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THE EFFECTS OF *Tetrapleura tetraptera* DRY FRUIT FRACTIONS ON THE HISTOPATHOLOGICAL CHANGES OF THE ORGANS IN BARIUM CHLORIDE INDUCED ARRHYTHMIA AND ISOPRENALINE-INDUCED MYOCARDIAL INFARCTION ON WISTAR RATS.

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ABSTRACT

Histopathological changes in organs are being studied through microscopic examination of tissue samples from biopsies or autopsies to determine damages caused by diseases, toxins, or environmental stresses. Medicinal plants have long played important roles in the treatment of diseases all over the world and contain pharmacologically active ingredients of which have been associated with adverse effects ranging from therapeutic benefits to severe toxicity, depending on dosage and usage. Tetrapleura tetraptera fruit in literature has been documented to have many health benefits, providing anti-inflammatory and antioxidant benefits of which misuse can lead to severe hapatotoxicity, nephrotoxicity gastrointestinal distress and potential carcinogenesis. Studies have explored its impact on heart morphology, blood pressure regulation and inflammatory markers in animal models but none has been seen documented on organs of induced arrhythmia and myocardial infarction in animal experiments after administering daily pretreatment for some days with the herbal fruit fractions. It is therefore very necessary to motivate studies looking at pharmacological agents that will provide protective effects on the organs while providing therapeutic treatment. Aim: To determine the effects of Tetrapleura tetraptera dry fruit fractions on the histopathological changes on the heart, liver, and kidney of the Barium Chloride-induced Arrhythmia and Isoprenaline-induced Myocardial Infarction in Wistar Rats. Objective: The objective of this study was to investigate the effects of fractions derived from the dried fruit of Tetrapleura tetraptera *on histopathological alterations in the hearts, livers, and kidneys of Wistar rats following the inducement of Arrhythmia and Myocardial infarction with barium chloride and Isoprenaline respectively. Methodology: The study was an experimental design. Ethical approval was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. Lethal dose (LDL_{50}) was determined by the methods of Lorke 1983. The study was conducted in the animal house of the College of Medicine, Enugu state University of Science & Technology Parklane, Enugu (ESUT), Nigeria. Tetrapleura tetraptera indehiscent fruits were bought from Ogbete market Enugu, washed with clean water and air dried for three days. The whole fruits together with the seeds were ground to get coarse forms for easy filtration after cold maceration using methanol as the menstrum. The extract obtained was also fractionated at the Chemistry department of the University of Nigeria Nsukka to obtain Methanol and Ethyl Acetate fractions used in the experiment. Result: The 120mg/kg Methanol Fraction had the highest organ protection after barium chloride induced arrhythmia and Isoprenaline induced myocardial infarction on the experimental rats. The*

histopathological analysis from the experiment revealed that pre-treatment with the different administered doses resulted to various degrees of damages and protections on the organs (Hearts, Livers and Kidneys) of the experimental Wistar rats. Conclusion: From the experimental evidence, it was found that the 120mg/kg Methanol Fraction of T. tetraptera fruit gave the highest protection on the organs of the experimental Wistar rats, therefore can be used in treating the conditions (Arrhythmia and Myocardial Infarction) but within a shorter demonstrated time frame and the study extended to human since the results were extrapolation from the used animal models.

Keywords: Histopathological changes, Arrhythmia, Myocardial infarction, *Tetrapleura tetraptera* fruit fraction.

1.0 INTRODUCTION

Histopathological evaluation of tissue samples remains a cornerstone for assessing organ damage induced by diseases, toxins, or pharmacological agents. In cardiovascular research, such evaluation is essential for determining the protective or deleterious effects of therapeutic interventions on vital organs including the heart, liver, and kidneys. Arrhythmia and myocardial infarction (MI) represent two common cardiovascular pathologies.

Arrhythmia refers to any cardiac rhythm deviating from normal sinus rhythm (Katzung, 2017), whereas myocardial infarction is defined as ischemic necrosis of myocardial tissue resulting from sustained impairment of blood flow and oxygen supply (Frangogiannis, 2015). Both conditions can be experimentally induced using pharmacological agents such as barium chloride (for arrhythmia) and isoprenaline (for myocardial infarction), and the ability of a test substance to restore normal histoarchitecture or reverse rhythm disturbances is widely regarded as evidence of cardioprotective potential (Ansawa, 2007).

Medicinal plants have long served as important sources of therapeutic agents, with their pharmacological properties largely attributed to their bioactive constituents (Harbone et al., 1999; Fallah-Hoseini et al., 2006). Despite the growing interest in herbal medicines, concerns regarding their safety remain paramount. Herbal products contain pharmacologically active compounds that may exert adverse effects, including hepatotoxicity, nephrotoxicity, and cardiotoxicity, particularly with chronic use or at high doses (Pittler & Ernst, 2003; Ernst et al., 2006; Kotsiou et al., 2024). Systematic reviews have documented serious adverse events associated with herbal medicine use, ranging from hepatic failure to cardiovascular collapse (Posadzki et al., 2013). *Tetrapleura tetraptera* commonly known as prekese or aidan fruit belongs to the Fabaceae family. It is indigenous to West Africa and the fruit is locally known as "Aridan" in southwestern Nigeria. It has been employed in traditional medicine for the treatment of various ailments. Studies have reported antioxidant, anti-inflammatory and cardioprotective properties of *T. tetraptera* fruit (Ojewole, 2005), while existing evidence suggests that *T. tetraptera* may confer cardioprotective benefits, no study to date has systematically investigated the effects of its fractions on histopathological changes of the hearts, livers, and kidneys in experimental models with induced

arrhythmia and myocardial infarction after days of pretreatment with herbal fractions on Wistar rats as demonstrated in this work. In possessing the therapeutic potentials, evaluating both the efficacy and safety of herbal preparations on key organs is essential for their rational therapeutic application. This study aimed to investigate the effects of fractions derived from the dried fruit of *Tetrapleura tetraptera* on histopathological alterations of the hearts, livers, and kidneys of Wistar rats subjected to barium chloride-induced arrhythmia and Isoprenaline-induced Myocardial infarction.

2.0 MATERIALS AND METHODS

2.1 Ethical approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. All animal procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

2.2 Study design and Site

This study employed an experimental design using albino Wistar rats. The research was conducted at the animal house of the Enugu State University of Science and Technology, Enugu State with extraction processed at Interceed Laboratory Nsukka and fractionation performed at the Chemistry department of the University of Nigeria, Nsukka.

2.3 Plant material and Authentication

Fresh indehiscent fruits of *Tetrapleura tetraptera* were purchased from Ogbete Main Market, Enugu, Nigeria. The plant material was authenticated at the Botany Department of the University of Nigeria, Nsukka. The fruits were thoroughly washed with clean water and air-dried under incandescent bulb lights at room temperature for three days.

2.4 Extraction and Fractionation

The dried whole fruits, including seeds, were ground into a coarse powder. Extraction was performed using the cold maceration method. The coarse powder of about 2500g gram was soaked in analytical grade methanol as the menstrum to cover the whole ground herbal fruit in an airtight stoppered container at room temperature for 72 hours with frequent agitation. The resulting extract was filtered and concentrated by evaporation using a rotary evaporator to obtain the extract. The dried methanol extract was subsequently fractionated using Column chromatography using silica gel as the stationary phase on the column and a methanol and ethyl acetate solvents as a mobile phase to obtain methanol and ethyl acetate fractions respectively at the Chemistry department of the University of Nigeria, Nsukka.

2.5 Acute toxicity study

The median lethal dose (LD50) of the extract was determined using the method described by Lorke (1983) and the LD50 was found to be 1,265 mg/kg. This two-stage procedure involved intra-peritoneal administration of graded doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg of extract

followed by higher doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract to animals. Each stage was observed for mortality within 24 hours of administration of the extract.

2.6 Experimental animals

Adult healthy albino Wistar rats of both sexes weighing 160 – 350g to rule out gender differences in the animals were used and were allowed to acclimatize in a clean, well-ventilated house for two weeks at uniform temperature with 12hrs dark/light periodically and were fed with standard pellets and water ad libitum prior to the commencement of the experiment. 54 rats of 6 rats per group of animals were used in arrhythmia experiment and 27 Wistar rats of 3 animals per group for the Myocardial infarction experiment were used and the dissolved fractions in distilled water were administered intraperitoneally according to body weight.

2.7 Drugs and chemicals

All the chemicals used in the experiments were of analytical grade, Methanol (Honey well company > 99.8% purity), Ethyl acetate (Honey well company > 99.9% purity), the chemical salts and drugs used are as follows; Barium chloride (Bacl2), Isoprenaline (hydrochloride) (Cat. # HY-B0486/CS-2582, Lot # 79092, Size: 20g, MedChemExpress.com, MCE, USA), Metoprolol tablet and Phenobarbitone injection.

2.8 Induction of Arrhythmia

Arrhythmia was induced on the 14th day by intravenous injection of 10% Barium chloride at a dose of 20 mg/kg body weight via the tail vein, as previously described. Animals were pre-treated for 13 consecutive days with the fruit fractions prior to arrhythmia inducement. A total of 10 Albino Wistar rats died during the daily drug administration in the Arrhythmia Experiment. Group 7 started dying on the 6^{th} day and by the 13^{th} day was remaining two that was sacrificed and one used for ECG recording while group 8 were dying daily then on the 5th day have all died remaining one. Group 5 that were given 120mg/kg Methanol Fraction (M.F), towards the end of the experiment made the animals lose some weights probably because of the lipid regulation of the fruit of *Tetrapleura tetraptera* according to Ajayi et al 2011.

Group Allocations for Arrhythmia Experiment

Animals were randomly assigned into nine groups (n = [6] per group) as follows:

Group	Description	Treatment (Days 1–13)	Induction (Day 14)
1	Normal control	Standard pellets and water	None
2	Negative control	Standard pellets and water	$Bacl_2$ 20 mg/kg I.V.
3	Methanol Fraction (MF) 30 mg/kg	MF 30 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
4	Methanol Fraction (MF) 60 mg/kg	MF 60 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
5	Methanol fraction (MF) 120 mg/kg	MF 120 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
6	Ethyl acetate fraction (EA) 30 mg/kg	EA 30 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
7	Ethyl acetate fraction (EA) 60 mg/kg	EA 60 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
8	Ethyl acetate fraction (EA) 120 mg/kg	EA 120 mg/kg I.P.	$Bacl_2$ 20 mg/kg I.V.
9	Positive control	Metoprolol 25 mg/kg P.O.	$Bacl_2$ 20 mg/kg I.V.

Abbreviations: I.P., intraperitoneal; I.V., intravenous; P.O, per oral (by oral gavage). On the 14th day, animals were sacrificed by cervical dislocation and organs (livers, hearts and kidneys) harvested for histological examination.

NOTE: Metoprolol is an established drug designed clinically for oral use whereas the herbal fractions were administered intraperitoneally to enhance absorption and ensure consistent bioavailability.

2.9 Induction of Myocardial Infarction

Myocardial infarction was induced by intraperitoneal administration of Isoprenaline at a dose of 85 mg/kg body weight on the 12th and 13th days, as previously described. Animals were pretreated with the fractions for 11 consecutive days prior to the first inducement with Isoprenaline for 48hours, with treatment continuing through the induction period. A total of 11 Albino Wistar rats died during the daily drug administration in the Myocardial Infarction Experiment. Group 7 started dying on the 5th day and by the 13th day was remaining two that was sacrificed and one used for ECG recording while group 8 were dying daily then on the 4th day have all died remaining one. Group 5 that were given 120mg/kg Methanol Fraction (M.F), towards the end of the experiment made the animals lose some weights probably because of the lipid regulation of the fruit of *Tetrapleura tetraptera*.

Group Allocations for Myocardial Infarction Experiment
Animals were randomly assigned into nine groups (n = [3] per group) as follows:

Group	Description	Treatment (Days 1–11)	Induction (Days 12–13)	Assessment (Day 14)
1	Normal control	Standard pellets and water	None	ECG & sacrifice
2	Negative control	Standard pellets and water	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
3	MF 30 mg/kg	MF 30 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
4	MF 60 mg/kg	MF 60 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
5	MF 120 mg/kg	MF 120 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice

Group	Description	Treatment (Days 1–11)	Induction (Days 12–13)	Assessment (Day 14)
6	EA 30 mg/kg	EA 30 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
7	EA 60 mg/kg	EA 60 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
8	EA 120 mg/kg	EA 120 mg/kg i.p.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice
9	Positive control	Metoprolol 25 mg/kg p.o.	Isoprenaline 85 mg/kg I.P. (days 12–13)	ECG & sacrifice

On the 14th day, animals were sacrificed by cervical dislocation and organs (livers, hearts and kidneys) harvested for histological examination.

2.10 Tissue collection and Histopathological examination

At the end of each experimental period, animals were sacrificed by cervical dislocation. The heart, liver, and kidneys were rapidly dissected, washed immediately with ice-cold normal saline to remove blood, and fixed in 10% buffered formalin for 24 hours. Tissue samples were dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin wax using an automatic tissue processing machine. Sections of about 4–5 µm thickness were cut using a microtome, processed, mounted on glass slides, and stained with hematoxylin and eosin (H&E) according to standard protocols. Histopathological evaluation was performed by a consultant pathologist who was blinded to the group allocations. Representative photomicrographs were captured for each group to examine the histological changes.

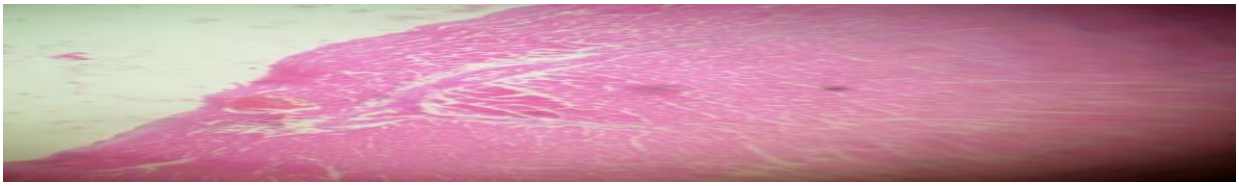
3.0 RESULTS AND DISCUSSION

3.1 Result:

The Intraperitoneal Lethal Dose 50% (I.P LD_{50}) of the crude extract was determined to be 1,265 mg/kg Body Weight (B.W). The histological features of the experiments are as follows:

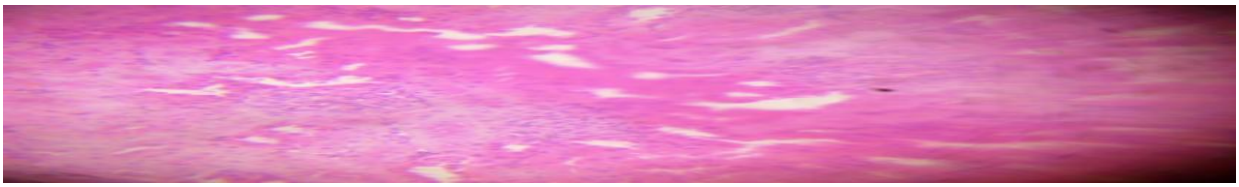
HISTOLOGICAL FEATURES OF MYOCARDIAL INFARCTION EXPERIMENT:

GROUP 1 M1 (HEART)



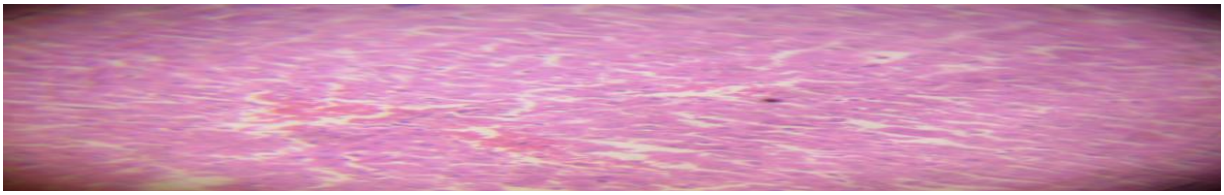
The histological section shows heart tissue with in which there are foci of short eosinophilic necrotic cardiac muscle cells surrounded by mixed inflammatory cells. These are surrounded by non-infarcted cardiac muscles.

GROUP 1 M1 (KIDNEY)



The histological section shows kidney tissue within which the tubules in some foci are infarcted with slough in the lumen. The glomeruli are spared.

GROUP 1 M1 (LIVER):



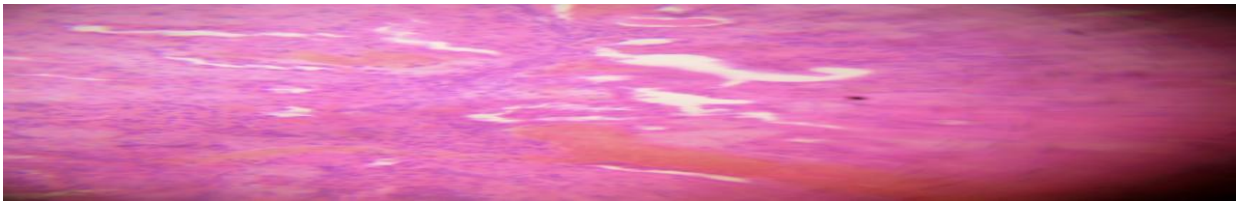
The histologic section shows liver tissue in which there is mild tissue necrosis with heamorrhages.

NOTE: These histopathological findings are consistent with the use of cervical dislocation method of sacrificing.

GROUP 2 M1 (HEART):

The histological section shows heart tissue in which the outer myocardium (beneath the epicardium) are moderately necrotic with lots of hemorrhagic lakes around them especially towards the epicardium.

GROUP 2 M1 (KIDNEY):

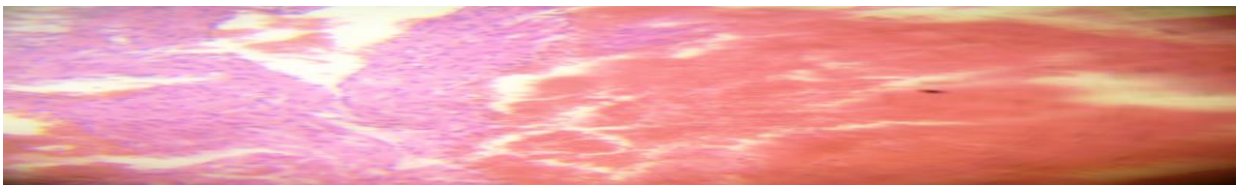


The histological section shows kidney tissue with severe necrosis of the tubules and interstitial inflammation. Most of the glomeruli are destroyed, with some been fibrotic and occluded in some foci.

GROUP 2 MI (LIVER):

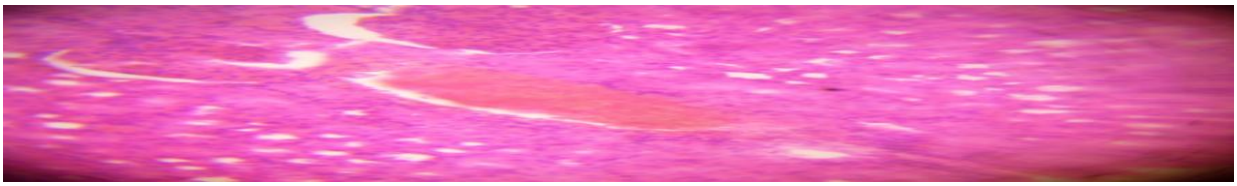
The Histological section shows a liver tissue with lots of congested blood vessels and minimal inflammation. Fibrosis is minimal and no tissue necrosis is seen.

GROUP 3 MI (HEART):



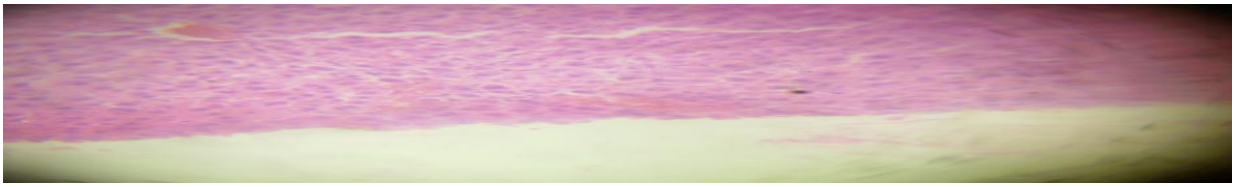
The histological section shows heart tissue with severe cellular necrosis and multiple hemorrhagic lakes or spaces in between the muscle layers and the epicardial layer.

GROUP 3 M1 (KIDNEY):



The histological section shows kidney tissue with severe tubular necrosis, lots of the glomeruli are destroyed with obliteration of the glomerular spaces. There were also multiple hemorrhagic lakes seen with congested blood vessels at the background.

GROUP 3 MI (LIVER):

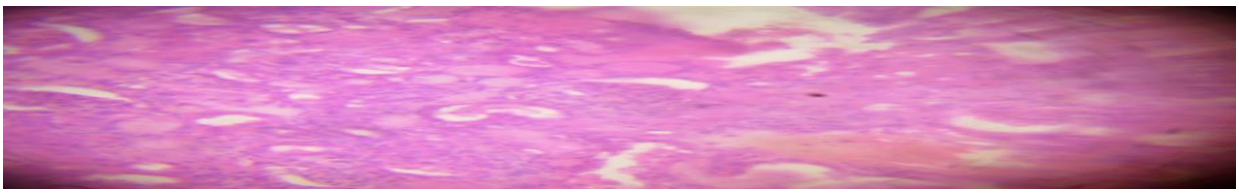


The histological section shows liver tissue with lots dilated blood vessel and few hemorrhagic spaces.

GROUP 4 MI (HEART):

The histological section shows heart tissue with lots of large hemorrhages trapped in between the muscle fibers. Some of the muscle cells are necrotic (amorphous and glassy).

GROUP 4 MI (KIDNEY):

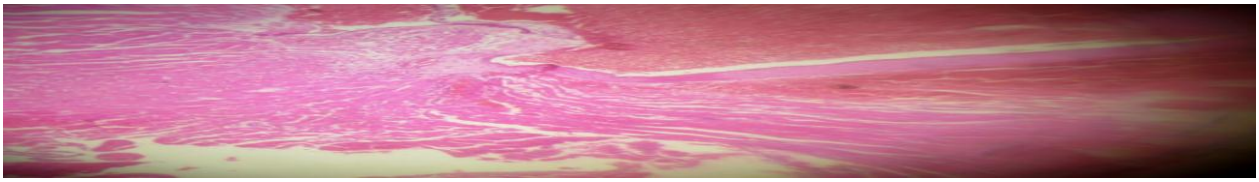


The histological section shows kidney tissue in which there is severe tubular necrosis with lots of eosinophilic cast in the tubular lumen. Some of the glomeruli are affected, cellular and fibrotic with occluded spaces. There are some hemorrhagic lakes in some foci.

GROUP 4 MI (LIVER):

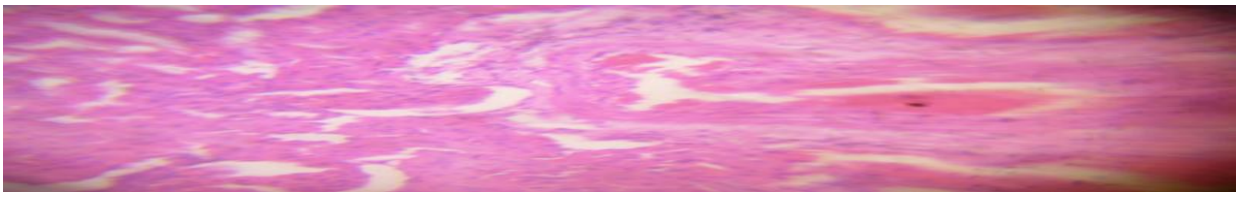
The histological section shows liver tissue with diffuse multiple hemorrhages in between the hepatocytes. The portal veins are dilated and congested.

GROUP 5 MI (HEART):



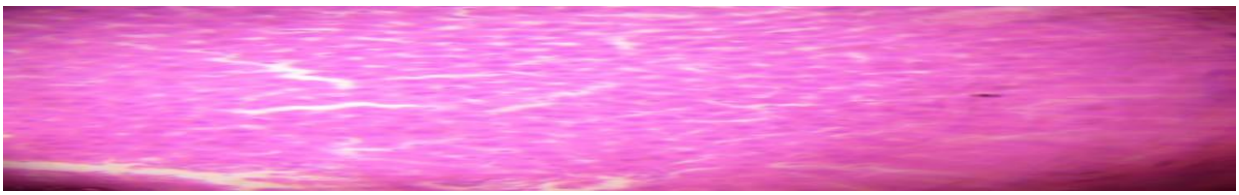
The histological section shows heart tissue in which there are massive hemorrhagic lakes with severe necrosis of the heart muscles.

GROUP 5 MI (KIDNEY):



The histological section shows kidney tissue with lots of dilated and congested blood vessels and tubular necrosis involving both the cortex and medullary parts of the kidney. Lots of hemorrhages are seen in-between the tubules.

GROUP 5 MI (LIVER):

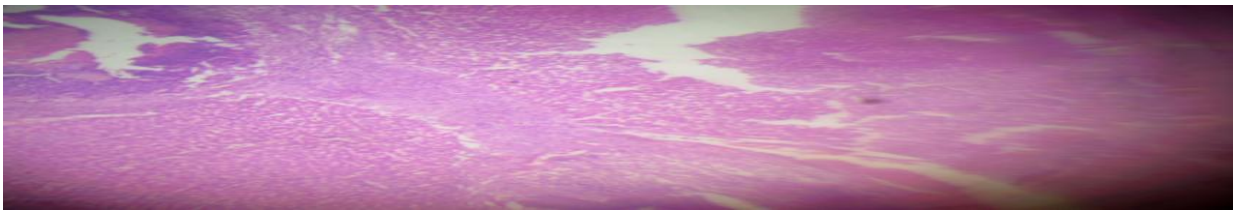


The histological section shows liver tissue in which there are massive necrosis and more around the portal veins. Bridging fibrosis is present. Lots of the vessels seen are dilated and large.

GROUP 6 MI (HEART):

The histological section shows lots of ruptured blood vessels with large hemorrhagic lakes dispersed in between the muscle fibers and cells.

GROUP 6 MI (LIVER):



The histological section shows liver tissue with necrosis walled off by inflammatory cells and ghost cells. In some foci there are tiny bleedings in between the hepatocytes. Lots of dilated and congested blood vessels are seen.

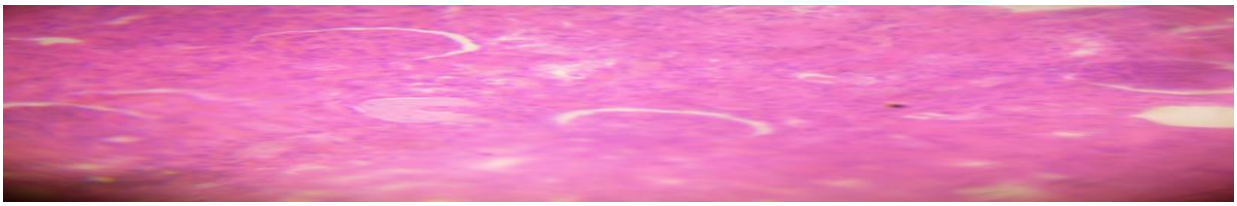
GROUP 6 MI (KIDNEY):

The histological section shows kidney tissue in which there are lots of hemorrhages and congested blood vessels. There is severe tubular necrosis seen with casts, besides most of the glomeruli are hemorrhagic.

GROUP 7 MI (HEART):

The histological section shows heart tissue in which there are multiple large hemorrhagic lakes splitting the cardiac muscles in many foci involving all the layers of the heart.

GROUP 7 MI (KIDNEY):



The histological section shows kidney tissue in which there are massive tubular necrosis and hemorrhages involving the cortex and medulla, Though the glomeruli were spared but some of the tubules are fibrotic.

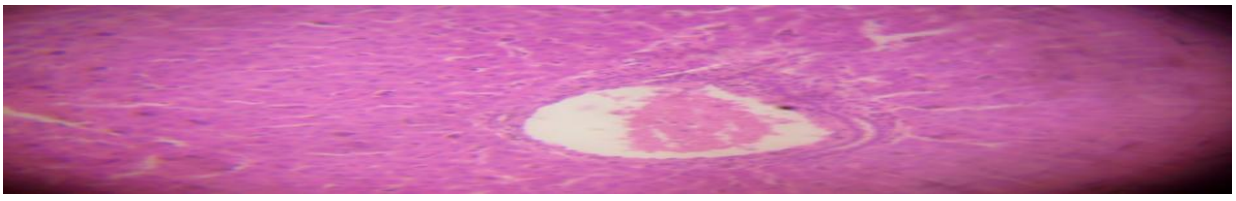
GROUP 7 MI (LIVER):

The histological section shows liver within which there are severe and extensive tissue necrosis involving most of the liver tissue with few viable cells around the portal vein in some foci with complete loss of architecture.

GROUP 8 MI (HEART):

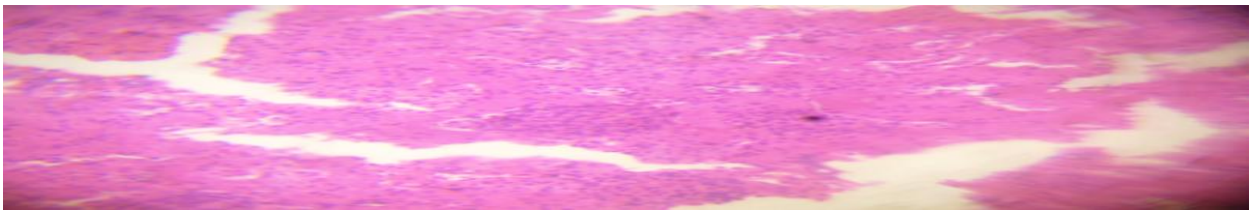
The histological section shows heart tissue in which there are hemorrhagic lakes splitting the muscles. Lots of dilated congested blood vessels are seen in the endocardium.

GROUP 8 MI (LIVER):



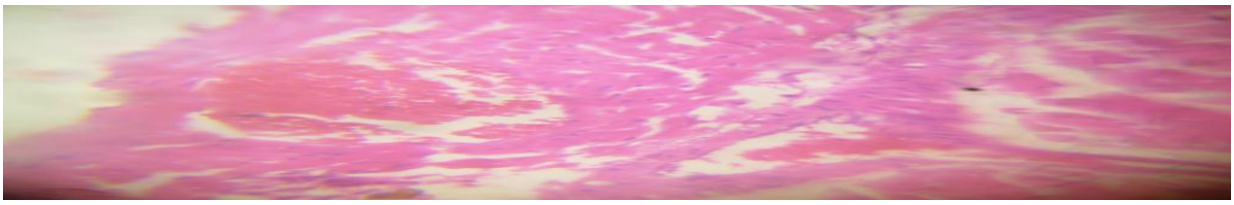
The histological liver section shows there are lots of dilated congested blood vessels dispersed throughout the tissue. Extensive tissue necrosis is seen in the capsular area.

GROUP 8 MI (KIDNEY)



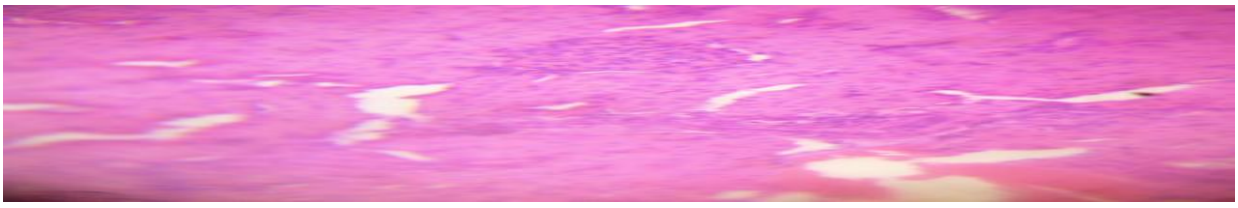
The histological section shows the kidney parenchyma in which there is extensive tissue necrosis involving the tubules. There are moderate hemorrhages in the cortex and medullary aspect of the kidney.

GROUP 9 MI (HEART):



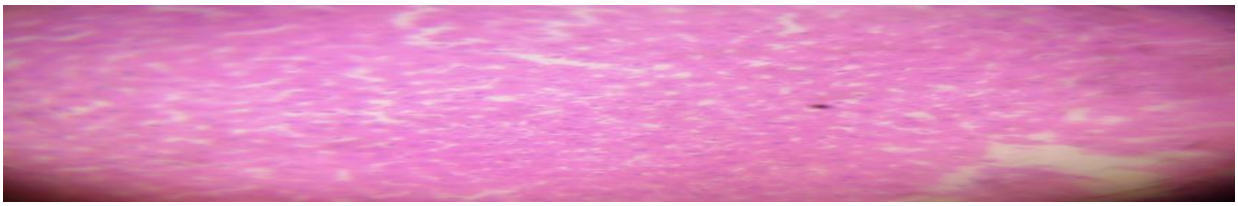
The histological section shows heart tissue in which there are hemorrhagic lakes involving the pericardial layer.

GROUP 9 MI (KIDNEY):



The histological section shows kidney tissue in which there are extensive necrosis involving the cortical and cortico-medullary area but sparing the medulla. The glomeruli are completely destroyed and lots of hemorrhages are seen.

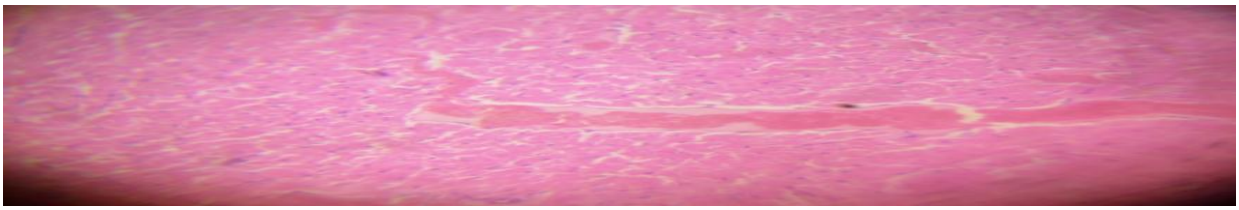
GROUP 9 MI (LIVER):



The histological section shows liver with intact architecture. Though there is tissue necrosis near the capsule and around the portal vein.

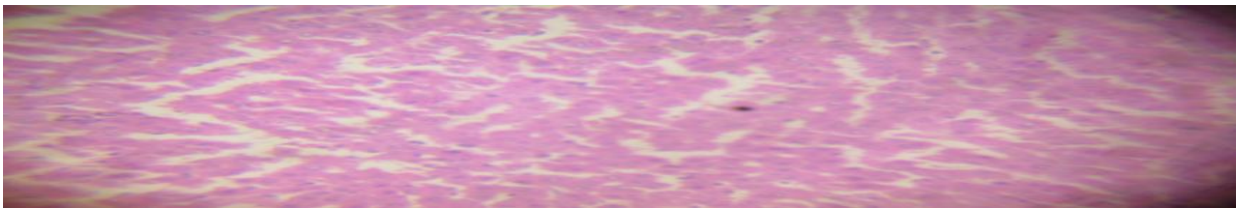
HISTOLOGICAL FEATURES OFARRHYTHMIA EXPERIMENT ARE AS FOLLOWS:

GROUP I ARR (HEART)



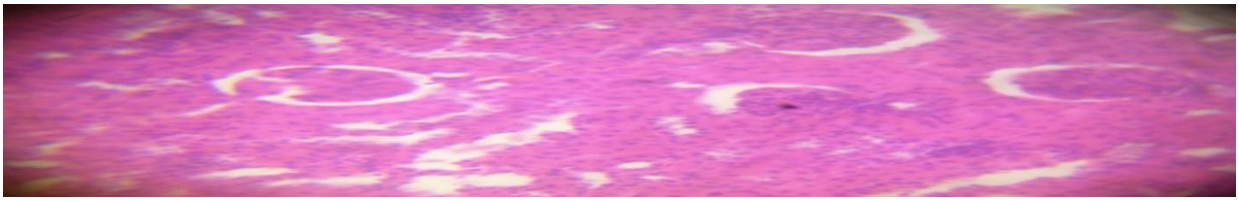
The histological section shows heart tissue with lots of large hemorrhagic lakes are present, Tissue necrosis with small hemorrhages are seen in the myocardial layer close to the pericardium.

GROUP 1 ARR (LIVER):



The histological section shows liver tissue in which there are multiple dilated and congested blood vessels.

GROUP 1 ARR (KIDNEY):

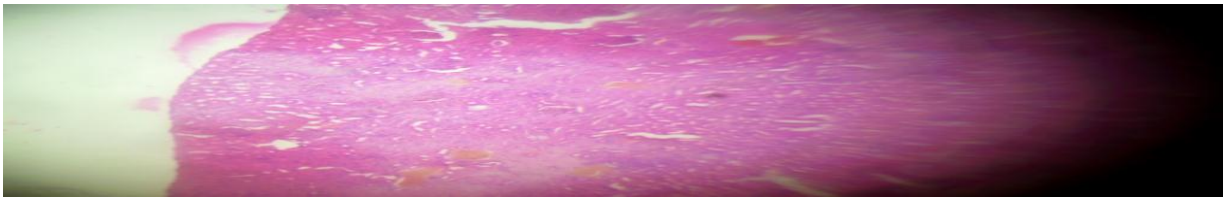


The section shows kidney tissue in which there are moderate tissue necrosis that involved structures in both the cortex and medullar.

GROUP 2 ARR (LIVER)

The histological sections show a liver tissue with lots of vascular congestion and mild inflammation with minimal fibrosis and absence of necrosis.

GROUP 2 ARR (KIDNEY)



The histological section shows extensive death of the tubule cells and inflammation in the surrounding tissue. Most of the filtering units (glomeruli) are destroyed, with some showing scarring (fibrosis) and blockage in specific areas.

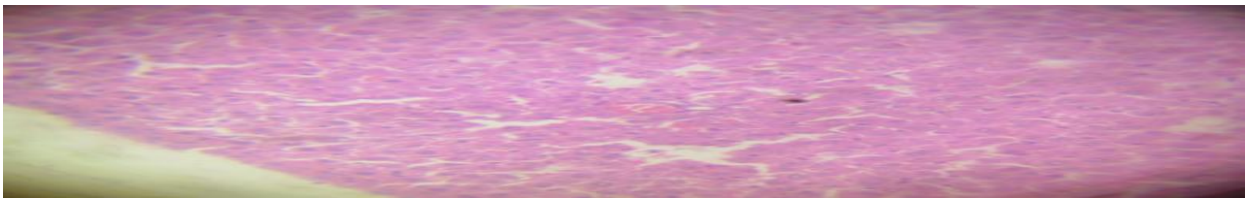
GROUP 2 ARR (HEART)

The histological section shows the heart tissue in which there are focal moderate necrosis of the outer myocardium (Sub-epicardium) accompanied by extensive hemorrhagic lakes, particularly concentrated near the epicardial surface.

GROUP 3 ARR (HEART):

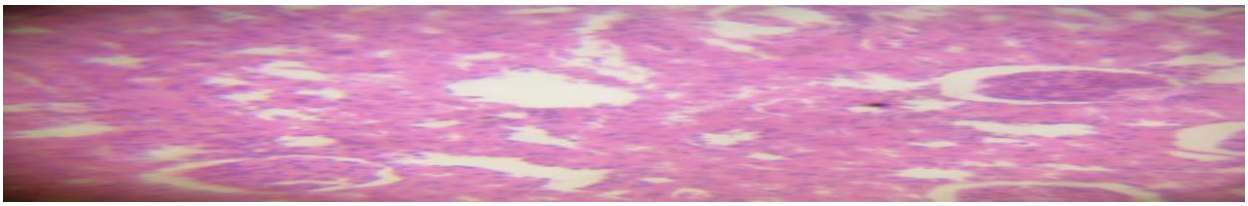
The histological section shows heart tissue in which there are islands of large hemorrhages. In the myocardial layer just below the pericardium are tiny flames of hemorrhages with mild tissue necrosis.

GROUP 3 ARR (LIVER):



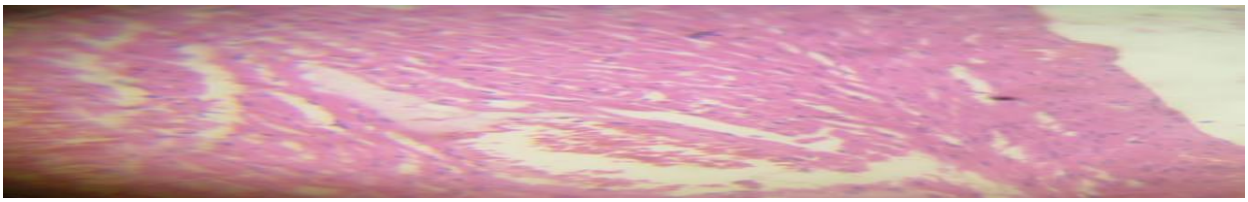
The histological section of the liver shows hepatocytes that are unremarkable though mild congestion of the blood vessels is noticed.

GROUP 3 ARR (KIDNEY):



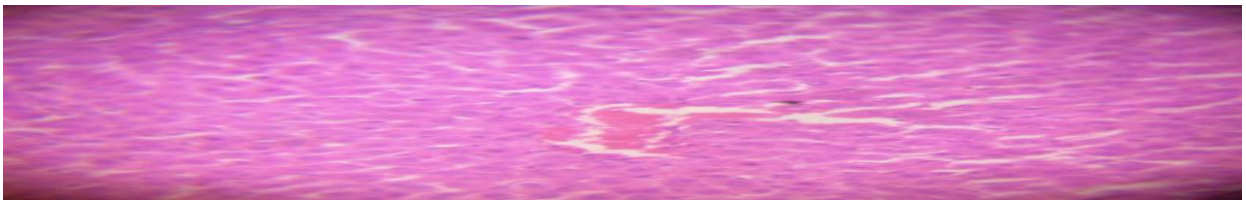
The histological section shows kidney tissue in which there is extensive tissue necrosis involving the cortex and the medullar to some extent. Both the tubules and collecting ducts are involved.

GROUP 4 ARR (HEART):



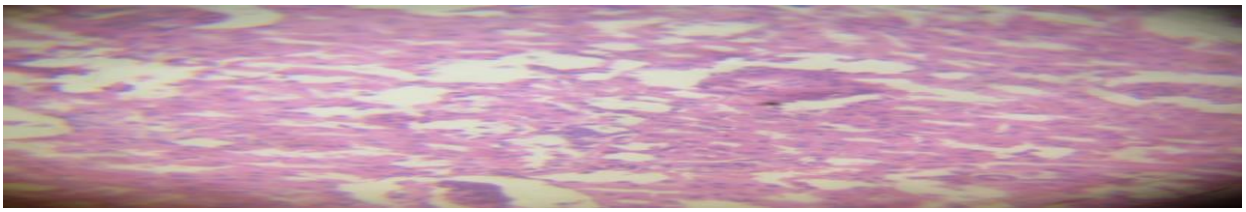
The histological section shows heart tissue within which there is mild tissue necrosis beneath the epicardium layer and few heamorrhages.

GROUP 4 ARR (LIVER):



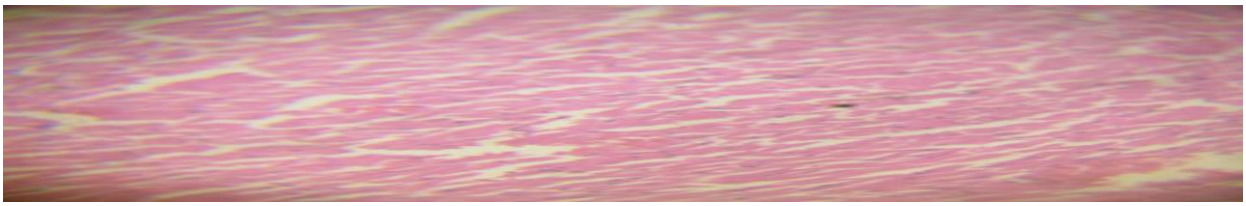
The histological section shows liver tissue in which there is minimal fibrosis underneath the Glisson's capsule layer with piece meal necrosis and blood vessel congestion been seen.

GROUP 4 ARR (KIDNEY):



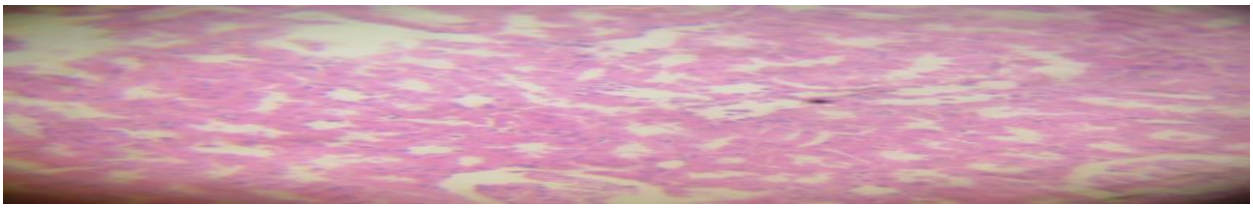
The histological section shows kidney tissue within which there are extensive and severe tissue necrosis involving the tubular, glomerular and interstitial components.

GROUP 5 ARR (HEART):



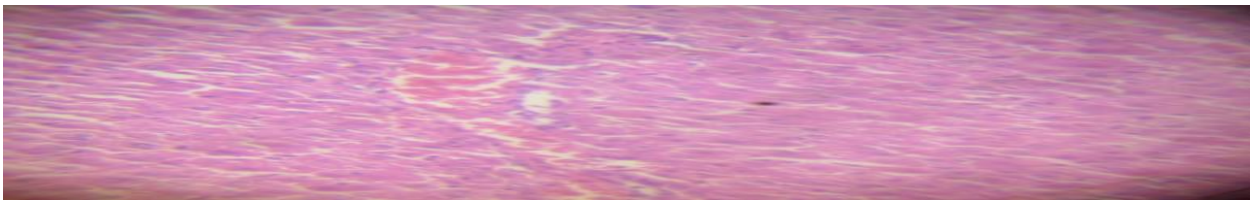
The histological section shows heart tissue in which there is tissue necrosis with hemorrhages in between the muscle fibers in some foci.

GROUP 5 ARR (KIDNEY):



The histological section shows kidney tissue in which there is necrosis beneath the capsule layer, the glomeruli and collecting tubules are spared.

GROUP 5 ARR (LIVER):

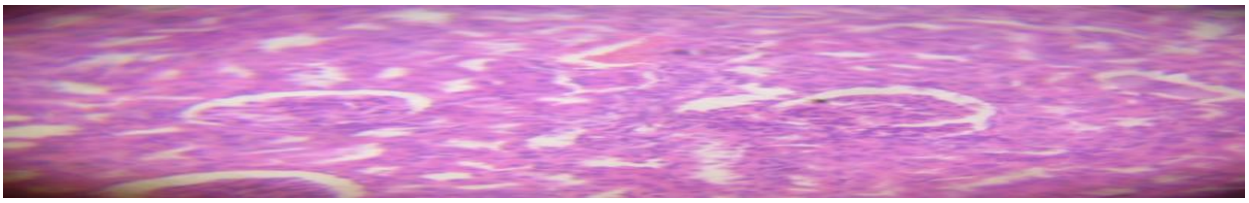


The histological section shows liver tissue in which there is mild tissue necrosis with few hemorrhagic around and in between the cells.

GROUP 6 ARR (HEART):

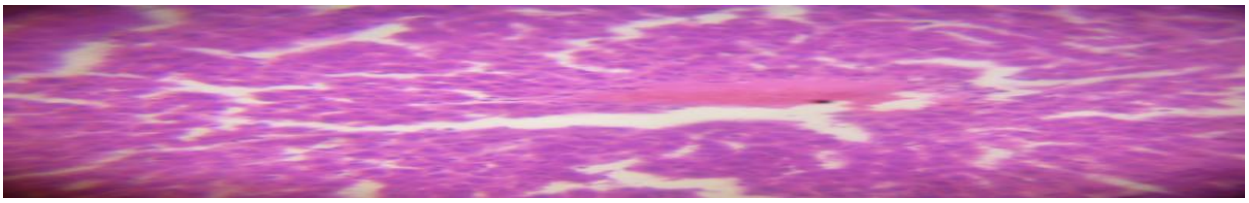
The histological section shows heart tissue with severe tissue necrosis involving all the levels of cardiac muscles.

GROUP 6 ARR (KIDNEY):



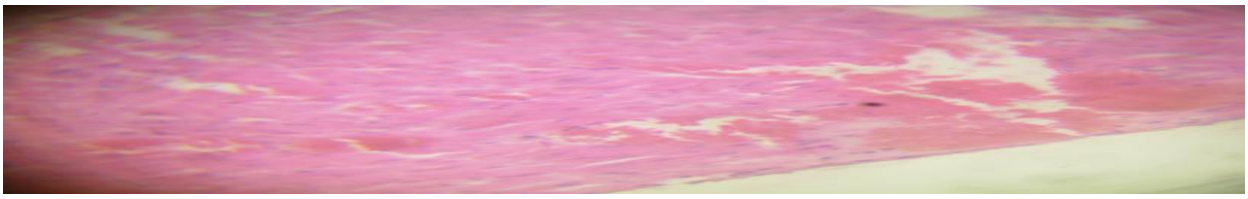
The histological section shows kidney tissue within which there are severe tissue necrosis involving the tubules, glomeruli and interstitial tissue. Some hemorrhagic lakes are seen.

GROUP 6 ARR (LIVER):



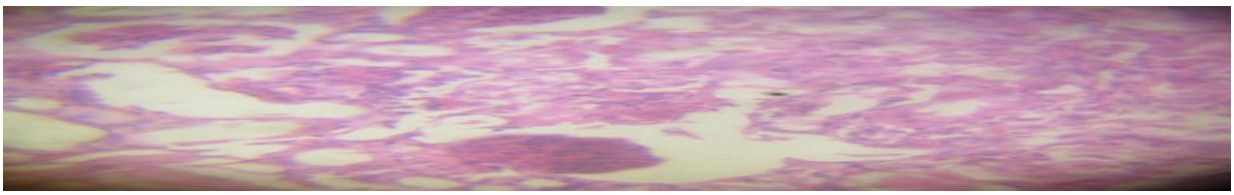
The histological sections show liver tissue in which there is moderate necrosis. Some hemorrhages are seen.

GROUP 7 ARR (HEART):



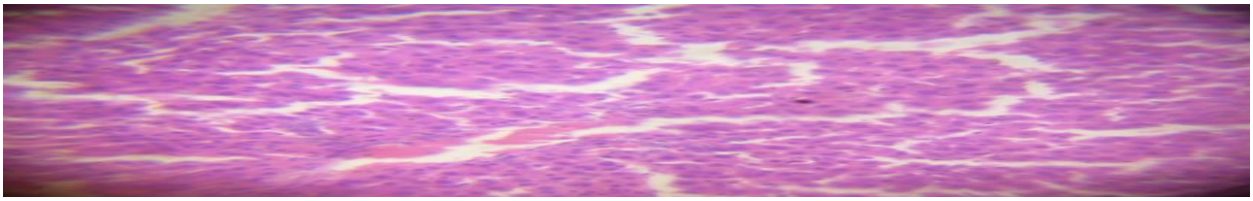
The histological section shows cardiac tissue with severe tissue necrosis involving all the layers. Lots of hemorrhagic lakes are seen.

GROUP 7 ARR (KIDNEY):



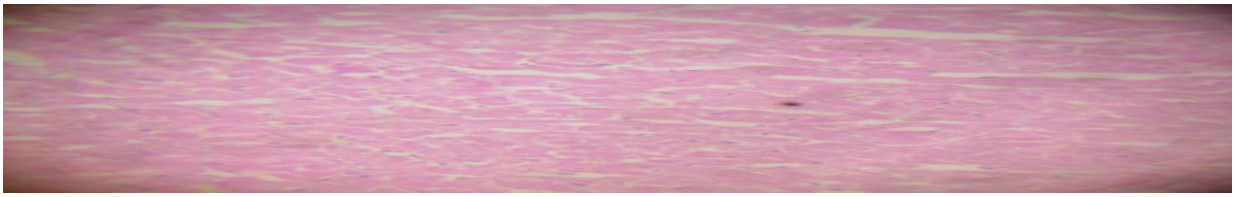
The histological section shows kidney tissue with severe tissue necrosis involving the tubules, glomeruli and interstitial tissue**.**

GROUP 7 ARR (LIVER):



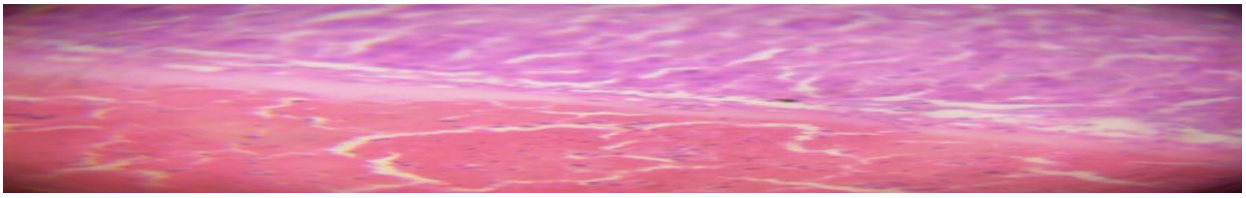
The histological section shows liver tissue within which there are moderate to severe tissue necrosis with some hemorrhages at the background.

GROUP 8 ARR (HEART):



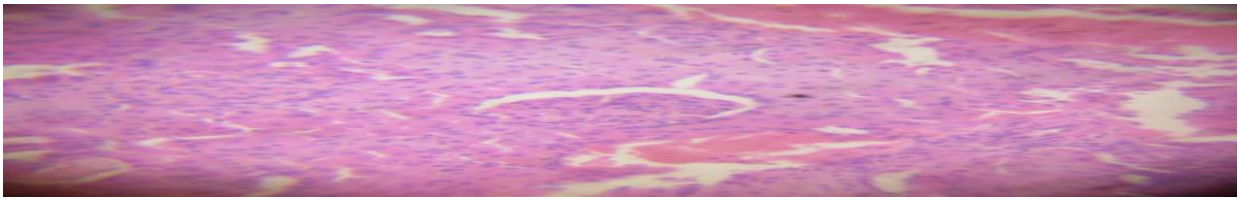
The histological section shows heart tissue within which there are large islands of hemorrhages with extensive and severe tissue necrosis.

GROUP 8 ARR (LIVER):



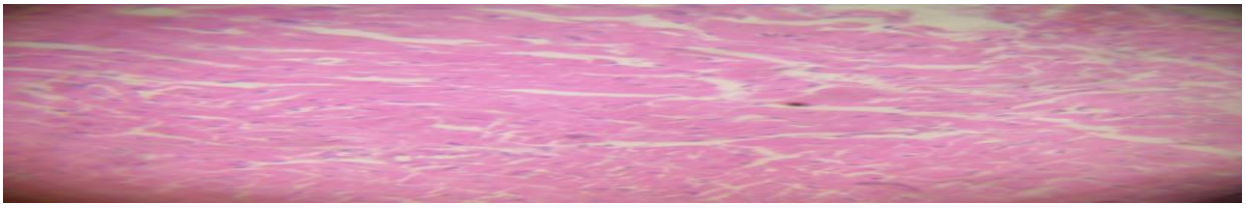
The histological section shows liver tissue within which there is moderate to severe necrosis of the cells beneath the capsular layer with inflammation. There is also tissue necrosis involving cells around the vessels with bridging fibrosis connecting the portal veins. Lots of dilated and congested blood vessels are seen.

GROUP 8 ARR (KIDNEY):



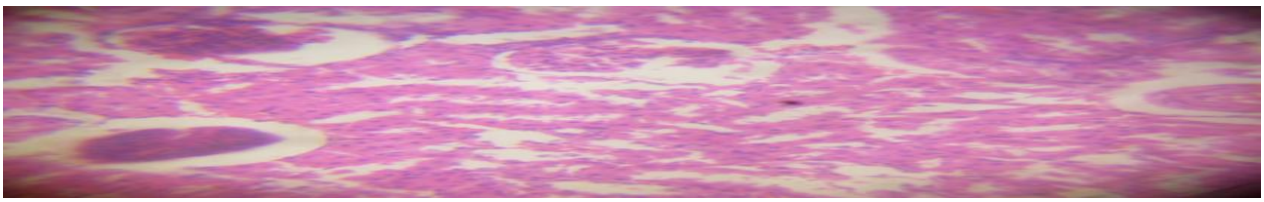
The histological section shows kidney tissue in which there is severe and extensive tissue necrosis that involved and destroyed the tissue structures in both cortex and medullar completely. Lots of hemorrhagic lakes are seen.

GROUP 9 ARR (HEART):



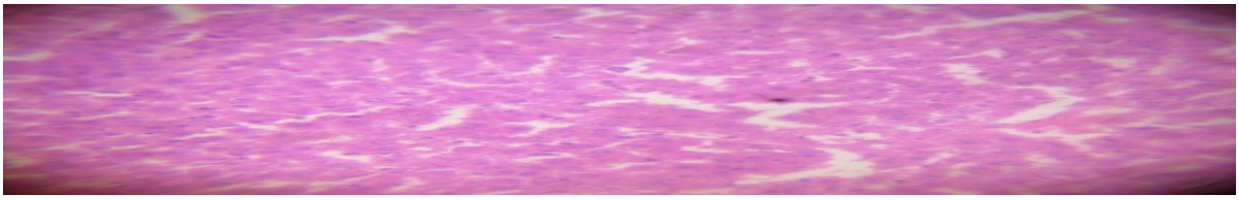
The histological section shows heart tissue within which there are moderate to severe tissue necrosis.

GROUP 9 ARR (KIDNEY):



The histological section shows kidney tissue within which there are tubular necrosis in the cortex and moderate hemorrhage. The glomeruli and collecting tubules are unremarkable.

GROUP 9 ARR (LIVER):



The histological section shows liver tissue in which there is mild capsular inflammation with blood vessel congestion in background of otherwise bland looking unremarkable liver cells.

3.2 Discussion

The LD50 got was 1,265 mg, meaning the herbal fruit extract is considered to be moderately toxic and may pose safety concerns particularly at high doses or with prolonged exposure. Cervical dislocation method was the method of euthanasia used in sacrificing the experimental rats. The **Histopathological analysis from arrhythmia experiment revealed** that pre-treatment with 30mg/kg(Group 3), 60mg/kg (Group 4), 120mg/kg MF (Group 5), 30mg/kg EA(Group 6), 60 mg/kg EA (Group 7), 120mg/kg EA (Group 8) and 25mg/kg Metoprolol (Group 9) exhibited some varying degrees of protections and damages on tissue architectures of the hearts, livers, and kidneys against barium chloride-induced arrhythmia, **However**, Group 3(30mg/kg MF) had unremarkable liver with mild congestion of blood vessel, the Group 5 (120mg/kg MF) kidney had glomeruli and collecting tubules spared, Group 8 (120mg/kg EA) kidney had cortex and medullary tissues completely destroyed and Group 9 (25mg/kg Metoprolol) had unremarkable liver cells with kidney tissue having unremarkable glomerular and collecting tubules. The **Histopathological analysis from Myocardial infarction experiment revealed** that pre-treatment with 30mg/kg (Group 3), 60mg/kg MF (Group 4), 120mg/kg M.F(Group 5), 30mg/kg EA(Group 6), 60 mg/kg EA (Group 7), 120mg/kg EA (Group 8) and 25mg/kg Metoprolol (Group 9) before the Isoprenaline induced myocardial infarction caused some varying damages and protections on the organs (Hearts, Livers and Kidneys) of the experimental rats, **However**, Group 3 (30mg/kg MF) had spared liver tissues, Group 7 (60 mg/kg EA) had complete loss of the Liver architecture with the kidney glomeruli spared but some tubules were fibrotic and Group 9 (pre-treated with 25mg/kg Metoprolol), the positive control exhibited a preserved liver tissue architecture with kidney glomeruli completely destroyed with lots of hemorrhages seen. From the two demonstrated experiments on Arrhythmia and Myocardial infarction the following are the obtained histological features on the organs (Hearts, Livers and Kidneys) of the experimental rats:

HISTOLOGICAL FEATURES FOR THE ARRHYTHMIA EXPERIMENT: GROUP 2 (The Negative control Group) histological finding suggested that barium chloride-induced vasoconstriction might have contributed to congestion which indicated increased blood flow or obstruction in blood vessel, then minimal scarring or tissue repair with no significant cell death were seen in liver section. The severe tubular necrosis indicated significant damages to the renal tubules, interstitial inflammation and destruction of glomeruli indicated significant damage to the filtration units with scarring and blockage of glomeruli of which the possible cause might be acute kidney injury (AKI) from loss of kidney function. The heart' necrosis indicated cell death in the outer myocardium while hemorrhages suggested damage to blood vessels leading to bleeding which could be as a result of physical injury to the heart from trauma or heart attack due to reduced blood flow to the heart muscle or from exposure to cardiotoxic substances that led to arrhythmia. There were varying degrees of damages on the organs that had pretreatment (cardio-protection) with the herbal fractions. **GROUP 3** had large hemorrhages and mild necrosis in the heart tissue, extensive tissue necrosis involving the cortex and the medullar to some extent indicated widespread damage in the kidney tissue and functional units and moderate tissue necrosis in the liver tissue. **GROUP 4** had Moderate tissue necrosis of the myocardium, mild necrosis in the liver and severe kidney damage. **GROUP 5** had minimal damages to the organs when compared with the normal control group 1 and other groups meaning it had some cardio-protection from the daily herbal fractions administration before arrhythmia inducement in them, the heart tissue showed there was tissue necrosis with hemorrhages in between the muscle fibers in some foci, the kidney tissue showed there was necrosis beneath the capsule layer, the glomeruli and collecting tubules are spared and the liver tissue showed there was mild tissue necrosis with few hemorrhagic around and in between the cells. **GROUP 6** had extensive tissue necrosis and multiple large haemorrhagic lakes in the heart tissue, the liver tissue had extensive severe necrosis and the kidney had extensive and severe tissue necrosis involving the tubules and some of the glomeruli that indicated loss of kidney function. **GROUP 7:** Tissue necrosis involving all layers indicated widespread cell death across the entire thickness of the cardiac tissue, potentially leading to significant impairment of cardiac function and lots of hemorrhagic lakes, Severe tissue necrosis suggested widespread cell death in the kidney tissue affecting the tubules, glomeruli and interstitial tissues causing significant impairment of the kidney function and Moderate to severe tissue necrosis in the liver tissue and hemorrhages. For the **GROUP 8**, the histological section showed heart tissue within which there were large islands of hemorrhages with extensive and severe tissue necrosis, The histological section showed liver tissue within which there is moderate to severe necrosis of the cells beneath the capsular layer with inflammation with tissue necrosis involving cells around the vessels with bridging fibrosis connecting the portal veins and lots of dilated and congested blood vessels in the liver and severe and extensive tissue necrosis widespread cell death affecting both the cortex and medulla which indicated loss of normal kidney architecture and hemorrhagic lakes. **GROUP 9 (The positive control),** Moderate to severe tissue necrosis in the heart tissue, severe tissue necrosis involving the tubules, glomeruli, the interstitial tissue and with moderate hemorrhages while the

liver tissue had mild capsular inflammation congestion or obstruction in the liver vessels and unremarkable liver cells which indicated that the liver cells appear normal.

HISTOLOGICAL FEATURES FOR THE MYOCARDIAL INFARCTION EXPERIMENT:

GROUP 2: The histological findings in the heart suggested that barium chloride-induced vasoconstriction might have contributed to congestion which indicated increased blood flow or obstruction in blood vessel and necrosis suggested cell death in the outer myocardium and hemorrhages from exposure to cardiotoxic substances that led to arrhythmia, for the liver section there was minimal scarring or tissue repair with no significant cell death were seen, then For the kidney there was severe tubular necrosis which indicated significant damages to the renal tubules, interstitial inflammation and destruction of glomeruli with scarring and blockage of glomeruli from loss of kidney function. **GROUP 3,** Large hemorrhages in the heart indicated significant bleeding in the heart tissue, tiny flames of hemorrhages suggested petechial hemorrhages which are small, pinpoints bleeds and mild tissue necrosis indicated some cell death in the affected areas. This causes cardiac dysfunction such as arrhythmia, heart failure or cardiac rupture. For the liver, the unremarkable hepatocytes indicated that the liver cells appeared normal and mild congestion suggested increased blood flow or pressure in the livers' blood vessels. For the kidney, extensive tissue necrosis involving the cortex and the medullar to some extent indicated widespread damage in the kidney tissue and functional units which could be as a result of acute kidney injury or ischemia or toxicity from nephrotoxic substances. **GROUP 4:** For the Heart, moderate tissue necrosis indicated cell death in the heart tissue, subepidermal involvement indicated damage beneath the outermost layer (epicardium) and few hemorrhages suggested bleeding or vascular damage possibly from toxicity or ischemia. In the liver, minimal fibrosis showed scarring in the liver tissue indicating chronic liver injury, the bridging fibrosis means fibrosis connecting the portal tracts or central veins in advanced liver damage, piece meal necrosis means death of liver cells at the interface between liver parenchyma and fibrotic tissue, blood vessel congestion indicated increased blood flow or pressure in the liver's blood vessels. Kidney; the extensive and severe tissue necrosis indicated a widespread cell death in the kidney tissue. Tubular, glomerular and interstitial involvement means damage affected multiple components of the kidney including tubules, glomerular and interstitium. **GROUP 5:** Tissue necrosis indicated cell death in the heart

muscle, hemorrhages suggested bleeding within the heart tissue. Kidney: Necrosis beneath the capsule layer indicated damage to the renal cortex, sparing of glomeruli and collecting tubules suggesting that the damage might be limited to specific areas. Liver: Mild tissue necrosis indicated some liver cell death, hemorrhages indicated presence of bleeding around and between cells. **GROUP 6**: The heart had lots of broken blood vessels and large hemorrhagic lakes means big pools of blood between the muscle fibers and cells, suggestive of trauma from cervical dislocation procedure used in sacrificing them, Liver tissue had necrosis walled off by inflammatory cells and ghost cells, some foci had tiny bleeding in between hepatocytes with lots of dilated and congested blood vessels and the kidney tissue had lots of hemorrhages, congested blood vessels, severe tubular necrosis seen with casts and many hemorrhagic glomeruli. **GROUP 7**: The heart tissue had large hemorrhagic lakes splitting the cardiac muscles in many foci involving all the layers of the heart, which may suggest traumatic cardiac injury or myocardial infarction with rupture, the kidney tissue had massive tubular necrosis and hemorrhages that involved the cortex and medulla, sparing glomeruli with some of the tubules being fibrotic while the liver tissue had severe and extensive tissue necrosis involving most of the liver tissue with complete loss of architecture and few viable cells around the portal vein in some foci. **GROUP 8**:The heart tissue showed there were hemorrhagic lakes splitting the muscles with lots of dilated congested blood vessels in the endocardium, This pattern of injury can be seen in conditions such as myocardial rupture and cardiac contusion, the liver showed dilated, congested blood vessels which indicated increased blood flow or obstruction, extensive tissue necrosis were seen in the capsular area, suggesting significant damage potentially due to ischemia or trauma (injury to the liver), the liver section showed extensive tissue necrosis, damage of the renal tubules (often caused by ischemia or toxins) with cortical and medullary involvement that indicated wide spread damage to the kidney tissue with moderate hemorrhages. The pattern of injury can be seen in conditions such as ischemia or toxicity due to exposure to toxin. **GROUP 9 (The positive control):** The heart tissue showed hemorrhagic lakes involving the pericardial layer of which the pattern of injury can be seen in conditions such as traumatic injury or myocardial infarction with rupture, the kidney tissue showed there were extensive necrosis involving the cortical and cortico-medullary area but sparing the medulla normally seen in severe shock (hypoperfusion), renal artery thrombosis or embolism or toxic injury while the glomeruli were completely destroyed with lots of hemorrhages, the liver

section showed intact architecture which indicated the liver's over all structure was preserved, the necrosis near the capsule and portal vein indicated focal necrosis.

4.0 CONCLUSION AND RECOMMENDFATIONS

4.1 Conclusion

From the pretreated groups after **Isoprenaline induced Myocardial infarction**, Group 3 had little protection on the liver with some protection on the heart but the kidney had lots of glomeruli destroyed, Groups 4 had some heart protection, some kidney glomeruli were affected with no protection and little protection on the liver tissue, Group 5 had little protections on the organs, Group 6 had some protections on the kidney, liver and heart, Group 7 did not protect the heart, glomeruli were spared but some of the tubules were fibrotic with complete loss of the liver architecture, Group 8 had no organ protections and Group 9 gave some protections to the heart, the kidney had extensive necrosis involving the cortical and cortico-medullary area but sparing the medulla and the glomeruli completely destroyed with intact liver architecture. **From the barium induced arrhythmia,** Group 3 had protection on the heart, the liver showed hepatocytes that were unremarkable and the kidney had extensive tissue necrosis involving the cortex and the medullar to some extent with both the tubules and collecting ducts involved, Group 4 had protections on the heart and liver with little protections on the kidney, Group 5 gave protections to the heart, liver and kidney with glomeruli and collecting tubules spared, Group 6 had some protections on the heart, kidney tissues and the liver, Groups 7 and 8 had no organ protections and Group 9 gave protection to the organs with unremarkable liver cell and the kidney glomeruli and collecting tubules unremarkable. From the experimental evidences, it was found that the use of 120mg/kg MF gave the highest protection on the organs of the experimental Wistar rats in the two experiments but highest in the arrhythmia experiment followed by 30mg/kg E.A that had some protections on the organs.

According to Onah Ifeoma .C et al, Ethyl acetate had more fatty acids esters and phytosterols than the Methanol fractions while Methanol fraction had more polyphenols than the ethyl acetate fraction from the demonstrated GCMS and FTIR results, therefore, the120mg/kg Methanol Fraction was able to protect the organs of the animals because of the bioactive compounds present in them and the some fractions could not protect some groups probably because of high doses or from the prolonged usage duration. Numerous studies by Kahkonem et al,2003, Shahidi et al, 2018, Lee et al, 2017 and Kumar et al, 201, stated that excess phytosterols can lead to accumulation of reactive oxygen species (ROS) causing oxidative stress and damage to cellular component causing apoptosis (programmed cell death) or necrosis which is a form of cell death, it could be the reason higher doses of the E.A was killing the experimental animals and affecting their organs than same doses with M.F. According to Ajayi et al 2011, excessive amount of the plant supplement could

pose a risk to the heart and the liver, in other words, the dry fruit of Tetrapleura tetraptera fractions should be administered in moderate amounts to prevent potential adverse effects. Some recent studies on the effect of antioxidant therapy according to Borut et al, 2013 indicated that in some circumstances the intake of antioxidants increased mortality because the oxidative stress is damaging and beneficial for the organism, as some Reactive Oxygen Species (ROS) are signaling molecules in cellular signaling pathways. Lowering the levels of oxidative stress by antioxidant supplements is not beneficial in such cases. The balance between ROS and antioxidants is optimal, as both extremes, oxidative and anti-oxidative stress, are damaging. Therefore, there is a need for accurate determination of individual's oxidative stress levels before prescribing the supplement antioxidants. When need arises to use the herbal fraction in the indicated conditions, it shall not be administered for a longer time as demonstrated in this experiment since it has moderate toxicity from the LD 50 value to avoid damaging the organs of the body while delivering therapeutic effects.

4.2 Recommendations

From the demonstrated experiments, the 120 mg/kg (Group 5) had the highest organ protection on the pre-treated group after arrythmia and Myocardial Infarction inducements, but gave highest protection on the organs of the experimental Wistar rats in arrythmia condition, Therefore, I recommend:

1. The use of 120mg/kg Methanol Fraction of *T. tetraptera* fruit fraction in treating arrhythmia since it gave the highest protection on the organs of the experimental Wistar rats in arrhythmia condition and also be used in Myocardial Infarction conditions with a little increase in the duration here to normalize the little infarcted tissues.
2. I recommend that the study be extended to human since the results were extrapolations from the used animal models.

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