

# Comparative study of teratogenic potentials of crude ethanolic root bark and leaf extract of *Rauwolfia vomitoria* (apocynaceae) on the fetal heart

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## Abstract

**Background:** *Rauwolfia vomitoria*, a tropical shrub, is a medicinal plant used in the treatment of a variety of ailments. It is popular to the locals because of its anti-hypertensive and sedative properties. **Aim:** This is to find the probable teratogenic effects of ethanolic leaf and root bark extracts of *Rauwolfia vomitoria* on the morphological and histological features of the fetal heart. **Material and Methods:** Twenty five female rats weighing between 170-200g were used for this study. The rats were divided into five groups labeled A, B, C, D and E, with each group consisting of five rats. Pregnancy was induced by caging the female rats with sexually matured males. The presence of vaginal plug and tail structures in the vaginal smear the following morning confirmed coition, and it was regarded as day 0 of pregnancy. Group A was given sham treatment of distilled water. Group B and C received respectively 150mg/kg and 250mg/kg body weight doses of ethanolic leaf extract of *Rauwolfia vomitoria*, and those in groups D and E received respectively 150mg/kg and 250mg/kg body weight doses of ethanolic root bark extract of *Rauwolfia vomitoria*. These treatments were on days 7-11 of gestation (5 days) with the aid of an orogastric tube. On the day 20 of gestation, the rats were sacrificed and the fetuses examined for gross anomalies, preserved and latter process for histological studies. **Results:** There were no mortality in this study, and no obvious gross malformations in the fetuses. Histological observations of the fetal heart showed marked distortion of the cardiac muscle nuclei and myocardial fibers in the treated groups particularly those whose mothers received 250mg/kg of the extracts. These effects were more pronounced in the groups whose mothers received the root extract when compared with the control and the groups whose mothers received the leaf extract. **Conclusion:** This result suggests that high doses of ethanolic leaf and root extracts of *Rauwolfia vomitoria* may be cardiotoxic to the developing rat's heart.

**Keywords:** Ethanolic leaf extract, *Rauwolfia vomitoria*, fetal morphology, heart

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## Introduction

During the latter part of last century, the practice of herbalism became mainstream throughout the world. This was due in part to the recognition of the value of traditional medical practice, particularly of Asian origin and the identification of medical plants from indigenous pharmacopeias. These herbs have been shown to have significant useful medicinal effects, either in their natural

state or as the source of pharmaceuticals [1].

One of the plants of medicinal value from the humid tropics is *Rauwolfia*. Well over 50 authenticated species of *Rauwolfia* have been named in memory of the great German physician and botanist, Professor Leonhart Rauwolf, who presented a wealth of information on the indigenous medicinal plants of Central Asia and Africa [2].

*Rauwolfia vomitoria* also called serpent wood, serpent snake root and swizzle stick, as well as, asofeyeje in Yoruba, ira in Hausa, akata in Bini and Mmoneba and utoenyin in Efik, is mostly found in the forest of the southern part of Nigeria [3]. Traditionally, it is used against snakebites, fever and nervous disorders. In Ghana and Nigeria, herbalists used it as an emetic and a purgative; in the same region, children are treated with this plant for cerebral cramps, jaundice and gastrointestinal disorders [4].

The pharmaceutical derivatives are used mainly as anti-hypertensive and sedative drugs. Its sedative property is attributed to its ability to balance body response to stress and anxiety and to increase oxygen delivery to the brain [5]. Decoctions of leaves of *Rauwolfia vomitoria* have a powerful emetic effect and chopped leaves stewed with animal fat are applied to swellings [6].

The active components of *Rauwolfia vomitoria* as reported by Gill [3] are alkaloids reserpine, rauwolfine, rescinnamine, serpentine, ajmaline, serpentinine, steroid-serposterol and saponin. Reserpine reduces the cardiac output by negative inotropic effect and chronotropic effects, thereby reducing sympathetic activity to the blood vessels and relaxing them which lead to reducing total peripheral resistance and blood pressure [7].

The developing cardiovascular system is a sensitive target of many environmental pollutants; laboratory research has utilized a variety of vertebrate models to elucidate potential mechanisms that mediate this cardiotoxicity and to establish the sensitivity of different species for predicting potential risk to environmental and human health [8]. The permanent cellular constituents of the heart include cardiac fibroblasts, myocytes and endothelial cells and vascular smooth muscle cells [9]. Previous studies have demonstrated that there are undulating changes in cardiac cell populations during embryonic development through neonatal development and into the adult. Transient cell populations include lymphocytes, mast cells and macrophages which can interact with these permanent cell types to affect cardiac function [9]. Current dogma states that the fibroblast makes up the largest cell population of the heart; however this appears to vary for different species, especially mice [9].

Cardiac fibroblasts play a critical role in maintaining normal cardiac function as well as in cardiac remodeling during pathological conditions such as myocardial infarct and hypertension [9]. Cardiac myocytes also called cardiomyocytes or cardiac muscle cells are responsible for generating the electrical impulses that control the heart rate; among other things. Co-ordinated contraction of cardiac muscle cells in the heart propels blood from the atria and ventricles to the blood vessels of the circulatory system. The muscle tissue of the heart (myocardium) is composed of this striated involuntary muscle cells connected to form the contractile pump to generate blood

flow [10]. In heart muscle disease (cardiomyopathies), there is deterioration of the function of the myocardium [10]. Hypertension is associated with a rise in arterial pressure and a compensatory increase in cardiac mass which if not treated effectively progresses to decompensate congestive heart failure [11]. Hence, this research work is to study the effect of ethanolic leaf and root bark extracts of *Rauwolfia vomitoria*.

## Materials and Methods

### *Preparation of ethanolic leaf extract of Rauwolfia vomitoria*

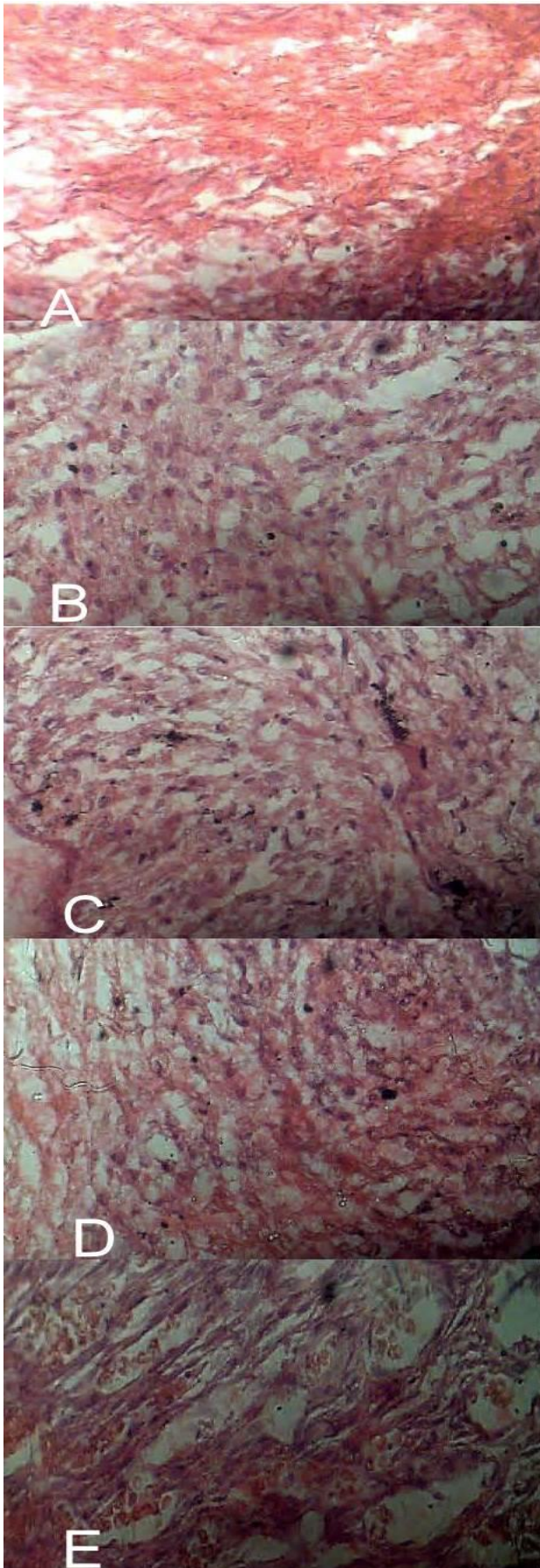
Fresh leaves and roots of *Rauwolfia vomitoria* plants were obtained from the University of Calabar botanical garden. The leaves were identified and authenticated by the botanist in the botanical garden of University of Calabar, Nigeria, with a sample deposited in the University herbarium with the voucher number MIA 2004. They were then washed with running tap water to remove the impurities, dried in carbolite moisture extraction drying oven (Grant instruments, Cambridge, England) at 45°C – 50°C for 3hours blender and kept in glass containers with plastic cover. Cold ethanolic extraction was carried out, where a known weight of the blended sample was soaked in ethanol for 24hours and then the extract was filtered and evaporated to dryness at room temperature to obtain the crude extract. The given quantities were diluted in distilled water and administered by oral gavage.

### *Procedures*

Twenty five female rats weighing between 170g-200g were used in this study. The rats were divided into five groups labeled A, B, C, D and E with each group consisting of 5 rats. Group A was the control group, Group B, C, D and E were the experimental groups. The female rats were caged overnight with sexually matured male rats. The following morning, vaginal smear was done to check the presence of sperm in the female tract. The presence of tailed structures (spermatozoa) signified day zero of pregnancy. Group B and C were given 150mg/kg and 250mg/kg body weight doses of ethanolic leaf extract of *Rauwolfia vomitoria* and those in groups D and E received 150mg/kg and 250mg/kg body weight doses of ethanolic root bark extract of *Rauwolfia vomitoria* on the day 7 through day 11 (5 days) of gestation respectively with the aid of an orogastric tube. The control, group A animals received corresponding volumes of distilled water on the corresponding days of gestation. The pregnancy was terminated on the 20<sup>th</sup> day of gestation and the fetuses were collected by uterotomy. The fetuses were blotted dry and examined for gross malformations. Fetuses were weighed on Libror EB-330H sensitive balance. The hearts were dissected out and fixed in 10% buffered formalin for 48 hours for routine histology using Haematoxylin and Eosin staining method.

## Results

There was no mortality in this study.



**Fig. 1** Photomicrographs of the heart of the control and treated groups, whose mothers received 150mg/kg and 250mg/kg leaf

and root bark extracts of *Rauwolfia Vomitoria* (H & E x400 for all plates). **A:** The control group (A) whose mothers received distilled water; **B:** Group B whose mothers received 150mg/kg of ethanolic leaf extract of *Rauwolfia Vomitoria*; **C:** Group C whose mothers received 250mg/kg of the leaf extracts of *Rauwolfia*. **D:** Group D whose mothers received 150mg/kg of ethanolic root bark extract of *Rauwolfia Vomitoria*. **E:** Group E whose mothers received 250mg/kg of the root bark extract of *Rauwolfia Vomitoria*.

#### *Morphological study*

There were no obvious gross malformations in the fetuses.

#### *Histological changes*

Histological study of the fetal heart using haematoxylin and eosin staining method showed in the control group A, branched cardiac muscle fibers arranged longitudinally with the nuclei of the cardiac muscle cells lying centrally (Fig.1).

Hypertrophy of cardiac muscle fibers, proliferation and prominence of the cardiac nuclei were seen in group B and D which received 150mg/kg of ethanolic leaf and root bark extracts of *Rauwolfia Vomitoria* (Fig. 1).

The fetal heart of group C and E animals that received 250mg/kg of the leaf and root bark extracts showed prominent nuclei with distortion and necrosis of the myocardial fibers (Fig. 1).

## **Discussion**

Histologically, proliferation and prominence of cardiac nuclei, hypertrophy of myocardial fibers was seen in the group administered with 150mg/kg dose of the extract, while in addition to the prominent nuclei, there was necrosis of myocardial fibers in the group administered with 250mg/kg of the extract, the effect being more pronounced in the group that received the root bark extract when compared with the control.

The period between the 7<sup>th</sup> and 11<sup>th</sup> day of gestation marked the time of organogenesis which is the period when fetuses are more susceptible to teratogenic agents and this was the same period in which the ethanolic extracts of *Rauwolfia vomitoria* was administered to the animals used for this study. Therefore, *Rauwolfia vomitoria* may have interfered with the proliferation of embryonic cells which was very rapid at the time the drug was administered. This was first noted by Perry and Mentzer [12] who discovered that apart from medicinal uses of most plants or tree species, their doses can be teratogenic during the first trimester and it has been noted by Cyril [13] that drugs may pass through the placental barrier and damage the fetus because the pharmacological effects are enhanced, as the enzyme systems responsible for the degradation are undeveloped in the fetus.

The muscle tissue of the heart is composed of striated involuntary muscle cells that are connected to form the contractile pump to generate blood flow [10]. Thus in this

study, increased number of central nuclei is non-specific but it may indicate muscle fiber disease as reported in "muscle diseases": Ed's Pathology notes [14] and hypertrophy of cardiac muscle as seen in hypertensive patients is characterized not only by increased myocardial mass but also by proliferation of fibrous tissue [15]; in the heart muscle disease, there is deterioration of the function of the myocardium [16].

## Conclusion

It is concluded from the result of this study that administration of ethanolic leaf and root bark extracts of *Rauwolfia vomitoria* on the 7<sup>th</sup> through 11<sup>th</sup> day of gestation may be cardiotoxic on the fetal heart of the developing rats and the extract of the root bark has more teratogenic potentials than the leaf extract .

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