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Effects of Short Term Oral Administration of Aqueous Extracts of Areca Nut (Areca Catechu) on Liver Profile 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 using chloroform anaesthesia. The results of the biochemical assay of the serum of the experimental rats revealed that the serum value of alkaline phosphatase (ALP) in group C was significant ($P \leq 0.05$) when compared with the control. There was however, no significant difference ($P \leq 0.05$) in the other treatment groups compared to control. The serum value of alanine transaminase (ALT) in group C and D was significant ($P \leq 0.05$) when compared with the control. There was no significant difference ($P \leq 0.05$) in group B compared to control. The serum value of aspartate aminotransferase (AST) in group B and C was significant ($P \leq 0.05$) when compared with the control. There was no significant difference ($P \leq 0.05$) in group D compared to control. The serum value of total protein (TP), albumin (ALB), total bilirubin (TB), and conjugated bilirubin (CB) in groups B, C and D were not significant ($P \leq 0.05$) when compared with the control.The changes in the blood levels of ALT, ALP, and AST showed that the aqueous extract of areca nut have damaging effects on the liver of adult Wistar rats.*

Keywords: *Areca nut, Aqueous Extract, Liver Profile, Biochemical, Wistar rat.*

I. 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].

Areca catechu is the most widely patronized species among the varieties of the species of the genus Areca. It is cultivated in many tropical countries for its valuable nuts. It grows in the savannah belt and in deciduous forests and secondary clearings. It is a major crop in North Africa and parts of South Africa[4].

The common names of Areca nut are Adike, Areca, Betel nut, Pinlang, Betel palm, Fobal, Tuuffel, Goorrecanut palm, Gouvaka, PaanSupari, Kamuku, Mak, Sopari, Tambul[5].After adequate research, I have named Areca nut in different dialects of Nigeria. The names given to Areca nut generally mean palm kernel of the whites, and are represented in table 1 below.

Areca nut is chewed regularly by at least 10% of the world population and it is the fourth most widely used addictive substance[6].

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[7].

Drugs that are cholinergic like areca nut produce series of side effects including excessive salivation, urinary and faecal incontinence, sweating, vomiting and diarrhoea.[8]

The lethal dose (LD50) 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.[9]

Pimolpan *et al.,* (2009)state that extracts from seeds of Areca nut have hepatoprotective potentials. Though they used nutgalls together with extracts of seeds of Areca nut but didn't state whether the hepatoprotective potential was caused by the nutgalls[10].

In humans, betel-quid chewing leads to metabolic syndrome, which indicates a higher chance of developing cardiovascular disease. Betel quid (Areca catechu) is used by close to 10 % of the world's population.[11]

The National Institutes of Health links regular betel nut chewing to cancers of the mouth. Additionally, compounds in betel nut can encourage the growth of cervical, oral, liver, prostate, lung, and stomach cancers[8].

Research has also shown that exposure of mice to aqueous extract of areca nut resulted in severe loss of ultrastructural integrity of cells in the liver lobules[12].

The liver damaged due to inflammatory changes was also found to be associated with rise in ALT level[13].

This study is conducted to evaluate the early biochemical changes that could be induced by aqueous extract of Areca nut on the liver profile of adult Wistar rats.

1.1 **Aim**

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

II. Materials and Methods

II.I Materials

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.

II.II 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 of the herbarium unit of the Department of Pharmacognosy of University of Benin, Benin City.

II.III 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.

II.IV 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 mash and distilled water daily, for four (4) consecutive weeks.

The administration of aqueous extract of Areca nut was done using orogastrictube.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 using chloroform anaesthesia. Five (5) ml of blood sample was obtained by cardiac puncture from each rat in each group and kept in lithium heparin bottles for biochemical analysis.

II.V Biochemical Analysis

Alanine aminotransferase (ALT) and Aspartate aminotransferase (ASP) assay kits used were obtained from Randox Laboratory Limited, UK. The assay methods used are for the quantitative in vitro determination of the ALT and ASP enzymes in serum.

ALT was measured using the principle of monitoring the concentration of pyruvate hydrazone. ASP was measured using the principle of monitoring the concentration of oxaloacetate hydrazone.

Alkaline phosphatase (ALP) assay kits were obtained from TECO Diagnostics, Canada. The Colorimetric Endpoint Method was used for the direct colorimetric determination of alkaline phosphatase in the serum. Albumin level was measured using Bromocresol Green (BCG) method for the quantitative in vitro determination of albumin in serum and plasma. The assay kits were manufactured by Randox Laboratory Limited, UK. Total protein level was measured using Buiret method for the quantitative in vitro determination of total protein in serum and plasma. The assay kits were also products of Randox Laboratory Limited, UK. The principle used was such that cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Serum Bilirubin level was ascertained using Evelyn and Malloy technique for the quantitative in vitro determination of serum bilirubin in the serum. The principle used was such that bilirubin was diazotized with sulphuric acid to form azo coupling. The resultant colour was read spectrophotometrically at 540 nm. The unconjugated bilirubin which is insoluble in water react at the addition of methanol.

II.VI Statistical Analysis

Statistical analysis of data was done using SPSS. P values of ≤ 0.05 were taken as significant and mean differences were compared using a multiple comparison test[14].

The mean and standard error of mean of the groups of animals were calculated and recorded.

The analysed data are presented with charts and tables.

III. Results

The results of the biochemical assay of the serum of the experimental rats revealed that the serum levels of alkaline phosphate (ALP) in the control group and experimental groups (B, C and D) were 36.4±2.48, 42.0±6.66, 29.0±2.77[a] and 43.8±4.21 (U/L) respectively. The serum value of ALP in group C was significant ($P \leq 0.05$) when compared with the control. There was however, no significant difference ($P \leq 0.05$) in the other treatment groups compared to control. (Table 2 and Fig. 1).

The serum levels of alanine aminotransferase (ALT) in the control group and experimental groups (B, C and D) were 21.8±1.83, 23.6±2.58, 27.0±0.95[a] and 31.0±1.48[b] (U/L) respectively. The serum value of ALT in group C and D was significant ($P \leq 0.05$) when compared with the control. There was no significant difference ($P \leq 0.05$) in group B compared to control. (Table 2 and Fig.1).

The serum levels of aspartate aminotransferase (AST) in the control group and experimental groups (B, C and D) were 53.00±4.04, 66.8±7.34[a], 43.6±2.62[c] and 55.4±1.86 (U/L) respectively. The serum value of AST in group B and C was significant ($P \leq 0.05$) when compared with the control. There was no significant difference ($P \leq 0.05$) in group D compared to control. (Table 2 and Fig.1).

The serum levels of total protein (TP) in the control group and experimental groups (B, C and D) were 7.18±0.26, 7.22±0.15, 7.58±0.27 and 7.02±0.11 (mg/dl) respectively. The serum value of TP in groups B, C and D were not significant ($P \leq 0.05$) when compared with the control. (Table 2, Fig.2).

The serum levels of total protein (ALB) in the control group and experimental groups (B, C and D) were 3.56±0.20, 3.86±0.24, 3.74±0.24 and 3.28±0.12 (mg/dl) respectively. The serum value of ALB in groups B, C and D were not significant ($P \leq 0.05$) when compared with the control. (Table 2, Fig.2).

The serum levels of total bilirubin (TB) in the control group and experimental groups (B, C and D) were 0.66±0.06, 0.50±0.04, 0.52±0.04 and 0.50±0.04 (mg/dl) respectively. The serum value of TB in groups B, C and D were not significant ($P \leq 0.05$) when compared with the control. (Table 2, Fig.3).

The serum levels of conjugated bilirubin (CB) in the control group and experimental groups (B, C and D) were 0.32±0.04, 0.20±0.03, 0.28±0.04 and 0.24±0.04 (mg/dl) respectively. The serum value of CB in groups B, C and D were not significant ($P \leq 0.05$) when compared with the control. (Table 2, Fig.3).

IV. Table and Figures

Table 1. Showing the name given to Areca nut in different Nigerian dialect.

S/N	NIGERIAN DIALECT	GIVEN NAME
1.	Yoruba	EkuọOyinbo
2.	Hausa	KwakwaMoinjaNbature
3.	Igbo	AkuNdiỌcha
4.	Igala	OmaẹkpẹOyibo
5.	Benin	IkpedinOkheEbo
6.	Urhobo	IbiediOyibọ Ẹ
7.	Isoko	EbiẹdeOyibo
8.	Ekoi Tub (Cross River)	EkepOkara
9.	Ekpeye (Rivers State)	OhuIgbeke

Table 2: The mean and standard error of mean values of liver enzymes (ALP, ALT & AST), total protein (TP), albumin (ALB), total bilirubin (TB), and conjugated bilirubin (CB), of Wistar rats treated with aqueous extract of Areca catechu compared with control.

GROUPS	ALP (U/L)	ALT (U/L)	AST (U/L)	TP (mg/dl)	ALB (mg/dl)	TB (mg/dl)	CB (mg/dl)
Control (A)	36.4±2.48	21.8±1.83	53.00±4.04	7.18±0.26	3.56±0.20	0.66±0.06	0.32±0.04
B(400mg/kg)	42.0±6.66	23.6±2.58	66.8±7.34[a]	7.22±0.15	3.86±0.24	0.50±0.04	0.20±0.03
C(800mg/kg)	29.0±2.77[a]	27.0±0.95[a]	43.6±2.62[c]	7.58±0.27	3.74±0.24	0.52±0.04	0.28±0.04
D(1200mg/kg)	43.8±4.21	31.0±1.48[b]	55.4±1.86	7.02±-0.11	3.28±0.12	0.50±0.04	0.24±0.04

Means with different alphabetical remarks are significantly different from control ($P \leq 0.05$).

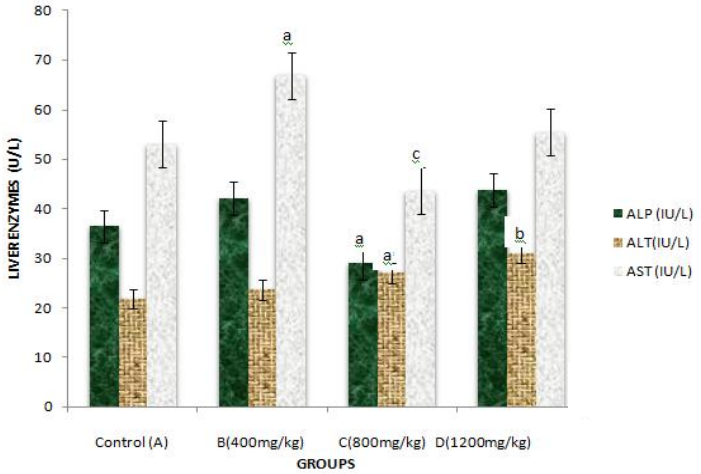

Fig.1: showing the value of liver enzymes levels in the control and experimental animals; means with alphabetical differences are statistically significant at p value ≤ 0.05 comparing that of the experimental groups to the control group.

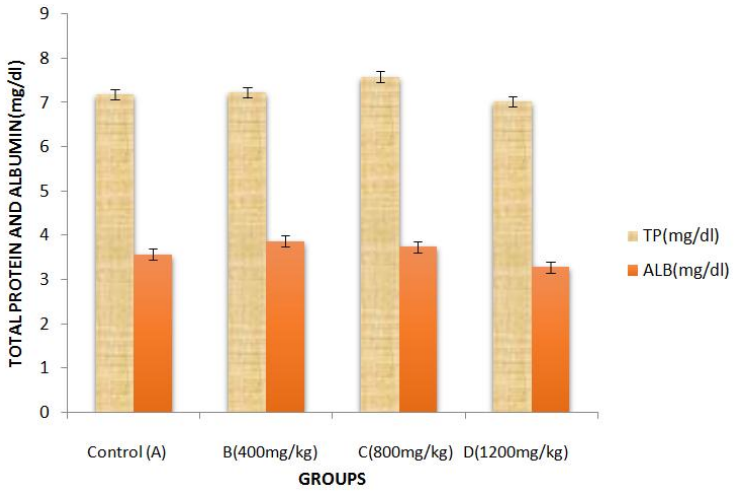

Fig.2: effects of aqueous extract of Areca catechu on total protein and albumin levels of experimental rats compared with control.

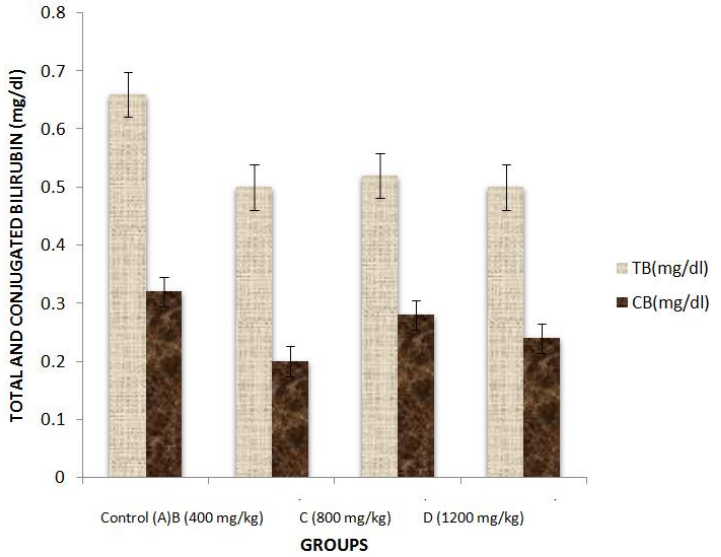

Fig.3: effects of aqueous extract of Areca catechu on total bilirubin and conjugated bilirubin levels of experimental rats compared with control.

V. Conclusion

The biochemical assay result from this study revealed that high doseof aqueous extract of Areca nut showed that the levels of parameters for liver enzymes were greatly altered compared with control and other treatment groups. This showed that high dose of aqueous extract of Areca nut has hepatotoxic effects on the liver profile of adult Wistar rats.

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