TOXICITY OF *Acalypha torta* (MUELL) LEAVES ETHANOLIC EXTRACT IN MICE AND RAT

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ABSTRACT

Herbal pharmaceuticals and nutraceuticals are growing in popularity worldwide. These herbal remedies, however natural, can cause some serious damaging effects on the vital organs of the body due to inadequacy in standardization and safety regulations. A 24 hour acute toxicity study was carried out to ascertain the risk of acute intoxication of selected doses (200 to 8,000 mg/kg body weight) of the Acalypha torta leaves ethanolic extract in mice. Subacute toxicity was also assessed following intraperitoneal administration of doses < 50% of the median lethal dose (LD_{50}) for 28 days in Wistar albino rats. Results of acute toxicity studies of extract given intraperitoneal to albino rat gave LD_{50} of 562.30 mg/kg body weight. All the doses of Acalypha torta extract administered reduced appetite in all the experimental animals used, whereas high doses, > 2000 mg/kg body weight caused loss of appetite, increased respiratory rate, convulsion and reduced responses to pains. Observed pathological changes after 28 day subacute toxicity study in rats were necrosis, follicular disorganization, inflammatory reactions, fibrosis and bronchial dilatation. These changes were seen in the liver, spleen, brain, heart, kidney and lung sections. These findings may suggest that prolonged use of the extract could lead to organ damage.

Keywords: *Acalypha torta*, Medicinal plants toxicology, Mice, Rat, Pathological changes, Inflammation, Necrosis

INTRODUCTION

Therapeutical application of herbal preparations are prevalent in many countries of the world (Venukumar and Latha, 2002; Malaya *et al.,* 2004). *Acalypha torta* (Muell), a member of *Euphorbiacae* family, is used in Nigerian folk medicine as a remedy for malaria, bacterial and fungal infections (Irobi and Banso, 1994). More recently, the ethanolic extract of *A. torta* leaves was reported to lower the mean arterial blood pressure in normotensive cats (Ezekwesili, 2007).

Herbal drugs, though natural, can still cause serious adverse effects on the body, ranging from cancer to dysfunctions of vital organs such as liver, heart, lung etc and even death (Aschwanden, 2001). Thus they could produce a wide range of deleterious effects especially where the plant extract is employed for the management of chronic diseases such as hypertension and diabetes mellitus. This study was therefore designed to investigate the toxicological responses following the intake of ethanolic extract of *A. torta* leaves, which is also used ethnomedically to alleviate the hypertension.

MATERIALS AND METHODS

Plant Materials: Mature leaves of the plant were collected from Abagana in Njikoka Local Government Area, Anambra State, Nigeria in March 2006. They were identified (Keay, 1989) and authenticated as *Acalypha torta,* family – Euphorbiacae in the Department of Botany, University of Nigeria, Nsukka by the herbarium curator. Voucher specimen, herbarium No. 8256, was prepared and kept in International Centre for Ethnomedicine and Drug Development (INTERCEED) herbarium.

Animals: Adult albino mice weighing 16 – 35 g and Wistar albino rats of both sexes weighing between 125 – 160 g were purchased from the animal house of the Department of Pharmacology, College of Medicine, University of Nigeria Teaching Hospital, Enugu. All the experimental animals used were kept in the Biochemistry Department animal house for two weeks to acclimatize before use.

Chemicals: All the chemicals used were of analytical grade and these included ethanol, chloroform and methanol from BDH Limited, Poole, England; whereas paraffin, haematoxylin and eosin dyes were made by May and Baker Limited, Dagenham, England.

Ethanolic Extract: Four hundred grammes of dried and pulverized leaves of *A. torta* were extracted three times by soaking in 2.0 L of chloroform : methanol (2:1) at room temperature for 18 hours. The extract was filtered and the filtrates discarded, whereas the residue was dried and re-extracted in 2.0 L of ethanol for 18 hours at room temperature, three times. After filtration, the filtrate was concentrated and used as crude extract.

Acute Toxicity: Forty two adult albino mice of both sexes used were starved overnight prior to administration of the extract. The animals were divided into seven treatment groups (0, 200, 500, 1000, 2000, 4000 and 8000 mg/kg body weight of extract) all equal mean weights and allowed free access to both water and feed (Guinea feed grower's mash) *ad libitum.* Mice in the control group received normal saline, 1 ml/kg body weight. Normal saline and extract were administered intraperitoneally. The ethanol extract of *A. torta* was solubilized in 1 ml normal saline prior to administration. The various groups of mice were housed in separate animal cages and were observed for 24 hour for any behavioral change and deaths. The LD_{50} of the extract was estimated from the graph of percentage mortality against log-doses of the extract (Litchfield and Wilcoxon, 1949).

Sub-Acute Toxicity and Histopathology: Toxicity of sub-lethal doses (<50 % of LD_{50}) of the extract was studied for 28 days using the method of Effraim *et al*. (2001). Eight mature Wistar albino rats of both sexes were divided into four groups of twenty rats each and housed in separate cages according to their body weights. Sub lethal doses of extract, 50 and 100 mg/kg body weight were administered daily to the first two groups of animals whereas 100 mg/kg was given, once, to the third group of rats to determine the effect of a single dose treatment with the extract. The control animals received ethanolic extract and normal saline were injected intraperitoneally. All the animals were allowed access to feed and water *ad libitum.*

At the end of the 28th day all the animals were weighed and sacrificed after chloroform anaesthesia. Sections of liver, kidney, brain, lung, heart and spleen were carefully excised from the rats post mortem and fixed in 10% formal saline for 72 hours. The organ sections were trimmed, processed and stained with haematoxylin and eosin (HE) stains for microscopic examination.

RESULTS

Acute Toxicity: In the acute toxicity study no lethality was recorded in the control group mice. Intraperitoneal administration of the ethanol extract of *A. torta*, at all doses tested, caused reduced appetite in mice. Other behavioral changes observed included drowsiness and increased respiration rate at 100 mg/kg body weight of mice and doses above. Animals that received 4.0 to 8.0 g of extract / kg body weight

mice exhibited twitching and convulsion at the point of death. The median lethal dose (LD_{50}) was estimated to be 562.3 mg/kg body weight of mice (Figure 1).

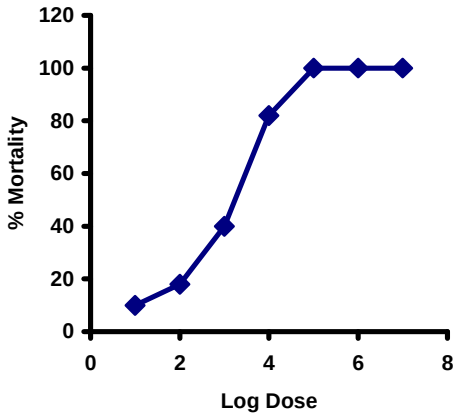

Figure 1: Percentage mortality against log of dose of ethanol extract of *A. torta* after 24 hours acute toxicity study in mice

Sub-Acute Toxicity and Histopathology: Normal organ architecture was observed in all the organs of the control rats and in the animals that received a single dose of 100 mg extract / kg body weight of rat (Figure 2).

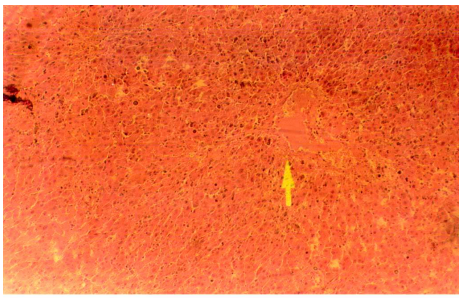

Figure 2: A liver section of the control group of rats showing normal architecture (H&E Stain, × 400)

Liver: Prominent histological changes found in the liver of rats after the 28 day treatment with the extract included pyknosis, hyperchromasia and mononuclear cell infiltration (Figure 3). These alterations were observed in all the rats that received *A. torta* extract. Necrosis and sinusoidal dilation were more pronounced in the liver of rats administered 100 mg of extract / kg body weight of rat daily (Figure 4).

Lung: The photomicrograph of the lung section of rats dosed 50 mg/kg body weight daily for 28 days revealed arterial hyalinization and attenuation (Figure 5). Lymphocytes were noted stromally. Lung section of rats given 100 mg/kg body weight displayed architecture with bronchial dilation, arterial congestion and ruptured vascular wall (Figures 6, 7 and 8).

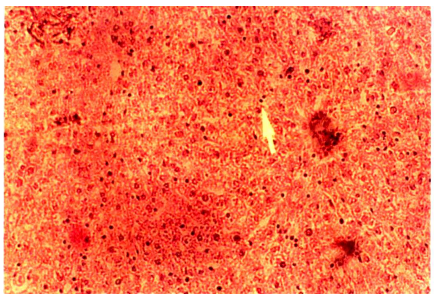

Figure 3: Liver section of rats treated with 50mg/kg wt of the extract daily for 28 days showing pykinosis (arrowed) and hyperchronesia. Mononuclear cell infiltration is noted indicating evidence of hepatitis. (H&E Stain, ×400)

Kidney: There were tubular necrosis and hyalinizations in the kidney section of the rats administered 50 mg/kg body weight daily of extract (Figure 9). In the group that received 100 mg/kg body weight of extract daily, glomerular degeneration was prominent. Droplets of hyaline materials were seen in the tubules (Figure 10).

Heart: The histological examination of the heart sections of rats dosed 50 and 100 mg/kg body weight of extract showed ventricular wall fibrosis with lymphoplasmacytic cells and perinuclear haloes (Figures 11 and 12).

Brain: A cross section of the brain of rats that were on daily injection of 50 mg/kg body weight of *A. Torta* extract displayed evidence of mild fibrosis in some parts. It also showed nuclear eosinophilia and chromatolysis, though these changes were mild. The same changes were seen in the brain of rats that received 100 mg/kg body weight. There was nuclear hyperchromasia (Figures 13, 14 and 15).

Spleen: Daily administration of both 50 and 100 mg of extract per kg body weight of rat caused vascular congestion and follicular

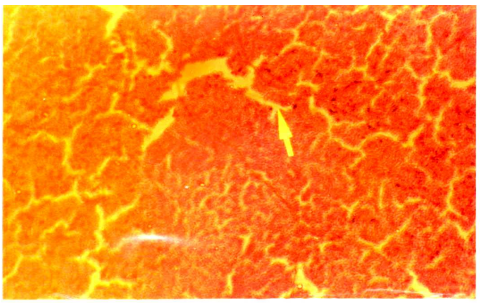

Figure 4: Liver Section of rats treated with 100mg/kg wt daily for 28 days showing necrosis and sinusoidal dilation. (H&E Stain, × 100)

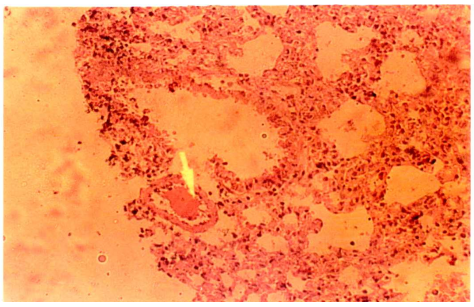

Figure 6: The lung section of rat dosed 50mg/kg wt daily for 28 days daily showing arterial hyalinization and attenuation. Lymphocytes are noted stromally (H&E Stain, ×100)

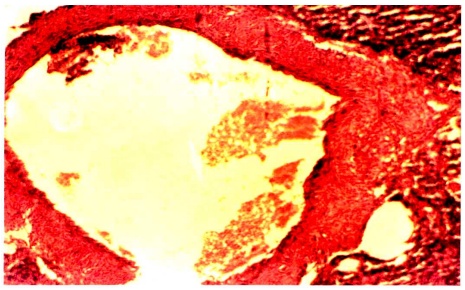

Figure 8: The lung section of rat dosed 100 mg/kg wt of extract showing architecture of vascular rupture, attenuation and congestion. (H.E. Stain × 400)

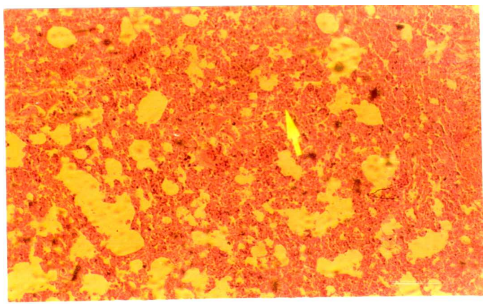

Figure 5: Lung section of the control group showing normal architecture (H&E Stain, ×100)

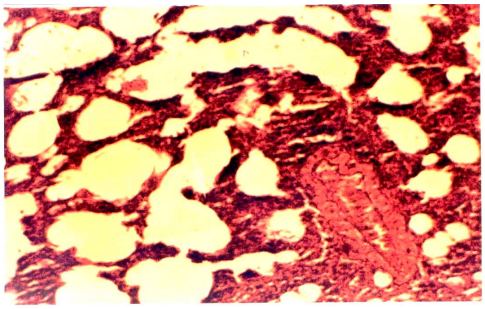

Figure 7: The lung section of rat that received 100mg/kg wt. showing bronchial dilation, arterial attenuation and congestion. (H&E Stain, ×400)

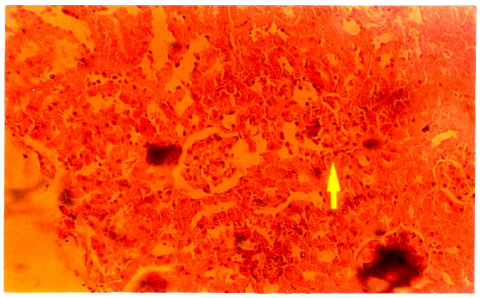

Figure 9: Photomicrograph of kidney section of rats in control group showing normal architecture. (H&E Stain, × 400)

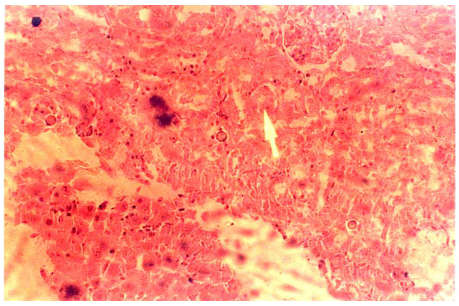

Figure 10: Kidney section of rats dosed 50 mg/kg wt daily for 28 days showing tubular necrosis and hyalinization. (H&E Stain, × 400)

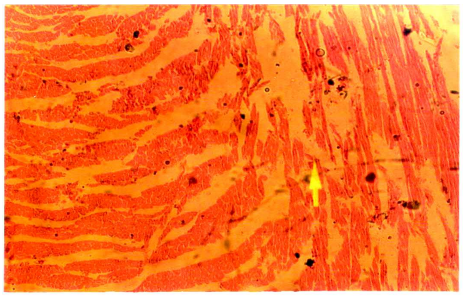

Figure 12: Photomicrograph of heart section of control rats showing normal architecture. (H&E Stain, ×400)

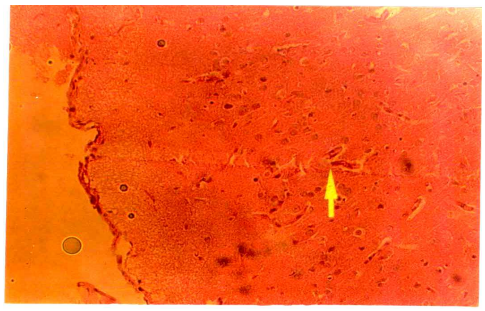

Figure 14: A cross section of brain of control rat showing nothing of pathological significance. (H&E Stain, × 400)

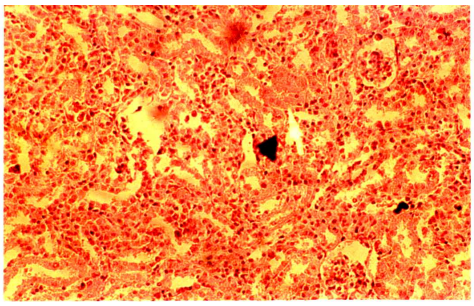

Figure 11: Kidney section of rats dosed 100 mg/kg wt. daily for 28 days showing evidence of glomerular degeneration (arrowed) and droplets of hyalin material in the tubules. (H&E Stain, × 100)

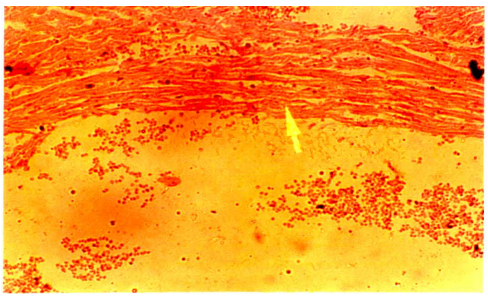

Figure 13: The heart section of rats dosed 50mg/kg and 100mg/kg wt respectively showing ventricular wall fibrosis with lymphoplasmacytic cells and perinuclear haloes (see arrow). (H&E Stain, ×400)

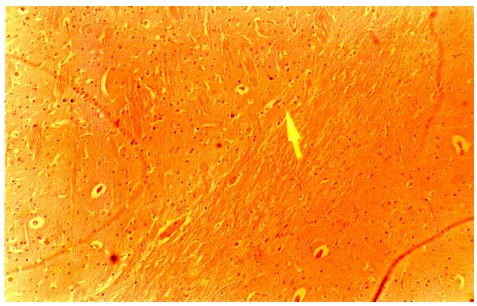

Figure 15: Photomicrograph of the brain of rat dosed 50 mg/kg wt Showing mild fibrosis, nuclear eosinophilia and chromatolysis indicative of neurotoxocity. (H&E Stain, ×100)

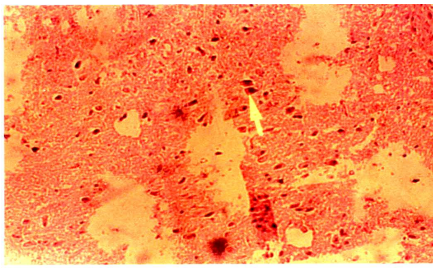

Figure 16: The brain section of rat dosed 100 mg/kg wt daily for 28 days showing the same nuclear hyperchromasia, chromatolysis and nuclear eosinophilia. Empty spaces represent celebral edema. (H&E Stain, ×400)

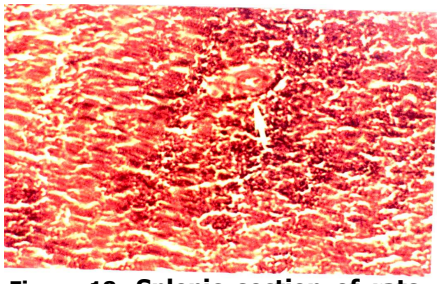

Figure 18: Splenic section of rats that received 50 mg/kg wt showing vascular congestion and follicular disorganization (H&E Stain, ×100)

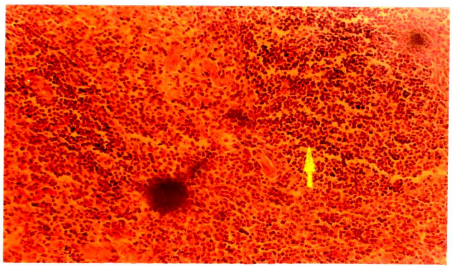

Figure 17: Splenic section of rats in control group showing normal architecture (H&E Stain, × 400)

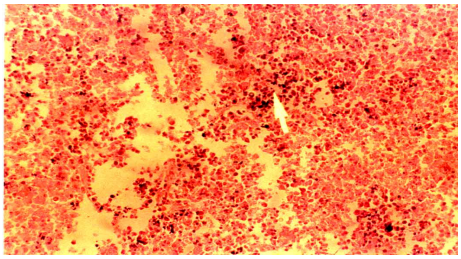

Figure 19: Splenic section of rats dosed 100 mg/kg wt of the drug showing follicular disorganisation, haemorrhagic areas and stromal infiltration of chronic inflammatory cells (arrowed). (H&E Stain, ×100)

disorganization when compared with the normal architecture of the spleen (Figures 16, 17 and 18). Haemorrhagic areas and stromal infiltration of chronic inflammatory cells were also noticed.

DISCUSSION

A. torta commonly known as copper leaf is a plant that is highly cherished in the ethnomedical practices of southern part of Nigeria. It's leaves are utilized in the management of the silent killer diseases such as hypertension and diabetes mellitus. But the damaging effects of the leaf extract on the functional organs of the body must not be ruled out completely considering the fact that such preparations are administered by the traditional medical practitioners without regards to standardization and safety guide. However no information has been documented on the toxicity or adverse effects of *A. torta* leaf extract in humans. The acute toxicity (LD_{50}) of *A. torta* extract in mice (562.3 mg/kg body weight) may not be regarded as an ideal index for toxicity since this parameter is dependent on the species of animal used as well as inherent physiological variations, but may act as baseline data for sub-lethal dose manipulations.

Histopathological examination showed that repeated intraperitoneal administration of the ethanol extract of *A. torta* leaves to rats caused some histological changes in the vital organs of the animals. The observed changes in the liver were necrosis and sinusoidal dilation suggestive of severe degeneration of liver hepatocytes. These changes are morphologically similar to necrotic lesion observed following exposure to certain toxic xenobiotics such as ethanol, acetaminophen, carbon tetrachloride and bromobenzene (Dapar *et al.,* 2007).

Hepatotoxicity of this extract could be a direct effect of the extract or as a result of the conversion of some phytoconstituents of the extract to toxic metabolites by the drug-metabolizing mixed function oxidase system of the liver.

Histological changes in the kidney section of rats treated with *A. torta* extract

showed marked tubular necrosis and glomerular degeneration. The observed effect did not vary with dose of the extract. These changes were usually observed following treatments with herbal preparations. *Securidaca longepedunculata* has been found to elicit tubular necrosis with diffused interstitial and glomerular haemorrhage (Dapar *et al.,* 2007), while oral administration of *Ocimum gratissimum* leaf extract caused renal tubular degeneration in rabbits (Effraim *et al.,* 2001).

Marked respiratory toxicity indicated by bronchial dilatation and vascular wall rupture was prominent. This corroborates the respiratory distress observed in the acute toxicity study at the point of death of rats that received very high doses of the extract. Infiltration of mononuclear cells (in the liver), lymphocytes (in the kidney), lymphoplasmacytic cells (in the heart), eosinophils (in the brain) and chronic inflammatory cells (in the spleen) all point to the fact that inflammatory reactions played a major role in the genesis of the observed pathological lesions. These cells responsible for combating foreign microorganisms and xenobiotics in the body have been found to produce damaging effects on the host tissues by the extracellular release of enzymes and reactive oxygen species. These oxidants attack cells, tissues and organs through the peroxidation of membrane phospholipids, DNA damage, oxidation of the sulfhydryl groups in proteins and degradation of extracellular matrix components (Pohlman and Harlan, 2000; Klings and Farber, 2001; Nwanjo, 2007). Evidence of mild lessons, displayed in parts of the brain also indicates the ability of the extract to cross the blood-brain barrier.

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