



PROTECTIVE POTENTIAL OF *PERSEA-AMERICANA* SEED EXTRACT AGAINST 5- FLUOROURACIL-INDUCED NEPHROTOXICITY IN WISTAR RATS

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Abstract

This study was carried out to evaluate the protective effect of *Persea americana* seed extract on 5-fluorouracil (5-FU)-induced nephrotoxicity in

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Introduction

One common chemotherapeutic medication used for the treatment of neoplasm is 5-fluorouracil (5-FU), an anticancer medication that is also a pyrimidine analog belonging to the class of anti-metabolite (Longley *et al.*, 2003). 5-Fluorouracil is an important anticancer drug, but studies have indicated that it also has the tendency of causing toxicity to the body. The toxic effect it causes on the body vary from one patient to another, depending on the patient gene and the dosage administered, which can occasionally result in reduction of its use. Al-Asmari *et al.* (2016) stated some of the severe adverse effects of the drug including: mucositis, hepatotoxicity, renal toxicity, myelosuppression, cardiotoxicity, alopecia, dermatitis, toxicity of sexual organs etc. The side effects could be acute or chronic and if not properly managed, can cause death. *Persea americana* is a very important plant having

Wistar rats. Of the 77 Wistar rats used for the study, 72 were treated intraperitoneally with a single dose of 150 mg/kg of 5-FU to induce toxicity. These animals were divided into six groups (2–7). Five rats were not exposed to 5-FU and served as the control group (Group 1). Group 2 served as the negative control, Group 3 was administered 500 mg/kg of vitamin C, while Groups 4–6 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract, respectively. Group 7 received 500 mg/kg of the extract in combination with 500 mg/kg of vitamin C

orally. Each group from Groups 2–7 contained 12 animals. Three animals from each group were sacrificed, and their blood and tissues were collected for biochemical and histological analyses. Administration of 5-FU caused renal toxicity, as evidenced by histological alterations as well as increased levels of kidney function parameters, including creatinine, urea, and bicarbonate. Most of the groups treated with the medium and high doses of the extract showed values lower than those of the negative control group, indicating the protective

effect of the extract and vitamin C on the kidney and a reduction in the level of toxicity. This decrease in toxicity could be due to the presence of phytochemicals such as flavonoids in the extract, which possess strong antioxidant properties, revealing the extract's potential in reversing the adverse effects caused by 5-FU. In conclusion, the study shows that there is potential normalization of renal tissue following the administration of the extract and, therefore, that it has a protective effect on the kidney.

both dietary and therapeutic properties. Some of the bioactive components found in the seed include polysaccharides, proteins, lipids, minerals, and vitamins and the seed makes up a significant portion (13–17%) of the fruit (Tremocoldi *et al.*, 2018). Lots of health benefits have been attributed to the seed, the seed extract has been examined for its bioactivities and some of the benefits of the seed are anti hyperglycemic (Tremocoldi *et al.*, 2018), antioxidants (Soledad *et al.*, 2021), anticancer and antimicrobial (Villarreal-Laraet *al.*, 2019), anti-inflammatory (Dabas *et al.*, 2019), anti-hypercholesterolemia (Uchenna *et al.*, 2017), antidiabetic, anti-neurodegenerative, analgesic effect, amongst others. *P. americana* seeds are also natural source of biologically active ingredients for the food, pharmaceutical, and cosmetic sectors because they contain no harmful compounds (Tremocoldi *et al.*, 2018). It is because of the phytochemicals contained in the seed extract and its antioxidant property (Unlu *et al.*, 2005) that this study was carried out to evaluate its effects on the kidney of Wistar rats following the exposure to 5-FU. While 5-FU remains one of the cornerstone treatments for various forms of cancers due to its anti-proliferative efficacy, it has been seen to cause toxicity to the kidney bringing about various renal diseases which are often identifiable clinically by changes in kidney function parameters useful to ascertain the state of a person's kidney. Some of the kidney function tests include test for creatinine, urea and bicarbonate levels, glomerular filtration rate, creatinine clearance, blood urea nitrogen, urine protein and urine output. Therefore, the association of 5-FU with nephrotoxicity requires cautious utilization of the drug especially among vulnerable populations or those presenting pre-

existing renal impairments. Prior research has indicated that creation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to 5-FU-induced nephrotoxicity by increasing the synthesis of lipid peroxide and lowering the functioning of antioxidant enzymes (Zhang *et al.*, 2022). Blocking the paths of oxidative stress, preventing apoptosis and mitochondrial dysfunction in renal tubular cells and inhibiting NF- κ B like TNF alpha, interleukin 1 beta, interleukin 6 production, antioxidants provide a beneficial impact by blocking a range of processes (Lee *et al.*, 2024), including the activation and protection of intracellular antioxidant enzymes or interactions with reactive oxygen species and enhancing antioxidant enzymes like superoxide dismutase, catalase and glutathione as well as decreasing lipid peroxidation, which are some of the effects seen due to administering *Persea americana* seed extract (Oyedikachi *et al.*, 2024). The phenolic chemical derivatives in the seed directly neutralizes invading reactive oxygen species thereby drastically reducing malondialdehyde levels which decreases lipid peroxidation (Miramontes-Corona *et al.*, 2024). Although *Persea americana* seed contains numerous bioactive compounds with antioxidant and anti-inflammatory properties, there is currently limited evidence evaluating its protective effect against 5-FU induced nephrotoxicity. Existing studies have been able to characterize the photochemical composition of avocado seeds and demonstrated antioxidant, antimicrobial, cardiometabolic properties etc, but their specific role in chemotherapy induced renal toxicity remains largely under study. Therefore, this study aims to bridge this knowledge gap by providing evidence on the efficacy of the extract in preventing or reversing the toxicity caused by 5-FU on the kidney as well as determining the optimal dose, duration, and molecular mechanism of the extract in modulating 5-FU toxicity.

MATERIALS AND METHODS

Procurement of Animals

The seeds were obtained and taken to the Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, Rivers State for plant identification. 77 Wistar rats of both genders weighing 200g on average were used for the study. These animals were made to acclimatize for a period of 2 weeks after separating the males from the females. They were housed in a spacious and adequately conditioned cage with an appropriate temperature and relative humidity under a 12-hour day and night period and they had free access to food and water. Ethical approval was obtained for the purpose of the study from University of Port Harcourt, Rivers State, Nigeria.

Method of *Persea americana* Seed Extraction and LD50 Determination

The extraction method used for the *Persea americana* seed was maceration, were finely grounded powdered seeds of about 100g were placed in a large container and some hot distilled water solvent of about 500mls was added into it amidst stirring. It was thereafter allowed to stand for a period of 24 hours until the soluble matter was dissolved. The mixture was then strained, pressed, and the combined liquid was filtered. The resultant filtrate was evaporated to dryness in an evaporating basin over hot water pot maintained at 50°C.

Method of LD50 Determination

LD50 was determined using Lorke's method of oral LD50 determination (Lorke, 1983). This method involved the use of 12 animals and it is usually in two phases – Phase 1 and 2. The weights of these animals were recorded and they were deprived of food for a period of 12 hours having only access to water before administering the extract to them at appropriate doses.

Experimental Design

Seventy-seven experimental Wistar rats (77) were randomly selected and grouped into seven groups (1-7) of twelve animals (n =12) each aside from the group 1 (control group) that had five (5) animals. They were thereafter treated under the following groups;

Group 1: Served as control, received feed and water only

Group 2: Served as the negative control

Group 3: 500mg/kg of Vitamin C (0.5ml)

Group 4: 250mg/kg of *Persea americana* seed extract (0.25ml)

Group 5: 500mg/kg of *Persea americana* seed extract (0.5ml)

Group 6: 1000mg/kg of *Persea americana* seed extract (1ml)

Group 7: 500mg/kg of Vitamin C + 500mg/kg of the *Persea americana* seed extract

Three rats from each group were sacrificed at weekly intervals for 4 weeks of extract administration except for the control group in which all five rats present in the group were sacrificed on the last day (28th) day. The blood of the rats was collected for biochemical evaluation of kidney function parameters, while the kidney tissue was collected and preserved with formalin for histological analysis.

Measurement of Kidney Function Parameters

Urea and Creatinine concentrations were measured using Urease-Berthelot method (Bethelot, 1859) and direct end point method (Jaffe, 1886) and their units were expressed in mmol/L. Sodium levels were measured in accordance to Maruna method (Maruna, 1958) and the units expressed in mmol/L while potassium was measured in accordance to Tietz method (Tietz, 1986), and the values were expressed in mmol/L. Bicarbonate using back titration method (Pauus *et al.*, 1990), and their values were expressed in mmol/L.

Method of Sacrificing Animals

The process of sacrificing animal was done based on the guidelines for humane killing of animals used for scientific purposes. Animals were sacrificed by placing them in a jar containing diethyl ether to induce anesthesia in order to reduce pain before they were dissected and the required tissues collected.



Figure 1: Process of dissection and organ collection in the animal

Histopathology Examination

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the liver tissue which was later on transferred to about 10% of formaldehyde and thereafter, the liver tissue was trimmed down to reduce its thickness, to allow the fixative to readily penetrate. The tissues were exposed to different stages of processing by standard method as described by Carleton *et al.*, 1967. The stages include fixation, dehydration, embedding, sectioning, staining with hematoxylin and eosin (H&E) and thereafter mounting.

Method of Statistical Analysis

Results were expressed in mean and standard deviation (mean + or - standard deviation. It was analyzed using Analysis of variance (ANOVA). Duncan multiple range test (DMRT) was used to separate the means at 95% confidence interval. The differences between groups at $P < 0.05$ were considered to be statistically significant (Mead *et al.*, 1982).

RESULTS

The kidney function parameters checked for include K; Potassium, Na; Sodium, UR; Urea, CR; Creatinine and HCO_3 ; Bicarbonate.

Table 1 reveals the result for effect of *Persea americana* seed Extract on 5-FU-induced toxicity on potassium (K) levels in Wistar rats over four weeks. The control group maintained consistent K levels of 4.87 ± 0.21 mmol/L across all weeks. The negative control group had significantly higher K levels (Week 1: 6.63 ± 0.12 , Week 2: 6.07 ± 0.23 , Week 3: 5.50 ± 0.00 , Week 4: 6.40 ± 0.00) compared with the control group ($p < 0.05$). The Vitamin C group maintained similar K levels to reference group throughout the study. 250mg/kg and 500mg/kg dose groups displayed significantly higher K levels compared to reference group in weeks 1 and 2 ($p < 0.05$), while the high dose group had similar K levels to the control group. The combination of Vitamin C and 500mg/kg dose extract-maintained K levels similar to the control group, indicating a potential protective effect. Table 2 shows the

results of the effect of *P. americana* seed Extract on 5-FU-induced toxicity on sodium (Na) levels in Wistar rats over four weeks. The control group maintained consistent Na levels of 145.0 ± 3.0 mmol/L across all weeks. The negative control group had significantly higher Na levels (Week 1: 161 ± 2.31 , Week 2: 156 ± 1.15 , Week 3: 152 ± 0.00 , Week 4: 156 ± 0.0) compared with control group ($p < 0.05$). The Vitamin C group showed lower Na levels in weeks 1 and 2, but similar levels to the control group in weeks 3 and 4. The 250mg/kg and 500mg/kg dose groups had significantly higher Na levels compared with control in week 1 and 2, but levels normalized in later weeks. The 1000mg/kg dose group showed significantly higher Na levels in weeks 2 and 4 ($p < 0.05$). The combination of Vitamin C and 500mg/kg dose extract-maintained Na levels similar to the control group, suggesting a potential protective effect. Table 3 shows effect of *P. americana* seed Extract on 5-FU-induced toxicity on urea (UR) levels in Wistar rats over four weeks. The control group maintained consistent UR levels of 4.43 ± 0.47 mmol/L across all weeks. The negative control group had higher UR levels in weeks 2 and 4 compared to reference group, but these values were not statistically outstanding. The Vitamin C group had lower UR levels in week 1, but similar levels to the control group in other weeks. The 250mg/kg extract group had higher UR levels in week 1, while the 500mg/kg and 1000mg/kg dose groups-maintained UR levels similar to the reference group. The combination of Vitamin C and 500mg/kg dose extract showed UR levels like the reference group throughout the study, indicating a potential protective effect. Table 4 shows effect of *Persea americana* Seed Extract on 5-FU-induced toxicity on creatinine (CR) levels in Wistar rats over four weeks. The control group maintained consistent CR levels of 92.00 ± 3.89 mmol/L across all weeks. The negative control group had significantly higher CR levels in week 2 (106.3 ± 9.81) when weighed up with reference group ($p < 0.05$). The Vitamin C group had lower CR levels in week 1 but higher levels in week 4 than reference group. The 250mg/kg group displayed significantly higher CR levels in all weeks weighed up with the reference group ($p < 0.05$). The 500mg/kg and 1000mg/kg groups had CR amounts like the reference group. The combination of Vitamin C and 500mg/kg dose extract showed varying CR levels, with no consistent significant differences from the control group. Table 5 shows effect of *P. americana* Seed Extract on 5-FU-induced toxicity on bicarbonate (HCO_3) levels in Wistar rats over four weeks. The control group maintained consistent HCO_3 levels of 22.57 ± 2.08 mmol/L across all weeks. The negative control group had significantly higher HCO_3 levels in weeks 2 and 4 compared to the control group ($p < 0.05$). The Vitamin C group had higher HCO_3 levels in week 1 but significantly higher levels in weeks 3 and 4. The 250mg/kg dose group showed significantly higher HCO_3 levels in weeks 2 and 3. The 500mg/kg dose group had similar HCO_3 levels to the control group in most weeks, while 1000mg/kg displayed remarkably great levels in week 3. The combination of Vitamin C and 500mg/kg dose extract showed varying HCO_3 levels, with significantly higher levels in weeks 2 and 4.

Table 1: Effect of *Persea americana* (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Potassium (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	4.87±0.21	4.87±0.21	4.87±0.21	4.87±0.21
Negative Control	6.63±0.12	6.07±0.23	5.50±0.00	6.40±0.00
VitaminC (500mg/kg)	4.67±1.39	4.97±0.23	5.50±0.00	5.00±0.00
250mg/kg of extract	5.90±0.87	6.53±0.29	6.40±0.00	5.50±0.00
500mg/kg of extract	5.20±1.18	6.00±0.17	4.83±0.12	5.60±0.00
1000mg/kg of extract	4.77±0.64	6.13±0.92	4.97±0.23	6.37±0.12
VitC + 500mg/kg extract	5.47±0.21	5.30±0.44	4.57±0.12	4.90±0.00

Values are presented in Mean and Standard deviation.

a = value is significantly different at p<0.05 when compared to control group

b = value is significantly different at p<0.05 when compared to negative control

c = value is significantly different at p<0.05 when compared to vitamin C

Source; Authors Fieldwork, 2024.

Table 2: Effect of *Persea americana* (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Sodium (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	145.0±3.0 ^{bc}	145.0±3.0 ^b	145.0±3.0 ^{bc}	145.0±3.0 ^b
Negative Control	161±2.31 ^{ac}	156±1.15 ^{ac}	152±0.00 ^a	156±0.0 ^a
VitaminC (500mg/kg)	139±17.52 ^{ab}	148±4.04 ^b	149±0.00 ^a	146±0.0 ^b
250mg/kg of extract	153±0.87 ^{abc}	154±2.89 ^{ac}	153±0.00 ^a	147±0.0 ^b
500mg/kg of extract	144±12.67 ^{bc}	150±4.62 ^b	150±1.73 ^a	144±0.0 ^b
1000mg/kg of extract	147±5.50 ^{bc}	159±2.89 ^{ac}	150.3±4.62 ^a	155±2.31 ^{ac}
VitC + 500mg/kg extract	143±8.89 ^b	151±3.06 ^{ab}	151.67±7.51 ^a	156±0.0 ^{ac}

Values are presented in Mean and Standard deviation.

a = value is significantly different at p<0.05 when compared to control group

b = value is significantly different at p<0.05 when compared to negative control

c = value is significantly different at p<0.05 when compared to vitamin C

Source; Authors Fieldwork, 2024.

Table 3: Effect of *Persea americana* (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Urea (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	4.43±0.47	4.43±0.47	4.43±0.47	4.43±0.47
Negative Control	4.53±0.40	5.23±0.46	3.70±0.00	3.90±0.00
VitaminC (500mg/kg)	4.10±0.70	4.07±0.06	4.80±0.00	4.90±0.00
250mg/kg of extract	5.40±1.14	5.17±0.64	4.60±0.00	5.70±0.00
500mg/kg of extract	5.40±0.79	4.60±0.17	5.50±0.17	4.80±0.00
1000mg/kg of extract	4.63±0.15	4.53±0.12	4.47±0.29	4.00±0.17
VitC + 500mg/kg extract	4.30±0.20	4.97±0.67	5.33±0.29	4.80±0.00

Values are presented in Mean and Standard deviation.

a = value is significantly different at $p < 0.05$ when compared to control group

b = value is significantly different at $p < 0.05$ when compared to negative control

c = value is significantly different at $p < 0.05$ when compared to vitamin C

Source; Authors Fieldwork, 2024.

Table 4: Effect of *Persea americana* (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Creatinine (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	92.00±3.89 ^c	92.00±3.89 ^{bc}	92.00±3.89 ^{bc}	92.00±3.89 ^c
Negative Control	95.00±8.66 ^c	106.3±9.81 ^{ac}	80.00±0.00 ^{ac}	92.00±0.00 ^c
VitaminC (500mg/kg)	83.33±14.22 ^{ab}	83.33±1.15 ^{ab}	102±0.00 ^{ab}	122±2.89 ^{ab}
250mg/kg of extract	110 ± 23.43 ^{abc}	110±8.66 ^{ac}	121.00±0.00 ^{abc}	117±0.0 ^{abc}
500mg/kg of extract	94.67±15.04 ^c	91.67±6.35 ^{bc}	101.67±4.62 ^{ab}	109±0.0 ^{abc}
1000mg/kg of extract	94.67±2.52 ^c	94±3.46 ^{bc}	96.67±1.15 ^{bc}	88.33±2.89 ^c
VitC + 500mg/kg extract	89.33±2.08 ^{bc}	92±15.87 ^{bc}	114.67±10.97 ^{abc}	97.00±0.0 ^{abc}

Values are presented in Mean and Standard deviation.

a = value is significantly different at $p < 0.05$ when compared to control group

b = value is significantly different at $p < 0.05$ when compared to negative control

c = value is significantly different at $p < 0.05$ when compared to vitamin C

Source; Authors Fieldwork, 2024.

Table 5: Effect of *Persea americana* (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Bicarbonate (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	22.57±2.08 ^c	22.57±2.08 ^b	22.57±2.08 ^c	22.57±2.08 ^{bc}
Negative Control	22.00±1.73 ^c	26.33±1.15 ^a	23.00±0.00 ^c	29.00±0.0 ^a
Vitamin C (500mg/kg)	25.00±2.00 ^{ab}	24.00±1.73	28.00±0.00 ^{ab}	32.00±0.00 ^a
250mg/kg of extract	23.00±2.65 ^c	28.67±1.15 ^a	27.00±0.00 ^{ab}	27.00±0.00 ^{ac}
500mg/kg of extract	23.33±2.52 ^c	23.67±1.15	23.67±0.58 ^{ab}	21.0±0.00 ^{bc}
1000mg/kg of extract	24.33±1.15	23.33±1.15	26.00±1.73 ^{ac}	21.67±0.33 ^{bc}
VitC + 500mg/kg extract	24.00±2.65	30.33±4.04 ^{ac}	24.00±1.73 ^c	28.0±0.00 ^{ac}

Values are presented in Mean and Standard deviation.

a = value is significantly different at $p < 0.05$ when compared to control group

b = value is significantly different at $p < 0.05$ when compared to negative control

c = value is significantly different at $p < 0.05$ when compared to vitamin C

Source; Authors Fieldwork, 2024.

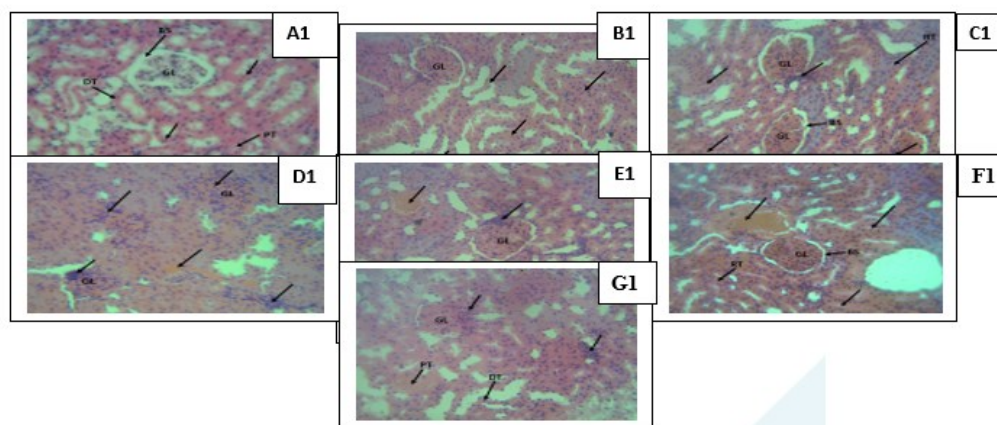


Figure A1-G1 shows the photomicrographs of the effect of the extract on the kidney. (A1) Normal control, (B1) Negative control, (C1) 500mg/kg of vitamin C, (D1) 250mg/kg extract, (E1) 500mg/kg extract (F1) 1000mg/kg extract, (G1) 500mg/kg of vitamin C +500mg/kg of Extract

Source; Authors Fieldwork, 2024.

Effect of *Persea americana* seed extract on Kidney Histopathology in Week 1

Figure A1 shows the photomicrograph (H&E X400) of the kidney from group one (normal control group) showing a well delineated glomerulus (GL), bowman's space (BS), proximal tubules (PT) and distal tubules (DT). Diagnosis reveals Normal Kidney tissue. Figure B1(Group 2; Negative control) shows the photomicrograph (H&E x400) of the kidney showing moderate interstitial edema of the renal tubules; mild inflammatory cell infiltration of the renal tubules and the diagnosis shows moderate interstitial edema of the renal tubules. Figure C1 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the Kidney showing moderate renal tubular degeneration associated with interstitial edema and minimal inflammatory cell infiltration of the glomerulus and thickening of the basement membrane and the diagnosis from this shows inflammatory cell infiltration. Figure D1 (Group 4; 250mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL) and renal tubules inflammatory cells infiltration with hemorrhagic deposit (arrows) and the diagnosis reveals Inflammation of the kidney tissue. Figure E1 (Group 5; 500mg/kg of extract) shows the photomicrograph (H&E X400) of the Kidney showing the glomerulus (GL), proximal tubules (PT) and distal tubules (DT): mild inflammatory cell infiltration, with blood deposit in the interstitium the histology reveals Mild inflammatory cell infiltration. Figure F1 (Group 6; 1000mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL), bowmans space (BS), proximal tubules (PT), renal tubules (RT) and distal tubules (DT): Moderate inflammatory cell infiltration and the diagnosis reveals that there was moderate inflammation of the kidney tissue. Figure G1 (Group 7; Vitamin C + 500mg/kg of extract) shows the photomicrograph (H&E x400) of the kidney showing interstitial edema of the renal tubules; mild

inflammatory cell infiltration of the renal tubules and glomerulus. Diagnosis: Interstitial edema and inflammatory cell infiltration of the kidney.

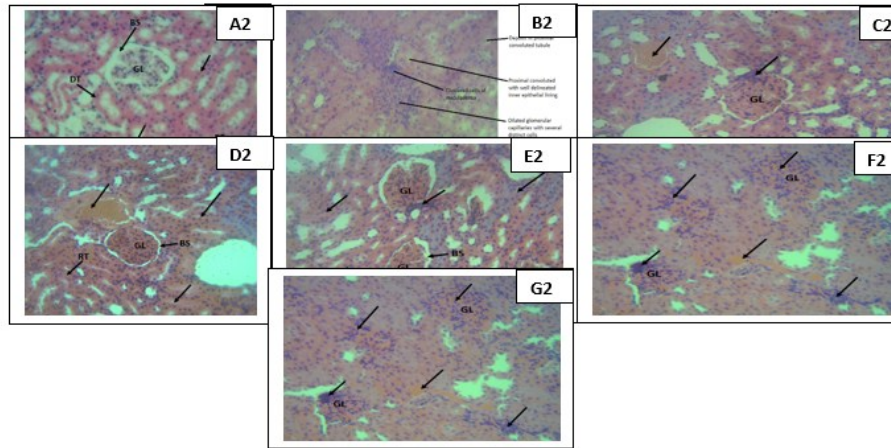


Figure A2-G2 shows the photomicrographs of the effect of the extract on the kidney. (A2) Normal control, (B2) Negative control, (C2) 500mg/kg of vitamin C, (D2) 250mg/kg extract, (E2) 500mg/kg extract (F2) 1000mg/kg extract, (G2) 500mg/kg of vitamin C +500mg/kg of Extract

Source; Authors Fieldwork, 2024.

Effect of *Persea americana* seed extract on Kidney Histopathology in Week 2

Figure A2 shows the photomicrograph (H&E X400) of the kidney from group one (normal control group) showing a well delineated glomerulus (GL), bowman's space (BS), proximal tubules (PT) and distal tubules (DT). Diagnosis reveals Normal Kidney tissue. Figure B2(Group 2; Negative control) shows the photomicrograph (H&E x400) of the kidney showing dilated glomerular capillaries with several cells closely related to clustered cells (macula densa), well delineated epithelial lining of distal convoluted tubules and congested proximal convoluted tubules and the diagnosis shows Mild distortion of cytoarchitectural appearance. Figure C2 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the Kidney showing the glomerulus (GL), proximal tubules (PT) and distal tubules (DT): mild inflammatory cell infiltration, with blood deposit in the interstitium (arrows) and the diagnosis from this shows mild inflammatory cell infiltration. Figure D2 (Group 4; 250mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL), bowmans space (BS), renal tubules (RT) with arrow showing moderate inflammatory cell infiltration (arrow) and the diagnosis reveals moderate Inflammation of the kidney tissue. Figure E2 (Group 5; 500mg/kg of extract) shows the photomicrograph (H&E X400) of the Kidney moderate degeneration of the renal tubules associated with minimal inflammatory cell infiltration of the glomerulus and thickening of the basement membrane (arrows) the histology reveals inflammation of the kidney tissue. Figure F2 (Group 6; 1000mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing the glomerulus (GL)

and also reveals renal tubules inflammatory cells infiltration with hemorrhagic deposit (arrows) and the diagnosis reveals that there was mild inflammation of the kidney tissue. Figure G2 (Group 7; Vitamin C + 500mg/kg of extract) shows the photomicrograph (H&E x400) of the kidney showing glomerulus (GL) and renal tubules inflammatory cells infiltration with hemorrhagic deposit (arrows). Diagnosis: Inflammation of the kidney tissue.

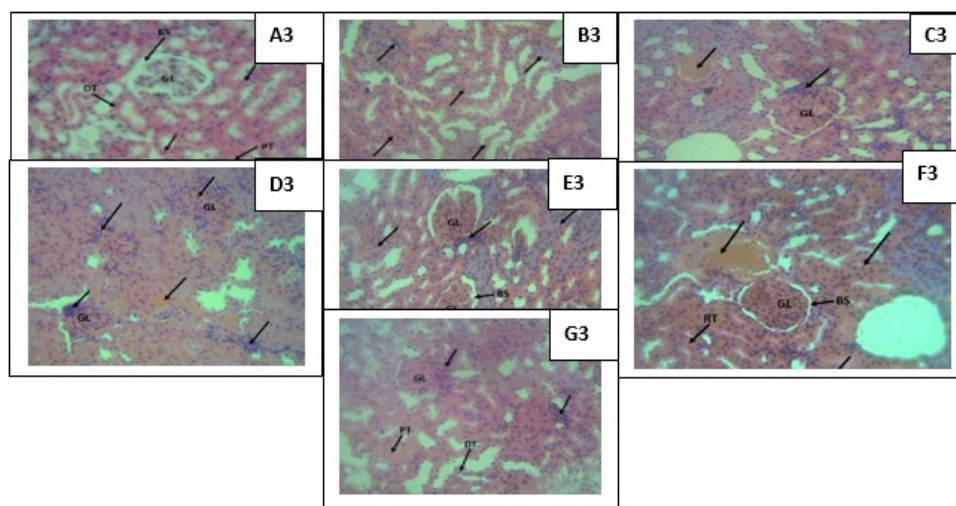


Figure A3-G3 shows the photomicrographs of the effect of the extract on the kidney. (A3) Normal control, (B3) Negative control, (C3) 500mg/kg of vitamin C, (D3) 250mg/kg extract, (E3) 500mg/kg extract (F3) 1000mg/kg extract, (G3) 500mg/kg of vitamin C +500mg/kg of Extract.

Source; Authors Fieldwork, 2024.

Effect of *Persea americana* seed extract on Kidney Histopathology in Week 3

Figure A3 shows the photomicrograph (H&E X400) of the kidney from group one (normal control group) showing a well delineated glomerulus (GL), bowman's space (BS), proximal tubules (PT) and distal tubules (DT). Diagnosis reveals Normal Kidney tissue. Figure B3(Group 2; Negative control) shows the photomicrograph (H&E x400) of the kidney showing moderate interstitial edema of the renal tubules; mild inflammatory cell infiltration of the renal tubules (arrows) and the diagnosis shows moderate interstitial edema of the renal tubules. Figure C3 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the Kidney showing the glomerulus (GL), proximal tubules (PT) and distal tubules (DT): mild inflammatory cell infiltration, with blood deposit in the interstitium (arrows) and the diagnosis from this shows inflammatory cell infiltration. Figure D3 (Group 4; 250mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL) and renal tubules inflammatory cells infiltration with hemorrhagic deposit (arrows) and the diagnosis reveals Inflammation of the kidney tissue. Figure E3 (Group 5; 500mg/kg of extract) shows the photomicrograph (H&E X400) of the Kidney showing moderate degeneration of the renal tubules associated

with minimal inflammatory cell infiltration of the glomerulus and thickening of the basement membrane (arrows) the histology reveals inflammation of the kidney tissue. Figure F3 (Group 6; 1000mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL), bowmans space (BS), proximal tubules (PT), renal tubules (RT) and distal tubules (DT): Moderate inflammatory cell infiltration (arrow) and the diagnosis reveals that there was moderate inflammation of the kidney tissue. Figure G3 (Group 7; Vitamin C + 500mg/kg of extract) shows the photomicrograph (H&E x400) of the kidney showing interstitial edema of the renal tubules; mild inflammatory cell infiltration of the renal tubules and glomerulus (arrows). Diagnosis: Interstitial edema and inflammatory cell infiltration of the kidney.

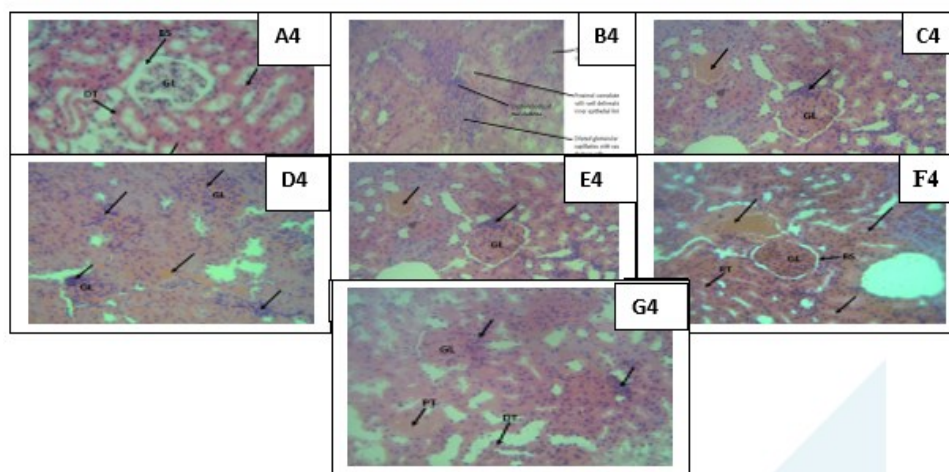


Figure A4-G4 shows the photomicrographs of the effect of the extract on the kidney. (A4) Normal control, (B4) Negative control, (C4) 500mg/kg of vitamin C, (D4) 250mg/kg extract, (E4) 500mg/kg extract (F4) 1000mg/kg extract, (G4) 500mg/kg of vitamin C +500mg/kg of Extract

Source; Authors Fieldwork, 2024.

Effect of *Persea americana* seed extract on Kidney Histopathology in Week 4

Figure A4 shows the photomicrograph (H&E X400) of the kidney from group one (normal control group) showing a well delineated glomerulus (GL), bowman's space (BS), proximal tubules (PT) and distal tubules (DT). Diagnosis reveals Normal Kidney tissue. Figure B4 (Group 2; Negative control) shows the photomicrograph (H&E x400) of the kidney showing dilated glomerular capillaries with several cells closely related to clustered cells (macula densa), well delineated epithelial lining of distal convoluted tubules and congested proximal convoluted tubules and the diagnosis shows mild distortion of cytoarchitectural appearance. Figure C4 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the Kidney showing the glomerulus (GL), proximal tubules (PT) and distal tubules (DT): mild inflammatory cell infiltration, with blood deposit in the interstitium (arrows) and the diagnosis from this shows mild inflammatory cell infiltration. Figure D4 (Group 4; 250mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney

showing glomerulus (GL) and renal tubules inflammatory cells infiltration with hemorrhagic deposit (arrows) and the diagnosis reveals Inflammation of the kidney tissue. Figure E4 (Group 5; 500mg/kg of extract) shows the photomicrograph (H&E X400) of the Kidney showing the glomerulus (GL), proximal tubules (PT) and distal tubules (DT): mild inflammatory cell infiltration, with blood deposit in the interstitium (arrows) the histology reveals Mild inflammatory cell infiltration. Figure F4 (Group 6; 1000mg/kg of extract) presents the photomicrograph (H&E x400) of the Kidney showing glomerulus (GL), bowmans space (BS), proximal tubules (PT), renal tubules (RT) and distal tubules (DT): Moderate inflammatory cell infiltration (arrow) and the diagnosis reveals that there was moderate inflammation of the kidney tissue. Figure G4 (Group 7; Vitamin C + 500mg/kg of extract) shows the photomicrograph (H&E x400) of the kidney showing interstitial edema of the renal tubules; mild inflammatory cell infiltration of the renal tubules and glomerulus (arrows). Diagnosis: Interstitial edema and inflammatory cell infiltration of the kidney.

DISCUSSION

Findings from this study demonstrated the impact of *Persea americana* seed extract on several renal parameters in different treatment groups after 5-FU was administered, in comparison to the control group. When related to the control group, unusual amounts of kidney activity biomarkers such as K, Na indicate an electrolyte and fluid imbalance, which suggests a degree of renal dysfunction, dehydration, malnourishment, etc (Guyton & Hall, 2016). The result from this study reveals that with the kidney function parameters, there was no consistent trend on the effect of the various doses of the extract and vitamin C on the kidney. However, a lot of the kidney function parameters had values lesser than the control group denoting protective effect of the extract and vitamin C on the kidney and decrease in the level of toxicity. This decrease in toxicity could be due to increase in the levels of flavonoids present in the pear seed which has a high antioxidant property. Flavonoids have the tendency to inhibit urolithiasis thereby reducing oxidative stress to the kidney which can lead to decrease in impaired kidney function and reduction in various kidney diseases like glomerulonephritis, lupus, interstitial nephritis, vasculitis, acute kidney injury, kidney artery stenosis etc. A few of the parameters had treatment groups that were a bit higher for some weeks when compared with the control group indicating slight disturbances with electrolyte, fluid and acid-base balance. With Na, K, UR and CR there was a significant increase in the values of the groups of animals that took the low dose of the extract when it was compared with the control group and this could be due to decrease in the protective effect of the 250mg/kg dose of the extract when compared to the 500mg/kg or 1000mg/kg dose. Result from these findings is supported by Olukemi et al. (2021), that investigated on the nephroprotective potential of aqueous extract from *Persea americana* seeds against cadmium-induced nephrotoxicity in Wistar rats. The study demonstrated that treatment with an aqueous extract from *P.americana* seeds notably reversed adverse alterations in serum creatinine, urea, uric acid levels as well as blood urea nitrogen caused by cadmium toxicity. Furthermore, it restored antioxidant status evidenced by significant improvements in superoxide

dismutase, catalase activities among others while also exhibiting pronounced anti-inflammatory effects via reduction in interleukin-2 and tumor necrosis factor α activities

CONCLUSION

This study evaluated the effect of *Persea americana* seed extract on 5-FU induced Renal toxicity in Wistar rats. The study revealed that the extract is useful in reversing toxicities to the kidney caused by the administration of 5-FU, which was accomplished by decreasing oxidative stress and inflammation to the kidney. However, further studies are required to unveil other molecular pathways by which the extract exerts its beneficial effect on the kidney in Wistar rats. Also, studies to evaluate if the knowledge from this research can be used as a guide by pharmaceutical scientist in the production of a new drug and to determine if the use of this extract would have an effect on the potency and efficacy of 5-FU in the treatment of cancer is also recommended.

There are however some limitations to this study, such as the relatively short duration of action of the experiment, also this study is basically a preclinical study and requires clinical trials to back the observed effect, further studies that would be carried out for a longer period of time to determine the effect of the extract over such period is therefore recommended.

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Author Contributions

All authors contributed to the study design and conception. Both authors contributed in one way or the other in data collection, analysis and material preparation.

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Availability of data and material

Data are available upon request.

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There was no conflict of interest by the authors to disclose.

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