



Research Paper

Histopathological Study of Ethanolic Extracts of *Rauwolfia Vomitoria* Leaves on the Lung and Liver.

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ABSTRACT: The histopathological effects of graded doses of ethanolic extracts of *Rauwolfia vomitoria* leaf(RVL), a plant used in the treatment of hypertension, schizophrenia and among other diseases was studied on the liver and lungs of wistar albino rats. Twenty (20) wistar rats of body weights between 160-180Kg were used, designated into group A(control), treatments groups B,C,D respectively administered orally 16mg,25mg, and 33mg per body weight extracts of RVL for a duration of 14 days respectively. Histopathological findings after H&E treatment of the organs indicated that the treatment groups B,C of the liver showed normal architecture as in the control group, whereas group D showed congestion of the hepatic vessels. In the lungs, group B showed mild inflammatory cells and coalition of alveoli spaces, C and D showed extensive inflammatory cells, congestion of the entire alveoli vessels. Caution should therefore be exercised in therapeutic use of RVL because there are still some equivocal evidences surrounding the totality of the benefits.

Keywords: *Rauwolfia Vomitoria*, Histopathology, Lungs, Liver.

I. INTRODUCTION

Herbal medicine is considered the oldest form of health care known to humanity and has been used in all cultures throughout history. The primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active. Even in cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias. Traditional medicines have evolved over centuries depending on local flora, cultures, and religion. (Mokutima A. Eluwa et al. 2013). Despite advance in and availability of orthodox medicine for the treatment of mental and other disorders in Africa, Japan and United State of America, herbal medicine is still relevant (Sunday Bisong et al. 2011).

RVL is used traditionally for psychiatric management; its extracts have anti-inflammatory, antipyretic, anti-diuretic effects (due to the B-carbolin alkaloid, alstonine). RVL has been reported to be relatively safe with LD50 of 17.5g/kg, by this wide therapeutic index has become favorites of the traditional medicine users. Besides reserpine, one of the alkaloid of the RVL species has been used for the management of hypertension, schizophrenia, and psychiatric disorders and even reported useful in cases like Huntington diseases (Osim et al. 2011).

RVL is mostly found in the rain forest of southern Nigeria. The plant is a small tree of 8m to 11m in length, grows in whorls of tree and are elliptic and pointed at the end, 5-12cm wide. Flowers are tiny, sweet scented, pale greenish-white, and some white hairy inside.

The generic name *Rauwolfia vomitoria*, is named after a 16th century German physician, Leohart Rauwolf, who travelled the world over, collecting and documenting medicinal plants (Mirko Beljanski 2014). The English name Swzzler stick, African snakeroot. RVL is a source of medicinal alkaloid especially and is grown commercial for this purpose worldwide. In Youuba *Rauwolfia Vomitoria* is known as asofeyeje, iri in Hausa, akanta in Igbo and mmoneba and utoeyin in Efik languages. (Mokutima Eluwa et al. 2013).

There is scarcity of information on histopathological effect of RVL which is an indication that it has not been worked on. Since it is widely consumed by people, it is therefore important to carry out a study with a view to finding out its safety to consumer; this forms the justification for this study using Albino Wistar rats.

II. MATERIALS AND METHODS

Twenty Wistar rats were used in the study. They were purchased from the animal house of the Department of Zoology, University of Calabar weighing between 167-180kg, were acclimatized for a period of two weeks at which they were feed with pelleted rat feeds with water ad libitum. The Abia state University Ethical Guideline on use of animals in Research was followed in this study.

RVL were collected from the sub-tropic bush in Calabar, Cross River State, Nigeria. The leaves were identified and authenticated by the botanist in-charge of the Botanical Garden of the University of Calabar, Nigeria.

Preparation Of Herb Extract

The leaves were washed with water to remove impurities, chopped into smaller size to enhance the drying process and were dried under shade using Laboratory mill for two weeks at room temperature. The dried RVL were then blended into powder

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form using a binatone kitchen blender and kept in a glass container with plastic cover. 50g of the ground sample was macerated in 250ml of ethanol and placed on a mechanical shaker for 24hours. After, the mixture was sieved with a sieve into a glass baker. The filtrate was decanted and evaporated to dryness using rotary evaporator. The extract was collected in powder form. About 1g of the extract was dissolved in 50ml of distilled water to form a concentration of the solution. The animals were weighed using a weighing balance and the required dose for each animal was calculated to make for oral administration.

Toxicity Level Studies

Toxicology of medicinal plants and their products formed important part of the early and late phase of drug development. (Gamaniel 2000). Their importance lies in the safety evaluation and risk assessment. Researchers have shown that RVL is safe up to 0.8ml/kg body weight administered for 28days (Ezejindu et al 20013).

Experimental Protocol And Administration

On commencement of the experiment the animals were randomly selected and distributed into four (4) groups of five (5) rats each as followed:

Control Group A. Received normal rat pellet feed with water *ad libitum*. feed and tap water all throughout the experimental period.

Treatment Group B. Received 16ml/kg body weight of the ethanolic extract of RVL with normal diet feed and tap water.

Treatment Group C .Received 25ml/kg body weight of the ethanolic extract of RVL with normal diet feed and tap water.

Treatment Group D .Received 33ml/kg body weight of the ethanolic extract of RVL with normal diet feed and tap water.

The administration of the extract lasted for 14 days (2 weeks), the ethanolic extract was orally administered using a sterile syringe.

Preparation Of Tissues.

The lungs and liver were harvested and fixed in 10% formalin for 72hours .They were then thoroughly dehydrated by passing them through graded solution of alcohol (30%,50%,70% and 100%) after which they were cleared in two changes of xylene at an interval of one hour each and impregnated with three changes of paraffin wax for an hour each and finally embedded in fresh molten wax. Section of 5µm thickness of each organ were prepared and stained with Haematoxylin and Eosin (H&E) staining techniques for photomicrograph (Bryan 2001).

III. RESULTS

Histopathological findings of the lungs

Photomicrograph 1 Group A (control)

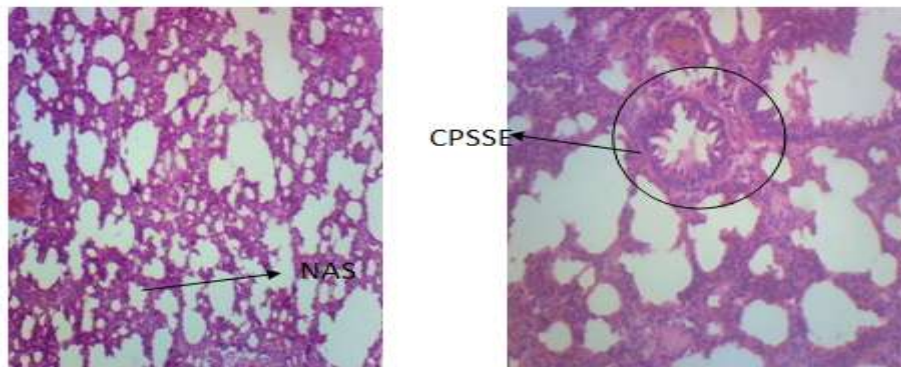


Fig. 1: Photomicrograph 1 Group A (control) section of the lungs. Photomicrograph of control section of the lungs (X150)(H/E) shows normal lung cytoarchitecture with normal aveoli spaces(NAS), tall columnar pseudo stratified ciliated epithelium (CPSSE)

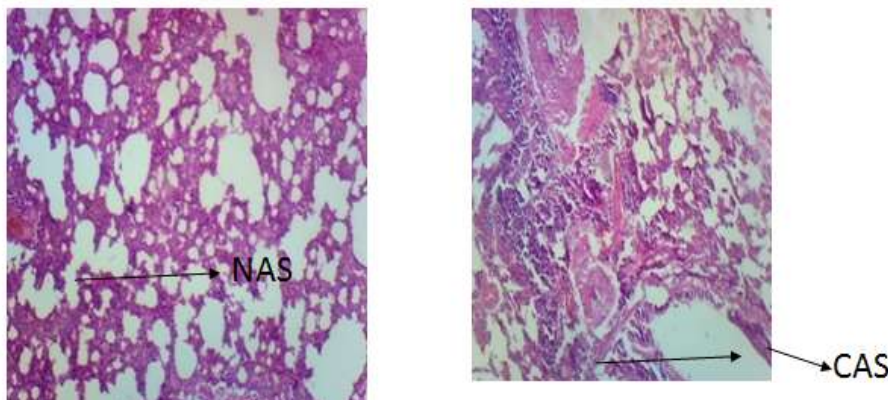
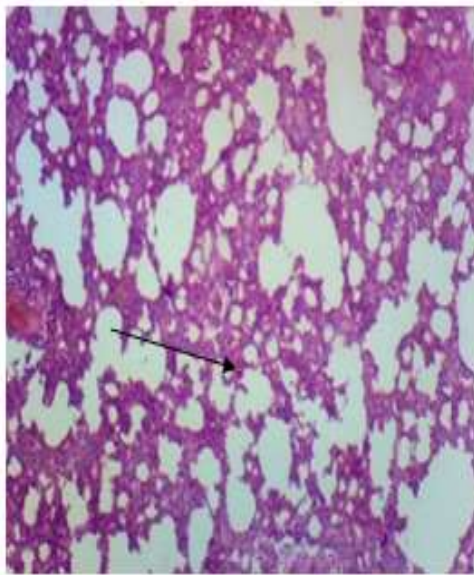


Fig.2: Photomicrograph 1 Group A (control) and treatment group B of the Lungs. Photomicrograph 1 group A shows normal lungs features, compared to photomicrograph 2 group B administered with 16ml/kg body weight of RVL for 2weeks (x150)(H/E) There are signs of mild aggregate of inflammatory cells(MAIC) ,coalition of alveoli spaces(CAS) and alveoli lymphocytes(AL).

Photomicrograph 1 Group A (control)
25ml of RVL extract)



Photomicrograph 3 Group C (Administered

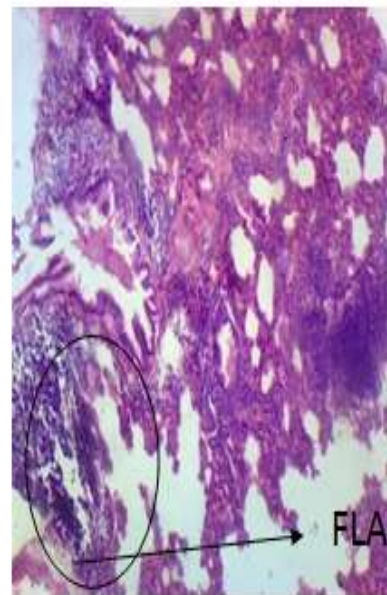


Fig.3: Photomicrograph 1 Group A and treatment group C of the lungs. Photomicrograph 1 group A shows normal cytoarchitecture of the lungs, compared to photomicrograph 3 group C administered with 25ml/kg body weight of RVL extract for 2 weeks. (x150)(H/E) shows extensive inflammatory cell aggregate (EICA) and focal lymphocyte aggregate (FLA).

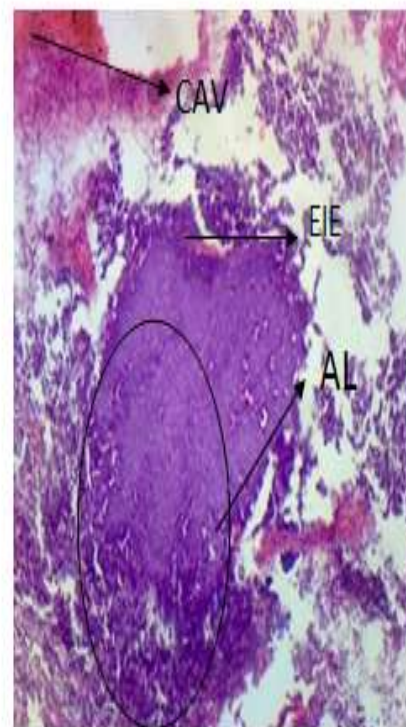
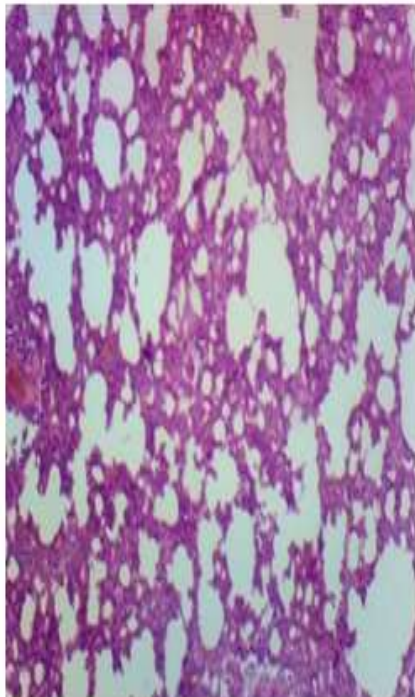


Fig.4: Photomicrograph 1 Group A and treatment group D of the lungs. Photomicrograph 1 control shows normal lung features, compared to photomicrograph 4 group D administered with 33ml/kg body weight of RVL extract for 2 weeks. (x150)(H/E) shows increased accumulation of lymphocyte (AL), congestion of alveoli vessel (CAV) with extensive inflammation exudates (EIE).

Histopathological findings of the liver treated with Rauwolfia Vomitoria.

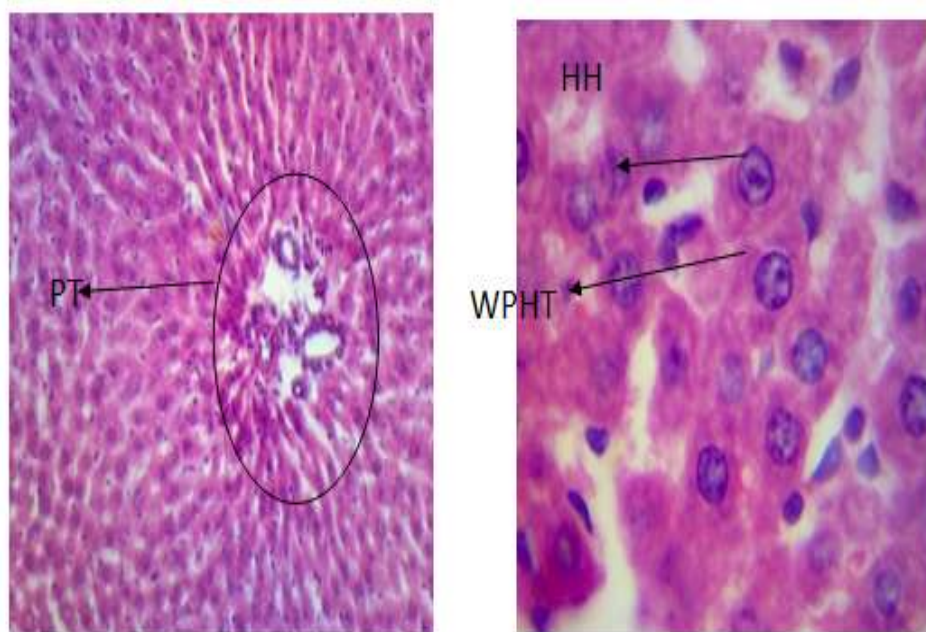
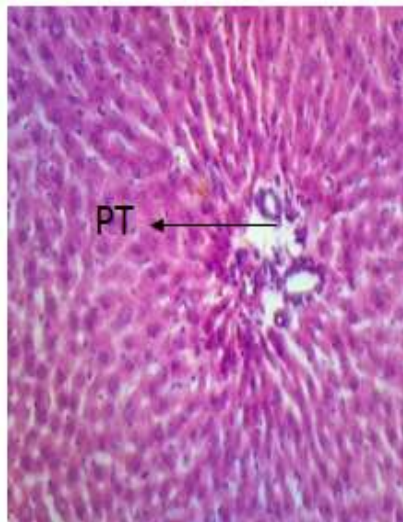


Fig.5: Photomicrograph 1 Group A (control) section of the liver. The photomicrograph shows normal hepatic cytoarchitecture composing of well perfused hepatic tissue (WPHT), healthy hepatocytes (HH) and centrally placed portal triad (PT). (H&E)(X150 and 600).

Photomicrograph 1 Group A (control)



Photomicrograph 2 Group B (Treated with 16ml of RVL extract)

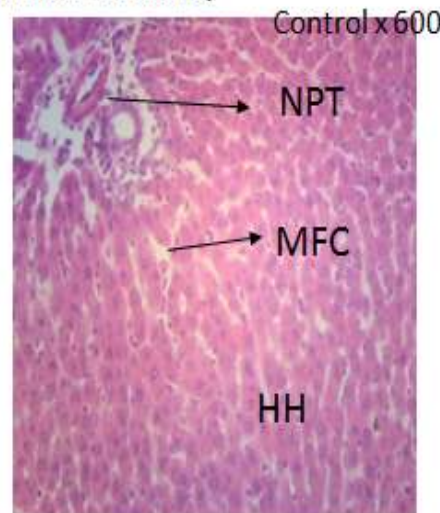
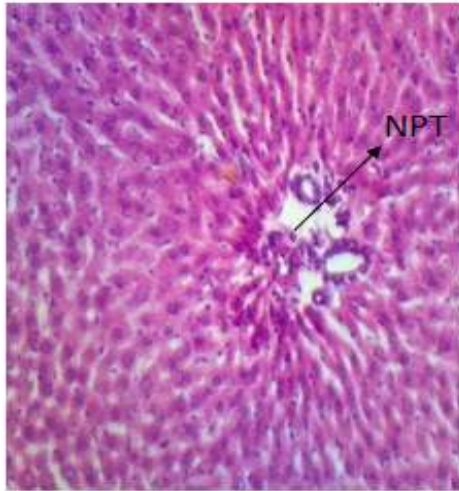


Fig.6: Photomicrograph 1 Group A and treatment group B of Liver section. Photomicrograph 1 Group A shows normal hepatic tissues, compared to photomicrograph 2 group B administered with 16ml/kg body weight of RVL extract for 2 weeks, shows normal hepatic architecture with well perfused hepatic (WPHT), healthy hepatocytes (HH) and normal portal triad (NPT). However there are areas of mild fatty change (MFC) and mild inflammation of hepatocyte (MIH). (H/E) (X150).

Photomicrograph 1 Group A (control)



Photomicrograph 3 Group C (Administered 25ml of RVL extract)

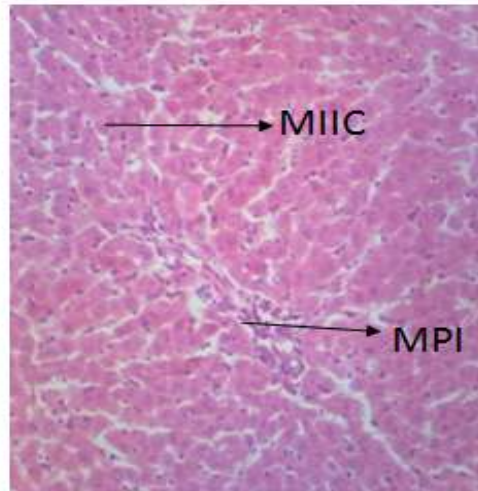


Fig.7: Photomicrograph 1 Group A (control) and treatment Group C of the liver section. Photomicrograph 1 group A (control) shows normal hepatic tissues compared to photomicrograph 3 group C administered with 25ml/kg body weight of RVL extract for 2 weeks, shows well perfused hepatic tissue (WPHT) and healthy hepatocytes (HH). However, there are areas of mild portal inflammation (MPI) and mild infiltrate of inflammatory cells (MIIC).

Photomicrograph 1 Group A (control)



Photomicrograph 4 Group D (Administered 33ml of RVL extract)

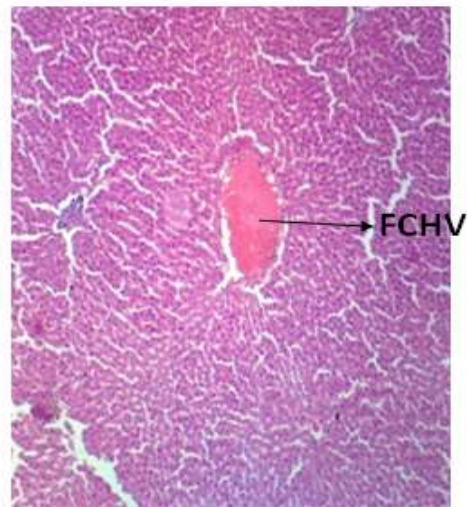
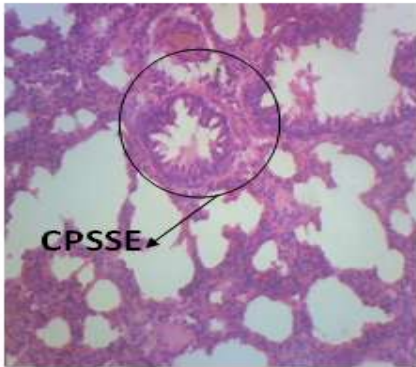


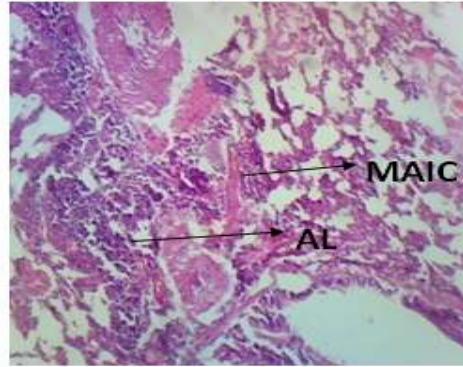
Fig.8 Photomicrograph 1 Group A (control) and treatment group D. The control group shows normal hepatic tissues, compared to group D administered with 33ml/kg body weight of RVL extract for 2 weeks, shows moderate infiltrate inflammatory

cells (MIIC), focal congestion of hepatic vessel (FCHV) and mild fatty changes (MFC).
Photomicrographs showing the effect of different doses of Rauwolfia vomitoria extract on the lungs.

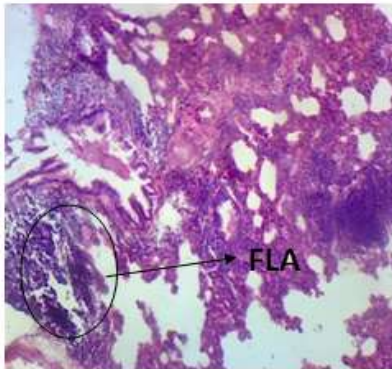
Photomicrograph 1 Group A (control)



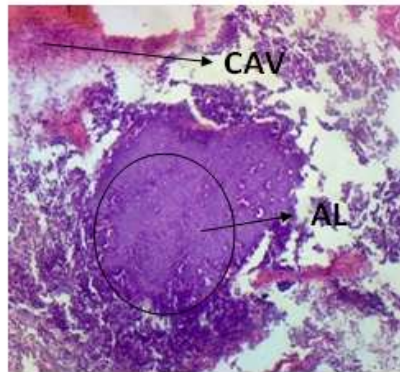
Photomicrograph 2 Group B (Administered with 16ml of RVL)



Photomicrograph 3 Group C (Administered with 25ml of RVL)



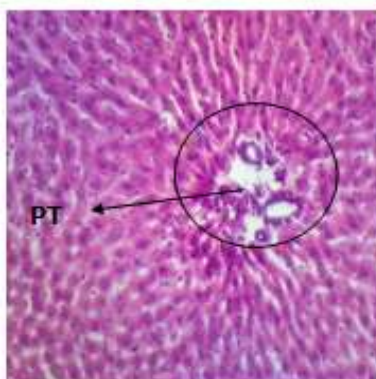
Photomicrograph 4 Group D (Administered with 33ml of RVL)



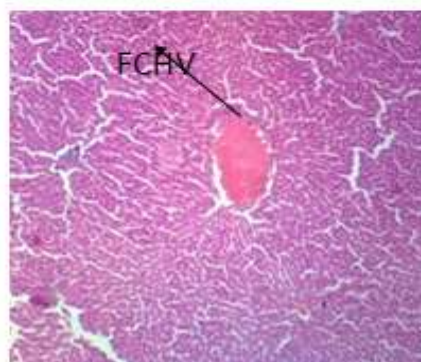
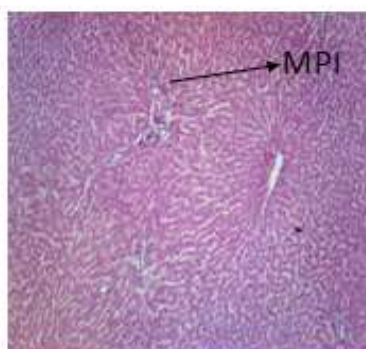
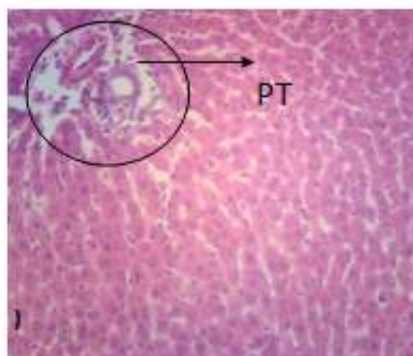
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Fig.10: Photomicrographs Showing The Effect Of Different Doses Of Rauwolfia Vomitoria Extract On The Liver.

Photomicrograph 1 Group A (control)



Photomicrograph 2 Group B (Administered with 16ml of RVL)



IV. DISCUSSION

From our experiment, we observed that there was no adverse effect or damage to the features in the liver, in group B and C administered 16ml and 25ml/kg body weight of RVL extract for two weeks when compared to the control. Abnormalities may possible not develop with RVL used for a short period. It is therefore, considered to be non-hazardous (Amole et al 2007). It was obvious that in the group D administered with 33ml/kg body weight of RVL for two weeks, there was moderate infiltrate inflammatory cells, focal congestion of hepatic vessel.

Histopathological observation of the lungs in both group B, C and D, showed marked distortions of the normal lungs cytoarchitecture when compared to the control. This implies that organs such as the lungs remain more vulnerable or susceptible to toxic effects of herbal products than others.

In all, our results have shown that the liver and lungs are affected by 16ml, 25ml and 33ml/kg body weight as the dose was increased probably beyond the therapeutic range (17.5+ 0.5). Organ such as the liver resist distortion or damage probably due to its microsomal or hydrolytic potentials. These findings indicate that the cytoarchetcural changes in tissues of animal fed with low doses of extracts of *Rauwolfia vomitoria* posed no serious consequence. Thus, RVL is safe for people as far the administration is within the therapeutic index.

RVL extract at lower doses had no adverse effect in the liver features but could impair liver function at high doses. In the lungs RVL extract showed mild distortions of cytoarchitecture and congestion of the alveoli vessels even at slightest increase in doses; therefore there should be proper cancelling on the use of this herbal medicine.

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