

TERATOGENIC EFFECT OF CRUDE ETHANOLIC ROOT BARK AND LEAF EXTRACTS OF *RAUWOLFIA VOMITORIA* (APOCYNACEAE) ON THE LIVER OF ALBINO WISTAR RAT FETUSES

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ABSTRACT

Objectives: *Rauwolfia vomitoria* is a medicinal plant which is used locally for the treatment of a variety of disorders including snake and insect bites and stings, insomnia and insanity. This study was to investigate the teratogenic effect of the ethanolic root bark extract of *R. vomitoria* on the fetal liver.

Material and methods: Twenty-five virgin female Wistar rats weighing between 180 – 200g were used. The animals were divided into 5 groups, labeled A, B, C, D and E, each consisting of 5 rats. Group A rats were the control, while Groups B, C, D and E were the experimental. The female rats at proestrous were mated with sexually matured male rats. The day after was designated as day zero of pregnancy. Groups B and C animals received oral doses of 150mg/kg and 250mg/kg body weight of the root bark extract while groups D and E animals received 150mg/kg and 250mg/kg body weight of the leaf extract respectively from 7th to 11th day of gestation. On the 20th day of gestation, the rats were sacrificed and the fetuses examined for gross anomalies. Each liver was routinely processed using Haematoxylin and Eosin method.

Results: Histological observations of the fetal liver showed marked distortion of normal liver architecture in the treated groups when compared with the control, the effect being more pronounced in the groups that received the root bark extract.

Conclusions: Our result suggests that ethanolic root bark and leaf extracts of *Rauwolfia vomitoria* maybe hepatotoxic to the developing rats liver.

Keywords: Root Bark Extract, Leaf Extract, Crude Ethanolic Extract, *Rauwolfia vomitoria*, fetal liver

“Histological observations of the fetal liver showed marked distortion of normal liver architecture in the treated groups that received the root bark extract”

INTRODUCTION

Drugs of herbal origin have served through the ages as the mainstay in the treatment of variety of diseases and preservation of human health in the sub-Saharan region of Africa. However, general acceptability to the rest of the world has been limited for lack of doses regiment and adequate toxicity test to evaluate their safety¹. *Rauwolfia vomitoria* is a medicinal plant which has been used for centuries in India and Africa for the treatment of a variety of disorders including snake bites, insect bites and stings, insomnia and insanity. The parts mostly used are the roots and leaves. The active principles in the *Rauwolfia vomitoria* are alkaloids, rauwolfine, reserpine, rescinnamine, serpentine, ajmaline, steroid-serposterol and saponin².

Reserpine one of its major alkaloid is widely distributed into the brain, liver, spleen, kidney and adipose tissues. It also crosses the placental barrier^{3,4,5,6}. The liver performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defense. It is largely composed of epithelial cells hepatocytes, which are bathed in blood derived from the hepatic portal veins and hepatic arteries. There is continuous chemical exchange between the cells and the blood. Hepatocytes are also associated with an extensive system of minute canals, which forms the biliary system into which products are secreted. The liver is important in the removal and breakdown of toxic or potentially toxic, materials from the blood. It regulates blood glucose and lipids, and plays a role in the storage of certain vitamins, iron, and acids⁷.

It is involved in a plethora of other biochemical reactions. Since the majority of these processes are exothermic, a substantial part of the thermal energy production of the body especially at rest, is provided by the liver. The liver is populated by phagocytic macrophages (Kupffer cells), which

form part of the mononuclear phagocytic system of the body, and are important in the removal of particulates from the blood stream. In fetal life the liver is an important site of haemopoiesis⁷.

Liver is a prime target for the toxic effect of some herbs. The safety and efficacy of the use of *Rauwolfia* root bark extract in pregnancy has not been established. In this research work, the effect of ethanolic extract of *Rauwolfia vomitoria* root bark extract investigated on the histology of the fetal liver by administering various doses of ethanolic root bark extract.

MATERIAL AND METHODS

Twenty five adult female Wistar rats were bred in the animal house of the Department of Human Anatomy, University of Calabar. They were fed with normal rat chow and water was provided *ad libitum* throughout the duration of the experiment. The rats were kept under standard room temperature of 25-27°C. The animals were divided into five groups designated A, B, C D and E, each consisting of five rats. The group A animals were the control and groups B, C, D and E were the experimental animals.

Preparation of the herb extract

The roots and leaves of *Rauwolfia vomitoria* tree were collected from Ekpene Obo, Esit Eket Local Government Area, Akwa Ibom State, Nigeria and was identified and authenticated by the botanist in the botanical garden of the University of Calabar, Nigeria. The roots and the leaves were washed with water to remove the impurities. The root bark and the leaves were defoliated, dried in carbolite moisture extraction drying oven (Grant Instruments, Cambridge, England) at 40°C- 50°C for 3 hours. The dried root bark and leaves were blended into powdered form using a Binatone kitchen blender and kept in glass containers with plastic cover. The extraction method involved cold

ethanolic extraction, where a known weight of the blended sample was soaked in ethanol for 24 hours and then the extracts were filtered and evaporated to dryness at room temperature to obtain the crude extract.

Experimental protocol

Twenty-five virgin female Wistar rats were caged with sexually matured male rats of the same strain overnight after ascertaining the estrous phase of the estrous cycle. The presence of tailed structures in the vaginal smear the following morning confirmed coitus and the sperm positive day was designated as day zero of pregnancy. Oral doses of 150mg/kg and 250mg/kg per body weight of ethanolic root bark and 150mg/kg and 250mg/kg per body weight of leaf extracts of *Rauwolfia vomitoria* were administered to pregnant rats in Groups B, C, D and E respectively on the 7th through 11th days of gestation 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 20th day of gestation by chloroform inhalation method and the fetuses were collected by uterectomy. The fetuses were blotted dry and examined for gross malformations. Fetuses were weighed on Libror EB-330H sensitive balance. The livers were dissected out and fixed in 10% buffered formalin for 48 hours for routine histology using Haematoxylin and Eosin staining method.

RESULTS

Histological study of the liver using haematoxylin and eosin staining method showed in the control group A, a preserved cytoarchitecture of the liver with hexagonal lobules consisting of central vein and radially arranged hepatocytes. The sinusoids lined with endothelial cells were also present (Plates 1a, 2a)

Constriction of the central vein containing

lyzed blood cells, dilatation of the sinusoids, hypoplasia and vacuolation was seen in groups that received 150mg/kg and 250mg/kg of the root bark and 150mg/kg and 250mg/kg of leaf extracts (Plates 1b, 1c and Plates 2a, 2c).

PHOTOMICROGRAPHS

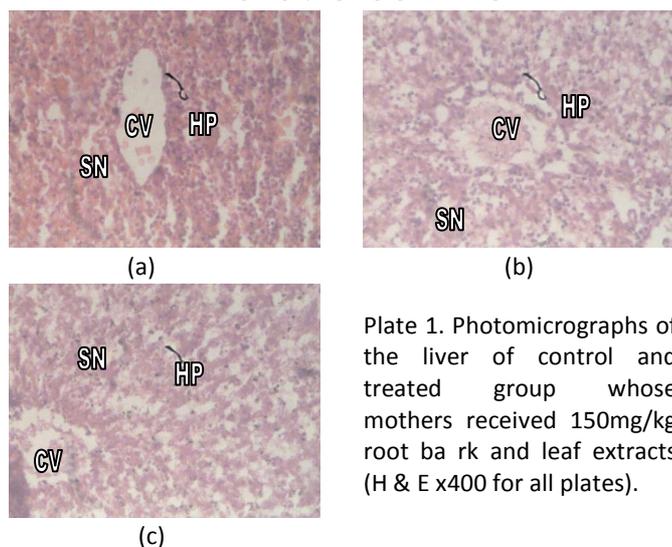


Plate 1. Photomicrographs of the liver of control and treated group whose mothers received 150mg/kg root bark and leaf extracts (H & E x400 for all plates).

- Control Liver showing the central vein (CV), well arranged hepatocytes (HP) and sinusoids (SN).
- Fetal Liver - 150mg/kg root bark extract showing constriction of the central vein containing lyzed blood cells, vacuolation (V), hypoplasia, distortion of the hepatocytes and dilatation of sinusoids.
- Fetal Liver – 150mg/kg leaf extract showing constriction of the central vein containing lyzed blood cells, vacuolation, dilatation of sinusoids and hypoplasia, distortion of the hepatocytes.

DISCUSSION

Histological results showed that the extract caused histological alterations of the liver structures such as constriction of the central vein, hypoplasia and hyperplasia of the cells, vacuolations, reduction in the size of the sinusoids, which were more pronounced in groups C and E. These may result in cellular degeneration, liver damage and impairment of hematopoietic functions of the liver. The toxicity of the extract

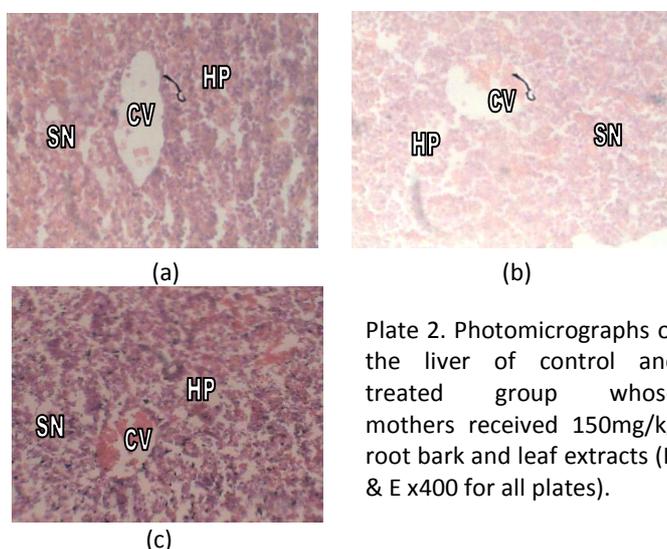


Plate 2. Photomicrographs of the liver of control and treated group whose mothers received 150mg/kg root bark and leaf extracts (H & E x400 for all plates).

- Control Liver showing the central vein (CV), well arranged hepatocytes (HP) and sinusoids (SN).
- Fetal Liver - 250mg/kg root bark extract showing constriction of the central vein, hyperplasia and distortion of the hepatocytes.
- Fetal Liver - 250mg/kg leaf extract showing severe distortion of the liver cytoarchitecture and constriction of the central vein.

may have caused the changes in sinusoids and other features of the liver.

The liver is a prime target for the toxic effect of some herbs. People with normal functioning livers with no history of prior liver disease have suffered adverse consequences to the liver as a result of taking certain herbs. The histological result revealed vacuolations, constrictions of the central veins, which contained lysed red blood cells in the groups that were treated with 150mg/kg of root bark and leaf extracts on 7th through 11th days of gestation. Cyto-architectural distortions of the hepatocytes, and hyperplasia were also evident in the treated sections. The effects being more severe in the groups that received 250mg/kg of the root bark extract and 250mg/kg of the leaf extract.

The liver is a very important organ in the

body which serves in the detoxification of metabolic waste products, various drugs and toxins. It also destroys spent red cells and reclaims their constituents⁸. Absorbed substances in the portal vein enter the liver sinusoids. If these substances are toxic, they may cause changes in the sinusoids and other parts of the liver⁹.

Distortion of the cytoarchitecture of the liver could be associated with the toxic effect of the alkaloid reserpine in the root bark, and saponin in the leaf extracts and this may result in functional changes that may be detrimental to the health of the rats. The proliferating cells of the liver, which produce red blood cells, are normally found between the hepatocytes and the walls of the vessels¹⁰. As a result of the distortion and dilatation the hepatocytes and their central vein, the hematopoietic function of the liver may have been affected as a probable toxic effect of *Rauwolfia vomitoria* root bark and leaf extract.

Eweka and Om'Iniabo¹¹ reported dilatations of the central veins, which contained lysed red blood cells and cyto-architectural distortions of the hepatocytes, centrilobular hemorrhagic necrosis, atrophic and degenerative changes in the liver treated with monosodium glutamate. Sarah et al.¹², and Jeong and Nah¹³ reported that steroidal saponins which share structural features with steroid hormones, possess numerous physiological activities, partly due to the nature of the steroid structure which can affect neonate development. Awang¹⁴ reported that when administered to pregnant rats; it causes histological changes in fetal liver. Histological examination revealed signs of hepatocyte degeneration.

In conclusion, this study suggests that the root bark and leaf extracts of *Rauwolfia vomitoria* had adverse effects on developing liver of fetal rats. These effects were more severe in the root bark than the leaf extract. Thus these two extracts may

than the leaf extract. Thus these two extracts may be hepatotoxic to the developing rats.

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