HISTOLOGICAL EFFECTS OF CHRONIC ADMINISTRATION OF *OCIMUM GRATISSIMUM* LEAVE EXTRACT ON SELECTED ORGANS OF ADULT WISTAR RATS

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ABSTRACT
Ocimum gratissimum leave extract is commonly used in traditional medical practice for the treatments of mental illness, epilepsy, high fever, diarrhoea, pneumonia, cough, and conjunctivitis. It is known to affect sexual behavioural score in animals. The leaves of the plant are also used culinarily in salads, soups, pastas, vinegars and jellies in many parts of the world. This study assessed the effect of chronic administration of ethanolic extract of the leaves of *Ocimum gratissimum* on the lungs, heart, pancreas and testes of adult wistar rats. Group 2, 3 and 4 received respectively 200mg/kg, 300mg/kg and 500mg/kg (W/V). The extracts of *Ocimum gratissimum* were administered orally once daily for 28 days. Group 1 was control. At the end of four weeks, the animals were sacrificed using the cervical dislocation technique. The lungs, heart, pancreas and testes were removed and fixed in 10% formal saline. Paraffin sections were prepared from these organs and stained with haematoxylin and eosin for histopathological assessment. Results showed that chronic administration of *Ocimum gratissimum* leave extract induced a dose dependent toxicity on the histology of the lungs and heart of male adult Wistar rats. While pancreatic and testicular histology did not depart from control. Hence consumption of extract of the plant may be toxic to humans and should be consumed with caution.

Key words: *Ocimum gratissimum*, toxicity, histology, lungs, heart, pancreas, testes.

INTRODUCTION
Ocimum gratissimum is a herbaceous plant of the Labiatae family. The plant is indigenous to tropical areas especially India and it is also found in West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan (Nadkarni, 1999). It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos (Effraim *et al.,* 2003). It is called ''Elah (Elaa)'' in part of Urhobo (Eboh and Ekundina, 2012). In the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim *et al.,* 2003).

Ocimum gratissimum is a small erect plumb plant with many branches usually not more than 1 meter (Vierra and Simon, 2000) to 2 meters high with a taproot and many adventitious side rootlets. The leaves are simple, opposite or whorled with several oil glands and possess a peculiar scent smell due to its composition of volatile essential oils. It produces an inflorescence that is capitate and reduces apical dominance while increasing branching. The flowers are zygomorphic, bilobal and bisexual with five petals and sepals and four stamens. The gynecium has two carpels ascending from the ovary. The fruit has a group of four nutlets each with a brown seed that has scanty or no endosperm (Okujagu *et al.,* 2005; Odebiyi and Sofowora, 1978). It is commonly called African basil or shrubby basil.

Ocimum gratissimum is commonly used in traditonal medical practice for the treatments of such ailments as mental illness (Abdulrahman, 1992), epilepsy (Sofowora,

1993), high fever (Oliver, 1980), diarrhea (Oliver, 1980; Sofowora, 1993). It is also used for pneumonia, cough, and conjunctivitis (Correa,1932; Onajobi, 1986). The leaves of the plant are used culinarily in salads, soups, pastas, vinegars and jellies in different parts of the globe.

The key roles of the lungs, heart, pancreas and testes as organs of the body are well known. Taking into cognizance that *Ocimum gratissimum* is widely used as vegetable in many local dishes or foods to achieve varied purposes, and bearing in mind that appropriate formulations and dosages are not considered during consumptions, not considering probable adverse effects, this study was necessitated to assess the effect of chronic administration of ethanolic extract of this plant on the histology of lungs, heart, pancreas and testes of adult Wistar rats. The results of the study will serve as guide to consumers of this herb regarding their quantitative application.

MATERIALS AND METHODS

Experimental animals

Twenty adult Wistar rats (200-250g) of about 12 weeks old bred in the animal house of the College of Health Sciences, Delta State University, Abraka, Nigeria, were used for this study. The animals were randomly selected and kept in four groups of five rats per group. The animals were fed with commercially formulated rat feed and water add libitum. The animals were acclimatized for 14 days.

Preparation of Extract

The leaves of *Ocimum gratissimum L* were dried at room temperature for two weeks. The dried leaves were pulverized into coarse form with a milling machine. Eight hundred grams (800g) of the pulverized form were soaked in absolute ethanol for 24 hours for extraction of the active ingredients before filtering with a white filter cloth. The resulting ethanolic extract was evaporated to dryness using a rotary evaporator at 40^{o}C. The weight of the dry extract was determined to be 35.05g.

Experimental Procedures

Animals in Group 1 (control) were given feed with water add libitum. The rats in groups 2, 3, and 4 were given 200mg/kg, 300mg/kg and 500mg/kg (W/V) extract of *Ocimum gratissimum* respectively for 28 days using an oro-gastric canular once daily. At the end of administration of extract of *Ocimum gratissimum*, the technique of physical euthanasia by cervical dislocation was adopted.

The tissues were harvested, fixed in 10% formal saline, processed by the paraffin wax technique, and sections cut with the microtome and stained with haematoxylin and eosin to demonstrate general tissue archetecture. The stained tissue images were captured using digital microscopic eyepiece "SCOPETEK"DCM ss500, 5,0 megapixel connected to USB 2.0 computer.

RESULTS

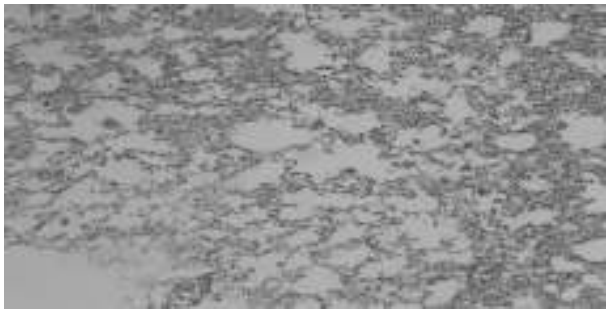

Fig 1: Control lungs x100

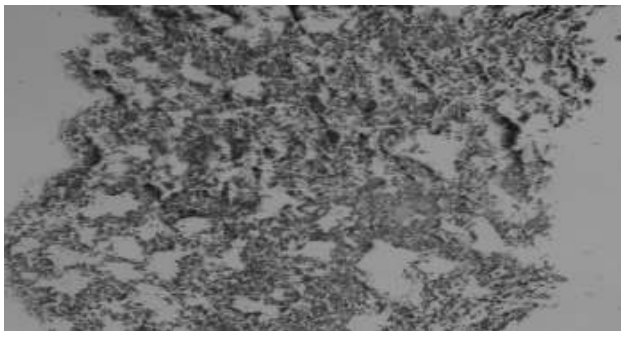

Fig. 2: 200mg lungs x100

Fig 3: 300mg lungs x100

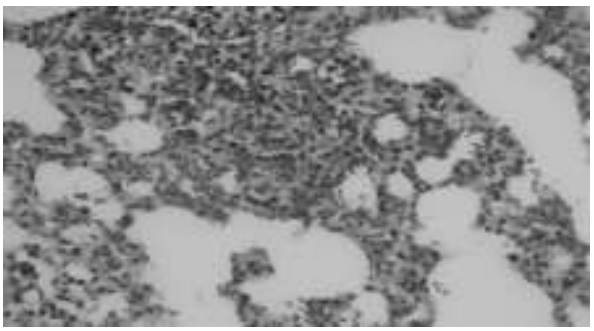

Fig 4: 500mg x100

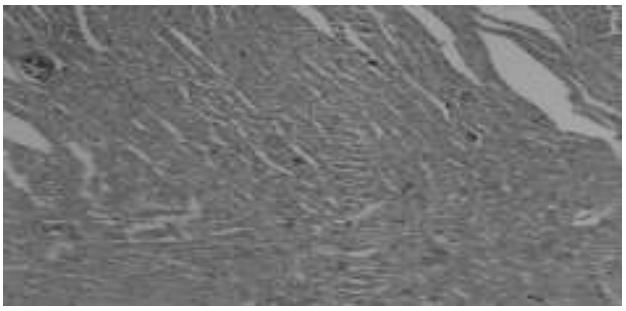

Fig 5: Control heart x100

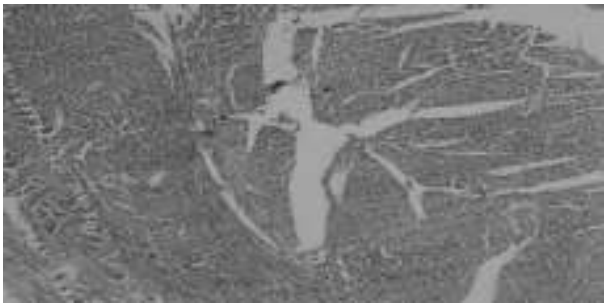

Fig 6: 200mg heart x100

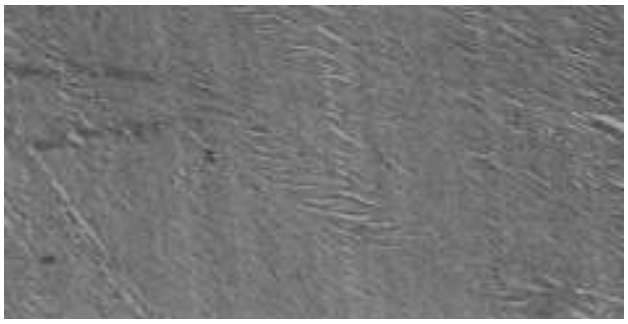

Fig 7: 300mg heart x100

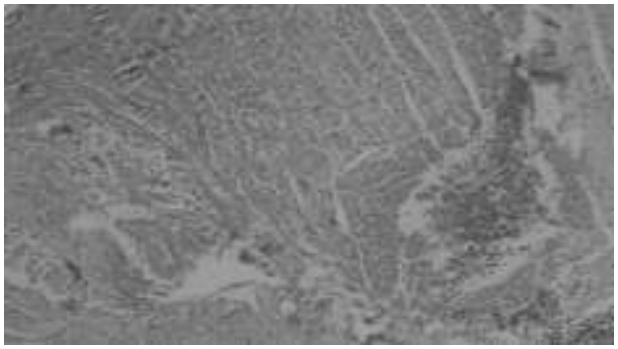

Fig 8: 500mg heart x100

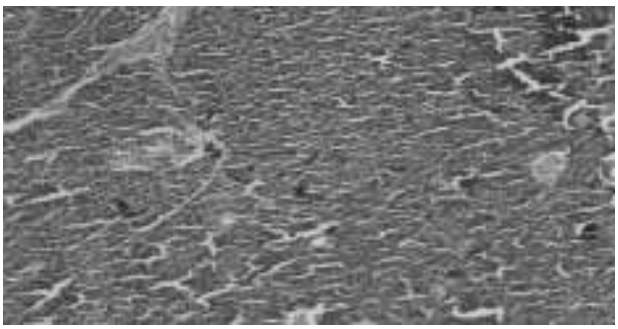

Fig 9: Control pancreas x 100

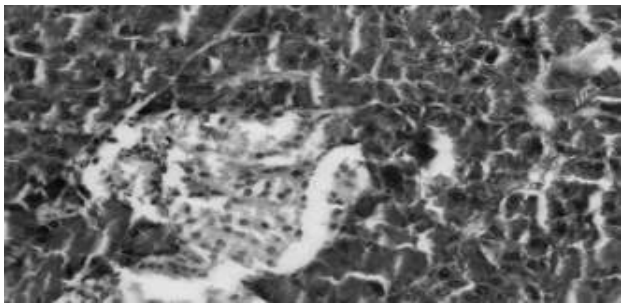

Fig 10: 200mg pancreas x100

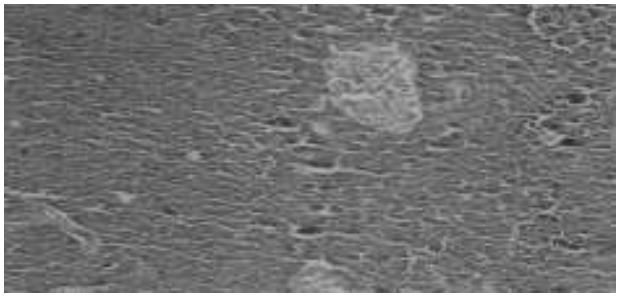

Fig 11: 300mg pancreas x100

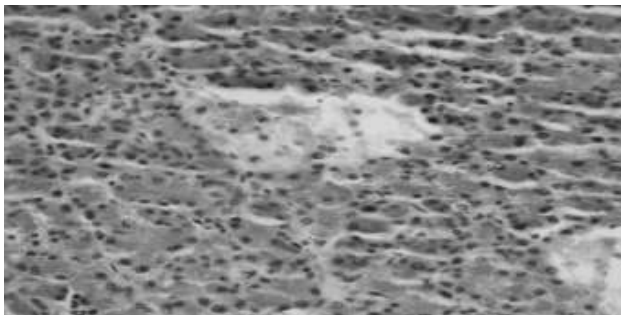

Fig 12: 500mg pancreas

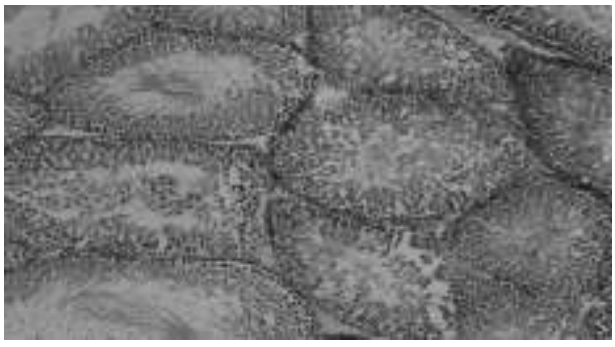

Fig 13: Control testis x100

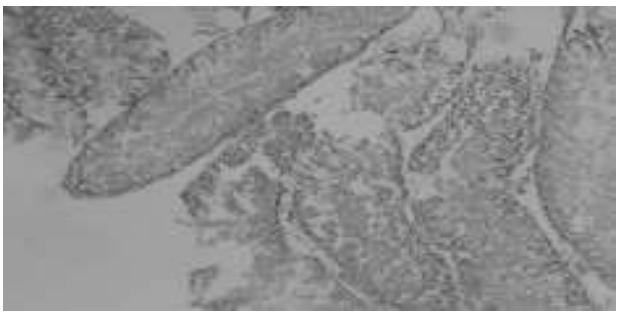

Fig 14: 200mg testis x100

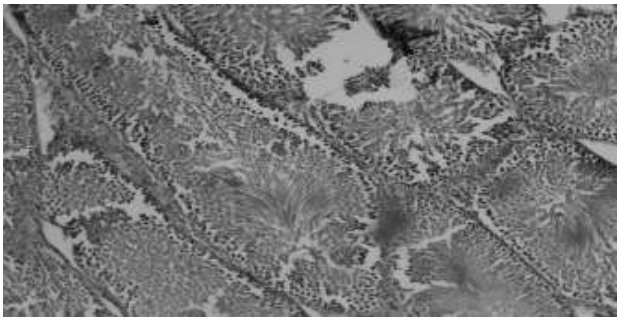

Fig 15: 300mg testis x100

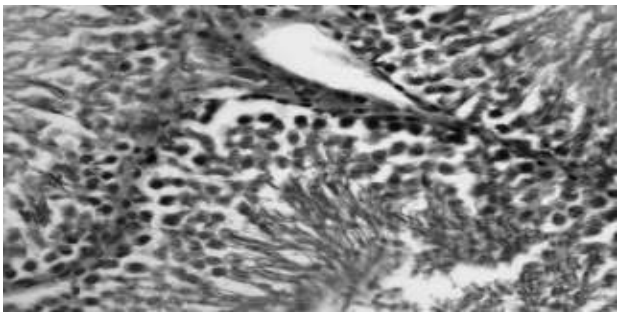

Fig 16: 500mg testis x400

Figure 1 (control lung tissue) showing normal alveoli and interstitial tissue. Alveoli are lined by locule epithelium.

Figure 2 shows histological features of lung of the animals treated with 200mg diet. Section shows a lung tissue with congestion of the capillaries.

Figure 3 shows histological features of lung of the animals treated with 300mg diet. Section shows lung tissue with moderate to heavy presence of acute to chronic inflammatory cells together with area of hemorrhage. It also revealed areas of lymphoid aggregate.

Figure 4 shows histological features of lung of the animals treated with 500mg extract. Section shows lung tissue with severe congestion and heavy infiltration of the Interstitium by polymorphornuclei leukocytes.

Figure 5 shows histological features of heart of the animals in the control group. Section shows cardiac muscles arranged in bundles. No inflammatory cells, congestion or oedema seen.

Figure 6 shows histological features of the heart of animals treated with 200mg extract. Section shows cardiac muscles arranged in bundles. No inflammatory cells, congestion or oedematous areas seen.

Figure 7 shows histological features of heart of the animals treated with 300mg diet. Section shows cardiac muscles arranged in bundles. No inflammatory cell, congested channels seen.

Figure 8 shows histological features of heart of the animals treated with 500mg diet. Section revealed cardiac tissue showing interstitial edema, vascular congestion and mild inflammatory cells.

Figure 9 shows histological features of pancreas of animals in the control group. Section shows a normal pancreatic tissue with sheath of exocrine portion of the pancreas and clusters of islet cells.

Figure 10 shows histological features of pancreas of the animals treated with 200mg diet. Section showed pancreas with normal exocrine portion with eosinophillic secretion and gland lined by cuboidal epithelium. Also revealed is endocrine portion with islet cells appearing essentially normal.

Figure 11 shows histological features of pancreas of animals treated with 300mg diet. Section of pancreas appeared essentially normal, no injury, and no inflammatory cells seen.

Figure 12 shows histological features of pancreas of animals treated with 500mg diet. Section of pancreas appeared essentially normal with exocrine and endocrine units intact and the Interstitium free of any collections and inflammatory cells.

Figure 13 shows histological features of testis of animals in the control group. Section shows normal testis tissue consisting of numerous seminiferous tubules of sizes that are lined by cells at different levels of maturation.

Figure 14 shows histological features of testis of animals treated with 200mg diet. Section shows thin seminiferous tubules, interstitial cells of leydig and sertoli cells were seen as essentially normal.

Figure 15 shows histological features of testis of animals treated with 300mg diet. Section

shows testis tissue consisting of numerous seminiferous tubules of sizes that are lined by cells at different levels of maturation. Interstitium is free of inflammatory cell and collection.

Figure 16 shows histological features of testis of animals treated with 500mg diet. Section showed testis tissue consisting of numerous seminiferous tubules of sizes that are lined by cells at different levels of maturation. Interstitium is free of inflammatory cell and collections.

DISCUSSION

The present study on ocimum gratissimum assessed the effect of chronic administration of ethanolic extract of the leaf on the histology of the lungs, heart, pancreas and testes in adult male wistar rats. The long term consumption of an *Ocimum gratissimum* diet by male Wistar rats had some varied significant effects on the histology of some of the organs, as seen in the results.

The histological effect of this extract on the lungs ranges from congestion of the capillaries at 200mg diet to acute-chronic inflammatory cell aggregation with areas of hemorrhage and lymphoid aggregation at 300mg. This pathology was marked with the administration of 500mg extract by infiltration of interstitium by polymorphonuclei leukocytes. These features (inflammation, congestion and lymphoid aggregates) may be as a result of tissue damage by bacteria, trauma, chemical, heart etc (Guyton, 2000). This observation corroborates Efiri (2012) who reported that animals given higher doses of this extract showed greater changes in their lungs histo-architecture when compared with those given lower doses. They observed congestion of blood vessels, inflammation of the lung tissue and widening of interstitium with areas of alveolar damage.

The histological effects of the extract on the heart of the animals that received 200-300mg extract showed essentially the same histological feature with control group. However, at high dose of 500mg extract, cardiac tissue pathology became evident (interstitial odema, vascular congestion and presence of mild inflammatory cell). There is no previous histological study on the effect of ocimum gratissimum leaf extract on the heart. Nonetheless the features shown in the present studies may be associated with failing heart.

In the present study, the histological appearances of the pancreatic tissues at different levels administration of *Ocimum gratissimum leave extract did not depart from the control group. This indicates that the leave extracts of Ocimum gratissimum do not have toxic effect on the pancreas when consumed for a long time. A search of the literature revealed that no previous study has been carried on the effect of Ocimum gratissimum leave extract* the histological appearances of the pancreatic tissues.

The effect of chronic administration of Ocimum *gratissimum leave extract on the testes revealed spermatogenic series at varied levels of maturation; histological appearances were not essentially different from control. In a related study,* Iweala and Obidoa (2010) reported increased number of sperm cells and greater number of spermatogonia and spermatocytes in the testes of the animals fed with *Ocimum gratissimum leave extract* when compared to the control group. They pointed out that consumption *Ocimum gratissimum leave extract* impacted a distinct effect on the histology of the testes which showed an increased synthesis of sperm cells and spermatozoa. They also opined that an increase in spermatogenesis is related to the possibility of increased reproductive capacity in the male rats fed with Ocimum *gratissimum leave extract*. It has also been reported that *Ocimum gratissimum* has been shown to affect sexual behavioral score in animals (Kantak and Gogate, 1992).

CONCLUSION

In the present study chronic administration of *Ocimum gratissimum* leave extract showed a dose dependent toxicity on the histology of the lungs and heart of male adult Wister rat. Long term administration of ocimum gratissimum leaf extract showed no toxic effect on the pancreatic and testicular tissues. Consequently, indiscriminate consumption of this leave by humans should be done with care. Further studies should be carried out on the functional and biochemical parameters in relation to lungs, heart, pancreas

and testis functions in order to confirm the histological findings.

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