The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats

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Abstract
Background: Oxidative stress plays a crucial role in the development of drug induced nephrotoxicity. The study aimed to determine the nephroprotective and ameliorative effects of Carica papaya seed extract in paracetamol-induced nephrotoxicity in rats.

Objectives: To carry out phytochemical screening of Carica papaya, measure serum urea, creatinine and uric acid and describe the histopathological status of the kidneys in the treated and untreated groups.

Methods: Phytochemical screening of the extract was done. Thirty two adult male Wistar rats were divided into four groups (n= 8 in each group). Group A (control) animals received normal saline for seven days, group B (paracetamol group) received normal saline, and paracetamol single dose on the 8th day; Group C received Carica papaya extract (CPE) 500 mg/kg, and paracetamol on the 8th day, while group D, rats were pretreated with CPE 750 mg/kg/day and paracetamol administration on the 8th day. Samples of kidney tissue were removed for histopathological examination.

Results: Screening of Carica papaya showed presence of nephroprotective phytochemicals. Paracetamol administration resulted in significant elevation of renal function markers. CPE ameliorated the effect of paracetamol by reducing the markers as well as reversing the paracetamol-induced changes in kidney architecture.

Conclusion: Carica papaya contains nephroprotective phytochemicals and may be useful in preventing kidney damage induced by paracetamol.

Keywords: paracetamol, papaya, kidney.

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Introduction
Paracetamol (acetaminophen) is one of the most popular and widely used drugs for the treatment of pain and fever. It occupies a unique position among analgesic and widely used drugs for the treatment of pain because of its wide availability paired with comparably low toxicity, (compared to ibuprofen and aspirin), there are no anti-inflammatory activity and does not produce gastrointestinal damage1. Paracetamol is contained in many preparations, available as both tablet and liquid forms. It is a much higher potential for overdose because it is a metabolite of the amino acid methionine and the rate of metabolism is influenced by a number of factors including, age, weight, and hepatic disease2. Paracetamol is metabolized by the liver into one of two products; N-acetyl-p-aminophenol (NAP), which is toxic and is rapidly cleared from the body, and an active metabolite, N-acetyl-p-quinonimine (NAPQI), which is highly reactive with sulfhydryl groups on cellular protein and is responsible for paracetamol toxicity3. Although the metabolism of paracetamol is usually rapid and this would result in toxic levels being built up in the body especially if overdose is taken, it is generally considered much safer than other analgesics, such as aspirin and ibuprofen due to the fact that at normal NSAID dosages, people with compromised liver function have little or no side effects would be exerted during their function in the body4,5. A number of herbs are traditionally used in different countries for mitigation of drug or toxin induced hepatic and renal disorders6.

Studies are going on throughout the world for the search of protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body6,7. Papaya tree is grown extensively all over the tropical regions including Uganda and it is cultivated for its fruits and latex papain, an enzyme that is used in the food industry. The papaya, pawpaw, or papawpaw is the fruit of the plant Carica papaya, the common species in the genus Carica of the plant family Caricaceae. Papaya is a fruit widely consumed and served either green or ripe. Its juice is also a popular beverage, while its leaves and young stems are sometimes steamed and served as a vegetable. Papaya has digestive, antibacterial, anti-inflammatory, anti-ageing, anti proliferative, contraceptive, anti-allergic, and anti-oxidant nature helps to reduce the likelihood of atherosclerosis, strokes, diabetes, and heart attacks8,9.

Phytochemical procedure: Phytochemical screening: A preliminary phytochemical screening of the seed extract of Carica papaya was also done using standard methods of analysis (13).

Dose administration and induction of nephrotoxicity
Animals were randomly assigned into four groups (A-D) of eight rats per group (14). Group (A) animals served as the control group and were treated with normal sa-
line (5 ml/kg by gavage daily) throughout the experimental period. Group (B) animals (paracetamol group) were also treated with normal saline (5 ml/kg by gavage) daily for 7 days. Animals in group (C) were treated with 500 mg/kg body weight of the aqueous extract of Carica papaya once daily by gavage for 7 days and group (D) animals were treated with 750 mg/kg body weight of the aqueous extract of Carica papaya once daily by gavage for 7 days. On the 8th day, paracetamol suspension was given by oral route at a single dose of 1g/kg body weight to all the animals except the animals in group (A). Treatments were done between 9.00 and 10.00 a.m to minimize possible diurnal effects.

**Sampling and biochemical analysis**

Forty-eight hours after paracetamol administration, rats were subjected to diethyl ether anesthesia after the 8th day, paracetamol suspension was given by oral route at a single dose of 1g/kg body weight to all the animals except the animals in group (A). Treatments were done between 9.00 and 10.00 a.m to minimize possible diurnal effects.

**Results**

**Phytochemical screening**

The crude extract of Carica papaya seed was tested for the most common phytochemical constituents of medicinal plants for which nephroprotective activity of other plants have been attributed. These included; saponins, tannins, flavonoids, glycosides, terpenoids, alkaloids, reducing sugars, steroids, proteins, fats and polyphenols. The results showed that the most abundant phytochemicals were tannins, alkaloids, phenols, vitamins, flavonoids and terpenoids as shown in table 1:

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
</tr>
<tr>
<td>Fats</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Vitamins</td>
<td>+</td>
</tr>
<tr>
<td>Sterols and Triterpenes</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: +++ abundant, ++ moderate, + trace

**Biochemical markers.**

Serum creatinine, uric acid and urea concentrations were significantly increased (p< 0.0001) in paracetamol treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. (Fig.1, 2 & 3). Treatment with the aqueous extract of Carica papaya showed significant decrease in concentrations of serum creatinine, uric acid and urea compared to the paracetamol treated group.

Comparison between the control group (group A) and the groups that were treated with the extracts (group C and D) did not show any significant difference in creatinine concentrations (Fig.1), neither did comparison between the control group (A) and group (D) show any significant difference for uric acid levels though these were slightly lower in group D (Fig.2).

**Histopathological examination**

Samples of kidney tissue from each group were fixed immediately in 10% formalin for a period of 48 hours, dehydrated in graded (50-100%) alcohol, embedded in paraffin, cut into 5µm thick sections and stained with Haematoxylin-eosin. These sections were evaluated for the pathological symptoms of nephrotoxicity such as leucocyte infiltration.

**Data analysis**

Data analysis was done using Graph Pad Prism 5. Results were expressed as mean ± standard error of the mean (SEM) and these were used to determine the differences in the serum urea, creatinine and uric acid quantities in the different groups. A One-Way ANOVA was done followed by Newman-Keuls Multiple Comparison test set at a significant level of p≤0.05 to compare the mean values of the different groups in order to identify the effect of the treatment on the biochemical markers, and comparison between treatment groups respectively. Graphs for the representation of the results were also drawn using graph pad software.

**Table 1: Phytochemical constituents of C. papaya seed aqueous extract**

![Image](image_url)
Biochemical results were confirmed by histological and the extract at 500 mg/kg did not show a significant difference although the urea levels were slightly lower in the 500 mg/kg extract than for the control group. It is therefore concluded from current results that Carica papaya is useful in protecting against paracetamol induced nephrotoxicity.

Figure 3 shows that comparison between the extract at 500 mg/kg and 750 mg/kg also showed a significant difference as well as between the control (group A) and group D while comparison between the control group and the extract at 500 mg/kg did not show a significant difference in urea levels. Serum urea concentration is often considered a more reliable renal function predictor than serum creatinine. Serum urea and creatinine are considered major nephrotoxicity markers, although serum urea concentration is often considered a more reliable renal function predictor than serum creatinine. Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatine breakdown. In the present study, administration of a nephrotoxic dose of paracetamol to rats resulted in a significant elevation of serum levels of urea, creatinine and uric acid in paracetamol administered group within 48 hours of exposure to it when compared to the normal control group.

These results are in agreement with those observed in Isik B et al. who noticed an elevation in serum urea and creatinine in rats after 1 g/kg body weight of paracetamol administration. Moreover, an elevation in serum urea and creatinine in a woman following therapeutic dose of paracetamol three days before hospital admission was reported. This elevation in the levels of urea and creatinine was explained by the presence of strong correlation between nephrotoxicity and oxidant stress. These biochemical alterations were corroborated by the histological findings of glomerular and tubulo-interstitial necrosis in the untreated control group. However, daily pretreatment with CPE for 7 days conferred nephroprotection in the paracetamol renal injured rats in a dose-dependent fashion and 750 mg/kg dose offered maximum protection.

The nephroprotective property of the extract was therefore confirmed by significant improvement of kidney architecture by reversing the nephrotoxic effects of paracetamol such as glomerular congestion, interstitial with inflammatory cells, tubular necrosis, peritubular necrosis and presence of intra-luminal casts suggesting massive total necrosis. Although, the possible mechanism of its protection against paracetamol-induced nephrotoxicity was not studied in the current study, it is possible that the protective effect of the extract is mediated through antioxidant and/or free radical scavenging activities. Literature has shown medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain. This is in agreement with the findings of this study. Summing these facts, it is plausible...
ble for the alkaloid, flavonoid and saponin components of CPE to be responsible for the observed biological effects. Flavonoids, tannins, and saponins have been reported to exert profound in vitro and in vivo stabilizing effect on the lysosomes of experimental animals. Plant flavonoids which show an antioxidant activity in vitro also function as antioxidants in vivo. Tannins and saponins stabilize the erythrocyte membrane by binding cations and other biomolecules. Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Again, a strong relationship between the total phenolic content and antioxidant activity in fruits, vegetables, grain products, and plant subjects of ethno-pharmacological treatments has also been reported.

Conclusion

The aqueous extract of Carica papaya seeds produced adequate nephroprotective activity on albino Wistar rats as evidenced by the reduction in the biochemical parameters and improvement of the kidney architecture. This supported the folklore use of the title plant in renal disorders. It is likely that the nephroprotective activities of the aqueous seed extract of the unripe mature fruits of Carica papaya in paracetamol-induced nephrotoxicity may involve its antioxidant and/or oxidative free radical scavenging activities which are provided for by the combined effect of active principles present in it.

However:

1. Financial limitations restricted us from investigating oxidative stress markers as one of the common pathogenic mechanisms for drug induced nephrotoxicity, thus future studies about the protective effects of papaya via antioxidants in ameliorating kidney damage are recommended.

2. The present study does not show the type of active phytochemicals responsible for the nephroprotective property of Carica papaya seed extract and this may be considered as one of the scope for future study on the plant.

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References