However, *Mimosa pudica* ethanolic root extract, which is highly aphrodisiac and toxic at high dose showed decreased spermatozoa number (http://www.hillgreen.com/pdf/mimosapudica).

**SUMMARY/CONCLUSION**

The current research demonstrated that *Mimosa pudica* has protective, as well as restorative capacity on the histoarchitecture of hypothalamic-pituitary-testicular axis in cadmium treated rats. This confirms its ethnomedical use as a therapeutic intervention in infertility. However, caution should be taken as regards the dose administered. Moreover, I recommend that biochemical, genetic as well as histochemical investigations should be carried out in subsequent studies for a more appreciable, detailed and confirmatory result.

**REFERENCES**


13. Dindyal, S. *“The sperm count has been decreasing steadily for many years in Western industrialised countries: Is there an endocrine basis for this decrease?”* The Internet Journal of Urology., 2007; 2(1): 1–21.


56. www.hillgreen.com/pdf/mimosapudica