



Histological Changes in Selected Tissues of Male Wistar Rats after Oral Administration of Aqueous and Ethanolic Extracts of *Phyllanthus amarus*

*Muritala, H. F., Akolade, J. O., Haruna, M. A., Tijani, H. A. and Yakubu, M. T.

Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

Abstract: The effects of oral administration of aqueous and ethanolic extracts of *Phyllanthus amarus* on the histology of brain, heart, liver, kidney and testes of male Wistar rats were studied. Thirty five rats (212.23 ± 16.14 g) were randomly assigned into seven groups designated A, B, C, D, E, F and G and were orally administered distilled water (control), 100, 200 and 400 mg/kg body weight (bw) of aqueous extract and 100, 200 and 400 mg/kg bw of ethanolic extract of whole *P. amarus*, once a day, for 15 days. The animals were anesthetized and sacrificed after which sections (1 cm thick) of each tissue was cut and processed for histological analysis using haematoxylin and eosin stains. The photomicrographs were observed under light microscope at $\times 100$ magnification. The histological examination of the tissues revealed dose-dependent mild to moderate glomerular inflammation in the kidney, stressed cardiac tissues in the heart, reduced germ cells in the testes and cytoplasmic degeneration in the liver of rats administered 100, 200 and 400 mg/kg bw of aqueous extract of *P. amarus*. In contrast, there was no evidence of histoarchitectural changes in the rats that were administered the ethanolic extracts of *P. amarus* except the 400 mg/kg bw that produced polymorph infiltrations in the hepatocytes, inflammation in the cardiac cells and testicular histoarchitecture when compared with those of the control rats. The brain histoarchitecture were preserved after the administration of the solvent extracts of *P. amarus*. The study revealed that administration of 100, 200 and 400 mg/kg bw of aqueous extract and 400 mg/kg bw of the ethanolic extracts of *P. amarus* on daily basis for 15 days exhibited structural toxicity on the selected organs of male rats. These histoarchitectural changes may lead to compromise of the normal functioning of the organs of male rats.

KEYWORDS: *Phyllanthus amarus*, phyllanthaceae, structural toxicity, histoarchitecture, germ cells, degeneration, inflammation

1.0 Introduction

Phyllanthus amarus (Schum. and Thonn.) belongs to the family, phyllanthaceae. It is an ethnomedicinal herb that is widely distributed as weed in tropical and sub-tropical regions (Fernand, 1998). In Nigeria, it is called commonly known as carry-me seed (Adjene and Nwose, 2010) and "Iyin Olobe" in Yoruba of South-West (Etta, 2008). In folklore medicine, traditional medicine practitioners of Nigeria use the *P. amarus* as decoction to treat dysentery diarrhoea, jaundice, hepatitis, kidney stones, gall bladder infections, menstrual or uterine cramps (Srividya and Perival, 1995; Patel *et al.*, 2011).

The antiviral, antimicrobial, antioxidant, anti-inflammatory, anti-hypertensive, anti-toxic, anti-

diabetic, sexual function enhancing and immunomodulatory activities and of various extracts from *P. amarus* have been investigated (Srividya and Perival, 1995; Karuna *et al.*, 2009; Bankole *et al.*, 2011; Lawson-Evi *et al.*, 2011; Ravikumar *et al.*, 2011; Oyewo *et al.*, 2012; Syed *et al.*, 2012; Babatunde *et al.*, 2014; Adedapo and Ofuegbe, 2015). A review of the ethnomedicinal usage, phytochemistry and the pharmacological activities have also been reported by Patel *et al.* (2011). Furthermore, the effects of administration of extracts from *P. amarus* on the histology of the kidney of rats have equally been documented in the literature (Etta, 2008; Eweka and Enogieru, 2011). However, most of these studies were carried out on the aqueous extract, without evaluating its alcoholic extract, which is the widely used form by traditional medicine practitioners within

*Corresponding Author

Tel.: +2348064459564

E-mail: hamdalatfolake@yahoo.co.uk

many localities in Nigeria and in several ethnobotanical surveys. In addition, information on the effects of solvent extracts of the plant on the histology of other organs like the liver, brain, testes and heart of Wistar rats remains scanty.

Therefore, this study was designed to evaluate the effects of aqueous and ethanolic extracts of *Phyllanthus amarus*, on the histology of selected tissues of adult male rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Material and Authentication

The plant material was obtained within the premises of University of Ilorin, Ilorin, Nigeria. The authentication was carried out at the herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, where voucher specimen (UIH/1051) was deposited.

2.1.2 Reagents

Ethanol and all other reagents used were of analytical grade obtained from British Drug House (BDH) Poole, England.

2.1.3 Experimental Animals

Thirty five adult male rats (212.23 ± 16.14 g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

2.2 Methods

2.2.1 Preparation of Aqueous and Ethanolic Extract of *Phyllanthus amarus*

P. amarus were uprooted and washed with distilled water to remove soil debris. The plants were then air-dried and pulverized using an electric blender. Dried plant samples (200 g) were extracted (1:10 w/v) separately in distilled water and ethanol (2 L) with intermittent stirring for 48 hours. The aqueous extract of *P. amarus* (AEPA) was filtered and concentrated on a water bath to give a yield 42.22 g; 21 % w/w

whereas the ethanolic extract of *P. amarus* (EEPA) gave a residue of 52.32 g (26 % w/w). The extracts were appropriately labelled, stored in a clean, air-tight bottles and refrigerated at 4°C.

2.2.2 Animal Grouping and Administration of Extracts

The animals were acclimatized for 15 days and were given rat pellets and clean tap water. They were then completely randomized into seven groups (A - G). Rats in group A were orally administered distilled water to serve as the control while those in groups B, C and D were administered 100, 200 and 400 mg/kg bw of the *P. amarus* aqueous extract. In addition, rats in groups D, E and F were also administered 100, 200 and 400 mg/kg bw of ethanolic extract of *P. amarus*. The administration was done once a day, for 15 days.

2.7 Tissue Preparation

On day 16 post-administration, the animals were sacrificed under diethyl ether anaesthesia. The liver, kidney, brain, heart and testes were fixed in 10% formalin, dehydrated through ascending grades of ethanol (70%, 90%, and 95% v/v), cleaned in xylene, and embedded in paraffin wax. Tissue sections were prepared according to the method described by Drury and Wallington (1973) and stained with haematoxylin and eosin. The photomicrographs were captured at x100. The extract-treated photomicrographs were compared with those of the control for the detection of any histological changes.

3.0 Results

The photomicrographs of the sections from the kidney, liver, testes, brain and heart of male rats orally administered distilled water, 100, 200 and 400 mg/kg body weight of the aqueous and 100, 200 and 400 mg/kg body weight of the ethanolic extracts of *P. amarys*, once a day, for 15 days are shown on Figures 1-5.

The kidney of rats that received 100, 200 and 400mg/kg bw of aqueous extract of *P. amarus*

showed normal architecture except for the dose dependent mild to moderate glomeruli inflammation (Figures 1A-D), whereas rats that received similar doses of the ethanolic extracts showed well preserved kidney histoarchitecture with no evidence of cytotoxicity (Figures 1E-G).

Normal liver architecture was observed in the rats administered (Figure 2A) whereas cytoplasmic degeneration of the hepatocyte was observed in the liver of rats that received all the doses of the ethanolic extracts of *P. amarus* (Figures 2B-G). In addition, polymorph infiltration was evident in the rats that were administered the 400 mg/kg body weight of ethanolic extract of *P. amarus* (Figure 2G).

Figure 3A shows the normal testicular histoarchitecture of the seminiferous tubules of rats with distinct germ and non-germ cells (spermatogenic and non-spermatogenic series). Testes of rats treated with the both extracts showed partly inflamed architecture with dose-dependent reduction in the number of germ cells (Figure 3B-G). There was preservation of the neurons within the cerebral cortex of all the extract treated animals (Figure 4A - G). Stressed, inflamed and degenerative cardiac tissues characterized the histoarchitecture of both the aqueous and ethanolic *P. amarus* extract treated animals (Figures 5B-G) as against the normal cardiac histoarchitecture in the distilled water treated control (Figure 5A).

4.0 Discussion

Histological examination is widely employed to ascertain the selective cyto-effects of drugs and medicinal plants on tissues of pathological interest. Previous histopathological studies have shown that chronic or sub-chronic administration of botanical extracts to experimental animals altered the structural and cytoplasmic architecture of tissues involved in detoxification like the kidney and liver (Afolayan and Yakubu, 2009; Adjene and Nwose, 2010). Photomicrographs of tissue sections of rats treated with aqueous extracts of *P. amarus* have been previously reported to disrupt the cyto-architecture and micro-anatomy of the kidney (Etta, 2008; Eweka and Enogieru, 2011). Furthermore, interstitial oedema, tubular necrosis or nephrosis with protein casts, cell

infiltration and inflammation were features of the *P. amarus* aqueous extract treated rat kidney (Adedapo *et al.*, 2005; Adjene and Nwose, 2010; Eweka and Enogieru, 2011).

In this study, similar findings of distortions in cellular morphology were observed not only in the kidney but also the liver, testes and heart of rats administered aqueous *P. amarus* extracts, whereas those of the brain were well preserved. The cytoplasmic degeneration with polymorphic and lymphocytic infiltrations of the portal areas in the liver of rats treated with both the aqueous and ethanolic extracts of *P. amarus* were indications of structural toxicity by the solvent extracts of the plants. These histoarchitectural changes which were also extended to the kidney, testes and heart of the animals might adversely affect the normal functioning of the organs. This is in line with previous studies which reported that tissue or cell lines when exposed to alcoholic or hydro-alcoholic extracts of *P. amarus* exerted milder or no net cytotoxic effect and that these structural toxic effects were more pronounced with the aqueous extracts of the plant (Ajaiyeoba and Kingston, 2006; Lawson-Evi *et al.*, 2011).

Variations in chemical compositions of the extracts prepared in either aqueous or ethanolic broth may be responsible for the marked and selective histological alterations in the present study. Secondary metabolites such as alkaloids, lignans, saponins, oxalates, cardiac and cyanogenic glycosides, cardenolides, phenols, flavonoids and tannins have been identified and quantified in *P. amarus* extracts (Patel *et al.*, 2011). Several of these chemical compounds such as the alkaloids and cardiac glycosides have been implicated to have pro-oxidant and toxic tendencies (Galati and O'Brien, 2004; Eweka and Enogieru, 2011). Indiscriminate and prolonged ingestion of the multi-component crude extracts may cause imbalance in redox status, promote oxidative stress and enhanced the toxic effects of such extracts which might culminate in the distortion of normal cellular morphology and its arrangement as observed in the present study. Such changes may adversely affect the normal functioning of the organs.

In conclusion, the consumption of aqueous and ethanolic extracts of *P. amarus* has inflicted structural toxicities on the organs investigated.

Consequently, the compromise in the normal structure of these organs will have adverse effects on the normal synthetic, secretory, metabolic and detoxifying abilities of the organs.

Therefore, *P. amaraus* is toxic to the structures of the organs of rats.

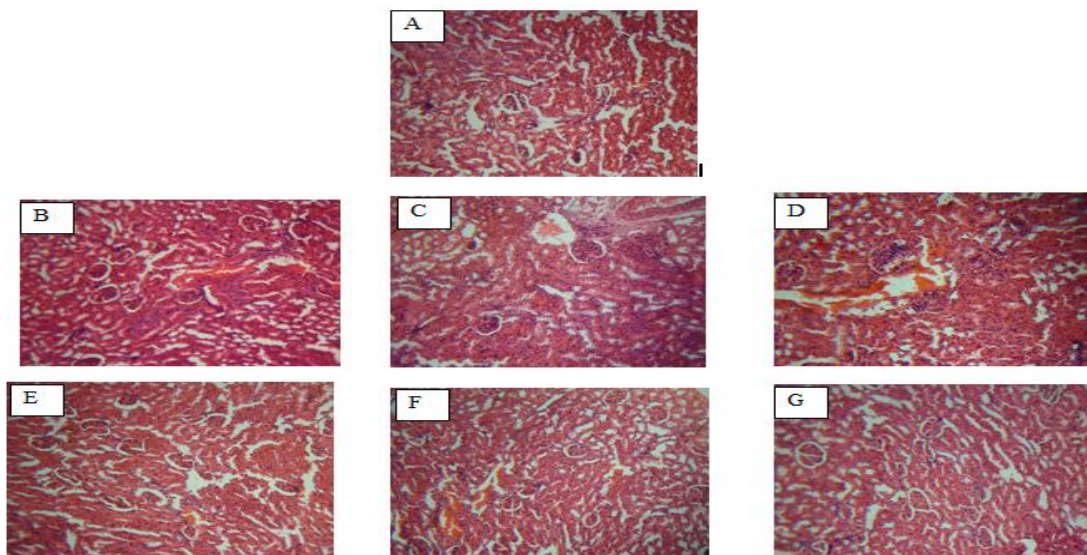


Figure 1: Cross section of the kidney of rats orally administered (A) distilled water; (B) 100 mg/kg body weight of AEPA; (C) 200 mg/kg body weight of AEPA; (D) 400 mg/kg body weight of AEPA; (E) 100 mg/kg body weight of EEPA; (F) 200 mg/kg body weight of EEPA; and (G) 400 mg/kg body weight of EEPA (H&E; x100).

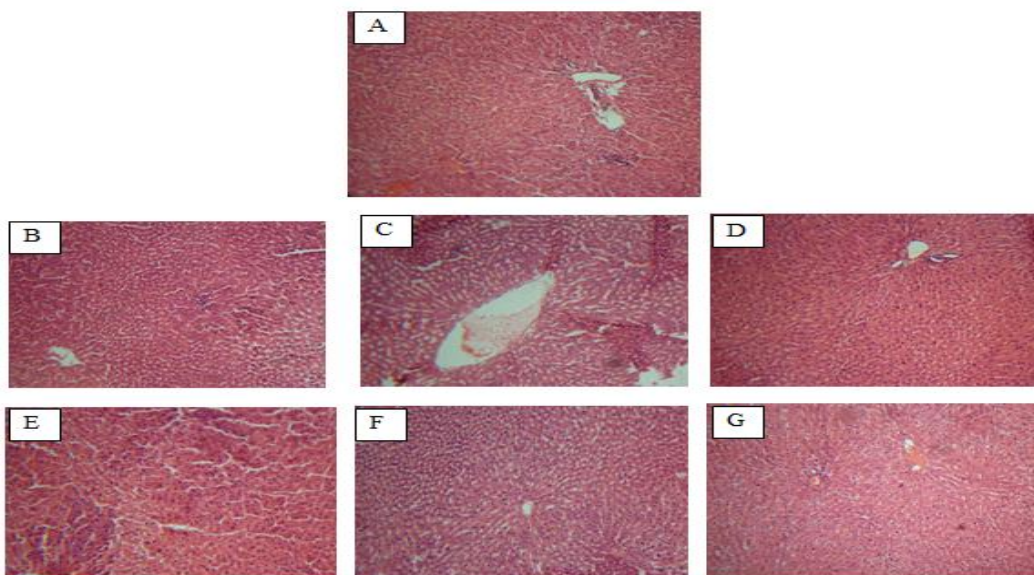


Figure 2: Cross section of the liver of rats orally administered (A) distilled water; (B) 100 mg/kg body weight of AEPA; (C) 200 mg/kg body weight of AEPA; (D) 400 mg/kg body weight of AEPA; (E) 100 mg/kg body weight of EEPA; (F) 200 mg/kg body weight of EEPA; and (G) 400 mg/kg body weight of EEPA (H&E; x100).

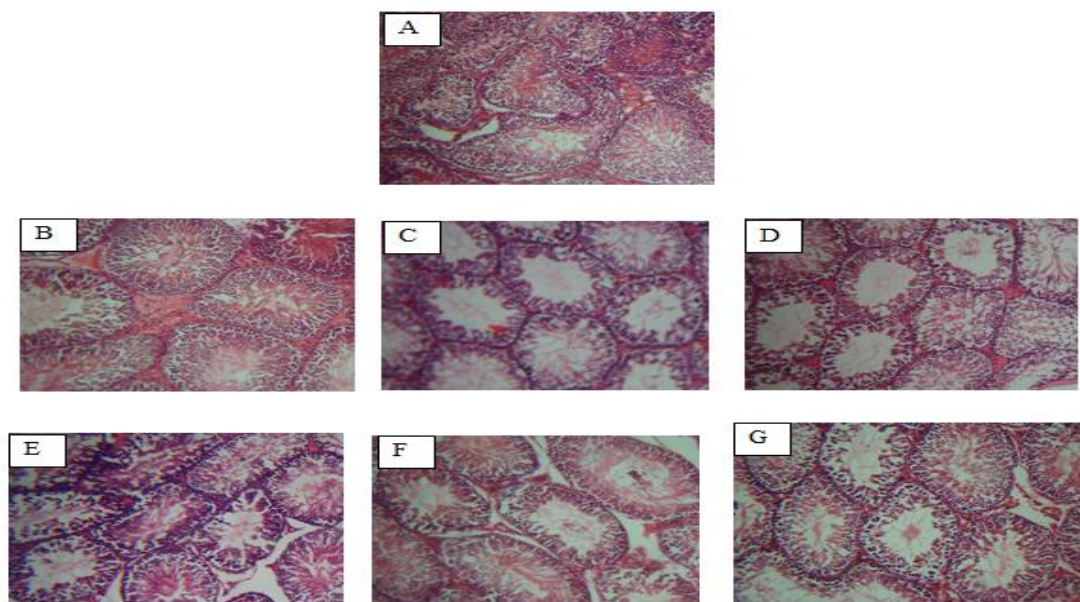


Figure 3: Cross section of the testes of rats orally administered (A) distilled water; (B) 100 mg/kg body weight of AEPA; (C) 200 mg/kg body weight of AEPA; (D) 400 mg/kg body weight of AEPA; (E) 100 mg/kg body weight of EEPA; (F) 200 mg/kg body weight of EEPA; and (G) 400 mg/kg body weight of EEPA (H&E; x100).

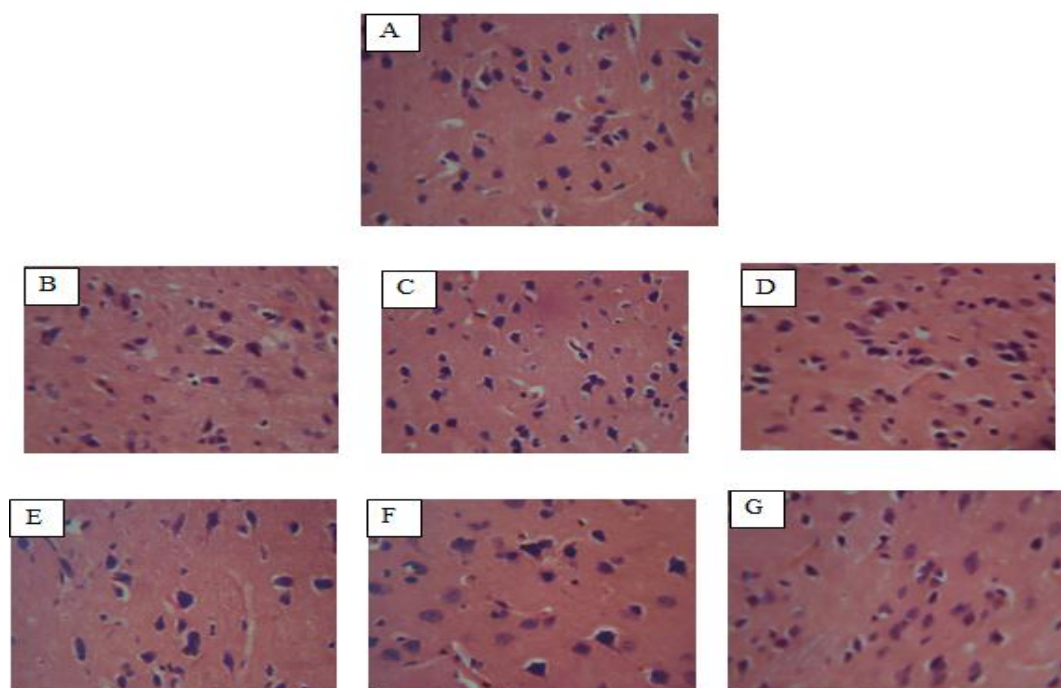


Figure 4: Cross section of the brain of rats orally administered (A) distilled water; (B) 100 mg/kg body weight of AEPA; (C) 200 mg/kg body weight of AEPA; (D) 400 mg/kg body weight of AEPA; (E) 100 mg/kg body weight of EEPA; (F) 200 mg/kg body weight of EEPA; and (G) 400 mg/kg body weight of EEPA (H&E; x100).

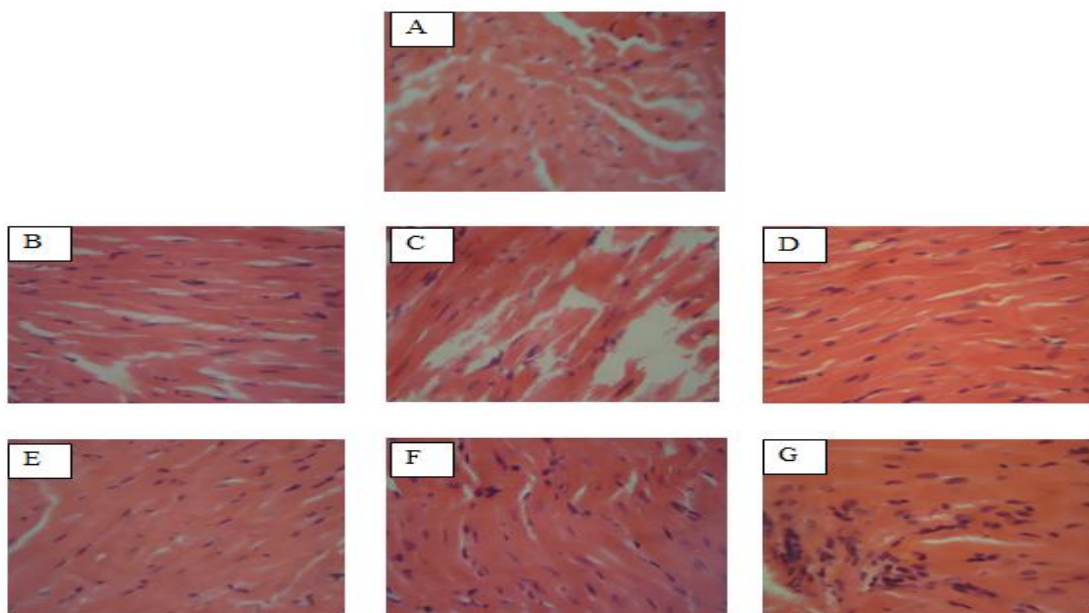


Figure 5: Cross section of the heart of rats orally administered (A) distilled water; (B) 100 mg/kg body weight of AEPA; (C) 200 mg/kg body weight of AEPA; (D) 400 mg/kg body weight of AEPA; (E) 100 mg/kg body weight of EEPA; (F) 200 mg/kg body weight of EEPA; and (G) 400 mg/kg body weight of EEPA (H&E; x100).

References

- Adedapo, A. A., Adegbayibi, A. Y. and Emikpe, B. O. (2005). Some clinico-pathological changes associated with the aqueous extract of the leaves of *Phyllanthus amarus* in rats. *Phytotherapy Research* 19: 971-976.
- Adedapo, A. A and Ofuegbe, S. O. (2015). Anti-inflammatory and antinociceptive activities of the aqueous leaf extract of *Phyllanthus amarus* Schum (Euphorbiaceae) in some laboratory animals. *Journal of Basic Clinical Physiology and Pharmacology* 26(1): 89-94.
- Adjene, J. O. and Nwose, E. U. (2010). Histological effects of chronic administration of *Phyllanthus amarus* on the kidney of adult Wistar rat. *North American Journal of Medical Sciences* 2: 193-195.
- Afolayan, A. J. and Yakubu, M. T. (2009). Effect of *Bulbine natalensis* Baker stem extract on the functional indices and histology of the liver and kidney of male Wistar rats. *Journal of Medicinal Food* 12(4): 814-820.
- Ajaiyeoba, E. and Kingston, D. (2006). Cytotoxicity evaluation and isolation of chromane derivative from *Phyllanthus amarus*. *Pharmaceutical Biology* 44(9): 668-671.
- Babatunde, S. K., Abubakre, A. A., Abdulraheem, Y. J. and Ajiboye, E. A. (2014). Antimicrobial activity of *Phyllanthus amarus* on some human intestinal facultatively anaerobic flora. *International Journal of Medicine and Biomedical Research* 3(1): 52-57.
- Bankole, H. A., Magbagbeola, O. A., Adu, O. B., Fatai, A. A. and James, B. A. (2011). Biochemical effect of ethanolic extract of *Phyllanthus amarus* (Euphorbiaceae) on plasma nitric oxide and penile cyclic guanosine monophosphate (cGMP) in mature male guinea pigs. *Asian Journal of Biochemistry* 6: 291-299.
- Drury, R. A. B. and Wallington, E. A. (1980). Preparation and fixation of tissues. In: Drury, R. A. B. and Wallington, E. A., (editors). *Carleton's Histological Technique*. Oxford: Oxford University Press; pp. 41-54.
- Etta, H. (2008). Effects of *Phyllanthus amarus* on litter traits in albino rats. *Scientific Research and Essay* 3 (8):370-372.
- Eweka, A. O. and Enogieru, A. (2011). Effects of oral administration of *Phyllanthus amarus* leaf extract on the kidneys of adult Wistar rats – A histological study. *African Journal of Complementary and Alternative Medicine* 8(3):307-311.
- Fernand, V. E., (1998). Initial characterization of crude extracts from *Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L. using normal phase thin layer chromatography [Thesis] Louisiana State University pp. 6-13.

- Galati, G., and O'Brien, P.J. (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radical Biology Medicine* 37: 287-303.
- Karuna, R., Sreenivasa, S., Reddy, R., Baskar, R. and Saralakumari, D. (2009). Antioxidant potential of aqueous extract of *Phyllanthus amarus* in rats. *Indian Journal of Pharmacology* 41(2): 64-67.
- Lawson-Evi, P., Eklun-Gadegbeku, K., Agbonon, A., Aklikokou, K., Creppy, E. and Gbeassor, M. (2011). Antidiabetic activity of *Phyllanthus amarus* Schum and Thonn (Euphorbiaceae) on alloxan induced diabetes in male Wistar rats. *Journal of Applied Sciences* 11(16): 2968-2973.
- Oyewo, E. B., Akanji, M. A. and Adekunle, A. S. (2012). Immunomodulation capabilities of aqueous leaf extract of *Phyllanthus amarus* in male Wistar rats. *Report and Opinion* 4 (1): 22-37.
- Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S. and Dixit, V. K. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *Journal of Ethnopharmacology* 138: 386-313.
- Ravikumar, Y. S., Ray, U., Nandhitha, M., Perween, A., Naika, H. R., Khanna, N. and Das, S. (2011). Inhibition of hepatitis C virus replication by herbal extract: *Phyllanthus amarus* as potent natural source. *Virus Research* 158: 89-97.
- Srividya, N. and Periwal, S. (1995). Diuretic, hypotensive and hypoglycaemic effect of *Phyllanthus amarus*. *Indian Journal of Experimental Biology* 33: 861-864.
- Syed, A. B., Iqbal, M. M., Kiranmai, M. and Ibrahim, M. (2012). Hepatoprotective activity of *Phyllanthus amarus* seeds extracts in CCl₄ treated rats. *Global Journal of Medical Research* 12(6):