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Effect of *Persea americana* seed extract on 5-Fluorouracil Induced Hepatic Histotoxicity on Wistar Rats

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ABSTRACT: *This study evaluated the effect of Persea americana seed extract on 5-fluorouracil (5-FU) induced hepatic histoxicity in Wistar rats. A total of 77 Wistar rats were used in this study, 72 were treated intraperitonially with a single dose of 150mg/kg of 5-FU to induce liver toxicity and these animals were thereafter divided into six groups (2-7). The five (5) animals in group 1 which were not exposed to 5-FU served as control, Group 2 served as negative control, those in group 3 were administered with the medium dose of vitamin C at 500mg/kg while groups 4-6 received 250mg/kg, 500mg/kg and 1000 mg/kg of the extract respectively and those in group 7 received the medium dose of vitamin C and the extract orally. Each group from 2-7 had 12 animals, 3 animals were sacrificed from each group at weekly interval for 4 weeks and their blood and tissues were collected for biochemical and histological analysis. It was observed that the administration of 5-FU caused hepatic toxicity which was evidenced by histological alterations as well as increase in the levels of liver function parameters including Aspartate transaminase (AST), Alkaline phosphatase (ALP), Alanine transaminase (ALT). On the contrary, administration of the extract reversed the adverse effects caused by 5-FU.*

KEYWORDS: 5-fluorouracil, *Persea americana* seed, hepatic-histotoxicity, liver function parameters

INTRODUCTION

One of the most frequently employed chemotherapeutic medications for the treatment of neoplasm is 5-fluorouracil (5-FU), an anticancer medication that is additionally a pyrimidine analog

(Longley et al., 2003). 5-Flourouracil is a helpful anticancer drug, but research indicates that it also has a potential to have dangerous and hazardous impacts on the body. These symptoms vary depending on the patient and the dosage administered, which can occasionally result in the stoppage in the use of this drug. According to Al-Asmari et al. (2016), mucositis, hepatotoxicity, renal toxicity, myelosuppression, cardiotoxicity, alopecia, dermatitis, and toxicity of sexual organs are all severe, painful, and intolerable adverse events associated with the drug. Less severe side effects include diarrhea, sore throat, mouth sores, loss of appetite, changes in taste etc. The severe side effects could be acute or chronic and could eventually lead to death if it is not properly managed.

Persea americana or avocado pear, represents a very valuable plant having both dietary and therapeutic properties. Polysaccharides, proteins, lipids, minerals, and vitamins are among the many essential and bioactive ingredients found in avocado seeds, which make up a significant portion (13–17%) of the fruit (Tremocoldi et al., 2018). There are alot of health benefits attributed to the seed, the seed extract has been examined for its bioactivities and some of these benefits which have been discovered in the phytochemicals present in the seed from other study's carried out includes anti hyperglycemic (Tremocoldi et al., 2018), antioxidants (Soledad et al., 2021), anticancer (Villarreal-Lara et al., 2019), anti inflammatory (Dabas et al., 2019), antimicrobial (Villarreal-Lara et al., 2019), anti-hypercholesterolemia (Uchenna et al., 2017), antidiabetic, anti neurodegenerative, analgesic effect, amongst others. They are also good natural source of biologically active ingredients for the food, pharmaceutical, and cosmetic sectors because they contain no harmful or dangerous compounds. (Tremocoldi et al., 2018). The powdered seed is taken as a dietary supplement in Nigeria to avert heart attacks and control hypertension (Imafidon & Amaechina, 2010). The usage of avocado seeds as nutraceuticals is becoming much more popular as people become more aware of its efficacy as a secure and antioxidant-rich fruit component (Araujo et al., 2018). Based on the phytochemicals that *Persea americana* seed contains and it's antioxidant property (Unlu et al., 2005), this study evaluated the effect of the seed extract on 5-FU induced hepato histotoxicity in Wistar rats.

According to Guyton and Hall (2016), the liver is the body's largest parenchymatous organ and is essential for intermediary metabolism. The numerous cellular activities that are taking place require it to be in close proximity to the portal blood in systemic circulation (Alberts et al., 2002). The arrangement and functionality of intermediate metabolism of dietary nutrients (proteins, fatty acids and glucose), removal of harmful or infectious factors, metabolic detoxification, and elimination of waste all depend on hepatocytes' spatial connection with the various cellular components of the liver, like fenestrated sinusoidal endothelial cells, Kupffer cells, and bile canaliculi (Roy-Chowdhury & Roy-Chowdhury, 2006). Metabolism of 5-FU mainly occurs in the liver but only a small proportion of the drug is metabolized in the kidney. 5 FU has a half life of 10 mins (Sobrero et al., 1997) and has been previously determined to have the tendency to cause liver damage (Ray et al., 2007).The enzyme dihydropyrimidine dehydrogenase is in charge of 5-FU's breakdown and according to a number of studies, 5-FU damages the liver and, like other anticancer medications, it produces too many reactive oxygen species and inhibits the body's

antioxidant protection system. As a result, antioxidants' possible function in preventing hepatotoxicity caused by chemotherapy has received a lot of interest (Behling et al., 2006). An increase in the amount of the aminotransferase enzyme is often a cause of the liver toxicity caused by 5-FU doses. This increase might be linked to portal inflammation and varying degrees of macrovesicular steatosis in the liver. Also, thymidylate synthase suppression might be a source of damage. Furthermore, the microsomal enzyme system has a vital duty in extensive hepatic metabolism of fluorouracil, and generation of a hazardous intermediate can result in liver damage. The evaluation and interpretation of liver function tests (LFTs) remains very important in the diagnosis and management of liver diseases. A series of clinical biochemistry laboratory blood tests known as liver function tests (LFTs) are intended to provide data regarding a patient's liver condition. Prothrombin time (PT/INR), albumin, bilirubin (direct and indirect), aPTT (activated partial thromboplastin time), and other markers are assessed. When a patient has a certain degree of preserved liver function, liver transaminases (AST or SGOT and ALT) are helpful indicators of liver damage (Mengel et al., 2005). These tests can be used to determine whether liver disease is present, differentiate between various liver conditions, determine the degree of known liver damage, and monitor the course of therapies.

MATERIALS AND METHODS

Procurement of Animals

The study was conducted in the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria. Fresh avocado pear seeds were obtained for the purpose of the study and were taken to the Department of Botany, University of Port Harcourt, Rivers State for plant identification. For this study, 77 Wistar rats of both genders weighing 200g on average were bought from a respectable animal house in Port Hacourt, Nigeria. In order to allow the animals to acclimatize over the course of 14 days, the males and females were separated and housed in a roomy and adequately conditioned cage with an appropriate temperature and relative humidity under a 12-hour day and night period. They were given unlimited supply of food and water but were starved of food for 12 hours before lethal dose (LD50 determination).
Ethical approval was obtained for the purpose of the study from University of Port Harcourt, Rivers State, Nigeria.

Method of Avocado pear seed Extraction and LD50 Determination

The extraction method combined maceration, digestion and decoction procedures. It involves boiling of the plant material in an open extractor which lasted for some time in order to obtain the final product. In the process, the finely grounded powdered seeds were placed in a large container and some hot distilled water solvent 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, the marc (the damp solid material) was pressed, and the solute was separated from the solvent via filtration. The resultant filtrate was evaporated to dryness in an evaporating basin over hot water pot maintained at 50^{o}C.

Method of LD50 Determination

LD50 was determined using Lorke's method of determination of oral acute toxicity (LD 50). Using this method, 12 animals were randomly selected and divided into two phases, Phase 1 and 2. The weight of these animals were recorded and they were starved for a period of 12 hours having only access to water before administering the extract to them at appropriate doses.

Phase l: Lorke's method of median lethal dose (LD50) determination was used (Lorke, 1983). A total of nine wistat rats were used. They were divided into three groups of three rats each. Doses of 10 mg/Kg, I00 mg/Kg, and 1000 mg/Kg of the extract were administered orally and then the rats were observed for behavioral manifestation of acute toxicity or death within 24 hours post administration.

Phase 2: This stage depended on the outcome of Phase I, whether or not death was observed. A total of three rats were used. They were divided into three groups of one rat each. The doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg were administered and the rats were observed again for death as the index of toxicity. It was observed that no death occurred both in phases one and two denoting that the extract is safe for administration. The table showing the result of the LD50 determination is as stated below;

Table 2.1: Mortality recorded in oral lethal dose (LD50) using Lorke's determination method of Avocado Pear Seed Extract

Experiment	Dose (mg/kg body weight)	No. of Dead rats after 24 hrs	No. of Dead rats after 72 hrs
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase 2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

Keys:
0 = Number of Deaths
3 = Number of Wistar rats used in the experiment
1 = Number of Wistar rats used in the experiment

Experimental Design

Seventy seven experimental Wistar rats (77) were randomly selected and grouped into seven groups (1-7) of twelve animals (n =12) each asides 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: Received medium dose of vitamin C i.e. 500mg/kg body weight of vitamin C (0.5ml)
Group 4: Received low dose of avocado pear seed extract i.e. 250mg/kg body weight of the avocado pear seed extract (0.25ml)
Group 5: Received medium dose of avocado pear seed extract i.e. 500 mg/kg body weight of the avocado pear seed extract (0.5ml)
Group 6: Received high dose of avocado pear seed extract i.e. 1000mg/kg body weight of the avocado pear seed extract (1ml)
Group 7: Received medium dose of vitamin C + medium dose of the avocado pear seed extract

Three rats from each group were sacrificed weekly i.e. on the 7th, 14th, 21st and 28th day of extract administration except for the control group in which all five rats present in the group was sacrificed on the last day (28th) day. The blood of the rats were collected for biochemical evaluation of liver function parameters, while the liver tissue of the rats were collected and preserved with formalin for histological analysis.

Measurement of Liver Function Parameters

Aspartate transaminase and Alanine transaminase concentrations were determined according to Reitman and Frankel method (Reitman & Frankel, 1957), using a readymade auto analyzer kit for this purpose. The measured levels were expressed in IU/L.

Alkaline phosphatase concentrations were measured according to Kochmar and Moss method (Kochmar & Moss, 1976), by a ready-made auto analyzer kit. The levels measured were expressed in IU/L.

The concentration of bilirubin was measured in accordance to Jendrasik and Grof method (Jendrasik & Grof, 1938). The measured levels were expressed in mg/dL.

Total protein level was measured using Biuret method (Plummer, 1988), and the unit was expressed in g/dL.

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.

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 liver function parameters checked for were AST;Aspartate transaminase, ALT;Alanine transaminase, ALP;Alkaline phosphatase, TP;Total protein, ALB;Albumin, TB;Total bilirubin and CB;Conjugate bilirubin.

Table 1 shows the results of the effect of Avocado pear (*Persea americana)* seed extract on 5-FU-induced toxicity on Aspartate transaminase levels in Wistar rats over four weeks. It was observed that the control group maintained a consistent AST level of 27.33±2.52 IU/L across all weeks. The negative control group had significantly lower AST levels (Week 1: 17.67±2.89, Week 2: 18.33±2.89, Week 3: 17.00±0.00, Week 4: 19.00±0.00) compared to the control and Vitamin C groups ($p<0.05$). High doses of the extract-maintained AST levels similar to the control group (Week 1: 23.67±1.53, Week 2: 24.33±1.15, Week 3: 25.33±0.58, Week 4: 24.33±0.58). The combination of Vitamin C and medium dose extract also demonstrated significantly lower AST levels (Week 1: 16.00±2.65, Week 2: 17.66±2.08, Week 3: 19.00±3.46, Week 4: 17.00±0.00) compared to the control and Vitamin C groups, suggesting a synergistic effect ($p<0.05$).Table 2 reveals the results of the effect of Avocado Pear (*Persea americana)* Seed Extract on 5-FU-induced toxicity on Alanine transaminase levels in Wistar rats over four weeks. It was observed that there were no significant differences in ALT levels among the control (21.33±2.52), negative control, and extract-treated groups in Week 1. However, by Week 2, Vitamin C (25.33±1.15) and low dose groups (24.33±1.15) had significantly higher ALT levels compared to the negative control (20.00±1.73) ($p<0.05$). The combination of Vitamin C and medium dose extract showed the highest ALT levels throughout the study (Week 1: 31.67±2.89, Week 2: 27.33±3.06, Week 3: 28.67±5.77, Week 4: 23.00±0.00), significantly different from the control, negative control, and Vitamin C groups ($p<0.05$).Table 3 shows the results of the effect of Avocado Pear (*Persea americana)* Seed Extract on 5-FU-induced toxicity on Alkaline phosphatase levels in Wistar rats over four weeks. The negative control group had significantly lower ALP levels (Week 1: 49.67±1.15, Week 2: 69.00±19.05, Week 3: 48.00±0.00, Week 4: 50.00±0.00) compared to the control group (56.67±2.08) in weeks 1 and 3 ($p<0.05$). The high dose extract group showed similar ALP levels to the control group throughout the study (Week 1: 50.33±1.53, Week 2: 54.67±0.58, Week 3: 49.33±2.31, Week 4: 54.33±0.35). The combination of Vitamin C and medium dose extract resulted in significantly lower ALP levels compared to the control group in week 1 (48.33±2.08, $p<0.05$), but similar levels to the control. Table 4 shows the results of the effect of Avocado Pear (*Persea americana)* Seed Extract on 5-FU-induced toxicity on Total protein levels in Wistar rats over four weeks. The result revealed that the control group maintained consistent TP levels (67.33±2.52), while the negative control group had significantly lower levels in weeks 1, 2,

and 4 (58.67±2.89, 58.67±1.15, 60.00±0.00) ($p<0.05$). The Vitamin C group showed lower TP levels compared to the control group in weeks 1 and 2 (57.67±3.06, 55.00±1.73) but similar levels in week 4 (64.00±0.00). The high dose extract group demonstrated TP levels comparable to the control group in week 4 (56.33±0.58), suggesting a protective effect. The combination of Vitamin C and medium dose extract showed no significant differences compared to the control group, indicating potential normalization (Week 1: 52.00±2.65, Week 2: 58.67±1.15, Week 3: 57.67±0.58, Week 4: 60.00±0.00).Table 5 shows the results of the effect of Avocado Pear (*Persea americana)* Seed Extract on 5-FU-induced toxicity on Albumin levels in Wistar rats over four weeks. The result reveals that the control group maintained stable ALB levels (43.00±2.00), while the negative control group had significantly lower levels in weeks 1 and 2 (39.67±2.89, 38.33±2.31) ($p<0.05$). The Vitamin C group and all doses of the extract showed lower ALB levels compared to the control group throughout the study, with no significant differences observed among them. The combination of Vitamin C and medium dose extract had the lowest ALB levels in week 1 (36.67±1.53, $p<0.05$) but showed a significant increase by week 4 (40.00±0.00).Table 6 shows the results of the effect of Avocado Pear (Persea americana) Seed Extract on 5-FU-induced toxicity on Total bilirubin levels in Wistar rats over four weeks. No significant differences were observed in TB levels among the control (5.87±0.32), negative control (Week 1: 3.63±0.58, Week 2: 3.80±0.69, Week 3: 3.00±0.00, Week 4: 4.00±0.00), and extract-treated groups throughout the study. The combination of Vitamin C and medium dose extract showed a significant difference in week 2 compared to the Vitamin C group (3.77±0.38, $p<0.05$), but no significant changes were noted in other weeks. Table 7 shows the results of the effect of Avocado Pear (Persea americana) Seed Extract on 5-FU-induced toxicity on Conjugated bilirubin levels in Wistar rats over four weeks. No significant differences were observed in CB levels among the control (3.60±0.26), negative control (Week 1: 2.03±0.40, Week 2: 2.07±0.40, Week 3: 2.00±0.00, Week 4: 2.10±0.00), and extract-treated groups throughout the study. The combination of Vitamin C and medium dose extract showed lower CB levels in week 1 (1.87±0.15) compared to the control group, but no significant differences were noted in subsequent weeks.

Table 1: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Aspartate transaminase (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	21.33±2.52	21.33±2.52^{c}	21.33±2.52	21.33±2.52
Negative Control	18.67 ± 1.15	20.00±1.73^{c}	21.00±0.00	20.00±0.00
Vitamin C (500mg/kg)	22.67 ± 2.00	25.33±1.15^{ab}	23.00±0.00	22.00±0.00
Low dose (250mg/kg)	20.67 ± 0.58	24.33±1.15^{b}	21.00±0.00	20.00±0.00
Medium dose (500mg/kg)	21.00 ± 2.00	22.67±1.15^{c}	22.00±0.00^{b}	23.00±0.00
High dose (1000mg/kg)	22.00 ± 2.00	24.00±1.15^{b}	22.33±1.15	23.00±0.58
Vit C + medium dose	31.67 ± 2.89^{abc}	27.33±3.06^{ab}	28.67±5.77^{abc}	23.00±0.00

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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 Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Alanine transaminase (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	27.33 ± 2.52^{b}	27.33 ± 2.52^{bc}	27.33 ± 2.52^{b}	27.33 ± 2.52^{b}
Negative Control	17.67 ± 2.89^{ac}	18.33 ± 2.89^{a}	17.00 ± 0.00^{ac}	19.00 ± 0.00^{a}
Vitamin C (500mg/kg)	24.66 ± 11.71^{b}	19.33 ± 1.15^{a}	25.00 ± 0.00^{b}	23.00 ± 0.00^{b}
Low dose (250mg/kg)	21.33 ± 0.58^{ab}	21.33 ± 1.15^{a}	23.00 ± 0.00^{b}	25.00 ± 0.00^{b}
Medium dose (500mg/kg)	22.00 ± 5.56^{ab}	21.66 ± 1.15^{a}	24.33 ± 0.58^{b}	26.00 ± 0.00^{b}
High dose (1000mg/kg)	23.67 ± 1.53^{b}	24.33 ± 1.15^{bc}	25.33 ± 0.58^{b}	24.33 ± 0.58^{b}
Vit C + medium dose	16.00 ± 2.65^{ac}	17.66 ± 2.08^{a}	19.00 ± 3.46^{ac}	17.00 ± 0.00^{ac}

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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 Avocado Pear (Persea americana) Seed Extract on 5-FU-induced toxicity on Alkaline phosphatase (IU/L) in Wistar rats

Groups	67.33±2.52^{bc}	67.33±2.52^{bc}	67.33±2.52^{c}	67.33±2.52^{b}
Control	58.67±2.89^{a}	58.67±1.15^{a}	65.00±0.00^{c}	60.00±0.00^{a}
Negative Control	57.67±3.06^{a}	55.00±1.73^{a}	60.00±0.00^{ab}	64.00±0.00^{b}
Vitamin C (500mg/kg)	60.67±5.51^{a}	61.00±6.93^{ac}	64.00±0.00^{c}	58.00±0.00^{ac}
Low dose (250mg/kg)	52.67±6.66^{abc}	61.33±0.58^{ac}	55.00±1.73^{abc}	59.00±0.00^{ac}
Medium dose (500mg/kg)	58.33±2.52^{a}	62.00±1.73^{ac}	56.33±2.31^{ab}	56.33±0.58^{ac}
High dose (1000mg/kg)	52.00 ± 2.65^{abc}	58.67±1.15^{a}	57.67±0.58^{ab}	60.00±0.00^{a}
Vit C + medium dose				

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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 Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Total Protein (g/dL) in Wistar rats

Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	56.67±2.08^{bc}	56.67±2.08^{bc}	56.67±2.08^{bc}	56.67±2.08^{b}
Negative Control	49.67± 1.15^{a}	69.00±19.05^{ac}	48.00±0.00^{a}	50.00±0.00^{a}
Vitamin C (500mg/kg)	52.00±1.00^{a}	49.00±1.73^{ab}	50.00±0.00^{a}	53.00±0.00^{a}
Low dose (250mg/kg)	62.00±1.00^{abc}	60.67±2.31^{abc}	63.00±0.00^{abc}	60.00±0.00^{bc}
Medium dose (500mg/kg)	55.67±6.81^{b}	51.33±1.15^{abc}	51.33±0.58^{a}	60.00±0.00^{bc}
High dose (1000mg/kg)	50.33±1.53^{a}	54.67±0.58^{bc}	49.33±2.31^{a}	54.33±0.35^{b}
Vit C + medium dose	48.33± 2.08^{a}	51.33±1.53^{ab}	50.33±2.89^{a}	50.00±0.00^{a}

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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 Avocado Pear Seed Extract on 5-FU-induced toxicity on Albumin (g/dL) in Wistar rats

Values are presented in Mean and Standard deviation.

Groups	Week 1	Week 2	Week 3	Week 4
Control	5.87±0.32	5.87±0.32	5.87±0.32	5.87±0.32
Negative Control	3.63 ± 0.58	3.80±0.69	3.00±0.00	4.00±0.00
Vitamin C (500mg/kg)	5.13 ± 2.25	3.97±0.23	5.30±0.00	5.80±0.00
Low dose (250mg/kg)	4.60 ± 0.10	4.53±0.46	4.60±0.00	6.30±0.00
Medium dose (500mg/kg)	4.87 ± 1.19	5.00±0.17	5.10±0.35	5.20±0.00
High dose (1000mg/kg)	5.03 ± 0.15	4.83±0.12	5.37±0.12	4.93±0.23
Vit C + medium dose	3.63 ± 0.57	3.77±0.38^{c}	4.00±0.69	4.00±0.00

Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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 6: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Total bilirubin (mg/dL) in Wistar rats

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$)

Groups	Week 1	Week 2	Week 3	Week 4
Control	43.00±2.00^{b}	43.00±2.00^{b}	43.00±2.00^{c}	43.00±2.00^{c}
Negative Control	39.67 ± 2.89^{a}	38.33±2.31^{a}	42.00±0.00	45.00±0.00^{c}
Vitamin C (500mg/kg)	37.00 ± 3.61^{a}	37.67±2.31^{a}	41.00±0.00^{ab}	38.00±0.00^{ab}
Low dose (250mg/kg)	39.33 ± 2.52^{a}	38.33±2.89^{a}	38.00±0.00^{a}	36.00±0.00^{ab}
Medium dose (500mg/kg)	36.67 ± 4.04^{a}	40.33±2.31^{a}	39.00±3.46^{a}	39.00±0.00^{ab}
High dose (1000mg/kg)	37.33 ± 2.52^{a}	39.00±1.73^{a}	40.00±1.73^{a}	37.00±1.73^{ab}
Vit C + medium dose	36.67 ± 1.53^{a}	38.67±1.53^{a}	36.00±1.15^{ac}	40.00±0.00^{b}

from each other.
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 7: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on Conjugated bilirubin (mg/dL) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	3.60±0.26	3.60±0.26	3.60±0.26	3.60±0.26
Negative Control	2.03 ± 0.40	2.07±0.40	2.00±0.00	2.10±0.00
Vitamin C (500mg/kg)	3.03 ± 1.57	2.33±0.46	3.20±0.00	3.10±0.00
Low dose (250mg/kg)	2.93 ± 0.15	2.57±0.23	2.50±0.00	3.50±0.00
Medium dose (500mg/kg)	2.27 ± 0.45	2.37±0.13	3.00±0.17	3.00±0.00
High dose (1000mg/kg)	3.33 ± 0.15	3.33±0.46	3.57±0.12	3.57±0.06
Vit C + medium dose	1.87 ± 0.15	1.80±0.10	1.97±0.58	2.30±0.00

Values are presented in Mean and Standard deviation.
Mean with different superscript along the same vertical array are significantly different ($p<0.05$) from each other.
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.

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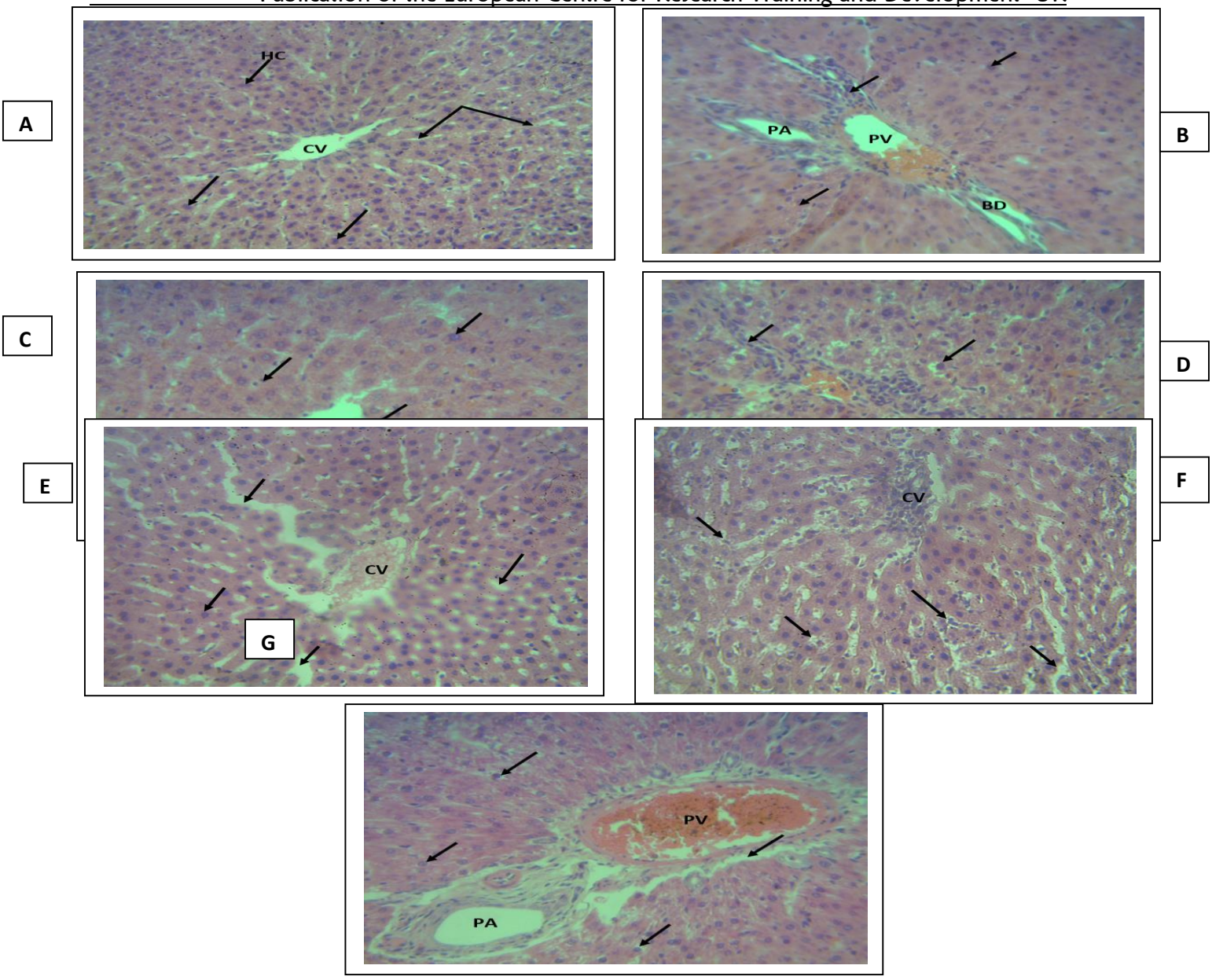

Figure A-G shows the photomicrographs of the effect of the extract on the liver.
(A) Normal control, (B) Negative control, (C) 500mg/kg of vitamin C, (D) 250mg/kg extract, (E) 500mg/kg extract (F) 1000mg/kg extract, (G) 500mg/kg of vitamin C +500mg/kg of Extract

Effect of *Persea americana* seed extract on Liver Histopathology

Figure A shows the photomicrograph (H&E X400) of the liver from group one (normal control group) with normal morphology of the central venules (CV); hepatocytes (HC), and sinusoids (SS) within zone 3 of the liver parenchymal (arrows). The histology revealed normal liver tissue.

Figure B (Group 2; Negative control) shows the photomicrograph (H&E x400) of the liver showing the bile duct (BD) with mild portal vessels congestion with reduced inflammatory cell infiltration activities within the liver parenchymal (arrows). The diagnosis reveals mild inflammation of the liver tissue.

Figure C (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the liver showing kupfer cell emptying into the central vein with normal hepatocytes (HC), and sinusoids (SS) (arrows) and the diagnosis from this shows normal liver tissue with mild distortion.

Figure D (Group 4; 250mg/kg (Low dose) of extract) presents the photomicrograph (H&E x400) of the liver showing a well delineated mononuclear infiltration within the portal vessels (arrows) and the diagnosis reveals Inflammation of the liver tissue.

Figure E (Group 5; 500mg/kg (Medium dose) of extract) shows the photomicrograph (H&E X400) of the liver showing the central vein (CV) and diffused cytoplasmic vacuolation of the liver parenchymal (arrows) and the histology reveals Moderate distortion of the liver tissue parenchyma.
Figure F (Group 6; 1000mg/kg (High dose) of extract) presents the photomicrograph (H&E x400) of the liver showing diffused lobular vacoulation associated with mononuclear activities within of the central vein (CV) and the diagnosis reveals that there was moderate inflammation of the liver tissue.

Figure G (Group 7; Vitamin C + Medium dose of extract) shows the photomicrograph (H&E x400) of the liver showing mild hepatocyte hypotrophy with sinusoidal constriction within the liver parenchymal (arrows) and the histology denotes mild sinusoidal constriction and hepatocyte hypertrophy

DISCUSSION

5-fluorouracil (5-FU), has demonstrated cancer treating effectiveness (Sakai et al., 2019), but because of the hepatotoxic and nephrotoxic adverse effects which arises with its administration, precautions are now been taken as regards its use clinically (Zhang et al., 2020).This investigation is required because, after the Wistar rats were injected with 5-FU, some practical changes in the organ's activity as well as alterations in biochemical indices were observed. Because this is essential for innovative drug search, the practical viability of organs must be evaluated by tracking the quantity of biochemical markers in the serum and evaluating the practical functionality of organs after this exposure.

The largest parenchymatous organ in the human system which is the liver is vital to mediate metabolism (Guyton & Hall, 2016a). The liver's close proximity to portal blood that returns from the gut and the systemic flow is necessary for various biological events (Alberts et al., 2002). The structuring and effectiveness of the liver's middle point metabolism of nourishment (proteins, glucose, and fatty acids), as well as elimination of harmful or infectious substances, metabolic cleansing, and contaminants flushing, are all facilitated by the spatial connections of hepatocytes with its different cellular components, including kupffer cells, bile canaliculi, and fenestrated sinusoidal endothelial cells (Roy-Chowdhury & Roy-Chowdhury, 2006). Elevated levels of the liver function biomarkers like AST, ALP, ALT, CB, TB, ALB as compared to the control groups indicates one form of liver toxicity or the other like jaundice, liver cirrhosis, or a bone disorder. Higher levels of ALB, TB and CB, denote elevated bilirubin which signifies liver or bile duct dysfunction. But only a decrease in TP amount compared with control group indicates liver disease and malnutrition. High levels of TP may be due to dehydration or certain bone disorders (Guyton & Hall, 2016). Specifically, the book states:"A decrease in the total protein concentration in the blood usually indicates either liver disease or malnutrition, because the liver is not producing enough albumin and globulins, or that the body is not absorbing enough amino acids to synthesize these proteins." Hepatic impairment is frequently evaluated by measuring enzyme phases, such as ALT and AST. Damage to the liver's membranes, or necrosis, makes intracellular enzymes available for circulation and serum detection. Since the region is altered by ALT-catalyzed processes and glutamate and pyruvate may be produced, raised AST concentrations are indicative of liver injury. Elevated levels of these enzymes in serum signify a loss in the integrity of the hepatic membrane. The amount of serum, total protein, ALP, and total bilirubin are also linked to the health of liver cells. This agrees with Sajid et al. (2016) that the elevated biliary pressure affects a spike in serum ALP.

Observation of the table shows increase in the extract's protective influence on the liver even though it wasn't consistent with all of the parameters but the safety of the extract against the adverse effect caused by 5-FU outweighs the toxicity on the liver. With AST, all values across the treatment groups from week one to four were lesser than the control group and this is in consonant with the work carried out by Kumar, (2022). In ALT activity, most of the groups had lesser values when compared with the control group and only the groups of rats that received vitamin C+MD of the extract had remarkably greater mean values than the control and the drop in values of this enzyme compared with the control group supports the protective influence; of the extract on the liver against liver cirrhosis, liver fat and other forms of non alcoholic fatty liver diseases (NAFLD) like steatosis and steatohepatitis. Decrease in NAFLD could be due to a reduction in mitochondrial dysfunction and decrease in oxidative stress levels which are a positive effect of the pear seed and this study is supported by a previous research carried out by Oboh, (2020). Specifically, the study found that the extract reduced mitochondrial dysfunction, decreased oxidative stress levels and attenuated NAFLD in rats. For ALP, though the trend was not consistent throughout the four weeks but it was noted that the groups of animals that received low dose of the extract had higher mean values when compared with control and this could likely be due a decrease in the effectiveness of the low dose of the extract and decrease in the antioxidant level when compared to the groups of

animals that took the high dose of the extract and the combination of the medium dose and vitamin C. With TP, the control group maintained a consistent TP levels while the negative control had significant lower levels in week 1, 2, and 4. However, the high dose extract group demonstrated TP levels comparable to the reference group suggesting a protective effect of the extract. Also, it was observed that the combination of vitamin C and the 500mg/kg dose extract showed no significant variation with control group, indicating potential normalization. For ALB, it was observed that the control group had higher mean values when compared with the rest of the groups but there was not much significant difference across the columns of treatment groups. The mean values of TB and CB decreased when it was compared to the control group denoting low toxicity on the liver and decrease in issues relating to the bile duct.

The results gotten from this study on the protective effect of avocado pear seed extract and vitamin C on the liver is supported by previous research carried out by Abdul et al. (2022), were a comprehensive study to explore the chemopreventive mechanisms offered by hydroethanolic extracts from avocado fruit and seeds against diethylnitrosamine (DEN)/2-acetylaminofluorene (2AAF)-induced hepatocarcinogenesis in rats. Their findings demonstrated significant reductions in liver enzyme activities, total bilirubin levels and also increased protein levels. Even though similar standard drug was not been used, this indicate improved hepatic function post-treatment with *Persea americana* seed extracts.

In the present study, following the liver tissue analysis, normal structure and histology were determined in the control groups but marked histological and ultra structural alterations were observed in groups (2-7) as well as mild to moderate distortion in the liver tissues of the Wistar rats following induction with 5-FU and treatment with various doses of the extract and vitamin C. The histological changes included mild to moderate inflammation of the liver and moderate distortion of the liver tissue parenchyma, mild sinusoidal constriction and hepatocyte hypertrophy and mild inflammatory cell infiltration. From the photomicrographs, there were cases of inflammation of the liver cells ranging from mild to moderate. This inflammation in the liver could be due to presence of fatty infiltrations which represents patterns of hepatic injury as a result of exposure to 5-FU. Lipid accumulation in the hepatocytes is well known as a pathological change that occurs in the cells under a variety of deleterious conditions and could be due to an alteration in the metabolism of the hepatic cells (Wheater et al., 1990). The increase in fat was explained by Mori (1983), who stated that increased lipid in the hepatocytes after exposure of drugs or toxins could be due to impaired synthesis of lipoproteins or due to the abnormal transport of lipoproteins via golgi apparatus and its secretory vacuoles. These findings were in accordance with the experimental studies conducted by others (Abdelmeguid et al., 2010). Also, with all of the photomicrographs, there were no severe effect on the liver; neither were there any form of tissue injury or necrosis. This shows likely protective effect of the on the liver tissues and this could be due the phytochemicals present in the extract and the antioxidant property of the extract in preventing oxidative stress on the liver tissue. Though, this research shows some positive effect of the extract on the tissues, more research on this is recommended.

CONCLUSION

This study evaluated the effect of avocado pear seed extract on 5-FU induced hepatic histotoxicity in Wistar rat. Results from the current study revealed that *Persea americana* seed extract is potent in reversing toxicities to the liver caused by the administration of 5-FU. Also, the combination of the medium dose of the extract with vitamin C also proved to be potent in reversing the toxic side effects of 5-FU which was accomplished by decreasing oxidative stress and inflammation to the liver tissue. However, further experiments are required to unveil other molecular pathways by which the extract exerts this beneficial effect on the liver of Wistar rats.

Recommendations

Based on the findings from this study, the following recommendations are made:

1. Further studies should be carried out to determine if the extract is clinically applicable
2. Research to evaluate if the knowledge from this study can be used as a guide by pharmaceutical scientist in the production of a new drug from the extract is required.
3. Also, studies to determine if the use of this extract would have an effect on the potency of 5-FU in the treatment of cancer is also recommended.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

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