Assessment of the impact of *Persea americana* seed extract on 5-Fluorouracil Induced Cardio Histotoxicity and Oxidative Stress in Wistar Rats

Naomi Akpevwe Mogborukor[1*]
[1]Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.
[1]Email Address:naomimogborukor@gmail.com

Joshua Charles Isirima[2]
[2]Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria.
DOI: 10.56201/ijhpr.v9.no4.2024.pg16.36

Abstract
This study assessed the impact of Persea americana seed extract on 5-fluorouracil (5-FU) induced cardio-histoxicity and oxidative stress in Wistar rats. 72 animals from a total of 77 were treated intraperitonially with a single dose of 150mg/kg of 5-FU to simulate toxicity in the heart and to cause oxidative stress. These animals were grouped into six (group 2-7). However, animals in group 1 served as control and received only feed and water. Group 2 was labeled negative control, animals in group 3 were administered with 500mg/kg of vitamin C while the animals in groups 4-6 received 250mg/kg, 500mg/kg and 1000 mg/kg of the extract and those in group 7 received the medium dose of vitamin C in combination with 500mg/kg of the extract. Groups 2-7 had 12 animals and 3 from each group were sacrificed weekly for 4 weeks. The animal's blood and tissue was collected for biochemical and histological analysis. It was observed that the administration of 5-FU caused cardio-toxicity and oxidative stress which led to some alterations in the histology of the heart and changes in oxidative stress markers like Glutathione, Catalase, Superoxide dismutase and Malondialdehyde. However, treatment with the extract ameliorated the adverse effects associated with the drug via enhancing antioxidant defense, decreasing inflammation and suppressing toxicity to the heart.

Keywords*: 5-fluorouracil, Persea americana seed, cardio-histotoxicity, oxidative stress markers*

INTRODUCTION

5-Fluorouracil is a commonly used anticancer drug belonging to the class of antimetabolite and also a pyrimidine analog for the management of neoplasms (Longley et al., 2003). Though useful in the management of head, neck and gastrointestinal tumors, research states that it has the potential of causing toxicity to the body. Some toxic effects common with the use of this drug includes hair loss, liver toxicity, toxicity to the heart, kidney and brain, mucositis, myelosuppression, dermatitis, and toxicity of genital organs (Al-Asmari et al., 2016). Some of

these toxicity's could range from been acute to chronic and could also cause death if it is not properly managed.

Persea americana seed is a very useful part of the avocado pear plant which is highly nutritional and medicinal and it represents about 13 %–17 % of the fruit. It is rich in various bioactive components, namely polysaccharides, proteins, lipids, minerals, and vitamins (Tremocoldi et al., 2018).Some of the important attributes of the seed according to Naomi & Joshua (2024), 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. The seed is also a good 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 seed powder in the management of various forms of heart diseases (Imafidon & Amaechina, 2010). With the awareness on the importance of avocado seed and its antioxidant-rich property, its use is gaining increasing acceptance (Araújo et al., 2018).

Due to the phytochemicals that the seed contains and it's antioxidant property (Unlu et al., 2005), this study evaluated the impact of the seed extract on 5-FU induced cardio-histotoxicity and oxidative stress on Wistar rats.

On the aspect of blood pumping, the heart is saddled with this responsibility. Pumping blood needed by the body for proper functioning of the body system. One of the most serious side effects seen with the use of 5-FU is cardiotoxicity. This cardiotoxicity can trigger various heart diseases in the body like angina, arrhythmia, myocardial infarction and sudden death (Labianca et al., 1982). The effect of 5-fluorouracil (5-FU) on the heart is a complex interplay involving direct cardiotoxic effects, metabolic reprogramming of resistance mechanisms in cancer cells, and potential strategies for mitigating these adverse outcomes associated with its administration. Thalambedu & Khan, (2019), through a case report, stated the clinical manifestation of 5-FU-induced cardiomyopathy, emphasizing its potential severity but also the ability of these toxic effects to reverse upon drug withdrawal. When 5-FU is used, cardiotoxic effects usually happen during the first cycle of medication administration (Saif et al., 2009). Although cardiotoxicity can happen at any point during the infusion or even up to 1-2 days after the start of the infusion, the median time to symptom onset is 12 hours after the infusion begins (Baselin et al., 2011). However, after stopping the medication, symptoms and alterations in the electroencephalogram may go away or persist for a few days.

These findings collectively suggest that while direct cardiotoxic effects of the chemotherapeutic agents 5-FU is of great concern, integrating targeted therapies or protective agents and also the use of antioxidants would be of advantage: enhancing anti-cancer efficacy while potentially mitigating adverse cardiovascular impacts and preservation of cardiac function. The mechanism of action underlying the cause of cardiotoxicity by 5 FU is not clearly understood but however, some studies suggest that vasospasm, presence of plaques or clot that blocks blood vessels (Heistad et al., 1984), thrombosis, arthritis, increased oxygen demand and decreased oxygen supply (Tsibiribi et al., 2006), endothelial dysfunction (which is a way of reaction to injury of the vasculature to variety of circumstances) (Bonetti et al., 2004) etc may be responsible for the cardiotoxicity associated

with the drug including a direct myocardial toxicity, activation of auto immune responses and direct coronary endothelial intima toxicity.

Dihydropyrimidinedehydrogenase breaks down 5-FU, reduce its rate of accumulation thereby reducing cardiotoxicity associated with it. Risk factors of 5-FU induced cardiotoxicity include age, presence of other cardiovascular diseases, smoking, drinking and diabetes.

5-FU induced cardiotoxicity is likely fatal so the first thing to do to prevent it's toxicity is to discontinue the drug use, treat symptoms empirically with anti angina agents like nitrites/ nitrates, calcium channel blockers, vasodilators, beta adrenoceptor blockers, sodium channel blockers etc.It is reported that this style was proven to stop systems in up to roughly 70% of patients of symptoms are known to be absorbed by this procedure (Jensen & Sorensen, 2006).Asides the use of these drugs, cardiac symptoms can also be traced by capturing transient arrhythmia with the use of agents like electroencephalogram, laboratory testing of cardiac enzymes and brain natriuretic peptides.

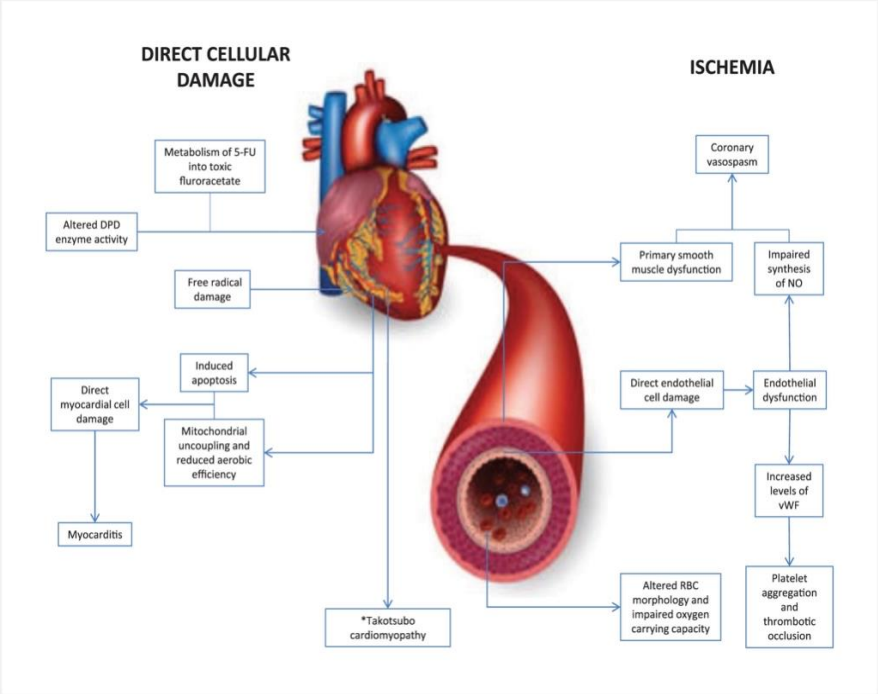

Figure 1.1: Diagram outlining the two potential; mechanisms by which 5-fluorouracil could lead to cardio toxicity: direct cellular damage and ischemia. (Jasvinder et al., 2018).

When there is an imbalance between reactive oxygen species and antioxidant activity, oxidative stress occurs. For example, too much hydroxyl radical and peroxynitrite (ONOO-) can lead to lipid peroxidation, which harms lipoproteins and cell membranes. A surplus of this free radical can also cause oxidative harm to proteins, DNA, and lipid peroxidation, as well as adverse effects on a number of cellular components, including membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) (Droge, 2002). Numerous tests unequivocally show that oxidative stress may harm both DNA and RNA. According to reports, DNA is thought to be a prominent

target, particularly in cancer and aging (Woo et al., 1998). Deoxythioguanosine (dTG), glycol, and 8-hydroxy-2-deoxyguanosine are examples of oxidative nucleotides that are reported to be elevated following oxidative harm to DNA caused by UV rays or free radical harm. According to reports, mitochondrial DNA is more prone to oxidative harm, which is linked to a number of illnesses, including cancer. According to Hattori et al. (1997), 8-hydroxy-2-deoxyguanosine may be employed as a biological marker for oxidative stress. Lipid peroxidation, also known as lipid oxidation, is a multifaceted chemical procedure that causes lipids to degrade (Joanna, 2016), producing peroxide and its components, including hydroperoxide (Ayala, 2014). Reactive oxygen species (ROS), a kind of free radical, interact with lipids found in cell membranes. Since PUFAs include carbon–carbon double bonds, they are commonly polyunsaturated fatty acids (PUFAs). Lipid radicals, also known as lipid peroxides or lipid oxidation products (LOPs) are created as a result of this process. These LOPs then react to additional oxidizing substances, setting off a series of events that cause oxidative stress and harm to cells. Proteins are susceptible to damage from reactive oxygen species (ROS), which can also result in the production of carbonyls, methionine sulfoxide, protein peroxide, and other amino acid modifications. Aging is caused by changes in the signaling transduction pathway, enzyme function, heat strength, and vulnerability to proteolysis caused by protein oxidation. Many investigations revealed that oxidative stress may have a role, albeit to varying degrees, in the development and/or advancement of a number of illnesses, like diabetes, cancer, metabolic conditions, atherosclerosis, and heart problems (Taniyama & Griendling, 2003).

Free radicals are atom, molecule, or ion that has at least one unpaired valence electron in them which is highly reactive (Hayyan et al., 2016). Radicals are extremely volatile due to their free electrons. They are very erratic molecules with accessible electrons for reactions with a wide range of organic materials, including proteins, lipids, DNA, and so forth. These free radicals include reactive oxygen species (ROS) which includes superoxide radicals (O2−), hydrogen peroxide (H2O2), hydroxyl radicals (OH), and singlet oxygen (O2). These radicals are produced as metabolic byproducts by living things (Sato et al., 2013). Many transcriptional variables protection, distinction, apoptosis, and protein phosphorylation are all reliant upon appropriate ROS formation and availability within cells, which must be maintained at a reduced rate (Gnanadhas et al., 2014). The free radicals also include reactive nitrogen species like nitric oxide and peroxynitrite ($ONOO^-$).

Antioxidants are molecules stable enough to donate electrons to the rampaging free radicals thereby neutralizing its toxic effect. Antioxidants are agents that have an intrinsic reactive oxygen species scavenging and neutralizing capacity. To defend against ROS-induced cell harm, antioxidant defense mechanism mostly composed of enzyme elements, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Deponte et al., 2013) are useful. Antioxidants are agents that obstruct oxidation, which is a chemical process that might birth free radicals (autoxidation). Organic materials, particularly living things, are degraded by autoxidation, which releases free radicals as outcomes. Antioxidants can reduce the chance of developing particular oxidative diseases associated with stress, such as cancer, liver, kidney, and cardiovascular disorders, as well as mortality. The real or purported therapeutic effects of several

antioxidants, including vitamin E, vitamin C, flavonoids, and polyphenols, against oxidative stress have been the subject of much research in recent years.

MATERIALS AND METHODS

Procurement of Animals

This research was carried out in the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria. For the purpose of this study, avocado pear seeds were were taken to the Department of Botany, University of Port Harcourt, Rivers State for seed identification. A total of 77 Wistar rats of both genders weighing 200g on average were used for the study. The males were separated from the females and were made to acclimatize for a period of 2 weeks and housed in a spacious cage under a 12-hour day and night period. They had unlimited supply of food and water but were deprived of food for 12 hours before lethal dose (LD50 determination).

Ethical approval was gotten for the purpose of the study from University of Port Harcourt, Rivers State, Nigeria.

Method of Avocado pear seed Extraction and LD50 Determination

According to Naomi & Joshua (2024), the seed was extracted using maceration, digestion and decoction procedures. The plant material was boiled in an open extractor to obtain the final product. The finely grounded powdered seeds were thereafter placed in a large container while hot distilled water was added into it as it was been stirred. The solute was allowed to stand for a period of 24 hours until the soluble matter was dissolved. The solution was then strained and the damp solid material was pressed while 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

Lorke's method was used in the determination of oral acute toxicity (LD 50) (Lorke, 1983). A total of 12 animals were used in this method. These animals were divided into two phases, Phase 1 and 2. Their weights were taken and recorded and they were deprived of food for a period of 12 hours with only access to water before the extract was administered at appropriate doses.

Phase l: In this phase, nine Wistar rats were used. The animals were divided into three groups with three rats each. 10 mg/Kg, I00 mg/Kg, and 1000 mg/Kg of the extract were given orally and the animals were thereafter observed for behavioral manifestation of acute toxicity or death within 24 hours of extract administration.

Phase 2: This phase depended on the observations from Phase I, whether or not death was observed. 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 basis of toxicity. **N**o death was observed in both phases denoting that the extract is a safe one.

Experimental Design

Seventy seven (77) Wistar rats used for the study had 12 animals in groups 2-7 while group 1 had 5 animals that were not exposed. They were thereafter treated under the following groups;

Group 1: Control

Group 2: Negative control

Group 3: Received 500mg/kg body weight of vitamin C

Group 4: Received 250mg/kg of extract (Low dose)
Group 5: Received 500 mg/kg of extract (Medium dose)
Group 6: Received 1000mg/kg of extract (High dose)
Group 7: Received 500mg/Kg of extract + 500mg/Kg of vitamin C

Three rats from each of the groups were sacrificed at weekly interval for a period of 4 weeks except for group 1 in which all five rats present in the group was sacrificed on the 28 day. The animal's blood was collected for biochemical evaluation of oxidative function test, while the tissue of the rats were collected and preserved with formalin for histological analysis.

Measurement of Oxidative Stress Parameters

Malondialdehyde concentration was measured in accordance to Ohkawa and Ohishi method (Okhawa et al., 1979) and the unit expressed in umol/L.

Sedlak and Linsay method (Sedlak & Linsay, 1968), was used to measure gluthathione levels and the unit was expressed in unit mmol/L

Clairborne method (Clairborne, 1985), was used for Catalase estimation and the unit expressed in unit U/mL

Misra and Fridovich method (Misra & Fridovich, 1972), was used to estimate superoxide dismutase levels and the unit was expressed in U/mL

Histopathology Examination

The animals were anaesthetized and dissected aseptically to remove the tissue which was later on transferred to about 10% of formaldehyde. The tissue was there after trimmed down to reduce its thickness, to allow for easy penetration of the fixative. The tissues were exposed to different stages of histological processing by standard method as described by Carleton et al., 1967. These stages include fixation, dehydration, embedding, sectioning, staining of the tissues with hematoxylin and eosin (H&E) and finally mounting of the tissues.

Method of Statistical Analysis

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

RESULTS

Oxidative stress parameters measured include GSH; Glutathione, CAT; Catalase, SOD; Superoxide dismutase, and MDA; Malondialdehyde.

Table 1 shows the result of the effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on GSH (mmol/L) in Wistar rats. The control group maintained consistent GSH levels at 2.02±0.46 throughout the study. The negative control group showed fluctuations, with levels increasing to 2.77±0.00 by Week 3 and then slightly decreasing to 2.51±0.00 by Week 4. The vitamin C group showed variability, with levels decreasing to 1.77±0.16 in Week 2 and slightly recovering to 1.93±0.00 in Week 4. 250mg/kg doses of the extract led to a consistent decrease in GSH levels, reaching 1.17±0.00 by Week 4. 500mg/kg doses caused fluctuations, with levels decreasing to 1.73±0.00 by Week 4. 1000mg/kg doses initially increased GSH levels to 3.25±0.50 in Week 2 before stabilizing at 2.10±0.35 by Week 4. The combination of vitamin C

with medium doses resulted in minimal fluctuations, ending at 1.92±0.00 by Week 4. Significant differences ($p<0.05$) were noted when compared to control, negative control, and vitamin C groups. Table 2 shows the result of the effect of Avocado Pear Seed (*Persea americana*) Extract on 5-FU-induced toxicity on CAT (U/mL) in Wistar rats. The control group maintained constant CAT levels at 2.32±0.74 throughout the study. The negative control group showed slight variations, with levels decreasing to 1.96±0.25 in Week 2 and then returning to 2.31±0.00 by Week 4. The vitamin C group exhibited variability, with levels increasing to 2.43±0.48 in Week 2 but decreasing to 1.84±0.00 by Week 4. The 250mg/kg doses of the extract generally decreased CAT activity, reaching 1.08±0.00 by Week 4. 500mg/kg doses showed a similar trend, with levels decreasing to 0.98±0.00 by Week 4. 1000mg/kg doses initially increased CAT levels to 2.61±0.17 in Week 2 before stabilizing at 2.06±0.23 by Week 4. The combination of vitamin C with medium doses led to increased CAT activity, peaking at 2.81±0.57 in Week 3 and slightly decreasing to 2.69±0.00 by Week 4. Significant differences ($p<0.05$) were observed when compared to control, negative control, and vitamin C groups.

Table 3 shows the result of the effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on SOD (U/mL) in Wistar rats. The control group maintained consistent SOD levels at 0.35±0.09 throughout the study. The negative control group exhibited fluctuations, with levels increasing to 0.49±0.00 by Week 3 and slightly rising to 0.51±0.00 by Week 4. The vitamin C group showed variability, with levels decreasing to 0.32±0.00 by Week 4. 250mg/kg doses of the extract led to a marked decrease in SOD levels, reaching 0.17±0.00 by Week 4. 500mg/kg doses caused initial decreases, stabilizing at 0.22±0.00 by Week 4. 1000mg/kg doses initially increased SOD levels to 0.54±0.11 in Week 2 before slightly decreasing to 0.50±0.04 by Week 4. The combination of vitamin C with medium doses resulted in minimal changes, ending at 0.39±0.00 by Week 4. Significant differences ($p<0.05$) were noted when compared to control, negative control, and vitamin C groups. Table 4 shows the result of the effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced toxicity on MDA (umol/L) in Wistar rats. The control group maintained consistent MDA levels at 0.44±0.10 throughout the study. The negative control group showed slight variations, with levels increasing to 0.50±0.05 in Week 2 and then slightly decreasing to 0.46±0.00 by Week 4. The vitamin C group exhibited variability, with levels increasing to 0.46±0.08 in Week 2 and stabilizing at 0.46±0.00 by Week 4. 250mg/kg doses of the extract resulted in higher MDA levels, peaking at 0.63±0.00 in Week 3 and slightly decreasing to 0.62±0.00 by Week 4. 500mg/kg doses caused initial increases, stabilizing at 0.58±0.00 by Week 4. High doses initially showed stable MDA levels, ending at 0.43±0.03 by Week 4. The combination of vitamin C with medium doses resulted in fluctuating MDA levels, ending at 0.42±0.00 by Week 4. Significant differences ($p<0.05$) were observed when compared to control, negative control, and vitamin C groups.

Table 1: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced histotoxicity on Gluthatione (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	2.02±0.46	2.02±0.46	2.02±0.46	2.02±0.46
Negative Control	2.13±0.30	2.14±0.73	2.77±0.00	2.51±0.00
VitaminC (500mg/kg)	2.34±1.14	1.77±0.16	2.32±0.00	1.93±0.00
Low dose (250mg/kg)	1.69±0.08	1.69±0.05	1.68±0.00	1.17±0.00
Medium dose(500mg/kg)	2.07±0.35	1.80±0.36	1.93±0.01	1.73±0.00
High dose(1000mg/kg)	2.25±0.36	3.25±0.50	2.34±0.66	2.10±0.35
VitC + medium dose	1.87±0.12	1.80±0.19	1.60±0.02	1.92±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 histotoxicity on Catalase (U/mL) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	2.32±0.74	2.32±0.74	2.32±0.74	2.32±0.74
Negative Control	2.20±0.70	1.96±0.25	2.10±0.00	2.31±0.00
VitaminC (500mg/kg)	1.72±0.78	2.43±0.48	1.65±0.00	1.84±0.00
Low dose (250mg/kg)	1.39±0.04	1.15±0.05	1.24±0.00	1.08±0.00
Medium dose(500mg/kg)	1.24±0.23	1.22±0.01	1.28±0.11	0.98±0.00
High dose (1000mg/kg)	2.13±0.17	2.61±0.17	1.73±0.45	2.06±0.23
VitC + medium dose	1.71±0.53	2.49±1.16	2.81±0.57	2.69±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 3: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced histotoxicity on Superoxide dismutase (U/mL) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	0.35±0.09	0.35±0.09	0.35±0.09	0.35±0.09
Negative Control	0.43±0.00	0.33±0.08	0.49±0.00	0.51±0.00
VitaminC (500mg/kg)	0.80±0.60	0.43±0.01	0.35±0.00	0.32±0.00
Low dose (250mg/kg)	0.21±0.12	1.15±0.12	0.19±0.00	0.17±0.00
Medium dose(500mg/kg)	0.17±0.02	0.16±0.01	0.22±0.01	0.22±0.00
High dose(1000mg/kg)	0.54±0.07	0.54±0.11	0.55±0.14	0.50±0.04
VitC + medium dose	0.43±0.07	0.38±0.08	0.35±0.14	0.39±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 4: Effect of Avocado Pear (*Persea americana*) Seed Extract on 5-FU-induced histotoxicity on Malondialdehyde (umol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Week 4
Control	0.44±0.10	0.44±0.10	0.44±0.10	0.44±0.10
Negative Control	0.36±0.08	0.50±0.05	0.32±0.00	0.46±0.00
Vitamin C (500mg/kg)	0.35± 0.05	0.46±0.08	0.45±0.00	0.46±0.00
Low dose (250mg/kg)	0.58±0.05	0.58±0.06	0.63±0.00	0.62±0.00
Medium dose(500mg/kg)	0.63± 0.06	0.60±0.01	0.60±0.05	0.58±0.00
High dose(1000mg/kg)	0.41± 0.05	0.40±0.05	0.44±0.02	0.43±0.03
VitC + medium dose	0.41±0.04	0.57±0.14	0.52±0.18	0.42±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.

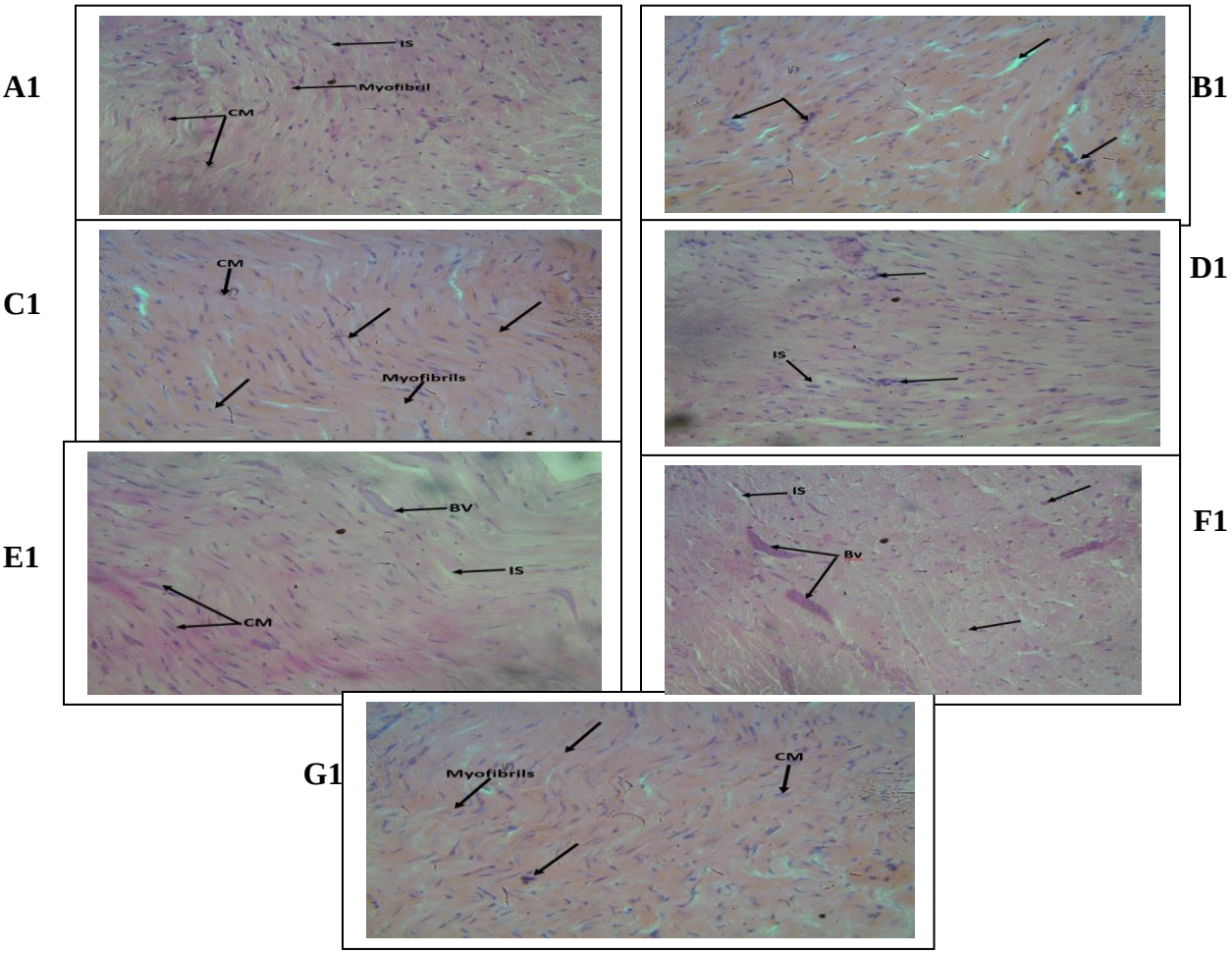

Figure A1-G1 shows the photomicrographs of the effect of the extract on the heart across different treatment groups for the first week.

(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 Cardio Histopathology in week 1

Figure A1 shows the photomicrograph (H&E X400) of the normal myocardium from group one (normal control group) with layers of striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS). The diagnosis reveals normal myocardium.

Figure B1 (Group 2; Negative control) shows the photomicrograph (H&E X400) of the myocardium showing minimal edema, mononuclear activities and cardiac myocytes (CM) interspersed within interstitium (IS) with diagnosis revealing minimal lymphocytic aggregation within the cardiac tissue.

Figure C1 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the myocardium showing wavy appearance with cardiac myocytes (CM) interspersed with interstitium (IS) and myofibrils. The histology reveals mild lymphocytic activities of the cardiac tissue.

Figure D1 (Group 4; 250mg/kg (Low dose) of extract) presents the photomicrograph (H&E X400) of the cardiac tissue with minimal mononuclear infiltration and interstitial edema. And the histology shows minimal inflammation of cardiac tissue.

Figure E1 (Group 5; 500mg/kg (Medium dose) of extract) shows the photomicrograph (H&E X400) of the cardiac muscle showing wavy layers of striated cardiac myocytes (CM), blood vessels (BV) and interstitium (IS). Diagnosis reveals normal myocardium.

Figure F1 (Group 6; 1000mg/kg (High dose) of extract) presents the photomicrograph (H&E x400) of the cardiac muscle showing layers of striated cardiac myocytes, blood vessels (BV) and interstitium (IS). Histology shows normal myocardium.

Figure G1 (Group 7; Vitamin C + Medium dose of extract) shows the photomicrograph (H&E X400) of the myocardium with wavy appearance with cardiac myocytes (CM) interspersed within interstitium (IS) and myofibrils, cardiac tissues appear normal and the diagnosis reveals decreased lymphocytic activities of the cardiac tissue.

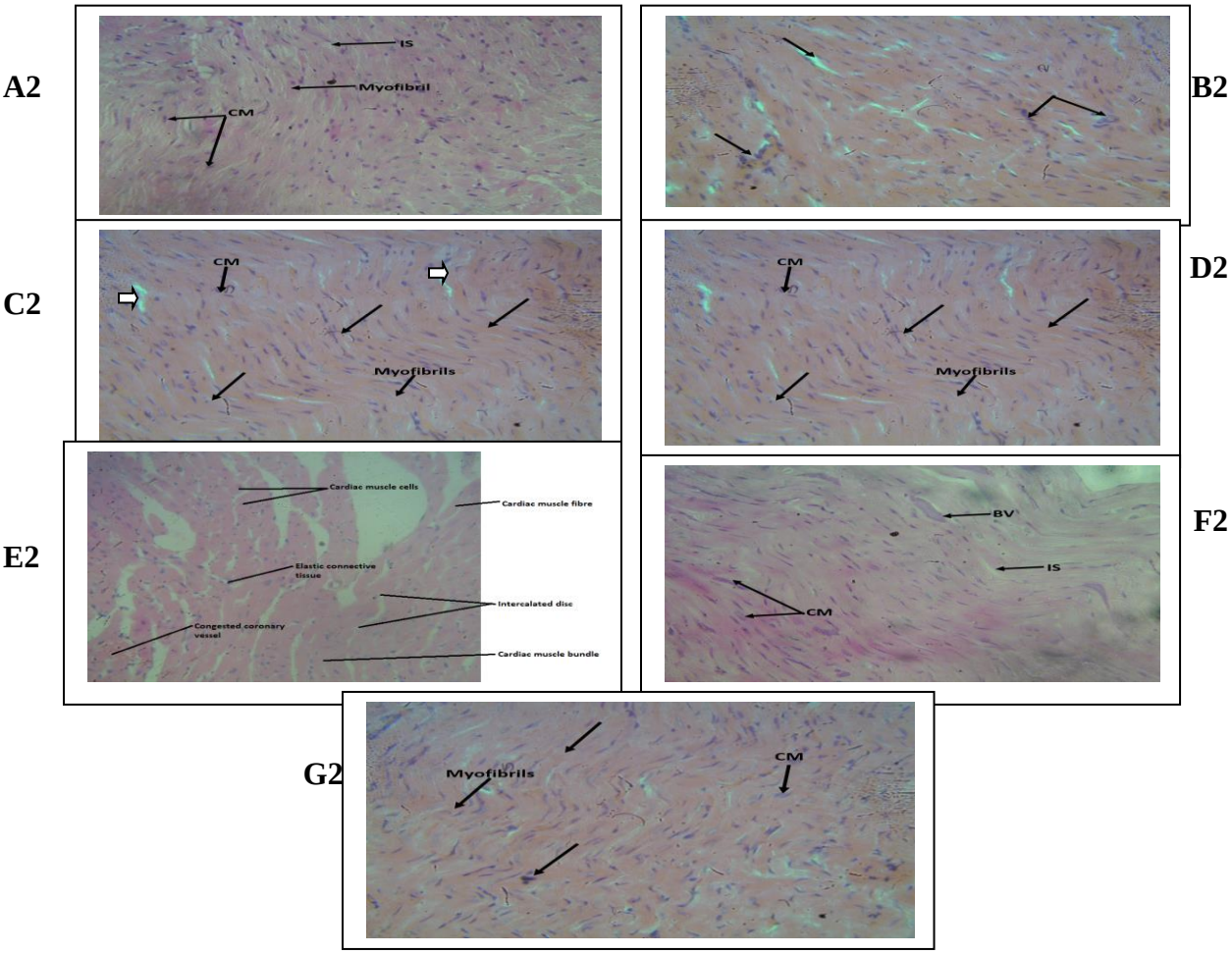

Figure A2-G2 shows the photomicrographs of the effect of the extract on the heart across different treatment groups for the second week.

(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 Cardio Histopathology in week 2

Figure A2 shows the photomicrograph (H&E X400) of the normal myocardium from group one (normal control group) with layers of striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS). The diagnosis reveals normal myocardium.

Figure B2 (Group 2; Negative control) shows the photomicrograph (H&E X400) of the myocardium showing minimal edema, mononuclear activities and cardiac myocytes (CM)

interspersed within interstitium (IS) with diagnosis revealing minimal lymphocytic aggregation within the cardiac tissue.

Figure C2 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the myocardium showing wavy appearance with cardiac myocytes (CM) interspersed with interstitium (IS) and myofibrils. The histology reveals mild lymphocytic activities of the cardiac tissue.

Figure D2 (Group 4; 250mg/kg (Low dose) of extract) presents the photomicrograph (H&E X400) of the cardiac tissue showing wavy appearance with cardiac myocytes (CM) interspersed with interstitium (IS) and myofibrils (arrows). And the histology reveals mild lymphocytic activity of the cardiac tissue.

Figure E2 (Group 5; 500mg/kg (Medium dose) of extract) shows the photomicrograph (H&E X400) of the cardiac muscle showing normal cardiac muscle fibers with intercalated discs, elastic connective tissue and congested coronary vessel. Diagnosis reveals normal myocardium.

Figure F2 (Group 6; 1000mg/kg (High dose) of extract) presents the photomicrograph (H&E x400) of the cardiac muscle showing layers of striated cardiac myocytes, blood vessels (BV) and interstitium (IS). Histology shows normal myocardium.

Figure G2 (Group 7; Vitamin C + Medium dose of extract) shows the photomicrograph (H&E X400) of the myocardium with wavy appearance with cardiac myocytes (CM) interspersed within interstitium (IS) and myofibrils, cardiac tissues appear normal.

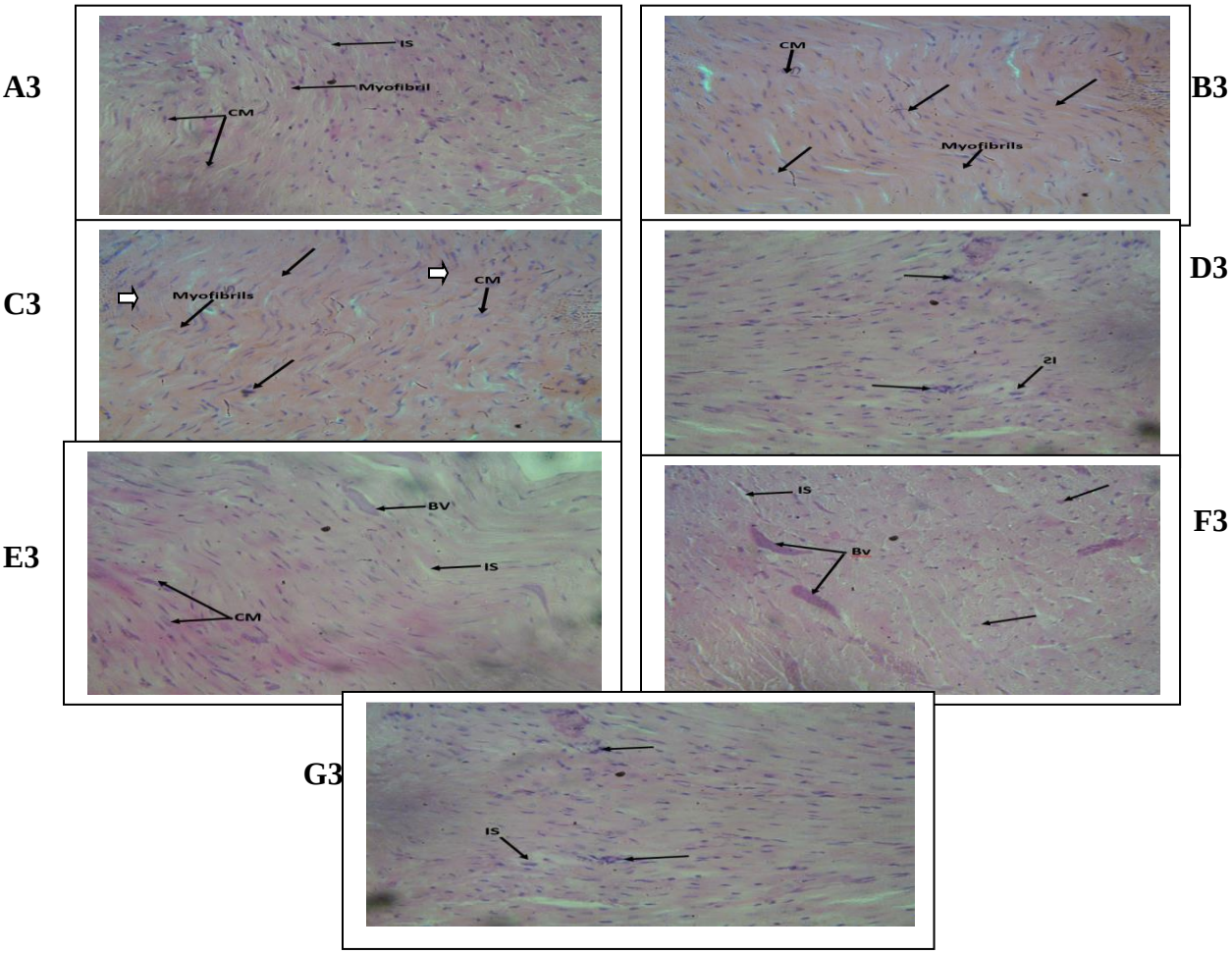

Figure A3-G3 shows the photomicrographs of the effect of the extract on the heart across different treatment groups for the third week.

(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 Cardio Histopathology in week 3

Figure A3 shows the photomicrograph (H&E X400) of the normal myocardium from group one (normal control group) with layers of striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS). The diagnosis reveals normal myocardium.

Figure B3 (Group 2; Negative control) shows the photomicrograph (H&E X400) of the myocardium showing minimal edema, mononuclear activities and cardiac myocytes (CM) interspersed within interstitium (IS) with diagnosis revealing minimal lymphocytic aggregation within the cardiac tissue.

Figure C3 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the myocardium showing wavy appearance with cardiac myocytes (CM) interspersed with interstitium (IS) and myofibrils. The histology reveals mild lymphocytic activities of the cardiac tissue.

Figure D3 (Group 4; 250mg/kg (Low dose) of extract) presents the photomicrograph (H&E X400) of the cardiac tissue showing minimal mononuclear infiltration and interstitial edema (arrows).And the histology reveals minimal inflammation of the cardiac tissue.

Figure E3 (Group 5; 500mg/kg (Medium dose) of extract) shows the photomicrograph (H&E X400) of the cardiac muscle showing wavy layers of striated cardiac myocytes (CM), blood vessels (BV) and interstitium (IS). Diagnosis reveals normal myocardium.

Figure F3 (Group 6; 1000mg/kg (High dose) of extract) presents the photomicrograph (H&E x400) of the cardiac muscle showing layers of striated cardiac myocytes, blood vessels (BV) and interstitium (IS). Histology shows normal myocardium.

Figure G3 (Group 7; Vitamin C + Medium dose of extract) shows the photomicrograph (H&E X400) of the myocardium with minimal mononuclear infiltration and interstitial edema (arrows). Diagnosis: Minimal inflammation of cardiac tissue.

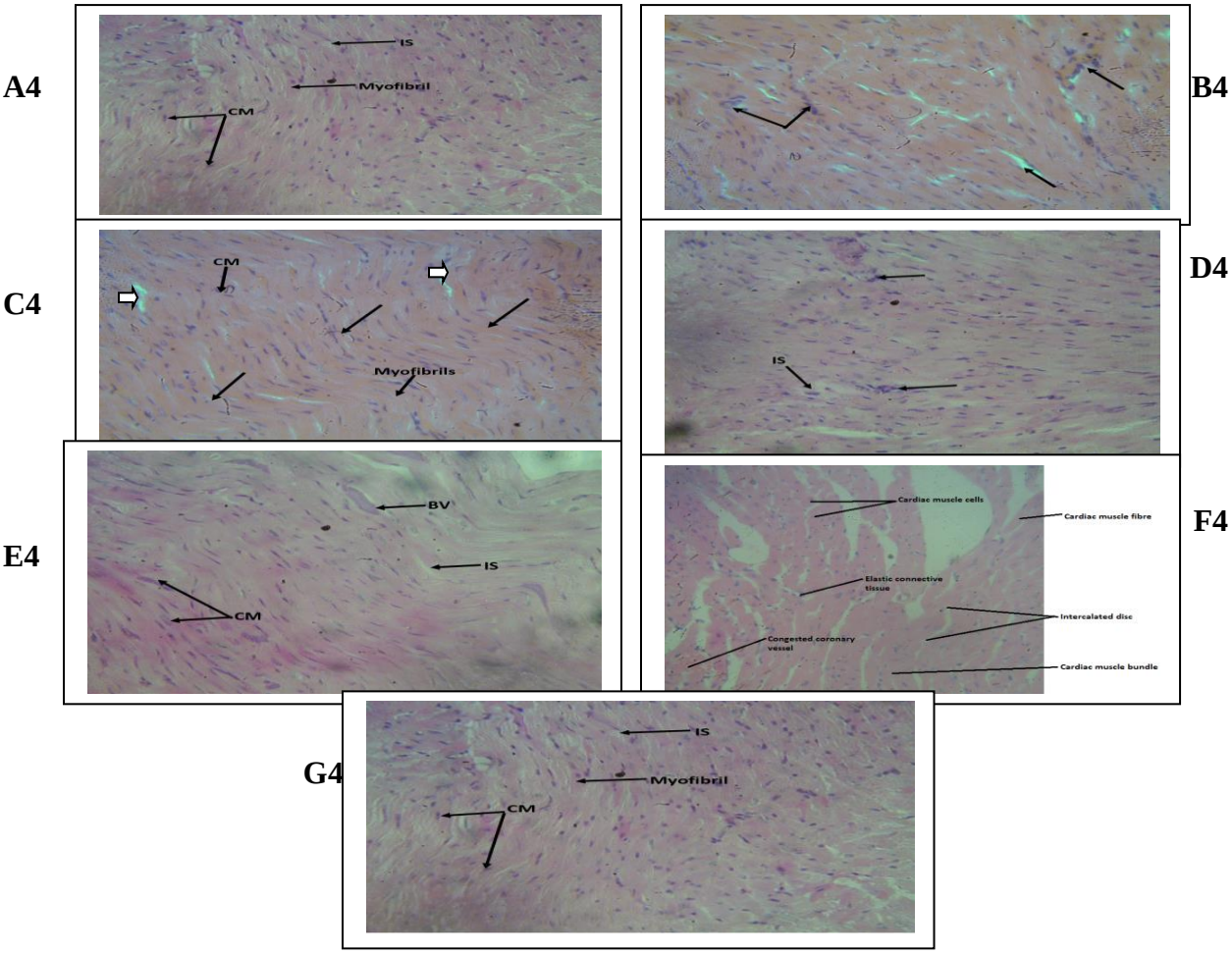

Figure A4-G4 shows the photomicrographs of the effect of the extract on the heart across different treatment groups for the third week.

(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 Cardio Histopathology in week 4

Figure A4 shows the photomicrograph (H&E X400) of the normal myocardium from group one (normal control group) with layers of striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS). The diagnosis reveals normal myocardium.

Figure B4 (Group 2; Negative control) shows the photomicrograph (H&E X400) of the myocardium showing minimal edema, mononuclear activities and cardiac myocytes (CM) interspersed within interstitium (IS) with diagnosis revealing minimal lymphocytic aggregation within the cardiac tissue.

Figure C4 (Group 3; 500mg/kg of Vitamin C) presents the photomicrograph (H&E X400) of the myocardium showing wavy appearance with cardiac myocytes (CM) interspersed with interstitium (IS) and myofibrils. The histology reveals mild lymphocytic activities of the cardiac tissue.

Figure D4 (Group 4; 250mg/kg (Low dose) of extract) presents the photomicrograph (H&E X400) of the cardiac tissue showing minimal mononuclear infiltration and interstitial edema (arrows).And the histology reveals minimal inflammation of the cardiac tissue.

Figure E4 (Group 5; 500mg/kg (Medium dose) of extract) shows the photomicrograph (H&E X400) of the cardiac muscle showing wavy layers of striated cardiac myocytes (CM), blood vessels (BV) and interstitium (IS). Diagnosis reveals normal myocardium.

Figure F4(Group 6; 1000mg/kg (High dose) of extract) presents the photomicrograph (H&E x400) of the cardiac muscle showing normal cardiac muscle fibers with intercalated discs, elastic connective tissue and congested coronary vessel. Diagnosis**:** Normal cardiac muscle.

Figure G4 (Group 7; Vitamin C + Medium dose of extract) shows the photomicrograph (H&E X400) of normal myocardium architecture: layers striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS). Diagnosis: Normal myocardium.

DISCUSSION

When there is inconsistency involving the production of free radicals and the potential of cells to eliminate them, oxidative stress results. For example, too much hydroxyl radical and peroxynitrite (ONOO-) can lead to lipid peroxidation, which harms lipoproteins and cell membranes. Excess of this free radical have consequential influence on structures of the cell like lipoproteins, lipids, proteins, DNA (Droge, 2002), leading to oxidative damage to proteins, DNA and lipid peroxidation. These free radicals are atom, molecules or ion that has at least on unpaired valence electron that is highly reactive with lipids, protein and DNA capable of causing an alteration in them leading to oxidative stress and potential cellular damage.Amongst four parameters of oxidative stress biomarkers, only an increased MDA amount in contrast to baseline group indicates a rise in oxidative stress due to a reduction in antioxidant level necessary to drop ROS influence by neutralizing free radicals in the body via the mechanism known as lipid peroxidation (Halliwell

& Gutteridge 2015). With CAT, SOD and GSH, a decrease in their levels indicates toxicity to the body as a result of reduction in the level of antioxidants necessary in donating electrons to free radicals thereby neutralizing their toxic effect.For the oxidative stress markers, no remarkable variance in mean values of treatment groups when compared to the control group. However, asides from the first week of treatment, there was a consistent increase in the levels of GSH, CAT and SOD and a decrease in MDA following the administration of a raised dose in contrast to the control group and this signifies likely protective effect of the high dose of the extract unlike the low and medium dose against oxidative stress and potential cellular damage across this weeks. This may be due to the level of phytochemicals like tannins, alkaloids, glycosides, oxalate, phytates present in this dose of avocado pear seed extract, and also bioactive agents like phenolics, flavonoids, carotenoids and vitamins that it contains which led to the increase in its antioxidant property. This finding is in consonant with that carried out by Akinyemi et al. (2019), who studied the defending influence of the extract against oxidative stress and cellular destruction and his findings showed the increase in the pear seed extract in reducing oxidative stress due to increase in the reduction of inflammation and apoptosis. More exploration into the phytochemicals and bioactive compounds contained in avocado pear seed needs to be unveiled as this would produce more insight into the antioxidant and potential health benefit of the seed and revolutionize our approach to utilizing agricultural by-products.

One of the study carried out that supports this research, demonstrated that ethanol and acetone extracts from avocado seeds exhibit remarkable actions of antioxidants through DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activities and reducing power assays (Soledad, 2021). Furthermore, these extracts had antimicrobial influence on Salmonella typhimurium and Staphylococcus auras, suggesting their potential application as natural additives with preservative qualities. In addition to direct antioxidant activity, avocado seed extracts have also shown protective effects against chemically induced liver cancer in rats through magnification of their stand against antioxidant. This includes elevating the activities of glutathione peroxidase, glutathione-S-transferase component in tissues of liver (Tugiyanti, 2013). Such findings indicate the role of avocado seed extract in modulating enzymatic antioxidants as part of its chemo preventive mechanisms.

From this study, following the heart 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). The histological changes included mild to moderate inflammation of the heart tissues, minimal edema, and minimal lymphocytic aggregation within the cardiac tissue. In the myocardium, the histology shows a more improved effect of the pear seed with minimal effect of 5-FU. There were little to no histological alterations in most of the groups and the control with mild and decreased lymphocytic activities in a few of the groups. This goes to show from the research that 5-FU reduced some of the toxicities to the heart tissue.
Though, this research shows positive effect of the extract on the tissue, more research on this is recommended.

CONCLUSION

This study evaluated the effect of avocado pear seed extract on 5-FU induced cardio histotoxicity and oxidative stress in Wistar rat. Results from the current study revealed that *Persea americana* seed extract ameliorated the toxicities to the heart caused by the administration of 5-FU for the management of cancer. Also, combination of the medium dose of the extract with vitamin C also proved potent in reversing side effects of 5-FU which was accomplished by decreasing oxidative stress and inflammation to the cardiac tissue. However, further studies are required to ascertain other molecular mechanisms by which the extract produces this beneficial effect on the cardiac tissue and how it reduces oxidative stress in Wistar rats is recommended. Also, research to evaluate if the findings from this study can be used as a guide by drug scientists in the production of a new drug from the extract and also to ascertain if it is clinically applicable is also required.

Ethical Approval

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

Funding

There was no external funding for this research

Disclosure

No conflict of interest by the authors to disclose

REFERENCES

Akinyemi, A.J., Adedayo, O.A., & Ganiyu, O. (2019). Protective effect of avocado pear seed extract against oxidative stress and cellular damage. *Journal of Pharmacy and Pharmacology, 71*(8), 1043-1053.

Al-Asmari, A.K., Al-Zahrani, A.M., Khan, A.Q., Al-Shahrani, H.M., & Ali Al Amri, M. (2016). Taurine ameliorates 5-flourouracil-induced intestinal mucositis, hepatorenal and reproductive organ damage in Wistar rats: A biochemical and histological study. *Human Exp Toxicol, 35*:10–20.

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology, 80*, 51-60.

Ayala, A., Muñoz, M.F., & Argüelles,S. (2014). "Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal". *Oxidative Medicine and Cellular Longevity*, 1–31.

Basselin, C., Fontanges, T., & Descotes, J. (2011). 5-Fluorouracil-induced Takoo-Tsubo-like Syndrome. *Pharmacotherapy, 31*, 226.

Bonetti, P.O., Lerman, L.O., & Lerman, A. (2003). Endothelial dysfunction: A marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol, 23*: 168–175.

Carleton, H.M., Drury, R.A.B., & Wallington, E.A. (1967). Carleton's Histological Technique. 4th ed. London: Oxford University Press; Chapter 3-7, pp.40-129.

Claiborne, A. (1985) Catalase activity. In: Greenwald, R.A., Ed., CRC . Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, 283-284.

Dabas, D., Elias, R. J., Lambert, J. D., & Ziegler, G. R. (2019). In vitro antioxidant and cancer inhibitory activity of a colored avocado seed extract. *International Journal of Food Science, 2019,* 1-7.

Deponte, M. (2013). Glutathione catalysis and the reaction mechanism of glutathione-dependent enzymes. *Biochimica et Biophysica Acta*, 3217–3266.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews, 82,* 47–95.

Gnanadhas, E.N., Lakshminarasaiah, U., Gopas, J., & Nishigaki, I. (2014). Antioxidants and human diseases. *Clinica Chimica Acta, 436*, 332–347.

Halliwell, B., & Gutteridge, J.M.C. (2015). Free Radicals in Biology and Medicine, 5th edition, pp 215-227.

Hattori, Y., Nishigori, C., Tanaka, T., Ushida, K. Nikaido, O., Osawa, T., Hiai, H., Imamuru, S., & Tyokuni, S. (1997). 8 Hydroxy-2-deoxyguanosine is increased in epidermal cells of hairless mice after chronic ultraviolet B exposure. *J Invest Dermatol, 89,* 10405–9.

Hayyan, M., Hashim, M.A., & Anjkut, I.M. (2016). "Superoxide Ion: Generation and Chemical Implications". *Chem. Rev, 116* (5), 3029–85.

Heistad, D.D., Armstrong, M.L., Marcus, M.L., Piegors, D.J., & Mark A.L. (1984). Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. *Circ Res, 54:* 711–718.

Imafidon, K., & Amaechina, F. (2010). Effects of aqueous seed extract of *Persea americana* Mill(avocado) on blood pressure and lipid profile in hypertensive rats. *AdvBiol Res, 4*(2), 116-121.

Jasvinder, K., Deepak, P., & Vinod, K. (2018). 5-Fluorouracil-induced cardiotoxicity: A review of the possible mechanisms and clinical implications. *Journal of Clinical and Experimental Cardiology, 9*(2), 1-9.

Jensen, S.A., & Sorensen, J.B. (2006). Risk factors and prevention of cardiotoxicity induced by 5-fluorouracil or capecitabine. *Cancer Chemother Pharmacol,58*(4):487–493.

Joanna, I. (2016). "Aging and Degradation of Printed Materials", Printing on Polymers. *Elsevier*, pp. 353–370.

Labianca, R., Beretta, G., Clerici, M., Fraschini, P., & Luporini, G. (1982). Cardiac toxicity of 5-Fluorouracil. A study in 1083 patients, *Tumori, 68*:505–10.

Longley, D.B., Harkin, D.P., & Johnston, P.G. (2003). 5-fluorouracil: Mechanisms of action and clinical strategies. *Nat Rev Canc,3*:330–338.

Lorke, D.A. (1983). New Approach to Practical Acute Toxicity. Testing. Archives *of Toxicology, 54*: 275-287.

Mead, R., Curnow, R.N., & Hasted, A.M. (1993). *Statistical methods in Agriculture and Experimental biology, 2.*

Misra, H.P., & Fridovich, I. (1972). The role of Superoxide Anion in the autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry, 247,* 3170-3175.

Naomi, A.M., & Joshua, C.I. (2024). Effect of *Persea Americana* seed extract on 5-Fluorouracil Induced Hepatic Histotoxicity on Wistar Rats. *International Journal of Public Health, Pharmacy and Pharmacology, 9*(3), 50-67.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351-358.

Saif, M,W., Shah, M.M., & Shah, A.R. (2009). Fluoropyrimidine-associated cardiotoxicity: Revisited. *Expert Opin Drug Safe 8*: 191–202.

Sato, H., Shibata M., Shimizu, T., Shibata, S., Toriumi, H., Ebine, T., Kuroi T., & Iwashita T. (2013). Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. *Neuroscience, 248*, 345–358.

Sedlak, J., & Lindsay, R. H. (1968). *Analytical Biochemistry, 25*, 1192-1205.

Soledad, C.P.T., Paola, H.C., Carlos, E., Israel, R., GuadalupeVirginia, N.M., & Raúl, Á.S. (2021). Avocado seeds (*Persea americana* cv. Criollo sp.): Lipophilic compounds profile and biological activities. *Saudi J. Biol. Sci, 28*, 3384–3390.

Taniyama, Y., & Griendling, K.K. (2003). Reactive oxygen species in the vasculature. *Hypertension, 42*, 1075–1081.

Thalambedu, N., & Khan, Y. (2019). Fluorouracil (5-FU) induced cardiomyopathy. (2019). *Cureus, 11*(7), e5162.

Tremocoldi, M.A., Rosalen, P.L., Franchin, M., Massarioli, A.P., Deny C., Daiuto, E.R., Paschoal, J.A.R., Melo, P.S,. & de Alencar, S.M.Augusto, J., Massarioli, A.P. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PLoS ONE, 13*, e0192577.

Tsibiribi, P., Bui-Xuan, C, Bui-Xuan, B., Lombard-Bohas, C., Duperret, S., Belkhiria, M, Tabib, A., Maujean, G., Descotes, J., Timour, Q. (2006). Cardiac lesions induced by 5-fluorouracil in the rabbit. *Hum Exp Toxicol 25*: 305–309.

Tugiyanti, E., Yuwanta, T., Zuprizal, Z., & Rusman, R. (2013). Supplentation of Vitamin E and C in Feed on meat Quality. *Vet World, 12*(10):1608 -1615.

Uchenna, U.E., Shori, A,B., & Baba, A.S. (2017). Inclusion of avocado (*Persea americana*) seeds in the diet to improve carbohydrate and lipid metabolism in rats. *Revista argentina de endocrinología y metabolismo, 54*(3), 140–148.

Unlu, N., Bohn, T., Clinton, S.K. & Schwartz, S.J. (2005) Carotenoid Absorption from Salad and Salsa by Humans Is Enhanced by the Addition of Avocado or Avocado Oil. *The Journal of Nutrition, 135*, 431-436.

Villarreal-Lara, R., Rodríguez-Sánchez, D.G., Díaz De La Garza, R.I., M.I. García-Cruz, M.I., Castillo, A., & Pacheco, A. (2019). Purified avocado seed acetogenins: Antimicrobial spectrum and complete inhibition of Listeria monocytogenes in a refrigerated food matrix. *CyTA-Journal of Food, 17* (1), pp. 228-239.

Woo, R.A., Melure, K.G., & Lee, P.W. (1998). DNA dependent protein kinase acts upstream of p53 in response to DNA damage. *Nature, 394*, 700–4.