Effect of *Cyperus esculentus* L. (Tiger Nut) Milk on Hepatic and Renal Indices of Wistar Rat

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Authors’ contributions

This work was carried out in collaboration between both authors and both authors read and approved the final manuscript.

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ABSTRACT

Background: Recently, death arising from hepatic and renal related diseases is on the increase; thus, several researches have focused on plant materials with hepato- and nephron-protective potentials.

Aim: This study therefore sought to investigate the effect of tiger nut on hepatic and renal indices of Wistar rats.

Materials and Methods: Twenty-four Wistar rats were divided into four groups of six each. Animals in group A were administered normal saline solution while those in groups B, C and D were administered 1, 2 and 4 mL/kg body weight of undiluted tiger nut milk respectively for twenty-eight days. Administration was done 12 hourly via oral route. At the end of the administration, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture. Hepatic and renal indices were determined using standard methods.

Results: Tiger nut milk was observed to decrease the activities of serum AST, ALT and ALP (hepatic biomarker enzymes). The decrease was only significant (p<0.05) when the activities of AST and ALT of animals treated with 4 mL/kg body weight of tiger nut milk were compared with those in control animals. Similarly, tiger nut milk was observed to decrease the serum concentrations of creatinine and urea. The decrease was only significant (p<0.05) when the concentration of urea of...
animals treated with 2 and 4 mL/kg body weight of tiger nut milk were respectively compared with those in control animals.

**Conclusion:** Observations from this study showed that tiger nut has positive effect on hepatic and renal indices; thus possesses hepatoprotective and nephroprotective potentials. Consumption of this plant tuber is recommended for healthy liver and kidneys.

**Keywords:** Hepatoprotective; nephroprotective; plant materials; tiger nut.

1. **INTRODUCTION**

Liver and kidney are the major organs which play key roles in processing critical biochemical and physiological phenomena including metabolism and detoxification of endogenous and exogenous compounds, such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply [1]. Hepatic and renal injury could occur by hepato/nephrotoxic agents such as drugs, alcohol, hydrocarbon and viral infections [2]. Liver diseases like jaundice, cirrhosis and fatty liver as well as renal diseases such as kidney stone have been public health concern across the world. Prevalence of chronic liver disease worldwide is 18.5% and cirrhosis is 4.5 to 9.5% while 2 million people die each year. In terms of medication, conventional or synthetic drugs are limited. Moreover they can have serious side effects [3,4]. Due to this fact, a huge number of medicinal plants have been used to figure out hepatic and renal protective activities [5]. Approximately 160 phytochemical constituents originated from 101 plants have been reported to be potentially hepatic and renal protective [6]. At present, medicinal plants have been a vital source of treatment of liver and renal diseases [7,8].

*Cyperus esculentus* L. (also called tiger nut, chufa, atadwe, yellow nutsedge, and earth almond) is a crop of the sedge family widespread across much of the world. It is found in most of the Eastern Hemisphere, including Southern Europe, Africa, as well as the Middle East and the Indian subcontinent [9]. In Nigeria, tiger nut is called “Ofio” by the Yorubas, “Akiausa” by the Igbos and “Aya” by the Hausas. It is cultivated for its edible tubers, called earth almonds or tiger nuts, as a snack food and for the preparation of *horchata de chufa*, a sweet, milk-like beverage. Tiger nut can be found wild, as a weed, or as a crop. It is an invasive species outside its native range, and is readily transported accidentally to become invasive. In many countries, tiger nut is considered a weed [10]. It is often found in wet soils such as rice paddies and peanut farms as well as well-irrigated lawns and golf courses during warm weather.

**Chart 1. The Scientific classification of *C. esculentus* is**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clade</td>
<td>Tracheophytes</td>
</tr>
<tr>
<td>Clade</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>Clade</td>
<td>Monocots</td>
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<tr>
<td>Clade</td>
<td>Commelinids</td>
</tr>
<tr>
<td>Order</td>
<td>Poales</td>
</tr>
<tr>
<td>Family</td>
<td>Cyperaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Cyperus</td>
</tr>
<tr>
<td>Species</td>
<td><em>C. esculentus</em></td>
</tr>
</tbody>
</table>

Despite its name, tiger nut is a tuber. However, its chemical composition shares characteristics with tubers and with nuts. This tuber is rich in energy content (starch, fat, sugar, and protein), and dietary minerals (mainly phosphorus and potassium). The oil of the tuber was found to contain 18% saturated (palmitic acid and stearic acid) and 82% unsaturated (oleic acid and linoleic acid) fatty acids [11].
Tiger nut is a good source of calcium, iron, magnesium, phosphorus, ascorbic acid (Vitamin C), tocopherol (Vitamin E), dietary fibre as well as fats like oleic acid [11]. Tiger nut can be used to make a drink called kunnu aya by the Hausas. Tiger nut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called tiger nut drink [11]. A good way of incorporating the nut into your diet for those asking how to eat tiger nut is by eating them or by blending it and draining to make Kunnu Aya (tiger nut milk) which can also be sweetened with honey or date. Ogbuagu and Airaodion [12] recently observed that tiger nut milk boosts male fertility. In another study, Airaodion and Ogbuagu [13] reported the haematopoietic potential of tiger nut. This study therefore sought to investigate the effect of tiger nut on hepatic and renal indices of Wistar rats.

2. MATERIALS AND METHODS

2.1 Extraction of Milk

Fresh tiger nut was purchased from a local market in Orita-Challenge Area of Ibadan, Nigeria. They were thoroughly washed in running tap water to remove contamination. The milk was extracted daily using electric juice extractor, and was preserved in the refrigerator at 4 °C before usage as described by Ogbuagu and Airaodion [12].

2.2 Experimental Design and Animal Treatment

Twenty-four adult male Wistar rats with body weight between 150 and 180 g were used for this study. They were acclimatized for 7 days during which they were fed ad libitum with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four groups of six rats each. Animals in group A were administered normal saline solution while those in groups B, C and D were administered 1, 2 and 4 mL/kg body weight of undiluted tiger nut milk respectively for twenty-eight days. Administration was done 12 hourly via oral route. At the end of the administration, the rats were sacrificed after an overnight fast under diethyl ether as an anesthesia. Blood samples were collected via cardiac puncture.

2.3 Determination of Hepatic Indices

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel [14]. Alkaline Phosphatase (ALP) activity was determined by Phenolphthalein Monophosphate method described by Babson et al. [15].

2.4 Determination of Renal Indices

Creatinine concentration was determined using Jaffe reaction described by Toora and Rejagopal [16]. Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus et al. [17].

2.5 Statistical Analysis

Data were subjected to analysis using Analysis of Variance (ANOVA) with the aid of graph pad prism. Data from each parameter was expressed as mean value ± standard deviation. Data were considered to be significantly different at 95% confidence level (P<0.05).

3. RESULTS

In this study, tiger nut milk was observed to decrease the activities of serum AST, ALT and ALP (hepatic biomarker enzymes). The decrease was only significant (p<0.05) when the activities of AST and ALT of animals treated with 4 mL/kg body weight of tiger nut milk were compared with those in control animals as presented in Table 1. Similarly, tiger nut milk was observed to decrease the serum concentrations of creatinine and urea. The decrease was only significant (p<0.05) when the concentration of urea of animals treated with 2 and 4 mL/kg body weight of tiger nut milk were respectively compared with those in control animals (Table 2).

4. DISCUSSION

The liver is the main metabolic organ in the body and is considered a viable defense system against environmental toxicants (xenobiotics) and metabolic toxins [18]. In this study, tiger nut milk was observed to decrease the activities of serum AST, ALT and ALP (hepatic biomarker enzymes). The decrease was only
significant (p<0.05) when the activities of AST and ALT of animals treated with 4 mL/kg body weight of tiger nut milk were compared with those in control animals (Table 1). This means that tiger nut is not hepatotoxic. This corresponds to the findings of Ogwuike et al. [19] who reported the effects of *Cyperus esculentus* (tiger nut) on haematological and biochemical profile of male hypercholesteremic subjects in Uli, Anambra State Nigeria. This shows that tiger nut has similar effect in Wistar rats and human. Administration of tiger nut milk could have resulted in increased transcription of some genes involved in glucose uptake, glycolysis and lipogenesis [9,20]. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic adenosine monophosphate (cAMP) a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the Lac operon. cAMP is required to activate an allosteric protein called catabolite activator protein (CAP) which binds to the promoter CAP site and stimulates the binding of ribonucleic acid (RNA) polymerase to the promoter for the initiation of transcription, but cAMP must be available to bind to CAP which binds to deoxyribonucleic acid (DNA) to facilitate transcription [9,21]. In the presence of glucose, adenylase cyclase (AC) activity is blocked. AC is required to synthesize cAMP from Adenosine Triphosphate (ATP) [9,22]. Therefore if cAMP levels are low, CAP is inactive and transcription does not occur. Thus the effect of glucose in suppressing these inducible enzymes is by lowering cyclic AMP level. The administration of tiger nut in this study might have lowered cAMP in animals thus causing inhibition of these inducible enzymes. ALT is considered most reliable hepatocellular injury because it is solely confined to the liver, unlike AST and ALP which are also abundantly present in other body organs such as the kidneys, brain, and hearts [9,23]. The result of this study is in agreement with the findings of Nwawuba and Okechukwu [24] who reported the effect of *Cyperus esculentus* (tiger nut) oil on liver, kidney and hematological biomarkers in low dose streptozocin and high fat diet exposed male Wistar rats. It is also consistent with the observations of Hassan [25] who studied the potential effect of tiger nut oil on some haematobiochemical blood indices in male albino rats. This effect was exhibited by tiger nut due to its phytochemical composition reported by Ogwuike et al. [19]. It could also be due to its nutritional constituent reported by Roselló-Soto et al. [26] who investigated the nutritional and microbiological quality of tiger nut tubers (*Cyperus esculentus*), derived plant-based and lactic fermented beverages.

Alkaline phosphatase (ALP) is involved in the hydrolysis of a wide range of phosphononoester substrates. It is a marker enzyme for the plasma membrane and endoplasmic reticulum of tissues [27]. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculi, located in the plasma membrane. Increase in the activity of ALP could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital functions.

### Table 1. Effect of tiger nut milk on hepatic indices of Wistar rats after 28 days of administration

<table>
<thead>
<tr>
<th>Hepatic Indices</th>
<th>Control</th>
<th>1 mL/kg of undiluted Tiger nut Milk</th>
<th>2 mL/kg of undiluted Tiger nut Milk</th>
<th>4 mL/kg of undiluted Tiger nut Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>101.34±5.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.99±5.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.73±4.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.34±5.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>53.05±2.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.84±3.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.37±2.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>44.94±1.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>17.88±2.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.22±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.94±2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.46±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SD, where n = 6. Values with different superscripts along the same row are significantly different at P<0.05

**LEGEND:** AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, ALP = Alkaline Phosphatase

### Table 2. Effect of tiger nut milk on renal indices of Wistar rats after 28 days of administration

<table>
<thead>
<tr>
<th>Renal Indices</th>
<th>Control</th>
<th>1 mL/kg of undiluted Tiger nut Milk</th>
<th>2 mL/kg of undiluted Tiger nut Milk</th>
<th>4 mL/kg of undiluted Tiger nut Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.13±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>42.22±2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.34±1.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.82±0.94&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>34.02±1.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SD, where n = 6. Values with different superscripts along the same row are significantly different at P<0.05
process as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver [28]. The non-significant effect observed in the activity of ALP in animals treated with tiger nut is an indication that consumption tiger nut is safe to the life of cells that are dependent on a variety of phosphate esters for their vital process.

The kidneys are mainly involved in excretion of xenobiotics and related metabolites into urine. They are especially vulnerable to damage by xenobiotics [3,29]. Serum urea and creatinine levels are an indication of kidney function both in man and in rodents [30]. In this study, the integrity of the kidneys was assessed through serum creatinine and urea levels. Tiger nut milk was observed to decrease the serum levels of creatinine and urea. The decrease was only significant (p<0.05) when the concentration of urea of animals treated with 2 and 4 mL/kg body weight of tiger nut milk were respectively compared with those in control animals. This is suggestive that at these doses, tiger nut might have inhibited the synthesis of urea or limited it transport to the blood. This result is in agreement with the findings of Hassan [25] who reported a decrease in the concentrations of creatinine and urea when animals were treated with tiger nut oil. Creatinine is derived mainly from the catabolism of creatine found in muscle tissues and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. Serum creatinine is a measure of the glomerular filtration rate and is used as an index of renal function in clinical practice [4,31]. The effect of administration of tiger nut milk in the serum creatinine and urea levels observed this study might be an indication that the integrity of the kidney was not compromised. Tiger nut might have exerted this effect due to its phytochemical composition reported by Oguwike et al. [19]. This result is consistent with the findings of Nwawuba and Okechukwu [24] who reported that C. esculentus oil plays a beneficial role in mitigating renal dysfunction induced by exposure of the rats to high fat diet and low dose streptozocin.

5. CONCLUSION

Observations from this study showed that tiger nut has positive effect on hepatic and renal indices; thus consumption of this plant tuber is recommended for healthy liver and kidneys.

CONSENT

It is not applicable.
26. Roselló-Soto E, García C, Fessard A, Barba FJ, Munekata PES, Lorenzo JM, Remize F. Nutritional and microbiological quality of tiger nut tubers (Cyperus esculentus), derived plant-based and lactic


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