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Research Article

HISTOLOGICAL CHANGES INDUCED BY AQUEOUS EXTRACT OF ARECA NUT (*ARECA CATECHU*) ON THE LIVER OF ADULT WISTAR RATS

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ABSTRACT

Twenty-four (24) male and female adult Wistar rats weighing between 190 kg – 240 kg were randomly divided into four (4) groups: the Control group (A) received pelletized growers mesh and distilled water for four weeks while groups B, C and D were administered 400 mg/kg, 800 mg/kg and 1200 mg/kg of aqueous extract of areca nut respectively for four (4) weeks consecutively. The animals were sacrificed following 24 hours of fasting by cervical dislocation. Histologically, treatment of the rats with graded dose (400 mg/kg, 800 mg/kg, and 1200 mg/kg) of aqueous extract of Areca nut for four (4) weeks induced loss of cytoarchitectural integrity of cells in the liver lobules. This is manifested by observing mild chronic inflammation around the portal triad (periportal hepatitis) and activation of the local immune system (kupffer cell activation) except that at high dose (1200 mg/kg), the immune system was not activated. The hepatocytes expressed prominent nucleoli which suggest increase in intracellular enzyme induction. The results of this study generally revealed that aqueous extract of areca nut caused histological distortion in the liver of adult Wistar rats.

Keywords: Areca nut, Aqueous Extract, Liver, Morphological, Wistar rat.

INTRODUCTION

The word 'Areca' is derived from the word adakka or from adakeya, the Indian equivalent[1]. Areca nut is believed to have originated from Sri Lanka and Malaysia. It is cultivated in South-East Asia, in India and in some regions of Central Africa[2]. The areca palm is used as an ornamental and interior landscaping plant. Areca palm is usually been used in hotels and malls[3].

The common names of Areca nut are Adike, Areca, Betel nut, Pinlang, Betel palm, Fobal, Tuuffel, Goorrecanut palm, Gouvaka, Paan Supari, Kamuku, Mak, Sopari, Tambul[4]. Areca nut is chewed regularly by at least 10% of the world population and it is the fourth most widely used addictive substance[5].

It contains water 30 %, protein 5 %, fat 3 %, carbohydrate 47 %, and total alkaloids with arecoline been the major alkaloid constitute 0.2 % – 0.7 %.

The active alkaloid compounds present in areca nuts are guvacine, arecaidine, guvacoline, and arecoline. Arecoline is the principal alkaloid found in *Areca catechu*, while the other three alkaloid compounds are available in small quantities[2].

The husk fibres of areca cathecu (areca nut) has been reported to be used for cleaning of teeth[6].

Drugs that are cholinergic like areca nut produce series of side effects including excessive salivation, urinary and faecal incontinence, sweating, vomiting and diarrhoea.[7]

The lethal dose (LD_{50}) of raw areca nut extract in healthy male and female wistar rats was found to be 2321.96 mg/kg and 2257.52 mg/kg, respectively.[8]

Research has shown that exposure of mice to aqueous extract of areca nut resulted in severe loss of ultrastructural integrity of cells in the liver lobules[9].

It has been documented that treatment of rats with areca nut and nutgall extracts reversed oxidative damage in hepatic tissues induced by carbon tetrachloride[10].

The liver is the largest of the abdominal visceras, which occupies some portion of the upper abdominal cavity. It also occupies most of the epigastrium and right hypochondrium, and usually extends to the left hypochondrium. The liver weighs approximately 5% of the body weight in infancy and it decreases to approximately 2% in adults. The size of the liver also varies according to age, sex, and body size which is wedge shaped. Throughout life, the liver is reddish brown in colour[11]. The main functional cell in the liver is a form of epithelial cell called hepatocyte. These cells are arranged as thin plates separated by fine vascular sinusoids through which blood flows. The main blood vessels and ducts run through the liver within a branched collagenous framework termed the portal tracts. The portal tract contains three main structures which are: portal vein, hepatic artery and bile ductules. These three structures are always found in the portal tracts thus, the tracts are often referred as portal triad[12].

The sinusoids of the liver lack a basement membrane and are loosely surrounded by speacialized fenestrated endothelial cells and kupffer cells (phagocytic cells)[13].

Aim
To investigate the morphological changes induced by aqueous extract of Areca nut (Areca Catechu) on the liver of adult Wistar rats.

Objectives
To investigate the effects of aqueous extract of Areca nut on the cytoarchitecture of the liver of adult Wistar rats.

MATERIALS AND METHODS

The materials used for this investigation included twenty four (24) adult Wistar rats weighing 190 – 240 kg. Areca nut was obtained within the Ugbowo campus of University of Benin. The pelletized growers mash was obtained from Livestock feed factory in Benin City. Distilled water was also obtained from University of Benin Enterprise. The following equipment were used during the course of this study: rotary microtome, Leica brand of automated tissue processor, refrigerator, mettler balance, embedding machine, British milling machine, chromatography jar, evaporating dish, water bath, bowl, white man paper, wax, dissecting set, needle and syringe, oral cannula, specimen (universal) bottles. The analytical grades of reagents used in this study include formal saline, ethanol, xylene, H & E stain, DPX, and chloroform.

Plant Collection

Areca nut (*Areca catechu*) used in this study were collected within the premises of University of Benin (Ugbowo Campus) and identified by Mr. Nweke Sunday who is the head of herbarium unit of the Department of Pharmacognosy of University of Benin, Benin City.

Preparation of Extract

The extract was processed and prepared in the Department of Pharmacognosy, University of Benin, Benin City. The fresh matured seeds of Areca nut were collected and air-dried at room temperature (to prevent solar leaching). The husks of Areca nut was removed and discarded. The seeds of the areca nut was ground into 4.0 kg of powdered form of Areca nut using British Milling Machine. Two hundred and fifty (250) grams of powdered areca nut was dissolved in 1.5Litres of distilled water using a chromatography jar for 24 hours.

The mixture of powdered Areca nut and distilled water was then filtered using filter paper. The residue obtained was discarded while the filtrate was poured into the evaporating dish. This evaporating dish containing filtrate of Areca nut was placed on a water bath at 40^0C for three (3) days to convert the filtrate to concentrate after series of evaporation had taken place. The concentrate was further heated on the water bath till it became dry and was stored at room temperature for use.

Animal Grouping and Experimental Design

Twenty-four (24) adult Wistar rats were randomly divided into four (4) experimental groups of six (6) rats each.

The rats in the Control group A, were orally fed with pelletized growers mesh and distilled water only, for four (4) consecutive weeks. The rats in Group B were administered orally with 400 mg/kg body weight of aqueous extract of Areca nut, pelletized growers mash and distilled water daily, for four (4) consecutive weeks. The rats in Group C were orally administered with 800 mg/kg body weight of aqueous extract of Areca nut, pelletized growers mash and distilled water daily, for four (4) consecutive weeks. The rats in Group D were orally administered with 1200 mg/kg body weight of aqueous extract of Areca nut, pelletized growers mesh and distilled water daily, for four (4) consecutive weeks.

The administration of aqueous extract of Areca nut was done using orogastric tube. After the fourth consecutive week of administration of aqueous extract of Areca nut, the animals were fasted for 24 hours to ensure that they were at normal metabolic rate before been sacrificed.

The animals were sacrificed after 24 hours of fasting by cervical dislocation. The abdomen was excised and the liver from each rat in each group were immediately harvested for tissue processing.

RESULTS

The section of the liver in control group (A) showed portal vein, hepatocytes, sinusoids and bile duct (Figure 1.1a & 1.1b).

When compared to control, treatment of the rats in group B which received 400 mg/kg of aqueous extract of Areca nut for four (4) weeks induced a mild chronic inflammation around the portal triad (periportal hepatitis) and activation of the local immune system (kupffer cell activation). The section showed mild periportal infiltrates of lymphocytes. In addition, the hepatocytes expressed prominent nucleoli suggesting increase in intracellular enzyme induction (Figure 1.2a & 1.2b).

When compared to control, the section of the liver of group C which received 800 mg/kg of aqueous extract of Areca nut for four (4) weeks showed mild vascular congestion, mild kupffer cell activation and one or more prominent nucleoli (Figure 1.3a & 1.3b).

When compared to control, the section of the liver of group D which received 1200 mg/kg of aqueous extract of Areca nut for four (4) weeks showed mild kupffer cell activation and mild periportal infiltrates of lymphocytes. In this group, the immune system was not activated (Figure 1.4a & 1.4b).

CONCLUSION

Histologically, treatment of the rats with graded dose (400 mg/kg, 800 mg/kg, and 1200 mg/kg) of aqueous extract of Areca nut for four (4) weeks induced loss of cytoarchitectural integrity of cells in the liver lobules. This is in conformity with the work of Choudhury and Sharan, (2010)[9] and is manifested by observing mild chronic inflammation around the portal triad (periportal hepatitis) and activation of the local immune system (kupffer cell activation) except that at high dose (1200 mg/kg), the immune system was not activated. The inactivation of the immune system at high dose is in conformity with the work of Apurva et al., (2014)[14] which stated that Areca nut affects the immune system leading to suppression of T-cell activity and decreased release of cytokines. The hepatocytes expressed prominent nucleoli which suggest increase in intracellular enzyme induction. The findings from this study revealed that aqueous extract of Areca nut caused loss of cytoarchitectural integrity of cells in the liver lobules which showed that the liver was damaged to the extent that the immune system were not activated at high dose.

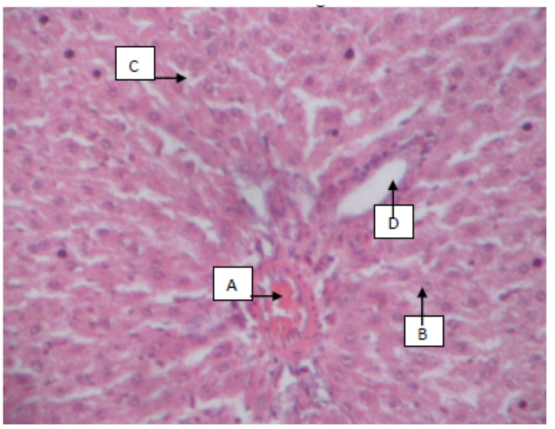

Figure 1.1a: Lower magnification of Section of the liver of Control Group (A) rat composed of portal vein = A; hepatocytes = B; sinusoids = C; and bile duct = D (H&E x 100).

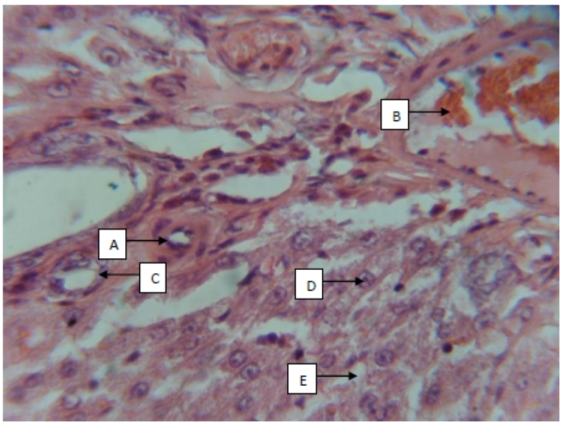

Figure 1.1b: Higher magnification of Section of the liver of Control Group (A) rat composed of hepatic artery = A; portal vein = B; bile duct = C; hepatocytes = D; and sinusoids = E (H&E x 400).

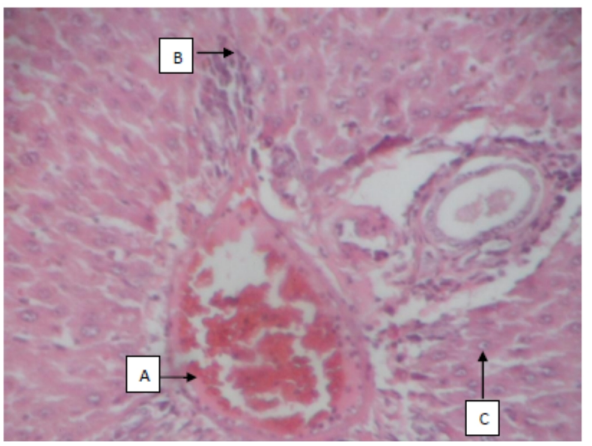

Figure 1.2a: Lower magnification of Section of the liver of Group (B) rat treated with 400 mg/kg Areca catechu for four weeks showing mild vascular congestion = A; mild periportal infiltrates of lymphocytes = B; and one or more prominent nucleoli = C (H&E x 100).

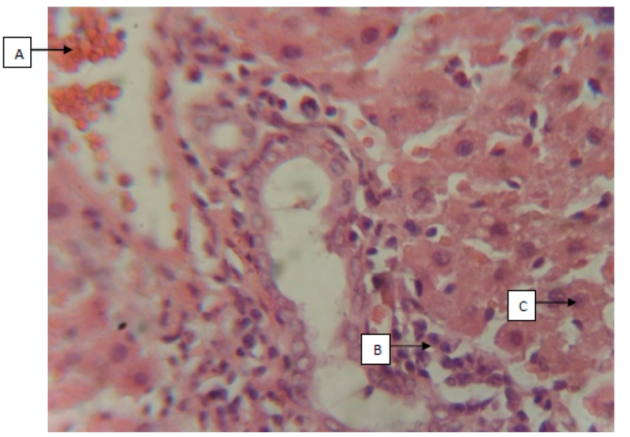

Figure 1.2b: Higher magnification of Section of the liver of Group (B) rat treated with 400 mg/kg Areca catechu for four weeks showing mild vascular congestion = A; mild periportal infiltrates of lymphocytes = B; and one or more prominent nucleoli = C (H&E x 400).

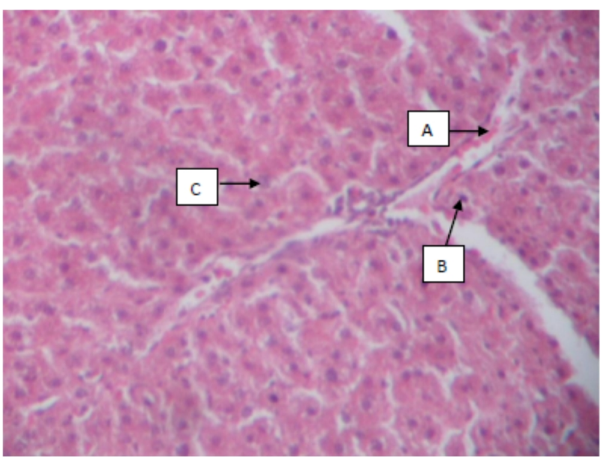

Figure 1.3a: Lower magnification of Section of the liver of Group (C) rat treated with 800 mg/kg Areca catechu for four weeks showing mild vascular congestion = A; mild kupffer cell activation = B; and one or more prominent nucleoli C (H&E x 100).

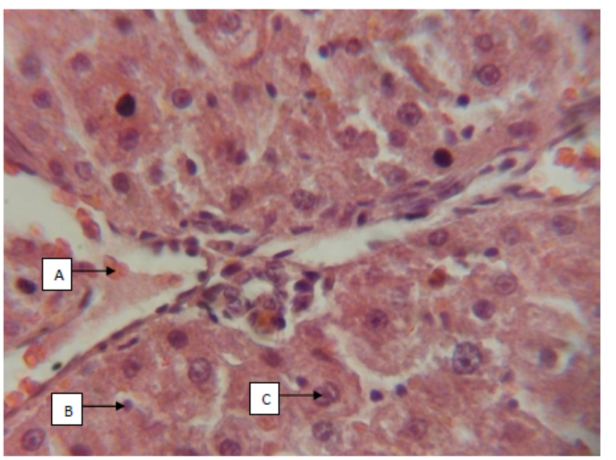

Figure 1.3b: Higher magnification of Section of the liver of Group (C) rat treated with 800 mg/kg Areca catechu for four weeks showing mild vascular congestion = A; mild kupffer cell activation = B; and one or more prominent nucleoli = C (H&E x 400).

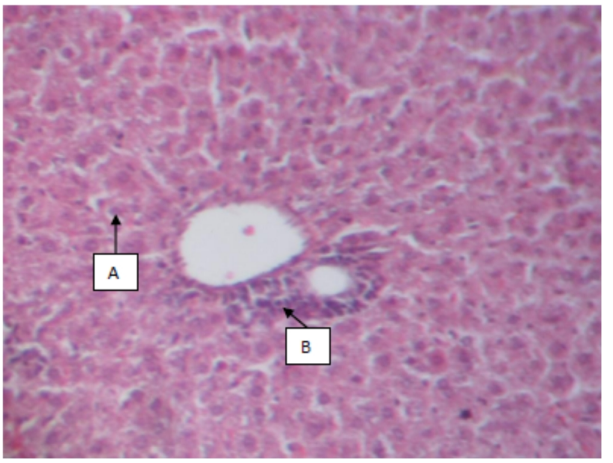

Figure 1.4a: Section of the liver of Group (D) rat treated with 1200 mg/kg Areca catechu for four weeks showing mild kupffer cell activation = A; mild periportal lymphocytosis = B (H&E x 100).

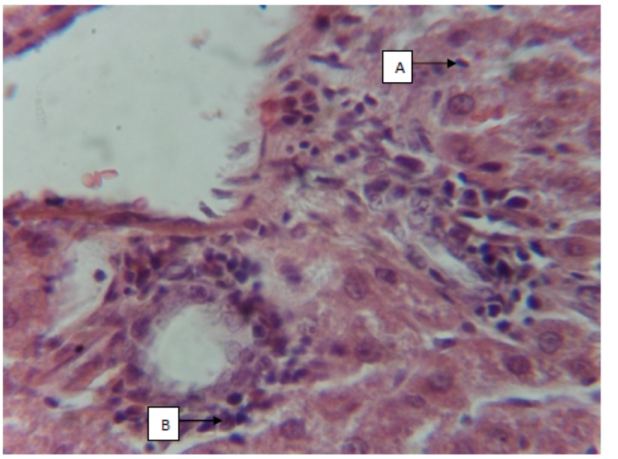

Figure 1.4b: Section of the liver of Group (D) rat treated with 1200 mg/kg Areca catechu for four weeks showing mild kupffer cell activation = A; mild periportal lymphocytosis = B (H&E x 400).

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