

Hepatoprotective Effects of Ipomoea Batatas Aqueous Tuber Extract Against Areca Catechu-Induced Liver Impairment in Albino Wistar Rats

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ABSTRACT

Background: Areca catechu, one of the world's most consumed psychoactive substances, has been reported to possess genotoxic, mutagenic and carcinogenic properties. Ipomoea batatas, while also commonly consumed, has several nutritional and functional properties. **Aim:** This study aimed to investigate Ipomoea batatas' tuber extract's hepatoprotective properties against Areca catechu-induced liver impairment in albino Wistar rats through analysis of enzymatic antioxidants, liver enzymes and histoarchitecture of the liver. **Methods:** Thirty adult Wistar rats weighing between 180g-260g, bred in the Animal House of the Department of Anatomy, University of Benin were randomly assigned into groups. Group A, B, C, D, E, and F received 1 ml of distilled water, 500 mg/kg of Ipomoea batatas aqueous extract, 1500 mg/kg of Areca catechu aqueous extract only, 1500 mg/kg of Areca catechu aqueous extract and left without further intervention, 250 mg/kg of Ipomoea batatas aqueous tuber extract with 1500 mg/kg of Areca catechu aqueous extract and 500 mg/kg of Ipomoea batatas aqueous tuber extract with 1500 mg/kg of Areca catechu aqueous extract, respectively. The study lasted for a period of eight weeks. **Results:** In addition to portal hepatitis and vascular distortion, significant increases ($P < 0.05$) in the mean concentrations of liver enzymes (ALP, AST, ALT) and MDA were recorded after the administration of *A. catechu* (in groups C and D) while *I. batatas* helped prevent such phenomena (in groups E and F). **Conclusion:** Results from this study re-affirms that Areca catechu has liver-impairing properties which could be attributed to its arecoline content while Ipomoea batatas is rich in antioxidants as depicted by the histology and biochemical assays

Keywords: Areca catechu, Ipomoea batatas, Liver, Wistar rats.



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INTRODUCTION

Areca catechu L. belongs to the Palmaceae family. It is a one-trunked, slender monoecious palm that has a noticeable crown shaft. Its endosperm or seed is called Areca nut.^[1] Areca palm can grow up to a height of 30 meters. Areca catechu is mainly planted in much of the tropics, Asia, and parts of Africa for its seed which is masticated in its raw, ripened or processed form, while its husks are used as herbal “chewing sticks” as an alternative to toothbrushes, for the maintenance of oral health and oral hygiene. Areca nut, in its dried form, has been reported by Indians to fortify the gums, purify the breath and could also be used as a dentifrice.^{[2],[3]} It is commonly found in the tropical and subtropical parts of the world.^[4] Due to its stimulating properties, approximately one-tenth of the world's population chew Areca nut, thereby making it one of the most consumed psychoactive substances.^[5] However, despite a copious amount of literature showing benefits of its consumption,^{[6],[7],[8]} Areca nut has been associated with the occurrence of cancer and lesions.^{[9],[10]} In animals fed Areca nut diet for twelve (12) months, hyperplastic changes were observed in oral, oesophageal, and stomach epithelium while a number of the experimental animals also developed oesophageal carcinoma.^{[9],[10]} Conversely, *Ipomoea batatas* (sweet potato) which is a member of the Convolvulaceae family is rated the seventh (7th) most important crop in the world as a result of its numerous nutritional and functional properties.^[11] *I. batatas* is a dicotyledonous plant. Its tubers stem from swollen roots. Its crude protein content is reportedly between 1 ~3% and 10%, but this includes 10~15% non-protein nitrogenous components.^[12] It is an important root vegetable with sweet taste

and high starch content.^[13] Vitamins such as riboflavin, niacin, thiamin (vitamin B1), pyridoxine (vitamin B6), pantothenic acid (vitamin B5) are present in *I. batatas*.^[14] In addition, other compounds such as polyphenols (anthocyanins) and phenols (caffeic, monocaffeoylquinic, dicaffeoylquinic, and tricaffeoylquinic acids)^{[11],[15]} proteins, trace elements (zinc, calcium and iron), and triterpenes (boehmeryl acetate and β -carotene) are also present.^[13]

The tubers and leaves of *I. batatas* have a minimum of six (6) polyphenolic compounds and fifteen (15) anthocyanins with the leaves and aerial region of the plant said to possess more polyphenols than other commercial vegetables such as lettuce, cabbage, and spinach.^[16] *Ipomoea batatas* have been reported to exhibit antidiabetic, anti-anaemic, anti-hypertensive,^[17] antifungal, bactericidal, laxative, anti-inflammatory and aphrodisiac properties.^[18] Studies of preliminary phytochemical screening of the aqueous extract of Areca nut showed a positive result for the presence of alkaloids, saponins, phenol, reducing sugar, triterpinoids, and glycosides and absence of flavonoids, protein, coumarin, tannins, phytosterols, anthroquinone, anthrocyanides, phlobatannins^[19] while that of *Ipomoea batatas* revealed the presence of phytoconstituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch and tannins.^{[20],[21]} Due to the aforementioned benefits of *Ipomoea batatas*, this present study has been designed to investigate its hepatoprotective effects against Areca catechu-induced liver impairment in Wistar rats by analysing enzymatic antioxidants, liver enzymes and histoarchitecture of the liver.

MATERIALS AND METHODS

Plant materials Nuts of Areca catechu were obtained from Areca palm in the University of Benin, while Ipomoea batatas was sourced from the local market in Egor Local Government Area of Benin City. Both plant materials were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. Specimens were also stored for record purposes. The tuber of I. batatas was used for this study. After the collection of the plants, they were air-dried for two (2) weeks, pulverized, and the aqueous extract of both plants were obtained according to the standard.^[10] 20 g of Areca catechu was dissolved in 200 ml of distilled water and 20 g of I. batatas was also dissolved in 200 ml of distilled water. Animals Thirty adult Wistar rats weighing between 180 g - 260 g, bred in the Animal House of the Department of Anatomy, University of Benin were randomly assigned into groups. The animals were acclimatized for two (2) weeks before the commencement of the study. They were housed in six (6) plastic cages containing five (5) animals each. Feed and water were provided ad libitum for

the entire duration of the research. The experimental protocols were carried out to conform to the acceptable guidelines on the ethical and humane use of animals in research.^[22] Ethical approval was sought from the Research and Ethics Committee, College of Medical Sciences, University of Benin, and REC approval number CMS/REC/2021/203 was obtained.

Experimental design

Sample collection Following the administration of A. catechu and I. batatas, the rats were sacrificed via chloroform anaesthesia method. Blood samples from the heart were collected in plain bottles, and serum was separated at 2500 rpm for fifteen (15) minutes and biochemical assays (liver function tests and oxidative stress) were carried out. The liver tissues from the experimental animals were harvested, weighed, and promptly fixed. The level of lipid peroxidation in tissues was assessed by quantifying malondialdehyde (MDA) concentrations with the thiobarbituric acid reactive substance (TBARS) method described by Varshney and Kale.^[23] Catalase (CAT) activity was

Table 1: showing experimental design

GROUPS	DOSAGE
GROUP A	1 ml of distilled water
GROUP B	500 mg/kg of Ipomoea batatas aqueous tuber extract
GROUP C	1500 mg/kg of Areca catechu aqueous extract only
GROUP D	1500 mg/kg of Areca catechu aqueous extract only + Withdrawal
GROUP E	250 mg/kg of Ipomoea batatas aqueous tuber extract + 1500 mg/kg of Areca catechu aqueous extract
GROUP F	500 mg/kg of Ipomoea batatas aqueous tuber extract + 1500 mg/kg of Areca catechu aqueous extract

**The animals in Group D were administered with the extract for four (4) weeks and left to recover naturally for the remainder of the experiment. This was done to determine if the changes induced by A. catechu were reversible without any intervention.*

The study lasted for a period of eight (8) weeks and all administrations were through a gavage.

determined by the method of Sinha.^[24] Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich^[25] while glutathione peroxidase (GPx) activity was determined by the method of Rotruck et al.^[26] ALT and AST activities were assayed by Reitman and Frankel^[27] method while ALP activities were measured by an optimized standard method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie.^[28] Serum protein levels were measured by the Biuret method^[29], serum albumin levels were measured by Doumas and Biggs^[30] method while the amount of globulins was calculated as a difference between total serum proteins and serum albumin. Total and direct bilirubin levels were assayed by Jendrassik and Grof^[31] method.

Histopathology

The liver tissues were excised and stored in bottles containing 10% neutral buffered formalin, ready for tissue processing. After fixation, the liver tissues were dehydrated in ascending grades of alcohol (70%, 90% and 100%), cleared in xylene, and embedded in paraffin wax. The deparaffinized sections were stained routinely with Hematoxylin and Eosin.^[32] Photomicrographs of the desired sections at x100 and x400 magnifications were taken by a histopathologist, using a standard photomicrography setup, namely: Olympus XSZ-107BN microscope and an attached 14MP Eakins 1080P microscopic camera.

Statistical analysis

Data were subjected to statistical analysis using the IBM SPSS statistical software (Version 25), New York, United States of America, and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean \pm SEM. LSD posthoc test

was used. Values of $P < 0.05$ were considered significant. The statistical values obtained were converted into tables.

RESULTS

Plates 1 and 2 are photomicrographs of the control group which received 1 ml of distilled water only at x100 magnification and x400 magnification, respectively. They showed normal histoarchitecture with distinct hepatocytes, sinusoids and normal portal vascular microstructure. Plates 3 and 4 are photomicrographs of the liver tissue of the experimental animals in Group B, at x100 magnification and x400 magnification, respectively. They exhibited activation of the local immune system (Kupffer cell activation) while also showing normal hepatocytes and normal portal vascular microstructure. Plates 5 and 6 are photomicrographs of the liver tissues of the animals in Group C, at x100 magnification and x400 magnification, respectively. The hepatic tissues showed heavy/intense periportal inflammatory infiltrates, (portal hepatitis) prominent nucleolus in hepatocyte nuclei and vascular distortion. In Plates 7 and 8, conspicuous nucleoli in the nuclei of hepatocytes, heavy periportal inflammatory infiltrates and severe Kupffer cell activation are seen. The plates (7 and 8) are photomicrographs representing Group D. Plates 9 and 10 are photomicrographs representing the hepatic tissues of the animals in Group E, at x100 magnification and x400 magnification, respectively. They show normal hepatocytes and normal portal microstructure. Plates 11 and 12 are photomicrographs representing the hepatic tissues of the animals in Group F, at x100 magnification and x400 magnification, respectively. They exhibit sinusoidal congestion and periportal inflammatory infiltrates.

Table 2: Levels of enzymatic antioxidants

	Control	<i>I. batatas</i> only	<i>A. catechu</i> only	<i>A. catechu</i> withdrawal	<i>I. batatas</i> (low dose) + <i>A. catechu</i>	<i>I. batatas</i> (high dose) + <i>A. catechu</i>	P- value
SOD (U/ml)	9.53±0.39	12.61±0.55*	7.17±0.48*	7.67±0.44*	10.41±0.55	10.75±0.41	0.001
MDA (x10 ⁻³ mmole/ml)	27.46±1.23	22.36±1.22*	34.52±1.25*	31.67±0.62	25.26±1.21	25.51±0.66	0.009
CAT (U/ml)	166.62±2.19	175.64±0.96*	159.35±0.77*	160.64±0.44*	165.51±1.26	167.61±1.23	0.002
GPx (U/ml)	123.44±2.18	136.63±0.79*	111.63±1.24*	115.35±1.20*	128.29±1.21	130.45±1.15*	0.000

*Significantly different from the control group at $P < 0.05$
The results are mean of five rats in each group \pm SEM

Table 3: Levels of liver function

	Control	<i>I. batatas</i> only	<i>A. catechu</i> only	<i>A. catechu</i> withdrawal	<i>I. batatas</i> (low dose) + <i>A. catechu</i>	<i>I. batatas</i> (high dose) + <i>A. catechu</i>	P- value
ALP (U/L)	208.67±12.35	145.50±24.50*	281.67±23.95*	159.00±6.00	195.00±10.00	178.00±22.00	0.008
AST (U/L)	129.67±6.89	125.42±15.77	164.46±9.31*	158.50±7.50	56.00±14.00*	99.50±33.50	0.108
ALT (U/L)	94.33±21.74	85.00±16.00	147.67±2.33*	115.50±2.50	106.86±5.23	101.50±3.50	0.014
Total protein (g/dl)	7.60±0.10	7.15±0.25	7.23±0.33	7.25±0.45	8.25±0.15	7.65±0.25	0.179
Albumin (g/dl)	3.27±0.12	3.55±0.15	3.27±0.12	3.40±0.10	3.70±0.10	3.15±0.15	0.349
Globulin (g/dl)	4.33±0.17	3.60±0.10	3.97±0.28	3.85±0.35	4.55±0.25	4.50±0.10	0.138
Total bilirubin (g/dl)	0.27±0.03	0.25±0.05	0.37±0.03	0.35±0.05	0.25±0.05	0.40±0.10	0.260
Conjugated bilirubin (g/dl)	0.17±0.03	0.10±0.00	0.17±0.03	0.15±0.05	0.10±0.00	0.25±0.05	0.164

*Significantly different from the control group at $P < 0.05$

DISCUSSION

The fourth (4th) most consumed social drug in the world is Areca nut, with only nicotine, ethanol and caffeine ranked higher.^[5] As several studies have reported, Areca catechu, though commonly masticated for various reasons, is also mutagenic and genotoxic.^{[33],[34],[35]} Areca nut components and arecoline, a main Areca alkaloid, have long been considered to be the major etiologic factors in the pathogenesis of oral and oropharyngeal cancers.^[36] As a matter of fact, arecoline has cell-transformation ability, which is a good predictor of animal carcinogenicity.^[37] Ipomoea batatas is

thought to be a highly nutritious food. Several people enjoy its health benefits and wonderful flavour. It is also a good source of vitamins and trace elements which are highly effective antioxidants.^[38] This research was designed and embarked upon in order to determine the effects of Ipomoea batatas on Areca catechu-induced liver impairment. Their effects were analysed individually and mutually. Results from the analysis of enzymatic antioxidants (Table 2) showed that the levels of superoxide dismutase, glutathione peroxidase and catalase were

significantly increased in the groups that received I. batatas only (Group B), while Groups C and D showed a decrease instead. However, for malondialdehyde (MDA), a significant decrease was observed in Group B while a significant increase was observed in Group C.

For liver function tests, the group that received Areca catechu only (Group C) showed significantly elevated levels of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, the group that received I. batatas only demonstrated a significant reduction in the level of alkaline phosphatase only, while in Groups E and F, results showed that the administration of I. batatas in low and high doses thwarted the effects of A. catechu on liver enzymes.

Reactive oxygen species play a crucial role in several pathological and physiological processes, including carcinogenesis.^{[39],[40]} Areca nuts have been convincingly demonstrated to produce reactive oxygen species which characteristically have deleterious effects.^{[41],[42]} When copious amounts of reactive oxygen species are produced, membrane lipids, protein and DNA are attacked and this causes damages to the tissues and cells, inevitably leading to a deterioration in cell function and eventually, clinical diseases.^{[40],[43]} It is therefore established that reactive oxygen species play a vital role in the initiation and promotion of cancer.^[44] Cells have been known to provide a bulwark to protect against attacks by reactive oxygen species. They do this through the incorporation of endogenous antioxidant enzymes such as glutathione peroxidase, glutathione, superoxide dismutase and catalase which provide a first line of defence inside the body against reactive oxygen species.^{[40],[45]} The group administered with I. batatas showed a significant increase in the levels

of these beneficial antioxidants, while also preventing a decrease in their levels, after the administration of Areca catechu, when compared with the control group. Liver function tests assess the functionality and integrity of the liver. Elevations in the serum concentrations of aminotransferases point towards liver impairment. Despite alanine aminotransferase and aspartate aminotransferase being present in the red blood cells, brain, kidneys, heart, skeletal muscle and liver, alanine aminotransferase is considered to be more specific for hepatocellular injury, as it has low concentrations in the kidneys and skeletal muscle.^[46] In the present study, the concentrations of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were significantly elevated after the administration of Areca nuts. However, the pre-treatment with I. batatas protected against such elevations. Bilirubin is known to have toxic effects at high concentrations^[47]. Bilirubin has been regarded for many years as cytotoxic, mainly because of its association with neonate jaundice and its possibility of provoking irreversible damage at high concentration^[48]. However, in the present study, there were no statistically significant differences in the levels of bilirubin across the groups. Furthermore, no statistically significant differences in the levels of total protein across the groups were recorded after administration. This suggests that A. catechu only affects the liver enzymes. The histology of chewers of Areca nuts has shown that inflammatory cell infiltration is a regular occurrence.^{[49],[50]} A similar phenomenon was observed in our study after the administration of Areca nut, and the administration of Ipomoea batatas prevented this incidence. Substantiating reports of the carcinogenicity, mutagenicity and genotoxicity of A. catechu, activated inflammatory cells have been

reported to release reactive oxygen species which results in the mutation of adjacent cells.^[51]

CONCLUSION

The results from this study indicate that Areca catechu has liver-impairing properties which could be attributed to its arecoline content while Ipomoea batatas is rich in antioxidants as depicted by the histology and biochemical assays. In view of the aforementioned, it may be beneficial if researchers and the pharmaceutical industry investigate further on nutritional value of I. Batatas.

Conflicts of interest

There are no conflicts of interest.

Legend for images

Plate 1 Liver of control group composed of A: hepatocytes, B sinusoids and C: central vein (H&E x100)

Plate 2 Liver of control group composed of A: hepatocytes, B sinusoids and C: central vein (H&E x400)

Plate 3 Liver given Ipomoea batatas only showing, A normal hepatocytes, B normal portal vascular microstructure and C Kupffer cell activation (H&E x 100)

Plate 4 Liver given Ipomoea batatas only showing, A normal hepatocytes, B: normal portal vascular microstructure and C Kupffer cell activation (H&E x 400)

Plate 5 Rat given Areca catechu only showing, A prominent nucleolus in hepatocyte nuclei, B heavy periportal inflammatory infiltrates and C vascular distortion (H&E x 100)

Plate 6 Rat given Areca catechu only showing, A prominent nucleolus in hepatocyte nuclei, B heavy periportal inflammatory infiltrates and C vascular distortion (H&E x 400)

Plate 7 Rat given Areca catechu and allowed reversal showing: A, conspicuous nucleoli in hepatocytes nuclei, B, heavy periportal inflammatory infiltrates and C, severe Kupffer cell activation (H&E x 100)

Plate 8 Rat given Areca catechu without intervention showing, A: conspicuous nucleoli in hepatocytes nuclei, B: heavy periportal inflammatory infiltrates and C: severe Kupffer cell activation (H&E x 400)

Plate 9 Rat given Low dose Ipomoea batatas + Areca catechu showing A: normal hepatocytes and B normal portal microstructure (H&E x 100)

Plate 10 Rat given Low dose Ipomoea batatas + Areca catechu showing A: normal hepatocytes and B normal portal microstructure (H&E x 400)

Plate 11 Rat given high dose Ipomoea batatas + Areca catechu showing, A: sinusoidal congestion and B periportal inflammatory infiltrates (H&E x 100)

Plate 12 Rat given high dose Ipomoea batatas + Areca catechu showing, A: sinusoidal congestion and B periportal inflammatory infiltrates (H&E x 400)

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