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ATELECTATIC AND DEGENERATIVE EFFECTS OF DATURA METEL LEAF EXTRACT ON THE MORPHOLOGY OF THE ALVEOLAR AND HEPATIC TISSUES

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ABSTRACT

Aim: This study was aimed at investigating the effects of Datura metel (D. metel) on the histology of the lungs and liver in rat models.

Methods: Twenty rats were divided into four groups of five each. D. metel extract was administered (50, 100 and 150mg/kg) to the rats through an orogastric tube once daily for 21days.

Results: The histomorphological parameters showed thin atrophic and collapsed alveolar sacs (Atelectasis) in the lungs. In the liver there was severe ballooning degeneration of hepatocytes. Biochemical parameters revealed significant decreases ($p<0.01$ and $p<0.05$) in chloride experimental groups (70.60±1.5 and 74.00±6.9) and $HCO^{-}3$ also increased significantly ($p<0.01$) in the second group (17.80±8.07). The level of urea concentration in serum increased but was not significant in all the experimental groups (7.38±0.3, 7.27±1.9 and 7.30±1.3) as well as Creatinine (11.00±1.22, 40.25±1.2, 58.00±30.5) when compared with their controls (6.13±0.2 and 47.50±3.4).

Conclusion: The result of administration of Datura metel in rat model is suggestive of pulmonohepatic and kidney toxicity at a concentration of 50mg/kg.

Key words: Pulmonary, Ballooning degeneration, Histopathology, Liver, Lungs

INTRODUCTION

History have it that plants are the oldest sources of medication and its use predates written human history (Fabricant and Farnsworth, 2001). They are either used alone or combined with other substances for the treatment of diseases (Avwioro, 2010) Datura metel (D. metel) belongs to the family Solanaceae, and includes about 2,400 species (Ahmad et al., 2012). It is called thorn apple (Abdullahi et al., 2003), devil's apple, stinkweed, Jimson weed and angels' trumpet (Ahmad et al., 2012). In Nigeria it is called different names by different ethnic groups. In Ogoni the southern part of Nigeria it is called "jegemi" (Wannang1 et al., 2009), in the eastern part (Igbo), it is called "myaramuo", in the western part (Yoruba) it is called "gegemu", "ewe ikan" or "Apikan" (Ahmad et al., 2012; Abdullahi et al., 2003), while the northern part (Hausa) calls it **"**zakami" (Abdullahi et al., 2003). D. metel has been reported to have hallucinogenic properties and these classes of plants are used as mind altering agents (Abubakaret al., 2009). It has a wide range of medicinal value which includes mental illness, epilepsy, insomnia, laryngitis, trachitis, catarrh, diarrhoea, skin disease, gonorrhoea, menstrual pain and many more (Babalola, 2014). D. metel plant contains dangerous levels of tropane alkaloids which are poisonous and may be fatal if ingested (Preissel and Preissel, 2002) though may be dose dependent, which is the reason for its prohibition in some regions. It is also used for psychoactive activities where it is abused. Atelectasis is due to hypoventilation of lung units. It may involve the entire lung or a lobe, segment, or a subsegment. It can be caused by intrinsic obstruction of an airway or external compression from lymph nodes, parenchymal masses, or other entities. When lung units are

atelectatic, ventilation-perfusion mismatch leads to hypoxemia. Infection may result from sustained atelectasis (O'donnell, 2012). The etiology and pathogenesis of atelectasis have been linked to impairment of surfactant (secreted by type II pneumocytes) function, compression of lung tissue and absorption of alveolar air (Duggan and Kavanagh, 2005).

MATERIALS AND METHODS

Plant Material

The plant was collected in July, 2014 at Akute-Odo, Ifo Local Government Area of Ogun State, and was confirmed by a botanist in the Department Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State.

Solvent Extraction of D. metel

The leaves were dried at 50^{o}C and pulverized. The powered material was extracted in 95% ethanol for 48hours. It was decanted, filtered and concentrated using a regulated water bath. The extract obtained was reconstituted with water to give the required dosage of 50,100 and 150mg/kg body weight.

Animal Care

Twenty adult Sprague Dawley rats weighing between 190 and 250g were obtained from the animal house of College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. All animals were allowed free access to commercially purchased grower's feed and water ad libitum. All animals were handled gently and calmly in accordance with the guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication 2009).

Experimental Design for in vivo study

The animals were divided into four groups of five rats each and the extract was administered with the aid of orogastric tube once per day for 21days as follows.

Group A (Control): Received water
Group B: 50mg/kg of D. metel extract
Group C: 100mg/kg of D. metel extract
Group D: 150mg/kg of D. metel extract

Histopathology

The rats were sacrificed 24 hours after the last dose, the lungs and livers were excised, fixed in 10% formal saline, dehydrated in ascending grades of alcohol, impregnated and embedded in paraffin wax. Paraffin sections (5µm thick) were stained with haematoxylin and eosin (H&E) method for general histological examination. Slides were viewed under a light microscope and digital photomicrographs were taken.

Biochemistry

Blood samples were obtained from the rats were kept in a plain vial and centrifuged at 5000 rpm for 10 min at room temperature to obtain serum. Serum urea and creatinine as well as electrolytes (chloride and bicarbonate ions) were determined using commercially available assay kits (AGAPPE product) following standard methods.

Statistical analysis

Data were expressed as mean ± standard deviation and were analysed using One-way Analysis of Variance (ANOVA) + Tukey-kramer multi comparison test, values are statistically different from control at $p<0.05$* and **0.01 using GraphPad Instat (Version 3.10). Charts were drawn using Paleontological Statistics (PAST) software package (Version 2.17c).

RESULTS

The results of the final weight (240.4±36.0, 214.8±11.3, 205.8±22.9 and 246±45 respectively) of animals increased but were not statistically significant when compared with their initial weight (234.6±31.53, 206.4±11.9, 199.6±3.57 and 206±2.9 respectively) (Table 1). There was significant decrease in chloride in both group B (70.60±1.5) and D (74.00±6.9), were as in group C there was decrease level of chloride when compared with the control group (90.75±5.3). In Table 2, bicarbonate ($HCO^{-}3$) increased in groups B (17.80±8.07), C (7.25±2.2) and D (6.80±2.5) but was only significant in group B when compared with the control group (4.00±1.8). The level of urea concentration in serum increased but was not significant in all the experimental groups (7.38±0.3, 7.27±1.9 and 7.30±1.3) when compared with the control (6.13±0.2) in Figure 1. Creatinine concentration decreased in group B (11.00±1.22) and group C (40.25±1.2) but increased in group D (58.00±30.5) when compared with the control (47.50±3.4) in Figure 2. In figure 3 (Micrograph of lung), Group A which is the control slide showed a well circumscribed alveolar sacs and duct .The pneumocytes are normal and distinguishable. Group B which was administered with 50mg/kg of D. metel extract showed collapsed alveolar sacs (Atelectasis), as well as collapsed alveolar duct. There was also presence of inflammatory cells in the stroma of the lung tissue when compared with the control. Group C (100mg/kg) showed few areas of loss of

connective tissue. Group D received 150mg/kg of D. metel extract and differed from the control due to its thin atrophic alveoli walls. Figure 4 (Micrograph of Liver), group A slide showed normal histology of the liver architecture with a well displayed central vein and radiating hepatocyte. The size of the canaliculi (sinusoid) is also normal. Group B (50mg/kg of D. metel extract) showed occluded central vein by blood. There are some hepatocytes with ballooned cytoplasm and markedly dilated canaliculi (sinusoid) are also prominent. There was also presence of ballooned cytoplasm in group C. While group D which was administered 150mg/kg of D. metel extract shows more severe ballooning degeneration and swollen hepatocytes have expanded cytoplasm. Few darkly stained cells are suggestive of Mallory bodies.

Table 1: Comparison of weight of animals

Group	Initial Wt (g)	Final Wt (g)	P value	
A (Control)	234.6±31.5	240.4±36.0	P>0.05	Not significant
B	206.4±11.9	214.8±11.3	P>0.05	Not significant
C	199.6±3.57	205.8±22.9	P>0.05	Not significant
D	206.0±2.9	246.0±5.1	P>0.05	Not significant

Each value represents the mean ± standard deviation, values are statistically different from initial weight at p<0.05* one-way analysis of variance (ANOVA) + Tukey-kramer multiple comparison test.

Table 2: Electrolyte analysis

Parameter (mmol/L)	Group A (Control)	Group B	Group C	Group D
Chloride	90.8±5.3	70.6±1.5**	82.8±14.1	74.0±6.7*
Bicarbonate	4.0±1.8	17.8±8.1**	7.3±2.2	6.8±2.5

Each value represents the mean ± standard deviation, values are statistically different from control at p<0.05* and ** 0.01One-way Analysis of Variance (ANOVA) + Tukey-kramer multi comparison test.

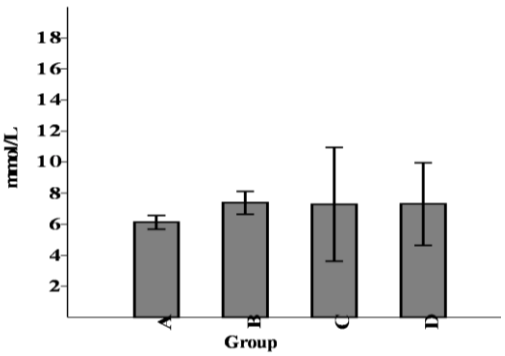

Fig. 1: Chart of urea

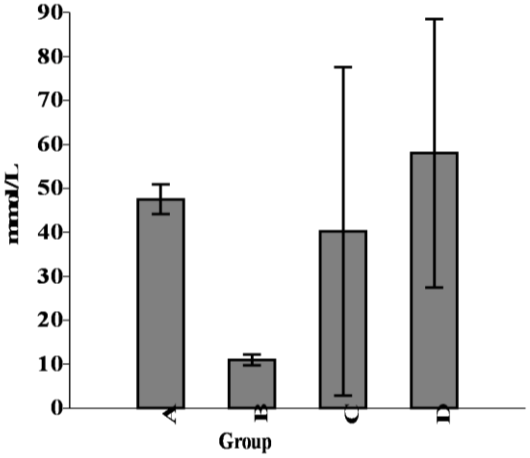

Fig. 2: Chart of creatinine

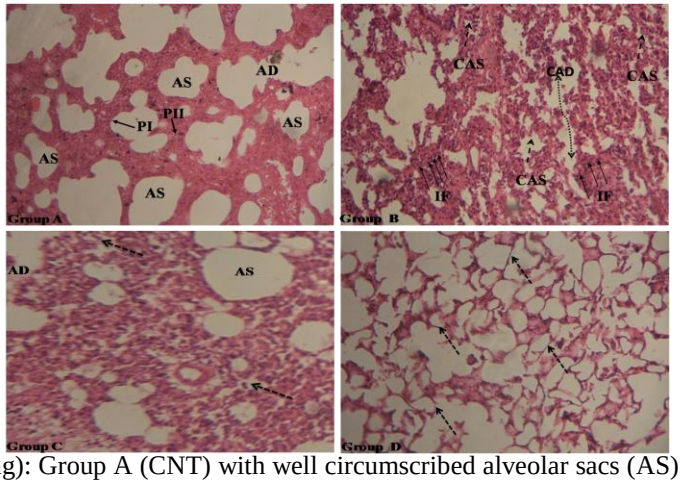

Figure 3 (Lung): Group A (CNT) with well circumscribed alveolar sacs (AS) and duct (AD). The Pneumocytes (PI and PII) are normal. Group B collapsed alveolar sacs (Atelectasis) (CAS), collapsedalveolar duct (CAD) and infiltration of inflammatory cells (IF).Group C shows loss of connective tissues in the alveolar (Dash arrows). Group CThin atrophic alveoli (Dash arrows). H&E x100

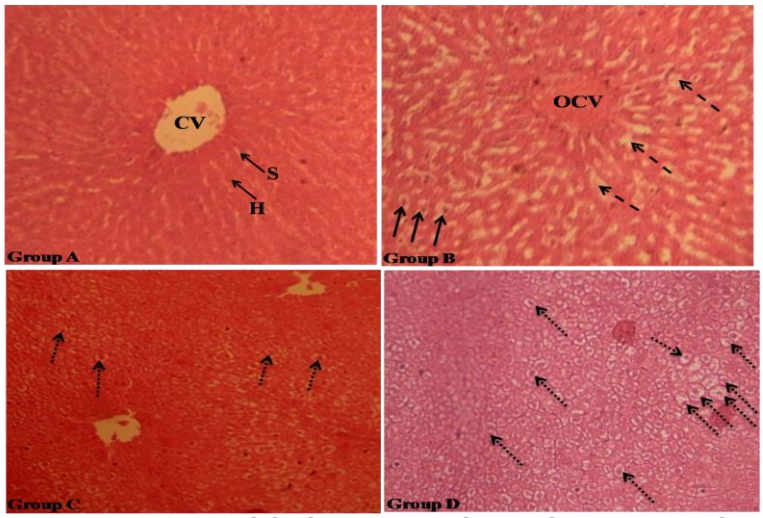

Figure 4 (Liver):Group A(CNT) slide showing normal Central Vein (CV) with well radiating Hepatocyte (H) and Sinusoid (S). Group B displays Central Vein occluded by blood (OCV). Hepatocytes cytoplasm are ballooned (Arrows) and markedly dilated Sinusoid (Dash Arrows) are also visible. Group C also shows presence of ballooned cytoplasm (Dash Arrows). Group D shows more severe ballooning degeneration, swollen hepatocytes having expanded cytoplasm (Dash Arrows). There is also presence of Mallory bodies (Dot Arrows). H&E x100

DISCUSSION

The appearance of ballooning degeneration in the histology of liver parenchyma is a predominant mode of hepatocyte injury in hepatitis (Puja, 2014)). There are several mechanisms by which toxins can cause hepatic injury which involves the expression of cytokines, growth factors and the extracellular matrix but these mechanisms are poorly understood (Michaelet al., 2013;Sharma et al., 2011; Kaplowitz, 2004).The mechanism that leads to the obstruction of the hepatic veins is thrombosis (Stuart, 1957) (thrombosis has to do with the formation of a blood inside a vessel, obstructing blood flow). The obstruction of a single hepatic vein is usually clinically silent but two or more may increase sinusoidal blood pressure and reduce sinusoidal blood flow (Parker, 1959), as a result of this may cause sinusoidal dilatation and congestion among others (Akiyoshi and Terada, 1999). The findings of this research revealed occluded central vein, markedly dilated sinusoid and different levels of ballooning degeneration of hepatocyte following the administration of 50,100 and 150mg/kg body

weight of D. metel as seen in figure 4. These findings are similar to some researches carried out with a similar species (Datura stramonium) such as Adekomi et al.,(2011)where they reported occlusion of the portal vein, degenerative and progressive cell death in rats exposed to fumes of Datura stramonium (0.5g and 0.74g). This present research is also similar to the work done by Zuhour and Samia (2014) who reported congestion, fatty changes and necrosis, infiltration of inflammatory cells in Sprague Dawley rats administered with 5 and 7.5mg/kg of Datura stramonium Seed. Contrary to the findings of this study, Dugan et al. (1989) reported normal liver in rats fed with diet containing 0.5, 1.58 and 5.0% Datura stramonium seed. Atelectasis which is the collapse or closure of the alveoli of the lungs (Wedding and Gylys, 2005) may be caused by pneumonia, obstruction from mucus plugging, tumour, general anaesthesia, surgery (Proto 1996; Ishikawa et al., 2002; Vargas et al., 1993), lack of surfactant that coat the lining of alveoli and prevent it from collapse, large dosage of opioids or sedatives, smoking etc. (Cedars, 2015). In the present research the lung tissue of the administered groups (50, 100,150 mg/kg of D. metel extract) showed atelectasis with inflammatory cells, loss of connective tissue and thin atrophic alveoli walls when compared to the control slide (group A) as seen in figure 3. This is corroborated by extreme significant increase ($p<0.01$) in serum $HCO^{-}3$ in group B (17.80±8.07) and other increases in groups C (7.25±2.2) and D (6.80±2.5) when compared with the control group (4.00±1.8), as seen in table 2. It is documented that increase level of serum $HCO^{-}3$ is seen in compensated respiratory acidosis and in metabolic alkalosis (Tietz, 1983; Friedman, 1980). This is also in agreement with the work of Adekomiet al. (2011) where there was a major alteration and enlargement of the alveoli sacs in rats administered with Datura stramonium leaf. Low serum chloride can be seen in salt-losing nephropathy, various acid base disturbances, conditions characterized by expansion of extracellular fluid volume and a lot more (Tietz, 1983; Friedman, 1980). There was significant decrease ($p<0.01$) in chloride in both group B (70.60±1.5) and D (74.00±6.9).The level of chloride in group C decreased but was not significant when compared with the control group (90.75±5.3). This result corroborates the serum urea increase of experimental groups (7.38±0.3, 7.27±1.9 and 7.30±1.3) which is an indication of acute and chronic intrinsic renal disease (Tietz, 1983; Friedman, 1980). Creatinine concentration decreased in groups B (11.00±1.22) and C (40.25±1.2) but increased in group D (58.00±30.5) when compared with the control (47.50±3.4) in Figure 2. Creatinine elevation is seen in renal functional impairment while decrease characterized by muscle wasting. The findings on the kidney in this research are closely related to the research carried out by Adekomi et al. (2011) where they reported major degenerative changes in the kidney in rats administered with Datura stramonium leaf.

Conclusion

The entire findings of this research are similar to the compelled work of U.S.-Australian symposium on the effects of poisonous plants on domestic livestock (Richard et al., 1978). Base on this research D. metel is not a nontoxic plant and its rampant abuse may even cause more harm.

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