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ORIGINAL RESEARCH ARTICLE

Dose-Dependent Hepatotoxic and Cardiotoxic Effects of Amlodipine in Wistar Rats

Iyare, G. I.[1], Omorodion, N. T.[2] and Momodu A.[1]
[1]Department of Medical Laboratory Science, Faculty of Applied Sciences, Edo University Uzairue, Edo State, Nigeria
[2]Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Nigeria

ABSTRACT
There is a high prevalence of premature deaths worldwide because of hypertension, a common condition that greatly raises the risk of heart disease, brain abnormalities, and renal malfunction. Reaching more than a billion people worldwide, low- and middle-income nations are the main areas where the illness is found. In order to lessen vascular smooth muscle contraction and increase vasodilation, amlodipine, a drug often used for hypertension, inhibits L-type calcium channels. Daily use is appropriate due to its extended half-life and excellent bioavailability. This study looks at how amlodipine affects the liver and cardiac tissues of Wistar rats from a histological, biochemical, and physical perspective. For the duration of the trial, a standard pellet diet and water were provided to the control group (Group A, n = 4) of the twenty rats utilised in this study. The drug was administered using a 0-10µl micropipette with appropriate tips, and an oral gavage was used to ensure accurate delivery into the animals' mouths. This study found significant dose-dependent increases in liver enzyme levels ($p<0.05$) in the treatment groups compared to the control, indicative of potential liver toxicity indicative of potential liver toxicity. Histological analysis showed normal heart tissue at lower doses but mild degenerative changes at higher doses. Liver tissues exhibited minor inflammatory responses at higher doses. These findings emphasize the importance of ongoing safety assessments for antihypertensive medications to ensure their long-term safety.

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Amlodipine, Histological analysis, Liver tissues, Heart tissues, Wistar rat

INTRODUCTION
High blood pressure is a critical health issue that greatly elevates the chances of heart disease, neurological problems, kidney issues, and various other health concerns. It is a major factor in early mortality worldwide, impacting more than a billion individuals, with over 25% of men and 20% of women affected by this condition. The burden of hypertension is particularly high in low- and middle-income countries, which account for two-thirds of the cases, due to the increasing prevalence of risk factors in these populations over recent decades (WHO, 2022).

Many people with high blood pressure experience no symptoms and may not realize they have the condition. When symptoms do present, they might include early morning headaches, nosebleeds, irregular heartbeats, vision changes, and ringing in the ears. Severe cases can cause fatigue, nausea, vomiting, confusion, anxiety, chest pain, and muscle tremors. If untreated, high blood pressure can lead to persistent chest pain, heart attacks, heart failure, and abnormal heart rhythms, potentially resulting in sudden death (AHA, 2023). It can also cause strokes by blocking or bursting the arteries that supply the brain with blood and oxygen, and it can damage the kidneys, potentially leading to kidney failure. High blood pressure harms the heart by stiffening arteries and reducing blood and oxygen flow to the heart. Detecting hypertension is straightforward and painless, requiring only a quick blood pressure measurement that can be performed at home or by a healthcare professional to evaluate risks or related conditions (NHLBI, 2022).

Heart failure (HF) and liver disease frequently coexist due to systemic conditions impacting both organs, such as substance abuse, inflammation, autoimmune diseases, and infections. Additionally, intricate interactions between the heart and liver contribute to this phenomenon (Andrew et al., 2019). The liver benefits from dual blood supply via the portal vein and hepatic artery, ensuring functionality even if one source is compromised. The hepatic arterial buffer response allows the hepatic artery to adjust for variations in portal flow, compensating for 25% to 60% of reduced portal flow (Ho et al., 2013). Total liver blood flow ranges from 800 to 1,200 ml/min, about 100 ml/min per 100 g of liver tissue (Eipel et al., 2010).

Correspondence: Nosa Terry Omorodioa. Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Nigeria. ✉ terry.omorodion@uniben.edu.
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Amlodipine is a widely used first-line antihypertensive drug, either alone or combined with other medications (McCormack and Williams 2012; Fares et al., 2016). It works by inhibiting L-type calcium channels, reducing calcium levels within cells, which decreases vascular smooth muscle contraction, promotes relaxation, and leads to vasodilation. This process enhances vascular endothelial function and lowers blood pressure by relaxing smooth muscles and dilating blood vessels (Ferrari et al., 2019). Amlodipine also treats stable angina by lowering the afterload, reducing myocardial oxygen demand during physical activity, and relieves Prinzmetal or variant angina by preventing coronary artery spasms and improving blood flow (Tang et al., 2016).

The bioavailability of amlodipine is between 64% and 90% and is not affected by food intake. Peak plasma levels are reached within 6 to 12 hours, and steady-state levels are achieved after 7 to 8 days of daily use (Rang *et al.,* 2016). Amlodipine binds highly to plasma proteins (93%) and is extensively metabolized in the liver into inactive forms (Rang *et al.,* 2016). t has a biphasic plasma half-life, with a terminal elimination half-life of 30 to 50 hours, which extends in cases of liver dysfunction. The drug is primarily excreted via the kidneys, with 10% of the unchanged drug and 60% of metabolites found in the urine (Kishen and Manouchkathe, 2023). Available in 2.5 mg, 5 mg, and 10 mg oral tablets, amlodipine can also be formulated into suspensions for those with difficulty swallowing. Its long half-life allows for once-daily dosing (Van, 1994).

Notable side effects of amlodipine include peripheral edema, heart failure, pulmonary edema, flushing, dizziness, headache, drowsiness, skin rash, nausea, abdominal pain, and constipation. Clinical studies indicate a dose-dependent increase in edema, dizziness, flushing, and palpitations at a 10 mg dose, with occurrences of 10.8%, 3.4%, 2.6%, and 4.5%, respectively. Other reported side effects include headaches (7.3%), fatigue (4.5%), nausea (2.9%), and abdominal pain (1.6%) (Vukadinović *et al.,* 2019). Amlodipine and other calcium channel blockers have been linked to rare cases of idiosyncratic drug-induced liver disease, usually presenting a mixed hepatocellular-cholestatic pattern, with recovery expected within 4 to 8 weeks after stopping the medication (Bethesda, 2016, 2017). Peripheral edema from amlodipine can sometimes lead to a prescribing cascade, where the edema is mistaken for a new condition, and diuretics are wrongly prescribed to address it (Savage, 2020).

Given amlodipine's widespread use to control blood pressure and its impact on hepatic and cardiac tissues, it is crucial to understand the long-term effects of prolonged administration and increasing dosage on these organs. This study aims to investigate the physical and histological effects of amlodipine on the liver and heart tissues of Wistar rats and establish correlations with some biochemical parameters. There is limited data on the cytological architecture changes induced by amlodipine in these organs.

MATERIALS AND METHODS

Ethical Approval

All experimental procedures complied with the ethical standards established by the Olabisi Onabanjo University Ethics Committee for animal care and use. The work was approved prior to commencement by the Olabisi Onabanjo University Ethics Committee for Animal Care and Use and issued a number: V.1561/67

Experimental Subjects

This study involved adult male albino rats with weights ranging from 152g to 194g. These rats were bred and housed at the Pre-Clinical Animal House of the Olabisi Onabanjo University College of Medicine and Health Sciences. They were maintained under standard laboratory conditions with unrestricted access to food and water. Prior to the start of the experiments, the animals were acclimated to the lab environment for one week. Each rat's weight was recorded weekly.

Drug

Amlodipine tablets (2.5mg, 5mg, 10mg) were procured from a pharmacy in Sagamu, Nigeria. The tablets were prepared at a concentration of 0.13%, as detailed in the Appendix.

Laboratory Equipment and Reagent

Automatic Tissue Processor: Model Tissue-Tek VIP 6, made by Sakura Finetek in Japan. Microtome: Model Leica RM2245, produced by Leica Biosystems, Germany, Floating Out Bath: Model FB-300, manufactured by Thermo Fisher Scientific, USA, Embedding Machine: Model Leica EG1150H, from Leica Biosystems, Germany, HPlate: Supplied by Thermo Fisher Scientific, USA, Grease-free Microscope Glass Slides: Provided by Thermo Fisher Scientific, USA, Microscope Cover Slips: Sourced from VWR International, USA, Forceps: Model Fine Tip Forceps, from World Precision Instruments, USA, Hot Air Oven: Model Thermo Scientific Heratherm,

produced by Thermo Fisher Scientific, USA, Embedding Moulds: Manufactured by Sigma-Aldrich, USA, Tissue Cassettes: Supplied by Thermo Fisher Scientific, USA, Xylene: Provided by Sigma-Aldrich, USA, Alcohol (70%, 80%, 95%, 100%): Produced by Fisher Scientific, USA, Paraffin Wax: Manufactured by Leica Biosystems, Germany, Hematoxylin Solution: Supplied by Thermo Fisher Scientific, USA, Eosin Solution (1%): Produced by Sigma-Aldrich, USA, Acidic Alcohol: Provided by Sigma-Aldrich, USA and DPX Mounting Medium: Sourced from Sigma-Aldrich, USA.

Drug Administration

A total of 16 wister rats were used for the experiment. They were divided into 4 groups of 4 rats each. The Control Group (Group A, n=4) received a standard pellet diet and water throughout the study. On the other hand, the other 3 groups, Experimental Groups (Groups B, C, and D, n=4 each), received daily oral doses of Amlodipine was chosen as recommended by Meyer and Barbier (2021). Besylate at 2.5 mg/kg, 5 mg/kg, and 10 mg/kg body weight, respectively, for 21 days, drug administration was carried out using a 0-10µl micropipette with an oral gavage.

Blood Sample Collection

Blood samples were collected from the medial canthus of each rat 24 hours after the final drug dose using heparinized capillary tubes. Samples were stored in lithium heparin bottles for liver function tests, focusing on Aspartate Transaminase (AST) and Alanine Transaminase (ALT).

Animal Sacrifice and Tissue Collection

Animals were sacrificed by cervical dislocation shortly after blood collection. The liver and heart were excised, weighed, and processed for histological examination using hematoxylin and eosin (H&E) staining.

Histological and Histochemical Analysis

The liver and heart tissues were processed using standard histological methods (Achukwu et al., 2019):

H&E Staining Protocol:

The tissues dewaxing and hydration was carried out by treating the tissues in descending alcohol concentrations (100%, 90%, 80%, 70%, 50%) and then in water, after which the tissues were stained with Harris hematoxylin for 5 minutes. The stained tissues were rinsed with water and differentiated in 1% acid alcohol for 30 seconds. The tissues were rinsed with with water again, birth in running tap water for 10 minutes, and counterstained with 1% eosin for 5 minutes. Finally, the tissues were dehydrated in ascending alcohol concentrations, and then the coverslips were mounted using a DPX medium.

Photomicrography

Structural changes in heart and liver tissues were observed at 400x magnification.

Liver Function Tests

Liver function parameters were analyzed using Randox Test Kits *Randox test kits for liver function* (Randox, 2023)

ALP Activity Measurement:

For the Alkaline Phosphatase (ALP) activity using the Rec. GSCC (DGKC) colorimetric method (Klein and Schwartz, 2000)

AST Activity Measurement:

The Aspartate Aminotransferase (AST) activity is measured using the Reitman and Frankel method (Reitman and Frankel, 1957).

Statistical Analysis

Data were analyzed using IBM SPSS v21. The mean, standard deviation, and standard error of the mean (S.E.M.) were calculated. One-way ANOVA with LSD and Duncan's Multiple Range Test, and a two-tailed t-test were used to compare control and experimental groups. Statistical significance was set at $p<0.05$.

RESULTS

Weight Analysis

Table 1 shows the mean weights of the control and experimental groups. Group A (control) had a mean weight of 193.94 ± 26.62g after 4 weeks. Groups B, C, and D showed significant weight increases (p=0.006) after administering 2.5 mg/kg, 5 mg/kg, and 10 mg/kg of amlodipine.

AST Levels

Table 2 showed a significant increase in AST levels with dosage, with a mean ± SD of 13.938 ± 0.565 and a highly significant value at 10 mg/kg (16.125 ± 0.854; p=0.001).

ALP Levels

Table 3 showed the significant differences in ALP levels between control (33 ± 0.00; p=0.01) and experimental groups (2.5 mg/kg: 39 ± 0.816, p=0.001; 5 mg/kg: 39 ± 1.414, p=0.001; 10 mg/kg: 39 ± 0.408, p=0.001), with a mean ± SD of 37.50 ± 0.603 (p=0.001).

Table 1: Effect of amlodipine administration (2.5 mg/kg, 5 mg/kg, 10 mg/kg) on animal weight.

Groups	**N**	Mean ± SD	Std.Error 3.328	Sig. P-value (***p≤0.05)**
Group A – nil	4	165.25 ± 23.940	5.985	.002
Grp B -2.5mg/kg	4	212.88 ± 25.838	6.459	.005
Grp C -5mg/kg	4	201.75 ± 16.707	4.177	.005
Grp D -10mg/kg	4	195.88 ± 11.278	2.819	.004
Total average weight	16	193.94 ± 26.621	3.328	-006

Table 2: Effect of amlodipine administration (2.5mg/kg, 5mg/kg, 10mg/kg) on Aspartate Aminotransferase (AST) - iu/

Group	**N**	AST Mean ± SDiu/l	Sig. P-value (***p≤0.05)**
Group A – nil	**4**	14 ± 0.0	**.002**
Grp B -2.5mg/kg	**4**	12.375 ± 0.479	**.001**
Grp C -5mg/kg	**4**	13.25 ± 0.957	**.001**
Grp D -10mg/kg	**4**	16.125 ± 0.854	**.001**
Total Average Drug dosage administered -AST_Values	**16**	13.938 ± 0.565	**.001**

Table 3: Effect of amlodipine administration (2.5mg/kg, 5mg/kg, 10mg/kg) on Alkaline Phosphatase Aminotransferase (ALP) - iu/

Group	**N**	AST Mean ± SDiu/l	Sig. P-value (***p≤0.05)**
Group A – nil	**4**	33 ± 0.00	**.01**
Grp B -2.5mg/kg	**4**	39 ± 0.816	**.001**
Grp C -5mg/kg	**4**	39 ± 1.414	**.001**
Grp D -10mg/kg	**4**	39 ± 0.408	**.001**
Total Average Drug dosage administered -ALP	**16**	39 ± 0.603	**.001**

Photomicrographs

The photomicrographs of the tissues (Plates 1, 2, 3, 4, 5, 6, 7 & 8) showed no degenerative changes in the heart and liver tissues across all dosages of amlodipine compared to the control group.

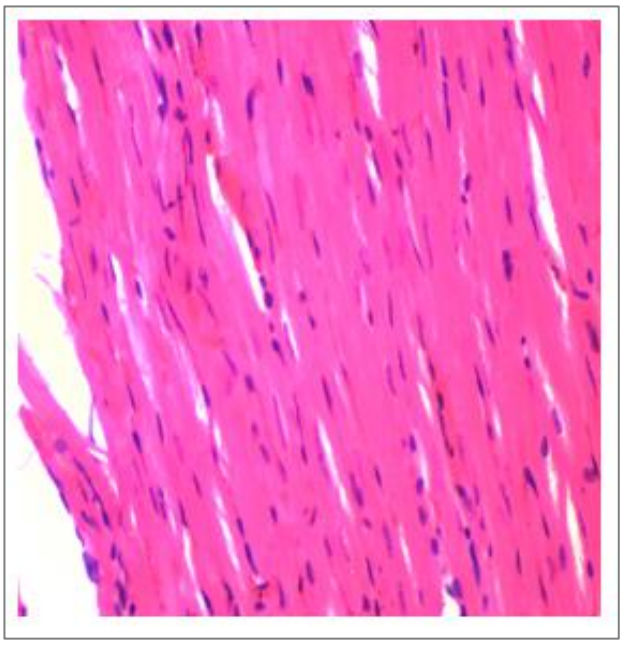

Plate 1: Section of cardiac muscle shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are indicative of normal heart muscle. Hematoxylin and Eosin X 400.

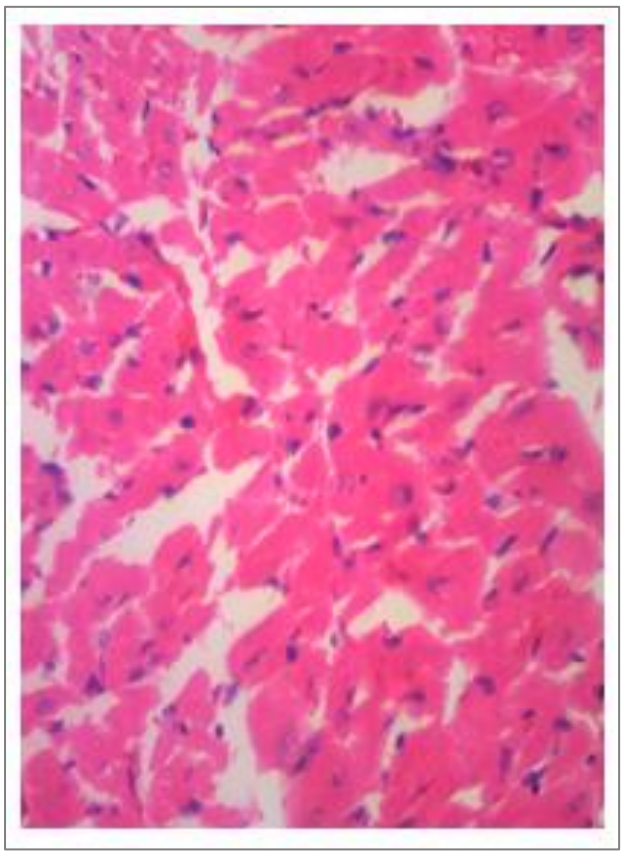

Plate 2: Section of rat cardiac tissue from Experimental Group B, treated with 2.5 mg/kg of TM. The section shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are indicative of normal heart muscle. Hematoxylin and Eosin X 400.

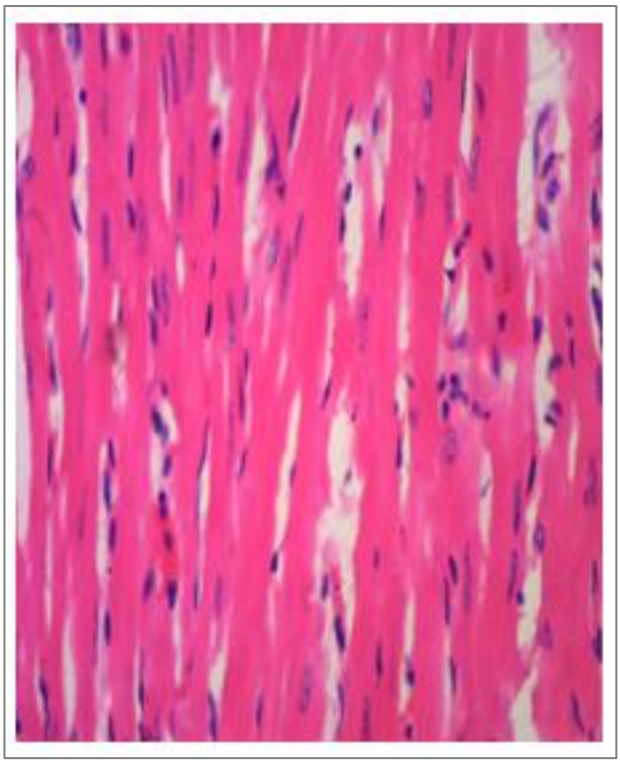

Plate 3: Section of rat cardiac tissue from Experimental Group C, treated with 5 mg/kg of TM. The section shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are indicative of normal heart muscle. Hematoxylin and Eosin X 400.

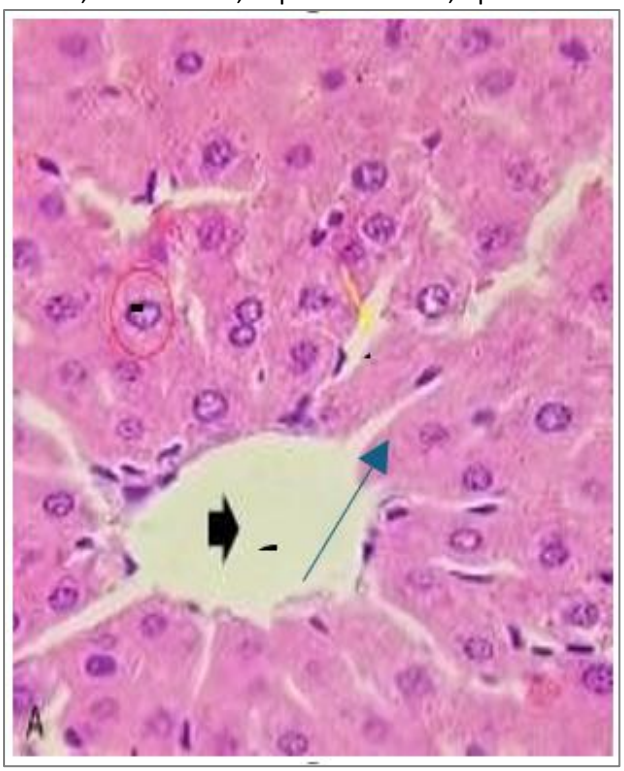

Plate 5: A section of hepatic tissue from Control Group A demonstrates well-organized and distinct hepatocytes (H) arranged on hepatic plates, with a central vein (CV) and sinusoids containing Kupffer cells (S). Stained with Hematoxylin and Eosin, magnification X 400.

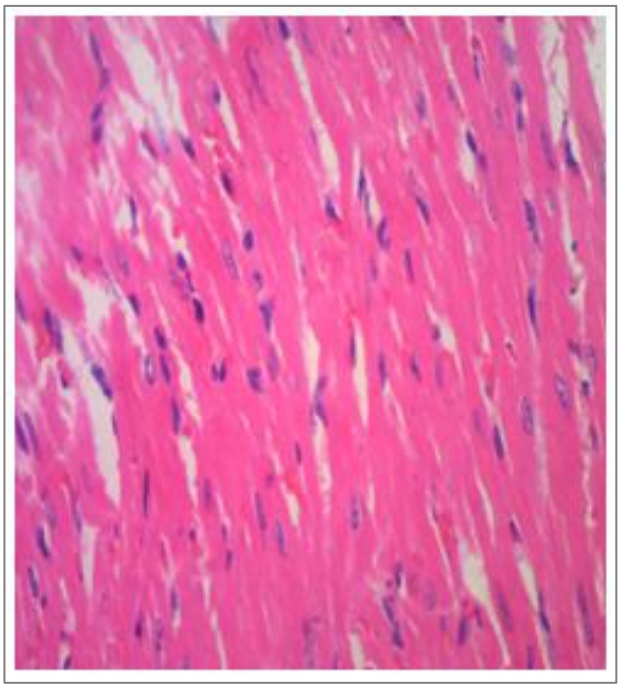

Plate 4: Section of rat cardiac tissue from Experimental Group D, treated with 10 mg/kg of TM. The section shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm but also exhibit slight degeneration of both the cardiac fibers (CF) and the nucleus (N). Hematoxylin and Eosin X 400.

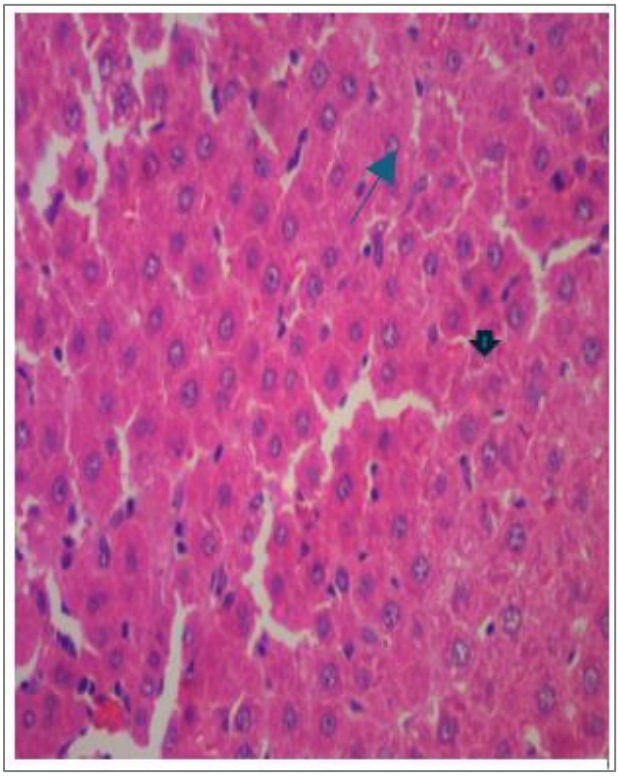

Plate 6: Section of rat hepatic tissue sample from Experimental Group B, treated with 2.5 mg/kg of TM. The liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding centrally placed normochromic nuclei with indistinct nucleoli. These features are

consistent with normal hepatocytes, showing irregularly distributed hepatocytes (H) on the hepatic plates, sinusoids (S), and a slightly congested central vein (CV). Hematoxylin and Eosin X 400.

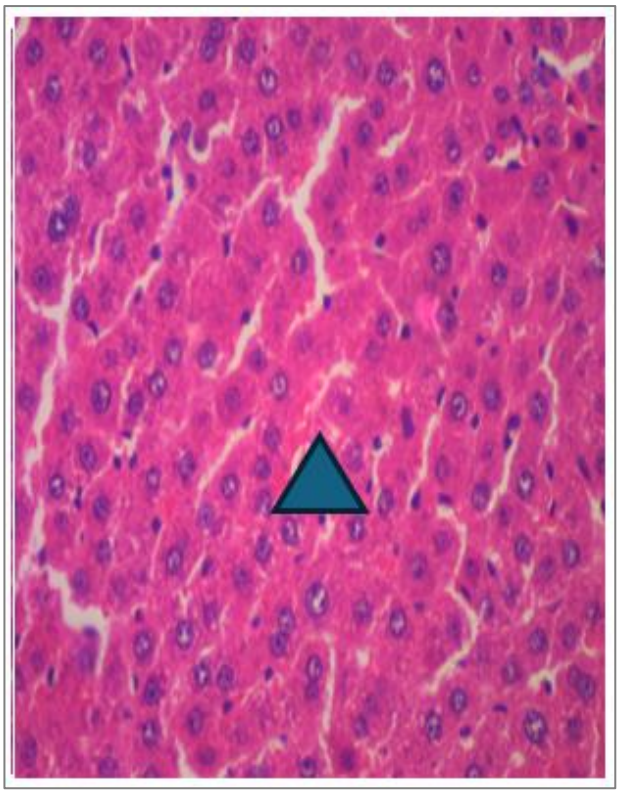

Plate 7: Section of rat hepatic tissue sample from Experimental Group C, treated with 5 mg/kg of TM. The liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding centrally placed normochromic nuclei with indistinct nucleoli. These features are consistent with normal hepatocytes. Hematoxylin and Eosin X 400.

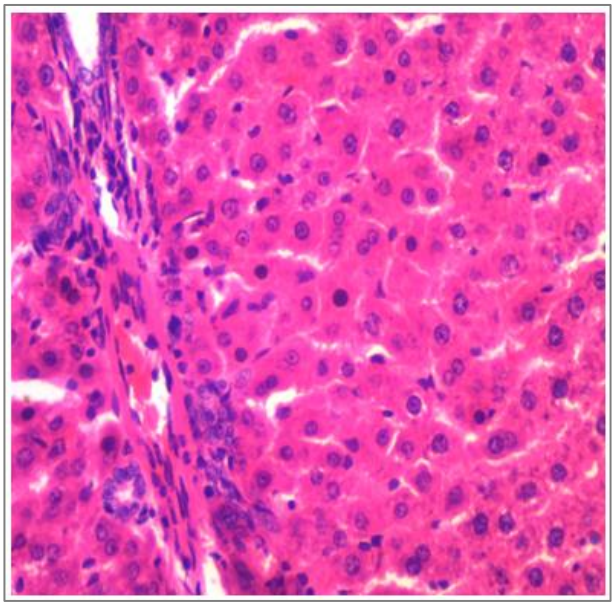

Plate 8: Section of rat hepatic tissue from Experimental Group D, treated with 10 mg/kg of TM. Hematoxylin and Eosin X 400

The liver (Plate 8) shows hepatocytes (thick arrow) with eosinophilic cytoplasm surrounding centrally placed normochromic nuclei with indistinct nucleoli. Also present are some lymphocytic infiltrates around the portal tract. These features are consistent with normal hepatocytes.

DISCUSSION

Over four weeks, the average body weight of albino rats was measured for both control and experimental groups. The control group (Group A) showed an average weight of 193.94±26.62193.94 \pm 26.62193.94±26.62 grams. Experimental groups treated with amlodipine at doses of 2.5 mg/kg, 5 mg/kg, and 10 mg/kg exhibited a notable increase in weight compared to the control, with a statistically significant difference ($p = 0.006$). This suggests that amlodipine treatment is associated with increased body weight in these rats (Bethesda, 2017).

After a one-week acclimatization period, AST (aspartate aminotransferase) levels were measured following three weeks of amlodipine administration. The mean AST level across experimental groups was 13.938±0.56513.938 \pm 0.56513.938±0.565. The group receiving the highest amlodipine dose of 10 mg/kg showed a significant rise in AST levels (16.125±0.85416.125 \pm 0.85416.125±0.854; $p = 0.001$). This increase suggests potential liver damage, as higher doses of amlodipine can elevate AST levels, indicating possible hepatocellular injury (Vukadinović *et al.*, 2019).

Alkaline phosphatase (ALP) levels were also assessed. The control group had an ALP level of 33±0.0033 \pm 0.0033±0.00. In contrast, all amlodipine-treated groups showed higher ALP levels: 39±0.81639 \pm 0.81639±0.816 for the 2.5 mg/kg dose ($p = 0.001$), 39±1.41439 \pm 1.41439±1.414 for the 5 mg/kg dose ($p = 0.001$), and 39±0.40839 \pm 0.40839±0.408 for the 10 mg/kg dose ($p = 0.001$). The overall mean ALP level was 37.50±0.60337.50 \pm 0.60337.50±0.603 ($p = 0.000$), indicating that amlodipine affects ALP levels significantly, suggesting hepatobiliary effects (Kishen and Manouchkathe, 2023).

The cardiac muscle showed normal myocyte structure with nuclei positioned peripherally and eosinophilic cytoplasm, indicating healthy heart muscle (Bethesda, 2017). Cardiac tissue in this group also displayed normal myocyte features. This finding supports the idea that low doses of amlodipine do not cause significant changes in cardiac tissue (McCormack & Williams, 2012). The cardiac muscle showed normal morphology, similar to Group B. This result suggests that moderate doses of amlodipine do not induce significant pathological changes in the heart (Ferrari *et al.*, 2019). The cardiac tissue showed normal myocyte structure but with mild degeneration of cardiac fibers and nuclei. This may indicate early signs of cardiac stress from higher doses of amlodipine (Vukadinović et al., 2019).

Liver tissue displayed well-organized hepatocytes with normal architecture, including the central vein and sinusoids populated by Kupffer cells (Ho *et al.*, 2013). Hepatocytes showed normal features, though some mild congestion in the central vein was observed. This could indicate an early response to amlodipine but is not suggestive of significant pathology (Eipel *et al.*, 2010). The liver tissue appeared normal with no significant histopathological changes, consistent with findings that moderate doses do not cause liver damage (Ferrari et al., 2019). The liver tissue showed normal hepatocytes but with some lymphocytic infiltration around the portal tracts, suggesting an inflammatory response. This is consistent with observations that higher doses may provoke mild inflammation in liver tissues (McCormack and Williams 2012).

The study indicates that amlodipine treatment in rats can significantly impact body weight and liver enzyme levels, potentially causing hepatotoxic effects. Lower to moderate doses of amlodipine generally maintain normal cardiac and liver tissue structure, while higher doses may lead to mild degenerative and inflammatory changes. These results align with previous research on the effects of calcium channel blockers and highlight the need for further investigation into their safety profiles (McCormack and Williams, 2012; Ferrari *et al.*, 2019; Kishen and Manouchkathe, 2023; Vukadinović et al., 2019).

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