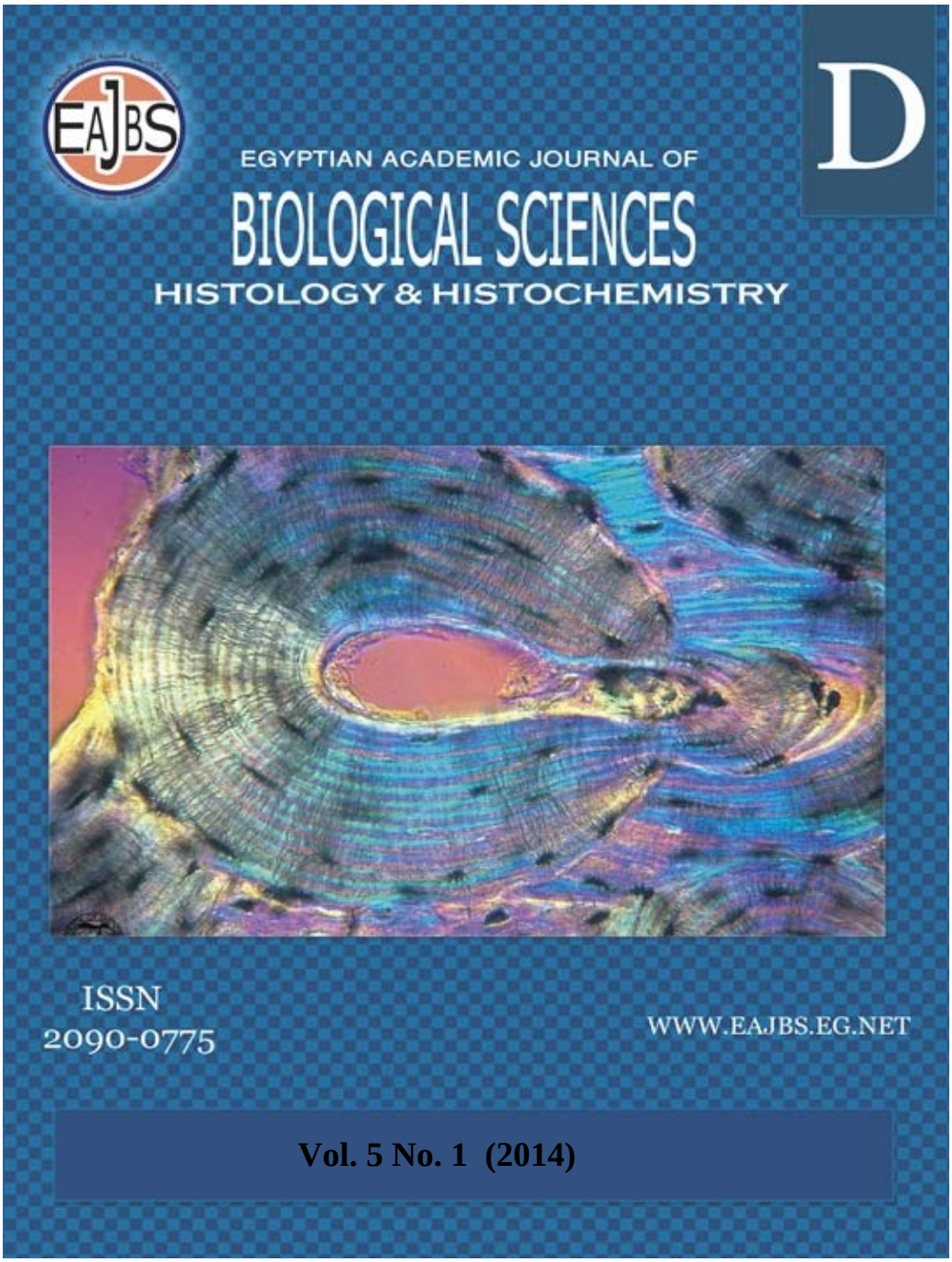


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# Effect of Crude Ethanolic Leaf Extract of Rauwolfia Vomitoria on the Fetal Lungs of Wistar Rats

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**ABSTRACT**

*Rauwolfia vomitoria* is a plant used in the treatment of hypertension, schizophrenia, and insomnia among others. This study investigated the effects of *Rauwolfia vomitoria* on the histology of the fetal lungs. Fifteen female Wistar rats weighing 180-200 g were divided into three groups (A, B, and C), consisting of five rats each. The animals mated overnight at estrous and the morning after coitus was designated day zero of pregnancy. Oral doses of 150 mg/kg and 250 mg/kg of ethanolic leaf extract of *Rauwolfia vomitoria* were administered to pregnant rats in groups B and C, respectively on day 7-11 of gestation, while group A served as the control group and were given distilled water. On day 20 of gestation, the rats were sacrificed and their fetuses examined and weighed. Fetal body weight, crown-rump and tail lengths were measured. Result showed significant ($P < 0.05$) higher anthropometric parameters, and the histological observations of the fetal lungs showed marked distortion of the normal lung cytoarchitecture in the treated groups compared to the control. These results suggest that doses of 150 mg/kg and 250 mg/kg body weight of the leaf extract of *Rauwolfia vomitoria* may cause intrauterine growth acceleration and may be pneumotoxic to the developing rat's lungs with the effect being dose dependent

## INTRODUCTION

Herbal medicine appears primitive and unscientific when compared to synthetic (conventional) drugs, which are thought to be more reliable. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care (Kamboj, 2000a; Kamboj, 2000b). This is primarily because of the general belief that herbal drugs are without any side effects, besides being cheap and locally available.

The use of plants for healing purposes predates human history and forms the origin of modern medicine. Many synthetic drugs originate from plant sources: a century ago, most of the few effective drugs were plant-based. Examples include: Aspirin, which is an analgesic chemical in the bark of willow trees; digoxin, from fox glove; guanine, from the bark of various cinchona tree species which was used in the treatment of malaria; and morphine, from the opium poppy (Kamboj, 2000b; Vicker and Zollman, 1999).

*Rauwolfia vomitoria* is a medicinal plant which grows in the humid tropical secondary forests of Africa and used traditionally to treat a variety of ailments (Sofowora, 1993). Extensive studies carried out on its chemical properties showed that the plant contained more than 50 active indole alkaloids, each possessing remarkable pharmacological activities (Pousset and Poisson, 1965; Iwu and Court, 1982). Some of its alkaloids include ajmalicine, alstonine, aricine, picricine, raugalline, residine, rescinnamine, reserpoxidine, stigmasterol, tetrahydroalstonine, and yohimbine (Lewis and Elvin-Lewis, 2003). The leaves also contain 40% heteroyohimbine, 52% oxindoles, rauvoxine, and rauvoxinine (Schmelzer and Gurio-fakim, 2009).

A bio-active β-carboline alkaloid and alstonine present in the root and leaf were previously shown to have anti-cancer activity (Pettit *et al*., 1994; Demis *et al*., 2006), while the antipyretic effect of the leaf extract has also been demonstrated (Amole, 1999). The pharmaceutical derivatives are used mainly as antihypertensive and sedatives. Folk medicinal uses of the roots are extensive particularly for their aphrodisiac, emetic, purgative, dysenteric, abortive, and insecticidal properties (Principe, 1989). Decoctions of the leaves of *R. vomitoria* have a powerful emetic effect, where chopped leaves stewed with animal fat have been applied to swellings (Burkill, 1994). The use of traditional remedies is very common in Nigeria, and pregnant women are the most vulnerable, as most of them frequent herbal homes. This research therefore examines the probable effects of the ethanolic leaf extract of *R. vomitoria* on the histological features of the fetal lungs.

## MATERIALS AND METHODS

Fifteen 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 25-27ºC. The animals were divided into three groups designated A, B and C, each consisting of five rats. The group A animals were the control, and groups B and C were the experimental animals.

### Preparation of the Herb Extract

Ethanolic leaf extract of *R. vomitoria* was prepared by obtaining fresh leaves of the plants from the University of Calabar botanical garden. The leaves were identified by the botanist in the botanical garden of University of Calabar, Nigeria. They were then washed with running tap water to remove the impurities after which they were dried in an oven at 45$^{o}$C–50$^{o}$C for 3 hours. The dried leaves were ground to powder using an electric blender. This was then soaked in ethanol for 24 hours. The extract was then filtered with a sieve and allowed to evaporate to dryness at room temperature leaving a crude extract.

### Experimental Protocol

The female 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 body weight doses of ethanolic leaf extract of *R. vomitoria* were administered to pregnant rats in groups B and C, respectively on the $7^{th}$ through $11^{th}$ 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 $20^{th}$ 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, crown rump length, as well as tail length was also measured. The lungs were dissected out and were fixed in 5% formaldehyde for Haematoxylin and Eosin staining (Drury and Wallington, 1978).

Statistical analysis using analysis of variance was carried out, with post-hoc student *t*-test. Experimental data were presented as mean ± standard error of mean (SEM). Values of ($P < 0.05$) were taken to be statistically significant.

## RESULTS

### Morphological Changes

The results showed that the fetal weight (2.77 ± 0.75), crown rump length (40.71 ± 2.69), and tail length (13.57 ± 2.50) of fetuses of rats given 150mg/kg of *R. vomitoria* ethanolic leaf extract were significantly ($P < 0.05$) greater than the fetal weight (2.53 ± 0.54), crown rump length (35.57 ± 1.13), and tail length (12.43 ± 1.39) of the control group. The fetal weight (3.22 ± 0.51), crown rump length (42.43 ± 3.73), and tail length (13.28 ± 2.99) of fetuses of rats given 250mg/kg per body weight of the ethanolic leaf extract of *R. vomitoria* were significant ($P < 0.05$) higher compared with those of the control group. However, no difference was observed between the fetuses in the group given 150mg/kg and 250mg/kg body weight of *R. vomitoria* (Table I).

Table I: Effect of ethanolic leaf extract of *R. vomitoria* on fetal body weight, tail length, and crown rump length.

| Parameters | Group A (Control) | Group B (150 mg/kg of *R. vomitoria*) | Group C (250 mg/kg of *R. vomitoria*) |
|---|---|---|---|
| Fetal Body Weight (g) | 2.53 ± 0.54 | 2.77 ± 0.75 | 3.22 ± 0.51 |
| Tail Length (mm) | 12.43 ± 1.39* | 13.57 ± 2.50* | 13.28 ± 2.99* |
| Crown Rump Length (mm) | 35.57 ± 1.13* | 40.71 ± 2.69* | 42.43 ± 3.73* |

Results are presented as Mean ± standard error of mean $n = 5$
*Significantly higher than the control group at $P < 0.05$

### Histological Observations

Histological study of the fetal lungs using the Haematoxylin and eosin (H & E) staining method in the control group showed the lung parenchyma characterized by numerous alveoli, alveolar duct, respiratory bronchiole and well defined inter-alveolar septum (Fig. 1).

Fetal lungs of group B whose mothers received 150mg/kg of the leaf extract of *R. vomitoria* showed enlarged respiratory bronchus, alveolar duct and alveoli, thin inter-alveolar septum, and detachment of epithelial lining and smooth muscle (Fig. 2).

The lung section of group C, whose mothers were treated with 250mg/kg body weight of the ethanolic leaf extract of *R. vomitoria* showed proliferation of the cells of inter-alveolar septum, constricted bronchiole, alveoli stained with eosinophilic materials, thickened walls of the pulmonary artery, and hyperplasia of the type II alveolar cells (Fig. 3).

## DISCUSSION

Body weight (Ordy *et al*., 1963; Davies, 1968), crown rump length (Goldman and Yakovac), tail length and trans-umbilical distance (Campbell, 1978; Singh and Padmanabhan, 1978) have been commonly used as indices of fetal growth and growth retardation in experimental animals. In this study, body weight, crown rump, and tail lengths were used to assess growth. Doses of 150mg/kg and 250mg/kg per body weight of the animals of ethanolic leaf extract of *R. vomitoria* administered on $7^{th}$ through $11^{th}$ days of gestation induced statistically significant ($P < 0.05$) intrauterine growth as evidenced in the increase in fetal weight, crown rump, and tail lengths when compared with the control.

A study reported by Sherman *et al.* (1958) showed that cattles fed on *R. vomitoria* feed efficiently and had improved growth rate. In another study conducted by Amole and Izegbu (2007) where the cumulative effects of the aqueous leaf extract of *R. vomitoria* on body tissues was studied, it was seen that body weight of the treated animals rose progressively as the period increased. It has also been noted that the leaves were generally rich in vitamins, and vitamins C and E are good antioxidants capable of preventing the buildup of free radicals with subsequent reduction in tissue damage (Abdel-Basset *et al.,* 1997).

Histologically, abnormally large bronchus, degeneration of alveolar cells, proliferation of cells of the interalveolar septal tissue, distortion of cells lining the epithelium of the bronchioles, and thickened walls of the pulmonary artery may lead to lungs hyperplasia and acute interstitial pneumonitis. These effects may be due to the various alkaloids present in the leaves. *R. vomitoria* have been reported to cause marked distortions of fetal liver architecture, cardiac muscle nuclei and myocardial fibers (Eluwa *et al*., 2010; Eluwa *et al.,* 2013a), Nissl substance disruption in fetal cerebral cortex (Eluwa *et al.,* 2013b), and alteration to adult cerebellum (Eluwa et al., 2009; Ekong *et al.,* 2014).

The thickening of the interalveolar septum may affect the type II pneumocytes that produce surfactant, thus leading to respiratory distress syndrome. It is reported that the deficiency of surfactant is the leading cause of mortality among premature infants (Luiz and Jose, 2003). There was degeneration of the lung tissues and distortion of the interalveolar septum especially in the group whose mothers received 200mg/kg body weight of the leaf extract. These may result in respiratory insufficiency as reported by Luiz and Jose (2013) that enlargement of the air space distal to the bronchioles with destruction of the interalveolar walls may result in respiratory insufficiency.

The histological effect observed in the present study is in consonance with a previous report (Effraim *et al*., 2003). They observed that *Ocimum gratissimum* induced alveolar damage, infiltration by inflammatory cells, interstitial pneumonitis, and congestion of blood vessels. Inflammation of the lungs result when there is injury to the tissue, which may be caused by bacteria, trauma, chemicals, heat, or any other phenomenon (Effraim *et al.,* 2003). This study is also in line with the report of Pua *et al.* (2007) on the histochemical analysis of altered fetal lungs development following single versus multiple course of antenatal steroids resulting in thinner alveolar walls, increased cell proliferation especially in the distal parenchyma.

In conclusion, the administration of ethanolic extract of *R. vomitoria* on the $7^{th}$ through the $11^{th}$ days of gestation causes intrauterine growth, and maybe pneumotoxic to the developing fetal lungs of Wistar rats, with the effect being dose dependent.

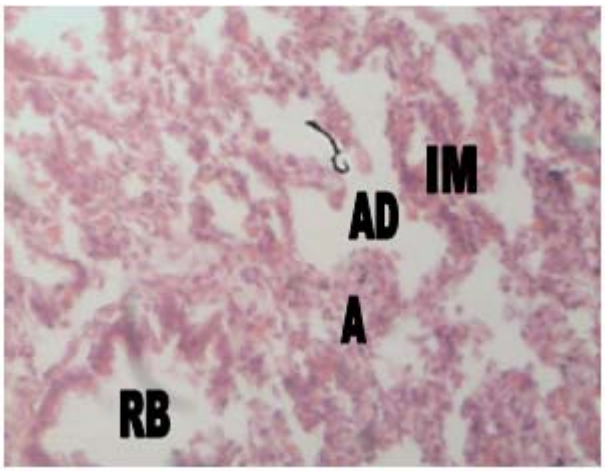


Fig. 1: Control: The lung parenchyma characterized by numerous alveoli (A), alveolar duct (AD), respiratory bronchiole (RB) and well defined interalveolar septum (IM). X400. H & E.

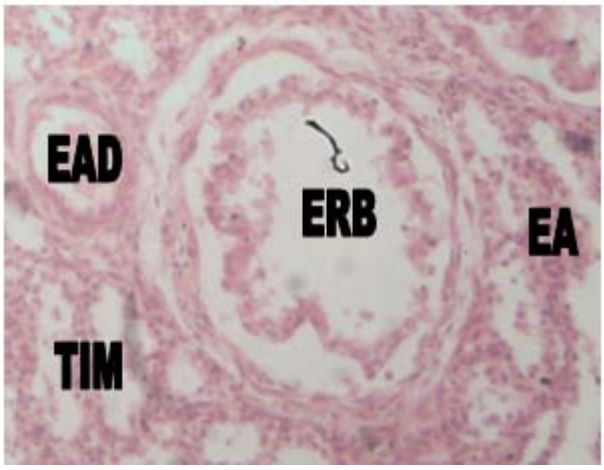


Fig. 2: 150mg/kg: Fetal lungs of Group B that the mothers received 150mg/kg of the leaf extract show enlarged respiratory bronchus (ERB), alveolar duct (EAD) and alveoli (EA), thin interalveolar septum (TIM), and detachment of epithelial lining and smooth muscle. X400. H & E.

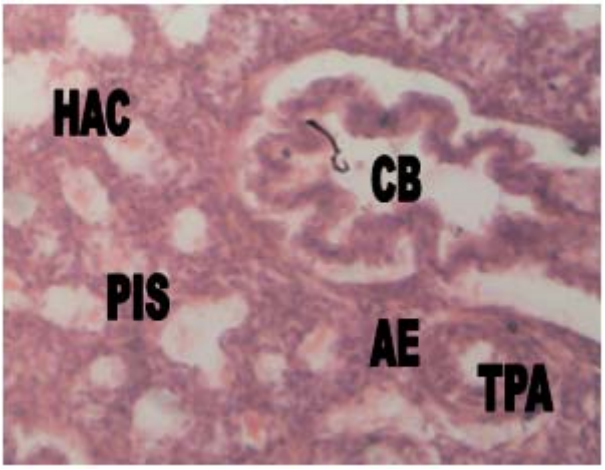


Fig. 3: Section of group C lungs, whose mothers were treated with 250mg/kg body weight of the ethanolic leaf extract of *R. vomitoria* showed proliferation of the cells of interalveolar septum (PIS), constricted bronchiole (CB), alveoli stained with eosinophilic materials (AE), thickened walls of the pulmonary artery (TPA), and hyperplasia of the type II alveolar cells (HAC). X400. H & E.

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