Effects of Aqueous Leaf Extract of *Lawsonia inermis* on Aluminum-induced Oxidative Stress and Adult Wistar Rat Pituitary Gland Histology

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**ABSTRACT**

**Objectives:** The aim of this study was to investigate the antioxidant effect of aqueous *Lawsonia inermis* leaf extract on aluminum-induced oxidative stress and the histology of the pituitary gland of adult Wistar rats.

**Methods:** Thirty-five adult male Wistar rats weighing between 100-196g and 15 mice of the same weight range were included in the study. The anterior pituitary gland was removed, and weighed on an electronic analytical balance.

**Results:** Decreased cell counts were observed in the pituitary gland micrographs of the Wistar rats given 0.5mg of aluminum chloride, whereas the Wistar rats given 0.5mg of aluminum chloride and varying doses of *Lawsonia inermis* had increased dose-dependent cell counts.

**Conclusion:** Aqueous *Lawsonia inermis* leaf extract increased the cell counts of the pituitary glands of adult male Wistar rats, in addition to alleviating aluminum-induced oxidative stress.

**Keywords:** adenohypophysis, neurohypophysis, asthenospermia, teratospermia

**INTRODUCTION**

The pituitary gland, often called the “master gland” of the body, physiologically regulates the endocrine function of several other glands and their associated activities. The pituitary gland can be functionally divided into two parts: the posterior (neurohypophysis) and the anterior (adenohypophysis). Lower animal species possess a third part that is not present in humans, known as pars intermedia. Developmentally, the anterior pituitary and the posterior pituitary have different origins. The anterior pituitary develops from Rathke’s pouch, an invagination from pharyngeal epithelium, while the posterior pituitary develops from a neural tissue outgrowth from the hypothalamus (Guyton & Hall, 2006).
MATERIALS AND METHODS

Aluminum chloride and ascorbic acid were procured from the Mich-Deson Hospital Equipment store, Upper Taiwo, Ilorin. Staining procedures were carried out in the Pathology Department, University Teaching Hospital Ilorin, Nigeria.

Preparation of Extracts

Plant samples were procured in Isanlu-Isin, Kwara State, Nigeria and had their identities confirmed identified and assigned herbarium number UPH/P/114 by the Taxonomist of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Rivers State, Nigeria. The Research Ethics Committee of the same institution approved the study on February 25, 2016, and gave it reference number UPH/CEREMAD/REC/04. The plant leaves were washed with water, cut into pieces, and dried in a cool environment. The dried plant leaves were then pulverized into a coarse powder with the aid of a grinding machine. The filtrate was concentrated using a rotary evaporator (Buchi) and further concentrated to dryness at 50°C in an electric oven (GallenKamp). After drying, the samples were stored in a refrigerator at 4ºC until the time they were used.

Acute Toxicity Testing (LD₅₀)

Fifteen mice were used in the tests to determine the safe and lethal dosages of the extract. The animals were split into five groups, each with three individuals. The acute toxicity of the aqueous extract of Lawsonia inermis leaves was assessed based on the LD₅₀ calculation, with a limit dose of 1000mg/kg of body weight of the extract administered orally (three animals per group) (OECD-OC-DE 425 Guide). The mice given the extract orally showed dose-dependent signs of toxicity, which ranged from lack of appetite, depression, immobility, and respiratory distress to death. The LD₅₀ for the Lawsonia inermis extract was 0.75g, while the safe dose was 0.1g/kg b.w.

Determination of the Dosage of the Extract to Administer

The choice of dosage was based on the acute toxicity test (LD₅₀) cited above, in which the safe dose of Lawsonia inermis was set at 0.1g/kg or 100mg/kg body weight. The highest dose was 100mg/kg, the medium dose was 75mg/kg, and the lowest dose was 60mg/kg.

Breeding of the Animals

Thirty-five adult male Wistar rats and fifteen mice were included in the study. The subjects weighed between 100g and 196g. After procurement, the rats were housed in cages (made with wood, wire gauze, and mesh) kept at room temperature and subject to cycles of natural light and darkness at the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The floor of the cages was made of wood to make it comfortable for the rats and was littered with sawdust to provide a soft floor for the rats. They were fed with pellets purchased from approved stores by the University of Ilorin and given water ad libitum. They were grouped and left to acclimatize for two weeks before the start of the study.

Grouping

A total of 35 animals were included in the study. They were grouped into one control and six case groups with consideration to size variations. Using a feeding tube (size-6), distilled water and portions of Lawsonia inermis extract were administered to the control and case animals respectively for a period of three weeks.

RESULTS

Histological Observation

Effect of Lawsonia inermis leaf extract and aluminum chloride on the histology of the pituitary glands (H&E/PAS/Orange G) of the groups are shown Plates 1 and 2 below.

Plates

- **Group 1**: (n=5): Given rat pellets and distilled water.
- **Group 2**: (n=5): Given 60mg/kg/d of extract of Lawsonia inermis and pellets.
- **Group 3**: (n=5): Given 0.5mg/kg/d of aluminum chloride in distilled water and pellets.
- **Group 4**: (n=5): Given 0.5mg/kg/d of aluminum chloride and 60mg/kg/d (low dose) of Lawsonia inermis in distilled water orally.
- **Group 5**: (n=5): Given 0.5mg/kg/d of aluminum chloride and 75mg/kg/d (medium dose) of Lawsonia inermis orally.
- **Group 6**: (n=5): Given 0.5mg/kg/d of aluminum chloride and 100mg/kg/d (high dose) of Lawsonia inermis in distilled water orally.
- **Group 7**: (n=5): Given 0.5mg/kg/d of aluminum chloride and 5mg/kg/d of ascorbic acid in distilled water orally.

Animal Sacrifice and Sample Collection

Twenty-four hours after the last administration, the animals were weighed and thereafter sacrificed with chloroform as a sedative. Their pituitary glands were located and removed.

Histology and Histochemistry Analyses

Tissue specimens were taken from the pituitary glands of subjects from each of the seven groups and were fixed in formaldehyde and calcium chloride for 24 hours. Then each specimen was sliced into small slabs (3-5mm thick) and further fixed in a change of the same fixative for another 15 hours. The fixed tissue specimens were trimmed and washed in tap water for 12 hours. An alcohol series (methyl, ethyl, and absolute) was used to dehydrate the tissue specimens. The tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned in 5-micron slices on a rotary microtome. The obtained tissue sections were collected on glass slides and stained with Hematoxylin and Eosin, Periodic Acid-Schiff, and Orange G.
Plate 1. Representative pituitary gland micrographs of Wistar rats from Groups 1-7 showing the anterior pituitary gland (AP), the posterior pituitary gland (PP), and the pars intermedia (IP). Staining: H&E. Magnification x100.

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stained cells, decreased number of chromophil and chromophobe cells, pas intermedia and pas nervosa. Stained with H&E/ PAS/Orange G; magnification x100 & x400.

- **Group 5:** Plates 1 and 2 show representative pituitary gland micrographs of Wistar rats given 0.5mg of aluminum chloride and 75mg/kg of *Lawsonia inermis* with the pars distalis with reddish-yellow large stained cells and a moderately increased number of chromophil and chromophobe cells, pas intermedia and pas nervosa. Stained with H&E/ PAS/Orange G; magnification x400.

- **Group 6:** Plates 1 and 2 show representative pituitary gland micrographs of Wistar rats given 0.5mg of aluminum chloride and 100mg/kg of *Lawsonia inermis* with a pars distalis with reddish-yellow large stained cells and increased counts of chromophil and chromophobe cells, pas intermedia and pas nervosa. Stained with H&E/ PAS/Orange G; magnification x400.

- **Group 7:** Plates 1 and 2 show representative pituitary gland micrographs of Wistar rats given 0.5mg of aluminum chloride and 5mg/Kg of ascorbic acid with a pars distalis with reddish-yellow large stained cells and increased counts of chromophil and chromophobe cells, pas intermedia and pas nervosa. Stained with H&E/PAS/Orange G; magnification x400.
Plate 2. Representative pituitary gland micrographs of Wistar rats from Groups 1-7 showing areas of degenerative change. The posterior pituitary (PP), the anterior pituitary gland (AP) with increase basophil (B) and acidophil (A) cell counts and few chromophobe cells (C). Staining: H&E. Magnification: x400.

DISCUSSION

Physiologically, the pituitary gland is made of two parts: the anterior pituitary (the adenohypophysis) and the posterior pituitary (neurohypophysis). Between these two parts lies a relatively small avascular zone called the pars intermedia. The hypothalamic-pituitary-testicular (HPT) axis is a hormonal axis of communication between the hypothalamus, the pituitary, and the testes that regulates male reproductive function.

In this study, the subjects in the control group had normal pituitary gland morphology (Plates 1 and 2) with a more cellular anterior pituitary gland and a posterior pituitary gland separated by the pars intermedia. The anterior pituitary gland contained clusters of cells grouped according to their affinities to dyes, with acidophil cells stained orange-brown, basophil cells stained dark-blue, and chromophobe cells stained light blue.

According to Plates 1 and 2, the histological architecture of the pituitary glands of the rats given 60mg/kg of aqueous extract of Lawsonia inermis could be easily distinguished by an anterior pituitary with numerous cells including basophil (gonadotropin secreting cells), acidophil, and chromophobe cells. Basophil cell counts were higher than the counts of acidophil and chromophobe cells, and were markedly increased when compared to the other groups. In addition, basophils and eosinophils that had recently undergone degranulation or were in the process of active hormone synthesis appeared as chromophobe cells.

In this study, the anterior pituitary of the rats given 0.5mg of aluminum chloride alone had few basophil,
acidophil, and chromophobe cells. Numerous areas with degenerative changes were also present. Secretory cells had generally decreased counts when compared to the other groups, as previously described by Olawuyi et al. (2017), leading to dramatic decreases on the level of gonadotropin hormones (FSH and LH), as also described by Mohammadirad & Mohammad (2011), in a study that found that aluminum toxicity increases oxygen free radical levels and lipid peroxidation, which in turn decreases glutathione (GSH) levels. Kara et al. (2005) reported that glutathione as a substrate directly reacts with free radicals in enzymatic antioxidant reactions, thus protecting cells against oxidative stress. According to Jensen et al. (2006), metals negatively affect the neuroendocrine system and the Leydig cells, thus potentially affecting the secretion of androgen. In our study, a marked drop was noted in the levels of FSH and LH.

When aluminum chloride and different doses of Lawsonia inermis aqueous leaf extract (60mg/Kg, 75mg/kg and 100mg/Kg) were concomitantly given, the counts of basophil cells surpassed the counts of acidophil and chromophobe cells and were markedly higher than the counts seen in the group given the low-dose protocol. The effect of Lawsonia inermis extract was found to be dose-dependent. Doses of up to 100mg/Kg improved cell counts, while doses greater than 100mg/kg possibly had a synergistic effect with aluminum to further decrease cell counts. The pars distalis had few areas with destructive changes and vacuolation areas (Plates 1 and 2). Moreover, as the dose increased the areas with destructive changes and vacuolation areas were significantly reduced. This finding is in agreement with Towolawi et al. (2010), in which an antioxidant role against heavy metal toxicity was described.

Lastly, the histology and morphology of the pituitary glands of the rats given 0.5mg of aluminum chloride and 5mg/Kg of ascorbic acid (Plates 1 and 2) revealed that the anterior pituitary gland could be well distinguished from the posterior pituitary gland by the elevated cell counts slightly greater than the counts seen in the subjects on the high-dose protocol (100mg/Kg of aqueous extract of Lawsonia inermis) with aluminum chloride for basophil (gonadotropin secreting cells), acidophil, and chromophobe cells. Basophil cell counts were greater than the counts of acidophil and chromophobe cells. There were few areas of destructive change related to aluminum intake. Periodic acid-Schiff (PAS) and Orange G staining were used to stain neuroendocrine cells. The numerous basophil cells present in the anterior pituitary stained magenta; acidophil cells stained yellow; the few chromophobe cells stained pale blue-grey; nuclei stained dark blue; and red blood cells stained yellow.

Lastly, acidophil cell counts were lower than the counts of basophil and chromophobe cells and are found as cords. Basophil cells are larger than acidophil cells and appear in clusters. Chromophobe cells have centrally-located nuclei, are round in shape, have granule-free cytoplasm, and are larger than other cells. In this study, Hematoxylin and Eosin (H&E) (Plates 1 and 2) and Periodic acid-Schiff (PAS)/Orange G staining (Plates 1 and 2) revealed similar increases or decreases in cell counts in the aluminum chloride and aqueous extract administration protocol. Similar pathological structures were also shown. The cells were more easily distinguished based on the colors they acquired after staining.

CONCLUSION

This study showed the effects of aqueous Lawsonia Inermis leaf extract in increasing the cell counts of the pituitary glands of adult male Wistar rats and alleviating the oxidative stress induced by aluminum poisoning.

ACKNOWLEDGEMENT

We would like to acknowledge Dr B. U. Enaibe of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria, for making their laboratory available and thus allowing this study to be performed.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

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