



Some morphological observations on the heart of adult wistar rats following exposure to aqueous extract of *ocimum gratissimum*

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ABSTRACT

Cardiovascular Diseases (CVD) continue to be one of the leading causes of deaths despite recent advancements in the medical therapies. This study assessed the effect of crude aqueous extract of *Ocimum gratissimum* commonly used for the treatment of diarrhoea, urinary infections, fever and dysentery on heart morphology. Twenty-four Wistar rats of both sexes with average weight of 175g were used. The rats were randomized into four groups each containing 6 rats. Groups B, C and D received the aqueous extract of *Ocimum gratissimum* orally at doses of 0.7g/kg, 1.4g/kg and 2.8g/kg body weight respectively for 3 consecutive weeks. Group A served as control group which received distilled water only. After the 3 weeks of administration, the rats were sacrificed by cervical dislocation and the hearts were dissected, blood samples were taken for haematological analysis for blood platelet and the organs were fixed in 10% formal saline for histological investigation by light microscopy. Histological observation was normal in control group. Treated sections showed distortions, disaggregation and loss of cellular components. Haematological findings showed significant differences between control and treated groups and revealed that *Ocimum gratissimum* is capable of invoking an inflammatory response that transits from acute to chronic on persistent administration. The heart weights of the treated rats also increased compared with the control.

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INTRODUCTION

The use of plant-based systems remains central to health care delivery. Several reports have shown that approximately 80% of the population in developing countries still depends on the practice of Traditional Medicine (TM) for their primary health care (WHO, 2011; Hostettman and Marston, 2002). In some African countries such as Nigeria, Ghana, Mali and Zambia. The use of herbal medicines at home is the first line of treatment for 60% of the children with high fevers, resulting from malaria (WHO, 2003). The African environment is probably the least explored in terms of available untapped resources. Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potentials of introducing new templates into modern medicine.

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into African continent (Akinyemi *et al*, 2005). Herbal medicine has been widely used and formed an integral part of primary health care in China (Liu, 1987) Ethiopia (Desta, 1993) Argentina (Anesini and Perez, 1993) and Papua New Guinea (Nick *et al*, 1995). A significant proportion of pharmaceutical products in current use are designed from plants (Cowan, 1999

and Raskin *et al.*, 2002). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effect on all types of micro-organisms in vitro (Cowan, 1999) and some plant extracts have shown activity on both gram negative and gram positive organism (Nascimento *et al.*, 2000). Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001). In Brazil alone, about 80,000 species of higher plants were described which offer enormous prospects for discovering new compounds with therapeutic property (Nakaruma *et al.*, 1999).

The use of various plant parts in the treatment of the sick developed into tradition which was handed down from one generation to another over the years verbally or written (Sofowora, 1982; Akinyanju, 1986). For thousands of years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients is extracted refined and made ready for consumption, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973). *Ocimum gratissimum* (African Basil) belongs to the family *Labiatae*. It is widely distributed in tropical and warm temperate regions. *Ocimum*

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gratissimum is called 'efinrin' by the yorubas of the southwestern part of Nigeria, 'nchanwu' by the Igbos and 'Dai'doya' by the Hausas. It has been reported to contain the terpenoids, eugenol and thymol, saponins and alkaloids (Gill, 1988). Aromatic oil from the leaves consist of thymol (32-65%). *Ocimum gratissimum* Linn (Labiatae) is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool B have been extracted from the oil (Sulistiarini, 1999). Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Dubey *et al.*, 2000). The essential oil also has an antinociceptive property (Rabelo *et al.*, 2003). The whole plant and the essential oil are used in traditional medicine especially in Africa and India. The essential oil is also an important insect repellent.

Ocimum gratissimum is germicidal and has found wide use in toothpastes and mouth washes as well as some topical ointments (Nakamura *et al.*, 1999; Holets *et al.*, 2003; Pessoa *et al.*, 2002). It is used as an excellent gargle for sore throats and tonsillitis. *Ocimum gratissimum* has been reported to be active against several species of bacteria and fungi (Nwosu and Okafor, 1995; Nakaruma *et al.*, 1999). The heart pumps blood ceaselessly because the body's tissues—especially the brain and the heart itself—depend on a constant supply of oxygen and nutrients delivered by the flowing blood. If the heart stops pumping blood for more than a few minutes, death will result. (Passamani, 2009). Traditional practitioners prescribe and administer decoction of the leaves of *Ocimum gratissimum* to patients without regard to its possible adverse effects. According to the world health organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Boyd *et al.*, 1994). *Ocimum gratissimum* has been reported to have antipyretic and antiarrhythmic activities (Oliver, 1980 and Sofowora, 1993). In folk medicine, *Ocimum gratissimum* is extensively used throughout West Africa as a febrifuge, anti-malarial and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities. (Ezekwesili *et al.*, 2004). The *Ocimum gratissimum* extract has been shown to have sedative activity (Effraim *et al.*, 2003) and to have therapeutic benefit in patient with inflammatory joint diseases. (Tanira *et al.*, 1988). However there is a paucity of information concerning the adverse effects of repeated oral administration of aqueous preparation of *Ocimum gratissimum* on the heart morphology. This study consequently reported the possible adverse effect of *Ocimum gratissimum* on morphology of the heart in wistar rats.

MATERIALS AND METHODS

24 albino rats of both sexes of average weight of 175g were purchased from the Department of Animal Production and Health Sciences, Faculty of Agriculture, Ladoko Akintola University of Technology Ogbomosho for the purpose of the research. The experimental animals used were cared for and they were acclimatized in the histology laboratory for three weeks. The animals were fed locally made feed portion, which consist of a mesh work of highly concentrate of carbohydrate.

They were given tap water at pleasure using water bottles or drinkers. They were kept in plastic cages with iron netting. The animals beddings were changed after three days of usage and the feeders and drinkers were also cleaned. The animals were weighed twice in a week to know if they were healthy. The rats were grouped into four of five animals per group according to the weight of the rats. Group B, C and D received the aqueous extract of *Ocimum gratissimum* at doses of 0.7g/kg, 1.4g/kg and 2.8g/kg body weight respectively, Group A served as control group which received distilled water. The method of Awuah (1989) was adopted. 10g of chopped fresh leaves of *Ocimum grassimum* was infused with 20ml of distilled water in 500ml laboratory flask. The flask with its content was heated in a water bath (60°C) for 2 hours. A crude aqueous extract was obtained by filtering the infusion through two layers of cheese cloth. The preparation of the plant extract was done in the Food Science Laboratory of Ladoko Akintola University of Technology, Ogbomosho.

The extract was administered orally by the use of syringe and canula. Animals received their doses everyday for a period of three weeks. At the end of the third week, the animals were sacrificed by cervical dislocation and the blood of the animals used was collected immediately after sacrifice for analysis of platelet count. The hearts were carefully removed and in fixed 10% formol saline for light microscopy, Digital photomicrography of the needed heart sections were obtained to show the morphologic changes that occurred in the treated groups as compared to the control group. The statistical analysis of the result obtained in this study was carried using ANOVA and tested for significance using student's t-test.

STATISTICAL ANALYSIS

All data were presented as Mean \pm SEM. Statistical analysis of the data of comparisons between the control and treated groups in this study were carried out using one-way analysis of variance and significance was tested for using student's t-test. P-Value $<$ 0.05 was considered statistically significant.

RESULTS

From table 2, the weight analysis for heart shows no significant difference ($p > 0.05$) in weight comparing control group to group B, also there was no significant difference ($p > 0.05$) in weight of the heart when comparing the control group to group C and also group D. Group D which received the highest dose had the highest heart weight followed by Group C which received the medium dose and Group B the low dose although there was no significant difference in the heart weight of all the groups. The increase in weight of heart can be attributed to the substance administered.

BODY WEIGHT ANALYSIS (n=6)

Table 1: showing the Mean \pm SEM of the initial (before treatment) and final body weight (after treatment) of rats

Weight (g)	Group A Control	Group B Low Dose	Group C Medium Dose	Group D High Dose
Initial	154.02 \pm 1.71	179.4 \pm 2.51	180.8 \pm 3.07	217.6 \pm 12.9
Final	192.6 \pm 2.52	187.06 \pm 3.62	202.7 \pm 5.79	248.27 \pm 14.8
Differences (Final- Initial)	+38.58	+7.66	+21.9	+30.6
% Difference in weight	25.04	4.26	12.11	14.06

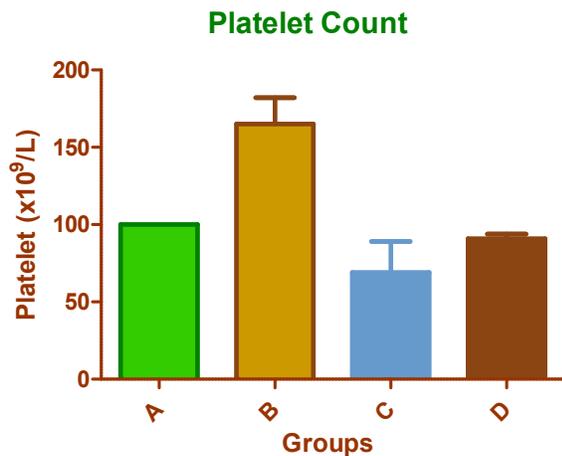
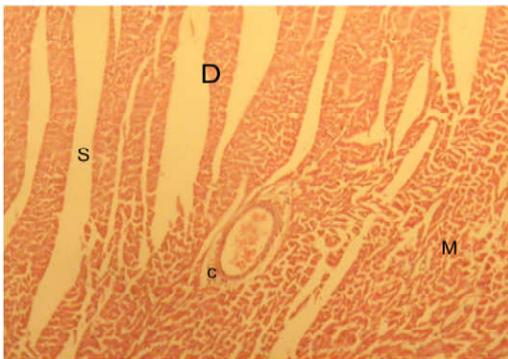
WEIGHT OF HEART (n=6)

Table 2: the Mean \pm SEM of heart weights of wistar rats after sacrifice

	Group A Control	Group B Low Dose(0.7g/kg)	Group C Medium Dose (1.4g/kg)	Group D High Dose (2.8g/kg)
Weight (g)	0.63 \pm 0.04	0.66 \pm 0.04	0.68 \pm 0.03	0.70 \pm 0.06

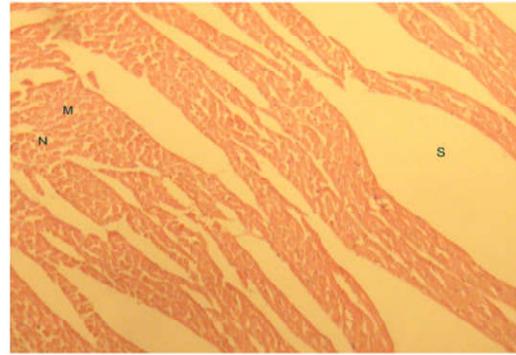
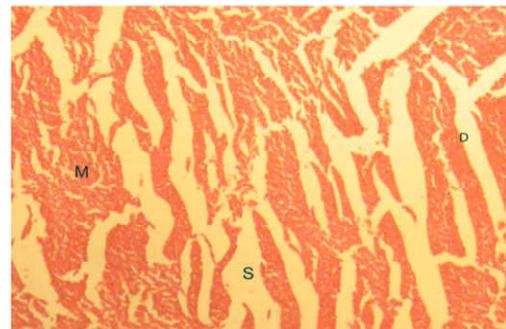
PLATELET COUNT ANALYSIS (n=6)**Table 3: showing the Mean \pm SEM of the platelet count of the rats**

	Group A Control	Group B Low Dose (0.7g/kg)	Group C Medium Dose (1.4g/kg)	Group D High Dose (2.8g/kg)
Platelet Count ($\times 10^9/L$)	100 \pm 0.0	165 \pm 17 ^{ef}	69 \pm 20 ^e	91 \pm 3 ^f

**Fig. 3: The graphical representation of effect of aqueous extract of *Ocimum gratissimum* on platelet count of rats****HISTOMORPHOLOGY OF THE HEART****Plate 1. Photomicrograph of the longitudinal section of the heart of wistar rat (control) showing normal cell and muscle, D=Intercalated disc, M= Muscle, C=Muscle cell nucleus, S= Sacroplasm, Mag. 100x, H&E stained.**

GROUP B (LOW DOSE): This group received 0.7g/kg body weight of the extract of *Ocimum gratissimum*. The histological examination shows enlarged sacroplasm and distorted Muscle structure as seen in plate 2.

GROUP C (MEDIUM DOSE): This group received 1.4g/kg body weight of the extract of *Ocimum gratissimum*. The

**Plate 2. Photomicrograph of the longitudinal section of the heart of wistar rat (treated with 0.7g/kg of the extract) showing enlarged sacroplasm and thorn Muscle structure. S=Sacroplasm, M= Muscle, N=Muscle cell nucleus. Mag. 100x, H&E stained.****Plate 3. Photomicrograph of the longitudinal section of the heart of wistar rat (treated with 1.4g/kg of the extract) showing enlarged cells and lysis of the muscle cells. D = intercalated disc, M =muscle, S= sacroplasm. Mag. X100, H&E stained****Plate 4. Photomicrograph of the longitudinal section of the heart of wistar rat (treated with 2.8g/kg of the extract) showing some oedematous cells as longitudinal cells become transverse section, there is great, S= Sacroplasm, Mag. 100x, H&E): This group received 2.8g/kg body weight of the extract of *Ocimum gratissimum***

The histological examination as it could be seen in the photomicrograph shows that the cells to become more enlarged and there is a great lysis of the muscle as seen in plate 3a and 3b as compared with the control group above. The histological examination shows that the cells also became oedematous as longitudinal cells become transverse section, there is a great lysis of the cells and enlarged sacroplasm as compared to control group.

DISCUSSION

The tribal groups of Nigeria use the leaf extract in treatment of diarrhoea, while the leaf infusions are used for the relief of stomach upset and haemorrhoids (Kabir *et al.*, 2005.) The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu *et al.*, 2005). The study of the toxic or adverse effects of crude drugs of plant origin is essential in order to provide guide to their safe usage and eventually standardization (Effraim *et al.*, 2003). This is especially pertinent, as traditional medicine practitioners and some individuals often administer such preparations without regard to their possible adverse effect. The present study showed that the *Ocimum gratissimum* extract can exert itself to cause some functional damages to the liver when applied at varying doses (0.7, 1.4 and 2.8g/kg body weight) of the extract.

It was observed from the histology results that sections of the heart showed different changes on treated organ when compared to control sections. As seen in plate 1 which is the control group, the cells appeared to be normal and the cells are well arranged with normal nucleus and there is no tearing of the muscle. As the doses increased according to each group, morphological changes were observed in the heart tissues. In plate 2 which received low doses (0.7g/kg), the cells seems to have enlarged sacroplasm and thorn Muscle structure, there also seems to be lysis of the cells, in plate 3 which received medium dose (1.4g/kg), the tissues become more enlarged and there is great lysis of the muscle, in plate 4 which received high dose (2.8g/kg), the cells also experienced oedematous changes as longitudinal cells become transverse section and this leads to tearing of the muscle, which may be the result of the inflammation of the heart tissues. Inflammation result when there is an injury to the tissue which is either caused by bacteria, trauma, chemicals, heat or any other phenomenon (Guyton, 2000).

From the result obtained, it was observed that the rats given higher doses of the extract, showed greater degenerative changes in their heart architecture; when compared to those given lower dose. Increase in heart weight was observed in all the treated groups as showed in Table 2. There was no significant difference between the body weight of the treated as compared with the control group ($p > 0.05$). The extract has no effect on the body weight of the rats. Therefore, it was observed that oral administration of *Ocimum gratissimum* extract resulted in increased weight gain in the animals which could be attributed to the nutrient composition of leaves of *Ocimum gratissimum*. *Ocimum gratissimum* elaborates nutrients that can increase weight such as carbohydrates, lipids, proteins mineral and vitamins (Edeoga *et al.*, 2006). The heart weight of the rats treated with crude extract of *ocimum gratissimum* as seen in Tab 2 shows increased in weight in a dose-dependent manner but statistically there was no significance difference between the treated groups compared with the control group. This study the effect of crude aqueous extract of *Ocimum gratissimum* administered at different doses shows that, there is a significant difference in the platelet counts of groups B and C. There was no significant difference between group C and D.

Therefore, the extract administered during the experiment suppressed the haemopoietic system, and the groups majorly affected were the group with high and medium doses respectively. The reduction of platelets may have occurred due to lysis of blood cells and probably suppression of blood cell synthesis by saponins found in the leaf extract (Irvine, 1961). Saponins are known to be toxic to body systems (Watt *et al.*, 1962). Despite the popularity of the plant as a condiment and herbal medicine, the extract has been observed to suppress the haemopoietic system. It is therefore suggested that chronic usage of the leaf is not advisable. Data analyses of haematological and histopathological findings showed significant differences between control and treated groups and revealed that *Ocimum gratissimum* is capable of invoking an inflammatory response that transits from acute to chronic on persistent administration (Orafidiya *et al.*, 2004).

CONCLUSION

This study concluded that aqueous extract of *Ocimum gratissimum* administered to wistar rats resulted in degenerative changes which may consequently impair some cardiac functions. We recommend that further studies should be carried out to corroborate these findings.

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