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# Effect of Areca Nut Extracts on Some Organ Markers from Rat Serum *in vivo*

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**Abstract:** Areca nut (*Areca catechu*) is one of the plant species that possess beneficial pharmacological properties. It had an important place as a pharmaceutical in Ayurveda (the ancient Indian system for medicine) as well as in the Chinese medical practices. The pharmaceutical importance of areca nut is due to the presence of an alkaloid. This study was performed to provide some scientific grounds to understand some of the biochemical and pharmacological properties of areca nut. Extraction of the active ingredients of areca nut was performed by using two solvents of different polarities (n- hexane and methanol). The extracts were tested on some organ markers from rat serum *in vivo*, including some key enzymes such as Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT), Gamma-Glutamyl Transferase (GGT), Creatine Phosphokinase (CPK); and other parameters which include glucose, cholesterol, total protein and urea. Those biochemical parameters were measured for both control and treated rats. The results showed significant changes in their activities compared to the control.

***Keywords:*** Areca nut, hexane, methanol, Wister white rat, ALP, LDH, GPT, GOT, GGT, CPK, glucose, cholesterol, total protein and urea.

## Introduction

Research interests have focused on various plant species that possess beneficial gastrointestinal properties, hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping to reduce the risk of cardiovascular diseases and cancer [1].

This study was then carried out to provide information for understanding the biochemical and pharmacological properties of areca nut.

Areca nut was known in China under the name Pinlang, from at least l00 BC. Immense quantities have been consumed in the east from very early times in the form of

a masticator known as betel which consists of a mixture of areca nuts, the leaves of *Piper betel*, and lime [2].

The pharmaceutical importance of areca nut is due to the presence of an alkaloid. Synthetic arecoline hydrobromide is also shown to possess numerous pharmacological properties [3].

Because the high incidence of oral cancers in South-East Asia is causally linked to the common habit of betel quid chewing [4, 5], the effect of an aqueous extract of areca nut on growth, differentiation, morphology and DNA damage were studied in cultured human buccal epithelial cells. The researchers concludes that betel quid carcinogenesis in the human oral cavity may involve cytopathic alterations of normal cells morphology, growth and differentiation, as well as DNA damage by areca nut-related agents extracted that are formed in saliva [4].

The modulator influence of areca nut, a masticator in several human populations, on the levels of biotransformation system enzymes in mouse liver has been studied. Areca nut-modulated profiles biotransformation enzymes and antioxidant levels are suggestive of its influence in the process of carcinogenesis induced by bioactivated electrophilic species of potential carcinogens among habitual areca nut chewers [6].

Recent works showed oxidative damage to DNA induced by areca nut extract [7]. Cytotoxic and DNA damaging effects in oral mucosal fibroblasts by areca nut extract was seen [8]. Areca nut extract alters the barrier properties of the epithelium, a factor which may play a role in the deleterious effects on oral mucosa [9], cardiac toxicities [10] and also affects the total and unscheduled DNA synthesis in cultured gingival keratinocytes [10].

Most of the work on areca nuts has been focused on its effect on the buccal cavity when used as a masticator, but few were consider its effect on the biochemical parameters in the serum. Recent paper describes its antidepressant property via monoamine oxidase type A [12].

The present paper discusses the effect of areca nuts extracts on some enzymes and biochemical parameters as organ markers from rat serum.

## Materials and Methods

### Materials:

### Spice

Samples of areca nut (*Areca catechu*) were obtained from a local market and used in this study.

**Extraction of samples**

Different dried weights of 43 and 50 gm areca nut (whole) was used for extraction. The samples were soaked for 20 days in dark bottle containing either 0.5 liter of n-hexane or 0.3 liter of methanol. By filtering through a filter paper, the residues were discarded and the crude n-hexane or methanol extracts were refiltered with Whatman filter paper (42 ashless, Whatman Ltd., UK). The solvents were removed by evaporation, at 80°C for n-hexane extract, and 90°C for methanol extract, using model R110 Buchi Rotary Evaporator [13]. The weights of the n-hexane extract and methanol extract were recorded and each sample was dissolved in 10 ml acetone each.

**Methods:**

**Animals**

Adult male Wister white rats aged 3-4 weeks and weighing 250-350 gm were obtained from the Animal House of King Fahd Medical Research Center, Jeddah, Saudi Arabia. The rat diet consisted of water and chow. The (chow) contains crude protein (20 %), fat (3 %), fiber (5.5 %), ash (6.5 %), calcium (0.8 %), salt (0.5 %), phosphorus (0.6 %), vitamin A (20 lU/g), vitamin D (2.2 IU/g), vitamin E (70 IU/kg), energy (2,850 kcal/kg) and the trace elements: cobalt, copper, iodine, manganese and zinc. The initial and final body weights were recorded for each experimental animal, and the animals were divided into three main groups as follows:

**Group one: Control rats**

a) A total of 5 normal rats were housed for 20 days and were used as control group (untreated) and was put under the same physiological conditions of test animals.

b) A total of another 5 normal rats were housed for 20 days in a separate animal cage, and were treated with corn oil only. The treatment involved a forced feeding technique of a 1.0 ml corn oil while under anesthesia by the use of a special feeding syringe (size 18 gauge) [13]. This treatment was conducted for 20 days (every other day).

**Group two: Rats treated with n-hexane extract**

A total of 5 normal rats were housed for 20 days in a separate animal cage, and were fed areca nut extract (forced feeding) at 586 mg/kg per day (the concentration that has noticeable effect) for a period of 20 consecutive days. The concentration used here is for the one seen to induce effect on markers under study.

**Group three: Rats treated with methanol extract**

A total of 5 normal rats were housed for 20 days in a separate animal cage, and were fed areca nut extract (forced feeding) at 775 mg/kg per day (the concentration that has noticeable effect) for a period of 20 consecutive days. The concentration used here is for the one seen to induce effect on markers under study.

**Collection of blood samples and separation of serum**

At the end of each experiment (3 trials), blood samples were collected from rats by cardiac punctures under mild ether anesthesia, into plain tubes. The serum was then separated by centrifugation at 3000 xg for 20 min and used for the determination of serum enzymes and other biochemical parameters.

**Determination of serum enzymes and other biochemical**

Six key enzymes were analyzed to determine the effect of the areca nut extract *in vivo*. These enzymes are: Alkaline phosphatase (ALP, EC 3.1.3.1), Lactate dehydrogenase (LDH, EC 1.1.1.27), Glutamate pyruvate transaminase (GPT, EC 2.6.1.2), Glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), γ- Glutamyltransferase (GGT, EC 2.3.2.2) and Creatinine phosphokinase (CPK, EC 2.7.3.2), and other biochemical parameters (glucose, cholesterol, total protein and urea) were measured for control and treated rats, by the use of an automated system (Boehringer Mannheim Hitachi Spectrophotometer, model 4020) at King Khalid Hospital, Jeddah-Saudi Arabia.

**Statistical analysis**

The data collected entered into a computer, and analysis of data was performed using SPSS statistical package. T-test was used for comparing means [14] and X2-test (chi s-test) was used for comparing the frequency of occurrence of variable in two or more occasions. P-value was considered to be statistically significant if <0.05.

## Results

Table 1 shows the dry weight of areca nut used in this study and its n-hexane and methanol extracts. Extraction of the active ingredients of areca nut was performed by using two solvents of different polarities (n- hexane and methanol). The yield of n-hexane extract was 7.20% whereas the yield of methanol extract was 7.28%. The experimental dosage used here (586 and 775 mg/kg per day, respectively) were chosen because of their noticeable effect on biochemical parameters under study.

**Table 1. The weight of various areca nut extracts used in the *in vitro* enzymatic studies**

| Samples | Dry weight of sample (gm)* | Dry weight of extract (gm)** | % |
|---|---|---|---|
| Areca (whole) extract in n-hexane | 43.30 | 3.12 | 7.20 |
| Areca (whole) extract in methanol | 49.30 | 3.59 | 7.28 |

Table 2 shows the effect of n-hexane and methanol extracts of areca nut on six serum enzymes namely; ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase), GPT (Glutamate pyruvate transaminase), GOT (Glutamate oxaloacetate transaminase), GGT (Gamma-glutamyl transferase) and CPK (Creatine phosphokinase).

**Table 2. Effect of Areca Nut Extracts (in corn oil) on Some Rat Serum Enzymes *in vivo***

| Sample[b] | | [a] ALP (U/ml) | LDH (U/ml) | GPT (U/ml) | GOT (U/ml) | GGT (U/ml) | CPK (U/ml) |
|---|---|---|---|---|---|---|---|
| Control | (mean) | 138.3 | 1173.3 | 62.0 | 195.3 | 10.67 | 1049.3 |
| | (SD) | ±0.2 | ±4.4 | ±0.8 | ±3.3 | ±0.70 | ±3.3 |
| | % | 100 | 100 | 100 | 100 | 100 | 100 |
| n-hexane extract | (mean) | 107.0* | 521.3** | 48.0* | 117.0** | 7.0* | 505.0** |
| | (SD) | ±1.7 | ±7.2 | ±3.6 | ±0.8 | ±0.2 | ±0.9 |
| | % | 77.36 | 44.43 | 77.42 | 59.9 | 65.6 | 48.12 |
| Methanol extract | (mean) | 118.0* | 270.33** | 37.0** | 111.0** | 1.33** | 382.0** |
| | (SD) | ±0.66 | ±0.65 | ±1.00 | ±0.21 | ±0.31 | ±0.51 |
| | % | 85.3 | 23.04 | 59.67 | 23.22 | 12.46 | 36.40 |

[a]ALP: Alkaline Phosphatase; LDH: Lactate Dehydrogenase; GPT: Glutamate Pyruvate Transaminase; GOT: Glutamate Oxaloacetic Trans-aminase; GGT: Gamma-Glutamyl Transferase; CPK: Creatine Phos-phokinase.
[b]Areca extract in n-hexane (586 mg/kg).
Areca extract in methanol (775 mg/kg).
(n.s) = Non-significant; P>0.05; * = Significant; P<0.05; ** = Highly significant; P<0.01.

Variations were observed in the enzyme activities of animals treated with hexane extract. A decrease in ALP activity (23 %; P<0.05), LDH (56 %; P<0.01), GPT (23 %; P<0.05), GOT (40 %, P<0.01), GGT (34 %; P>0.05) and CPK (52%, P<0.01) when compared to control animals was obtained. Whereas, animals treated with areca nut extract (methanol) dissolved in corn oil produced different results. A decrease in ALP activity (15 %; P>0.05), LDH (77 %; P<0.01), GPT (40 %; P<0.01), GOT (77 %; P<0.01), GGT (87 %; P<0.05) and CPK (64 %; P<0.01) when compared to control animals was obtained.

Table 3 shows the effect of n-hexane and methanol crude extract of areca nut on some biochemical parameters of rat serum (glucose, cholesterol, total protein and urea).

**Table 3. Effect of areca nut xxtracts (in corn oil) on some biochemical components of rat serum *in vivo*.**

| Sample | | Glucose mmol/l | Cholesterol mmol/l | Total Protein (g/l) | Urea mmol/l |
|---|---|---|---|---|---|
| Control | (mean) | 7.70 | 1.54 | 53.67 | 5.5 |
| | (SD) | ±1.39 | ±0.06 | ±0.06 | ±0.1 |
| | % | 100 | 100 | 100 | 100 |
| n-hexane extract | (mean) | 14.83* | 2.60* | 63.67* | 7.1 (n.s) |
| | (SD) | ±0.72 | ±0.05 | ±0.51 | ±0.04 |
| | % | 192.59 | 168.83 | 118.63 | 129.1 |
| Methanol extract | (mean) | 8.93 (n.s) | 1.26* | 61.00* | 6.9 (n.s) |
| | (SD) | ±0.85 | ±.25 | ±0.58 | ±0.3 |
| | % | 108.96 | 81.81 | 113.65 | 125.45 |

Areca extract in n-hexane (586 mg/kg).
Areca extract in methanol (775 mg/kg).
(n.s) = Non-significant; P>0.05; * = Significant; P<0.05; ** = Highly significant; P<0.01

Animals treated with areca nut extract (n-hexane) dissolved in corn oil produced different results. An increase in the glucose concentration (93 %; $P<0.05$), cholesterol (69 %; $P<0.05$), total protein (19 %; $P<0.05$) and urea (29%; $P>0.05$) was observed compared to control animals. Whereas, animals treated with areca nut extract (methanol) dissolved in corn oil produced different results. An increase in the glucose concentration (9 %; $P>0.05$), total protein (14 %; $P<0.05$) and urea (25 %; $P<0.05$) was observed. Whereas, reduction in cholesterol level by (18 %; $P>0.05$) was observed in comparison to control animals.

## Discussion

To understand some of the biochemical and pharmacological properties of areca nut, this study was conducted by extraction of the active ingredients using two solvents of different polarities and testing the effect of the extracts on some biochemical markers and parameters from rat serum *in vivo*.

The extraction of the areca nut by n-hexane solvent yielded (7% based on dry weight) which contains variable amounts of lipid soluble fraction. Areca nut is rich in fats [15]. In general, the lipid fraction of areca contains a major alkaloid (arecoline) and several other non-polar substances such as tannins, tannic acid and catechin, in addition to triacylglycerols, fatty acids, vitamin A and carotenes [3].

The use of a more polar solvent system such as methanol to extract the active ingredients from areca nuts which gave a slightly higher yield (7.28%). Methanolic fraction from areca contains carbohydrates (mainly sucrose, several reducing sugars, galactan, and mannan) [4]. In addition, proteins, saponins, gums and mineral salts are present in this extract. The methanolic extract also contains small amounts of other less polar compounds, such as arecoline [3].

Areca nut extract (in n-hexane and methanol) produced in general an inhibitory effect on most of the serum enzymes tested (Table 2). LDH was the most enzyme inhibited, whereas GPT was the least inhibited. Similar results were obtained with other spices such as garlic (*Allium sativum*) and ginger (*Zingiber officinale*) [16]. When these spices were fed to rats, they caused significant reduction in ALP activity as well as other blood constituents [16].

The activity of LDH on the other hand, was significantly reduced by the oily extracts of areca nut. LDH activity is elevated in general in a wide variety of diseases such as myocardial infraction, liver and kidney damage and cancer. Since the enzyme is present in high amount in red blood cells, the increase of activity is often associated with increased erythrocyte breakdown [17].

The methanolic areca extract tested showed no significant hypoglycemic effect. This finding is similar to a previous study made by Khan *et al*., (1995) [18] who tested several plants spices for their potential use as hypoglycemic factors. However, they

indicated that only cloves added to rat diet at 10 % (w/w) potentate insulin activity to a certain extent. Other spices are also known for their hypoglycemic action, such as curry leaf (*Murraya koenigii*) and mustard seeds (*Brassica juncea*) [19].

On the other hand, n-hexane extract produced a significant hyperglycemic effect, which caused an increase of 93% in glucose concentration. It is well known that serum glucose level increases in cases of stress, hypothyroidism, pregnancy and diabetes mellitus [17].

The normal value of total cholesterol in rat serum was found to be 1.54 mmol/L, which is significantly lower than that of a human serum (3.9-6.5 mmol/L). The areca extract (in n-hexane) which is rich in fatty substances, causes significant increase in total cholesterol (70 % increase at a concentration of 586 ppm) as compared to methanolic extract which had a slight hypocholestermic effect (18 % decrease at a concentration of 613 ppm). It was shown that feeding rats for 90 days on a standard diet plus 10 % mustard seeds, resulted in a reduction of total serum cholesterol and other lipids such as LDL (low density lipoprotein) and VLDL (very low density lipoprotein) [19]. It is suggested that this reduction in cholesterol may be due to the increased activity of lecithin cholesterol acyltransferase (LCAT). Other spices such as curcumin and red pepper, are known to increase both serum cholesterol and liver microsomal cholesterol [20]. Whereas spices such as ginger (*Zingiber officinale*) and fenugreek ( *Trigonella foenumgraecum*) had no significant effect on blood sugar or blood lipids [21].

In general areca extract increased the total protein content of serum by 12 %. No data is available in literature regarding the effect of spices on plasma protein. Yet, it is clinically known that serum total protein increases in case of certain diseases such as multiple myeloma [17].

The normal urea content of rat serum was found to be 5.5 mmol/L. In general, there are no significant changes of the rat serum urea when treated with areca nut extract.

Taken liver as an example, since liver is a versatile organ, which is involved in metabolism and independently involved in many other biochemical functions. Liver is the key organ and the principal site where the metabolism of carbohydrates, lipids and proteins take place. Liver is the organ where ammonia is converted to urea. It is the principal organ where cholesterol is synthesized and catabolized to form bile acid and bile salts. Since liver is involved in removal of sugars by glycogenesis or in conversion of other monosaccharides to glucose, therefore, a change in blood glucose level may indicate among another reason a liver damage. Since liver is the site of albumin synthesis and also some of $\alpha$- and $\beta$-globulins, therefore, determination of total plasma protein, albumin and A/G ratio might gave useful information in chronic liver diseases.

The liver plays an active and important role in the metabolism of cholesterol including its synthesis, estrification, oxidation and excretion. Total lipid cholesterol may

be an indication of obstructive jaundice and severe acute hepatic necrosis. Increases in both transaminases are found in liver diseases or in toxic hepatitis, with GPT is much higher than GOT. Alkaline phosphatase is used in differential diagnosis of jaundice. It is increased in both infectious hepatitis (viral hepatitis) and post hepatic jaundice (extra hepatic obstruction) but the rise is usually much greater in cases of obstructive jaundice, xantoniatous biliary cirrhosis and primary carcinoma. Lactate dehydrogenase increased activity might be an indication of an infectious hepatitis [17].

Generally, there are changes in most of the studied parameters. The biochemical and pharmacological properties of areca nut obtained from this study is considered preliminary. Each parameter should be taken separately and analyzed further in order to shed more light on its properties.

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# تأثير مستخلص نبات الفوفل على بعض المقاييس البيوكيميائية لمصل الفأر

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(قدّم للنشر في ١٤٢٠/١١/١٧هـ ؛ وقبل للنشر في ١٤٢٢/٧/٢٩هـ)

**ملخص البحث**. نبات الفوفل هو أحد أجناس النبات الذي يحتوي على خواص صيدلانية مفيدة. أجريت هذه الدراسة للحصول على أساس علمي لفهم بعض الخصائص البيوكيميائية والصيدلانية لنبات الفوفل. تم استخلاص الجزء الفعال لنبات الفوفل (*Areca catechu*) باستخدام مذيبين ذا قطبية مختلفة (الهكسان والميثانول).

تم اختبار تأثير المستخلص على بعض المقاييس البيوكيميائية لمصل الفأر داخل الكائن الحي، والتي شملت بعض الإنزيمات الرئيسية مثل الفوسفاتيز القلوي (ALP) ، لاكتيت دي هيدروجينيز (LDH)، جلوتاميت بيروفيت ترانس امينيز (GPT)، جلوتاميت اوكسال اسيتيت ترانس امينيز (GOT)، جاما– جلوتاميل ترانسفيريز (GGT)، كرياتين فوسفوكاينيز (CPK)، وبعض المقاييس الأخرى والتي شملت الجلوكوز ، الكوليسترول ، البروتين الكلي واليوريا. تم قياس هذه المقاييس البيوكيميائية لكلاً من مجموعة الفئران الضابطة والمعالجة وأظهرت النتائج تغيرات معنوية في النشاطية الإنزيمية مقارنة بالمجموعة الضابطة.