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Effects of Methanol Extract of Celery (*Apium graveolens*) Leaf on Ethanol-Induced Left Ventricular Changes in Wistar Rats

*Farinde Rukayat Adesewa[1,2]; Komolafe Omobola Aderibigbe[1]
[1]Department of Anatomy and Cell Biology,
Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria
[2]Department of Human Anatomy, Faculty of Allied Health Sciences, Elizade University Ilara-Mokin, Ondo State Nigeria

*Correspondence to: FARINDE, Rukayat Adesewa

Abstract:-

➢ ***Background:***

Alcohol consumption is associated with several cardiovascular diseases such as heart failure, left ventricular hypertrophy, as well as with other disorders. This study was designed to determine the cardioprotective effect of celery (*Apium graveolens)* extract in attenuating perturbations arising from alcohol ingestion on left ventricular injury in adult Wistar rats.

➢ ***Methods:***

Thirty male Wistar rats weighing 120g -160g were used for this study. The rats were divided into six (6) groups A, B, C, D, E &F five (5) rats each. The rats in group (A) served as control and received standard pellet, while groups B, C, D, E and F were administered orally with 8ml/kg b. w of ethanol and 100mg/kg, 150mg/kg, 200mg/kg of methanol extract of *Apium graveolens* (MEAG) were given to group C, D and E and 25mg of propranolol was given to group F orally. Group A and B were administered with 2ml/kg b. w distilled water.

➢ ***Results:***

The relative heart weight, level of serum cardiac troponin I and left ventricular wall thickness of the ethanol-induced rats were significantly increase ($F = 7.64$; $p = 0.002$), ($F = 22.66$; $p = 0.001$) and ($F = 85.29$; $p < 0.001$) when compared with the control and across all experimental groups in this study. Group treated with MEAG also showed regular striations.

➢ ***Conclusion:***

This study indicates that MEAG has cardioprotective effects on these perturbations.

Keywords:- *Methanol; Apium Graveolens; Heart; Ethanol; Left Ventricle; Celery; Wistar Rats.*

I. INTRODUCTION

Cardiovascular disease (CVDs) is a prevalent disease affecting the heart, capillaries, arteries, and veins [1]. Alcohol consumption is associated with several cardiovascular diseases such as heart failure, left ventricular hypertrophy, as well as with other disorders [1]. The decline in cardiovascular mortality rates has been reported in high-income countries, while low-income countries, like Nigeria, have seen a rapid increase in these rates [2]. Alcohol use affect cardiovascular health due to its complex effect on cardiovascular system [2]. There are many mechanisms that affect blood pressure with the effect of alcohol and they include the following: build-up of plaque on arteries due to impairments in cells (it is through availability of nitric oxide and alterations in endothelial cell function and also arterial vascular function disruption) [2].

Traditional practices and medicinal plants are widely used in developing countries for primary healthcare due to their accessibility and affordability, as reported by the World Health Organization [3]. The efficacy of these plants is attributed to their phytochemical components. *Apium graveolens* leaf contains various phytochemicals including tannins, resins, alkaloids, saponins, cardenolides, phlobatannins, terpenoids, and flavonoids [4]. Vitamins and phenolic compounds present in celery have been shown to scavenge reactive oxygen radicals and prevent cellular damage, highlighting the potential health benefits of celery consumption [5]. Celery contains flavonoids that can also suppress inflammation.

Ethanol induction causes cardiovascular diseases such as heart failure, hypertension which affected the cardiovascular system [6]. Propranolol is a medication belonging to the class of drugs known as beta-blockers, which work by blocking the action of certain natural chemicals that affect the heart and blood vessels. It is used to treat a variety of conditions including high blood pressure, irregular heartbeats, and tremors. Beta-blockers like propranolol reduce heart rate, blood pressure, and strain on the heart by blocking the receptor sites for the endogenous catecholamine epinephrine (adrenaline) and norepinephrine (noradrenaline) on adrenergic beta receptors of the sympathetic nervous

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system, which mediates the fight-or-flight response. This makes them an effective treatment option for high blood pressure.

II. MATERIALS AND METHODS

A. Animal Care and Management

Thirty male Wistar rats (8 weeks old) weighing 120-160g were obtained from animal holding unit, college of health sciences, Obafemi Awolowo University, Ile-Ife Nigeria. The rats were assigned into six groups (A, B, C, D, E, and F) of five animals each at random. The animals were housed in a well-ventilated plastic cage, acclimatized for a period of two weeks before the commencement of the experiment and were fed on standard rat chow and water *ad libitum*. They were maintained under natural day and night cycles and temperature. Random selection was used to ensure that the groups were similar in terms of age, weight, and other characteristics. Overall, the study was conducted in a controlled environment to ensure that the results were reliable and accurate. The animal procedures were performed in accordance with the guideline for the care and use of the experimental animals established by HREC, OAU,Ile-Ife.

B. Reagents & Drugs

Propranolol was obtained from Sigma chemical Co., USA.

Alcohol (Analar grade) was obtained from Faculty of Pharmacy, OAU, Ile-Ife. It was manufactured by Sigma Aldrich Fine Chemicals, USA. Other chemicals and reagent used for the analysis in this study met the quality criteria in agreement with international standard.

C. Plant Materials and Method

The study made use of fresh leaves of *Apium graveolens* (celery) which were collected from Molete garden in Ibadan, Oyo state. To ensure the authenticity of the plant species, a taxonomist in the Department of Botany at Obafemi Awolowo University, Ile-Ife was consulted, and a voucher specimen of the plant was deposited in the department's herbarium for future reference (IFE-17963). The collected leaves were thoroughly washed with running tap water, air-dried at room temperature, and pulverized to powder using an electric blender. The resulting powder was soaked in a methanol solution and left at room temperature for 48 hours with occasional shaking using an electric shaker. The mixture was then filtered, concentrated to dryness using a rotary evaporator, and stored in a desiccator for further use. The percentage yield of the powder was determined to be 19.53%.

D. Animal Treatment

Group A served as the control, while groups B, C, D, E, and F were orally administered 8ml/kg b. w of ethanol for five weeks. Following this, daily administration of 100mg/kg, 150mg/kg, and 200mg/kg of MEAG and 25mg/kg b. w of propranolol was given to group F orally for two weeks. Group A and B were administered with 2ml/kg B. w of distilled water. Administration lasted for seven weeks and was done via oral route; distilled water 2 mL/kg was used as vehicle.

E. Animals Sacrifice

All animals were sacrificed under ketamine anesthesia at the end of the experimental; midline incision was made on the thorax and organ of study (heart) were excised and weighed using the mettle P163 weighing balance.

F. Calculation of Relative Heart Weight and Percentage Body Weight

The heart weight was measured using a mettle P163 weighing balance and the relative heart weight was calculated as a percentage of the body weight at sacrifice.

$$Relative\ \ heart\ weight\ (\%) = \frac{weight\ of\ heart\ at\ sacrifice(g)}{body\ weight\ at\ sacrifice\ (g)} x\ 100$$

G. Sample Collection

Blood samples were collected via retro-orbital venous plexus using a microcapillaries and serum was obtained with the aid of a centrifuge machine.

H. Histological and Histochemical Analyses

The excised organs (heart) were fixed in 10% formal saline and processed using paraffin wax embedding method. Sections of 5um thickness were produced from paraffin embedded tissues and stained with haematoxylin and eosin to demonstrate general histoarchitecture of these organs. Masson's Trichrome was used to demonstrate collagen fibers in the left ventricle tissue.

I. Masson's Trichrome Staining Procedure

Masson's Trichrome staining was used to differentiate between collagen and smooth muscle in tumor, and the increase of collagen in diseases such as left ventricular hypertrophy, myocarditis

The sections were rehydrated through descending grades of ethanol and washed in distilled water. They were then re-fixed in Bouin's fluid for 1 hour at 56°C to enhance staining quality. Subsequently, the sections were rinsed in running tap water for 5-10 minutes to remove any yellow color. They were stained in Weigert's iron hematoxylin working solution for 10 minutes, followed by a rinse in running warm tap water for 10 minutes. After that, the sections were washed in distilled water for 2 minutes. They were then stained in Biebrich scarlet-acid fuchsin solution for 10 minutes, followed by another wash in distilled water for 2 minutes. Next, the sections were differentiated in phosphomolybdic-phosphotungstic acid solution for 10 minutes. They were then transferred directly to aniline blue solution and stained for 5-10 minutes. After a brief rinse in distilled water, the sections were differentiated in 1% acetic acid solution for 2-5 minutes. They were washed again in distilled water, dehydrated rapidly through 95% ethanol and absolute ethanol, and finally cleared in xylene. The sections were mounted in a resinous mounting medium to preserve them for further examination [7].

J. Orcein Staining Procedure

Orcein is an excellent stain for elastic fibres and is much favored by histopathologists with an interest in dermatology because of its ability to demonstrate the finest fibres.

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Sections were immersed in 70 percent alcohol and stained in a closed jar for 30 minutes at room temperature. They were then washed thoroughly in 70 percent alcohol and tap water. Nuclei were lightly counterstained with alum hematoxylin. The sections were dehydrated, cleared, and mounted in a synthetic resin medium to preserve them for examination [8].

K. Photomicrography

Stained sections were viewed under Leica research microscope (Leica DM750, Switzerland) with digital camera (Leica IC50) attached and digital photomicrographs were taken at x400 magnification and also scanned using a motic scanner. Photomicrographs of stained sections were imported on to the image J software for histomorphometric analysis.

L. Quantification of Staining Intensity

For the quantification of collagen and elastic fibers staining intensity, Image J was used, which is a software for image analysis and processing in Java. RGB images were imported and converted to grayscale images using ImageJ. The staining intensity was quantified by measuring the pixel value of each pixel in the grayscale images following the threshold of areas of staining activity and converting the pixel value to brightness value or gray value using the software. The statistical analysis was then performed on the obtained values [9].

M. Statistical Analysis

Statistical differences were examined by one-way analysis of variance (ANOVA), followed by Student Newman-Keuls (SNK) test for multiple comparisons. GraphPad Prism 5 (Version 5.03, Graph Inc.) was the statistical package used for data analyses. Data obtained were analyzed using descriptive and inferential statistics. Significant difference was set at $P< 0.05$. Data was expressed as mean ± SEM.

III. RESULTS

Data in table 1 illustrated the effects of *Apium graveolens* on relative heart weights of the rats. With the statistical analysis results, the highest RHW was observed in ethanol-induced group (group B) while the lowest was observed in rats treated with *Apium graveolens* extract and propranolol, according to this study. The relative heart weights of group b rats were significantly higher ($F=7.64$, $p= 0.002$) when compared with control and treated groups. The left ventricular wall thickness of the group B rats was significantly higher ($F=85.29$, $p<0.001$) when compared with the control (Fig.1)

One-way ANOVA followed by Student Newman-Keuls (SNK) test showed that the result obtained from the assay of cardiac troponin I level in this research was significantly higher ($F=22.66$, $p= 0.001$) in B as shown in (fig. 2).

Table 1: Effects of *Apium Graveolens* on Relative Heart Weight Across all Groups, n= 5.

Group	Heart's Absolute Weight (g)	Relative Heart Weight (%) (RHW=HW÷BWASx100)
A (Control)	0.49 ± 0.033^{b}	0.33 ± 0.027^{b}
B (Ethanol only)	0.62 ± 0.046^{ab}	0.50 ± 0.043^{ab}
C (Ethanol + 100mg/kg MEAG)	0.48 ± 0.036^{a}	0.28 ± 0.025^{a}
D (Ethanol + 150mg/kg MEAG)	0.47 ± 0.026^{b}	0.31 ± 0.025^{b}
E (Ethanol + 200mg/kg MEAG)	0.48 ± 0.029^{a}	0.29 ± 0.027^{a}
F (Ethanol + 25mg/kg Propranolol)	0.47 ± 0.026^{b}	0.32 ± 0.027^{b}

superscript a= C and E are not significantly different from each other but are significantly different from A, B, D and F, b = non-significant difference among A, D and F, ab =significantly different when compared with control(A) and treated groups (C, D, E and F) at $P<0.05$ (n = 5)

- **KEY:** RHW= relative heart weight, HW= heart weight**,** BWAS=body weight at sacrifice.

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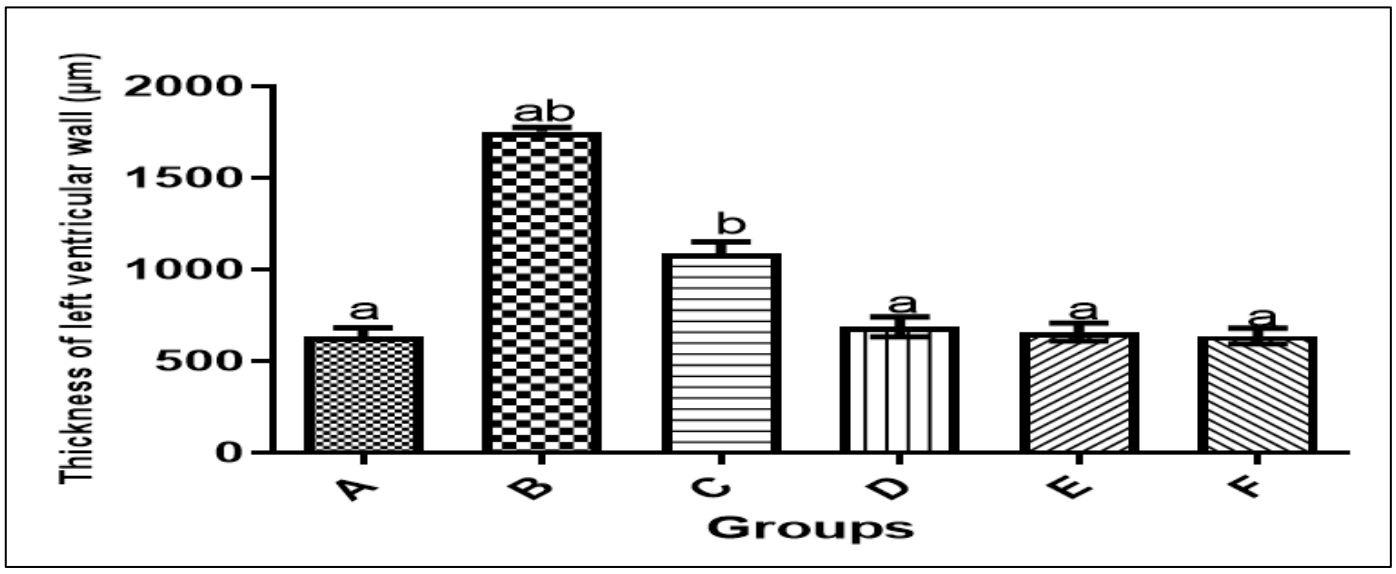

Fig 1: Effects of Apium Graveolens on the Thickness of Wall of Left Ventricle in Rats Induced with Ethanol. n=5. Values are Expressed as Mean Thickness of Myocardium of Left Ventricle (µm) + SEM. Superscript a =There was no Significant Difference When Thickness of the Wall in A (Control) was Compared with D, E and F (P>0.05), b = it was Statistically Higher when C (1090+64.03 µm) was Compared with Groups A, D, E and F. ab= Thickness of Left Ventricular Wall in Group B (1755 +24.19 µm) was Significantly Higher Than in Control Group (646+33.44 µm). MEAG- Methanol Extract of Apium Graveolens

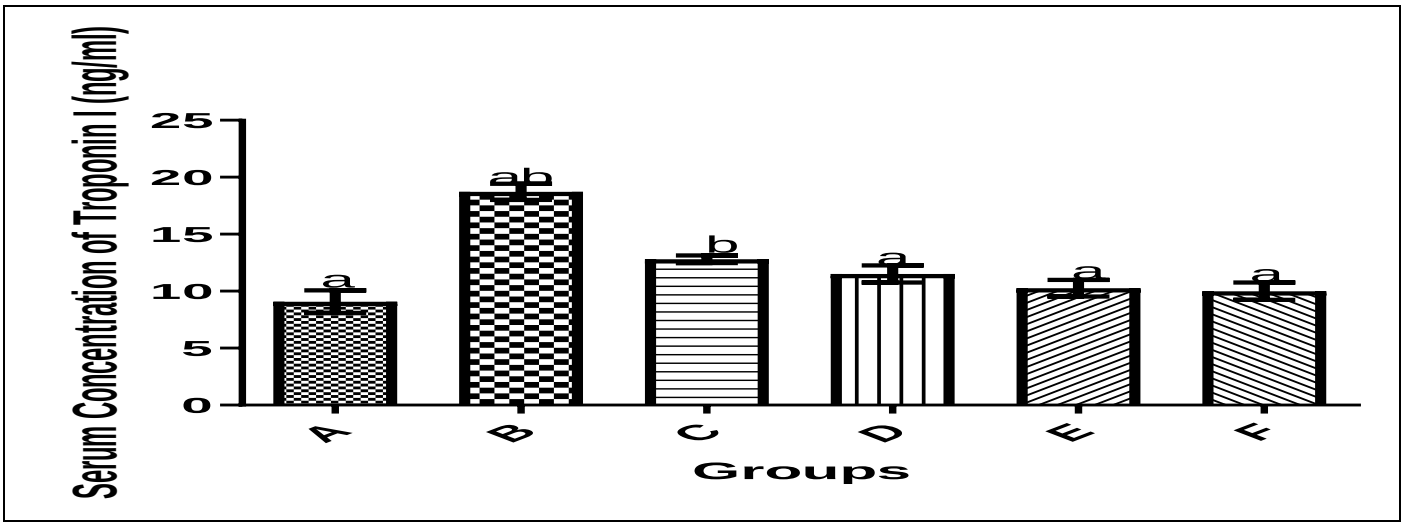

Fig 2: Serum Concentration of Cardiac Troponin in Control and Treated Groups. a= Non- Significant Difference among A, D, E and F but Significant Difference when Compared with B and C, b= Significantly Different when Compared with Groups A, D, E and F (P<0.05). There was no Significant Difference in Cardiac Troponin I Level Across the Treatment Groups (D, E and F) when Compared with Control (P>0.05), ab= Cardiac Troponin I Increased Significantly in B when Compared with Control (P<0.05). MEAG- Methanol Extract of *Apium Graveolens*

IV. HISTOLOGICAL ANALYSIS

In this study, the control group showed normal myocardial histology (fig. 3A) while ethanol administration resulted in left ventricular hypertrophy and distorted striations in group B (fig. 3B). However, treatment with *Apium graveolens* extract in groups C, D, and E prevented these distortions (figs. 3C, 3D and 3E) and propranolol prevented distortion in group F, resulting in a normal appearance (fig. 3F).

The use of Image J analysis of photomicrographs of stained sectioned (Masson's Trichome) showed that collagen fiber deposition in group B was significantly higher (fig. 4B) than that of the control (fig. 4A) and intervention groups (figs. 4C, 4D, 4E and 4F) (fig. 6). *Apium graveolens* extract protected against ethanol-induced myocardial damage due to its antioxidant potential. In contrast, there was a marked decrease (fig. 5B) in stained sectioned (orcein stain) of elastic fiber deposition in group B when compared with all groups. The control (fig. 5A) and intervention groups showed similar elastic fiber deposition (figs. 5C, 5D, 5E and 5F) (fig. 7).

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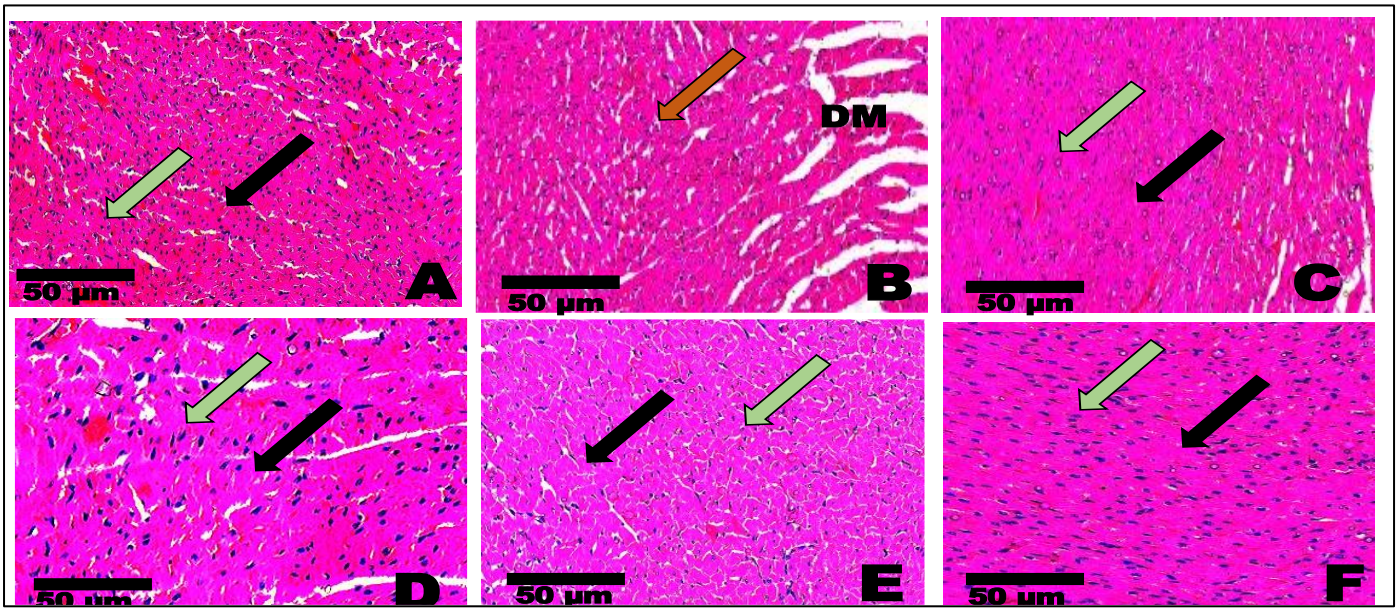

Fig 3: Photomicrograph of the Heart of Control and Treated Rat Grouped A-F. Green Arrow- Nucleus, Black Arrow- Cardiac Muscle *MEAG- Methanol Extract of Apium Graveolens*

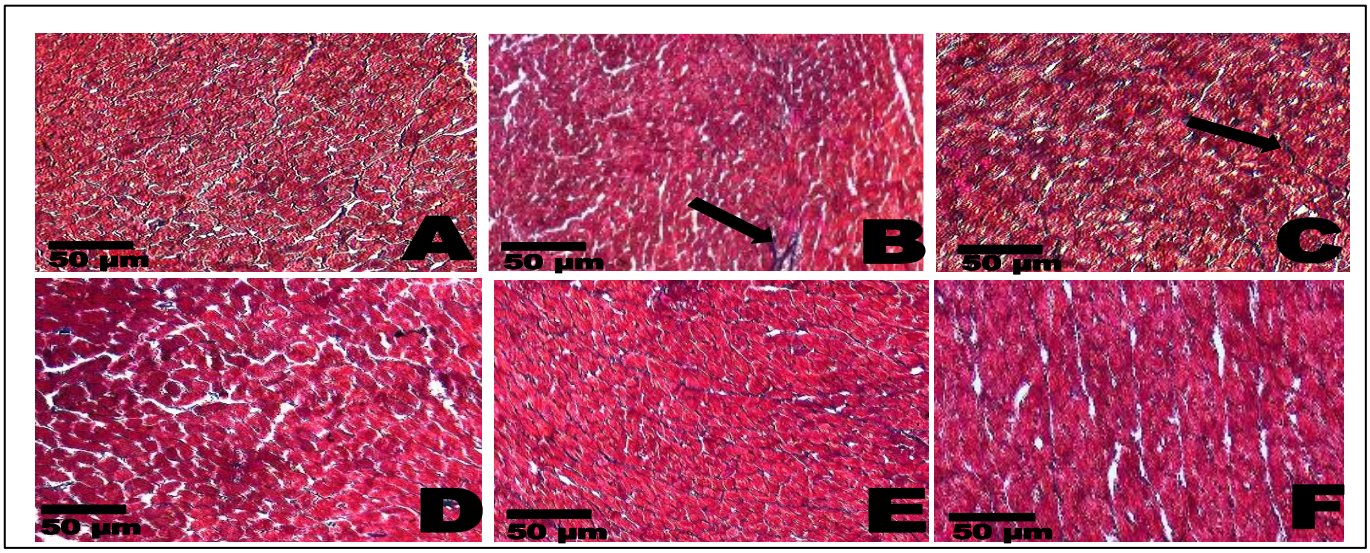

Fig 4: Photomicrographs of the Heart of Control and Treated Rats Grouped A-F. Black Arrow- Collagen Fiber Deposit. Masson Trichome X400 *MEAG- Methanol Extract of Apium Graveolens*

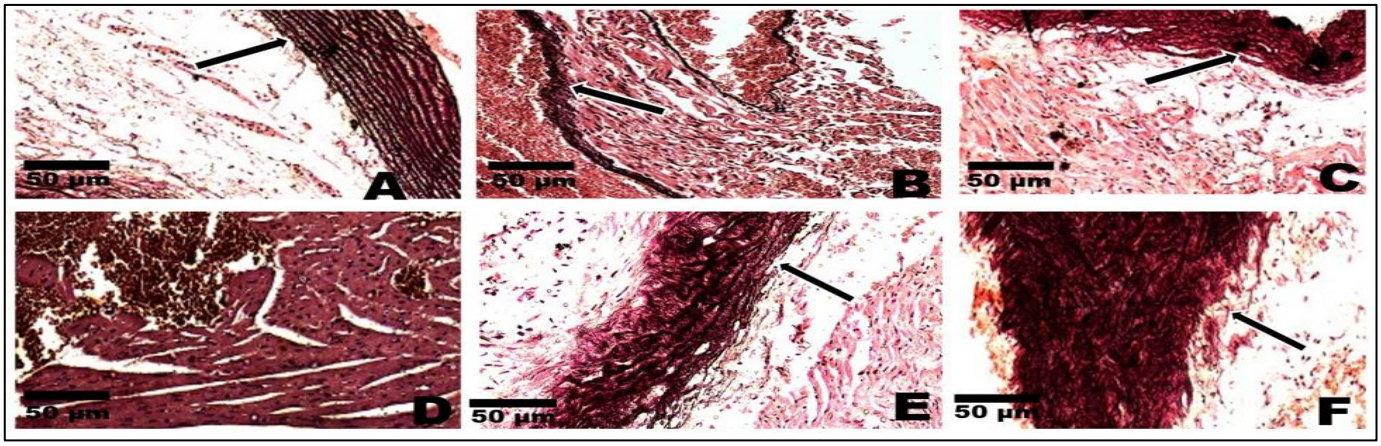

Fig 5: Photomicrographs of the Heart of Control and Treated Rats Groups A- F. Black Arrow- Elastic Fiber Deposit. Orcein X400 *MEAG- Methanol Extract of Apium Graveolens*

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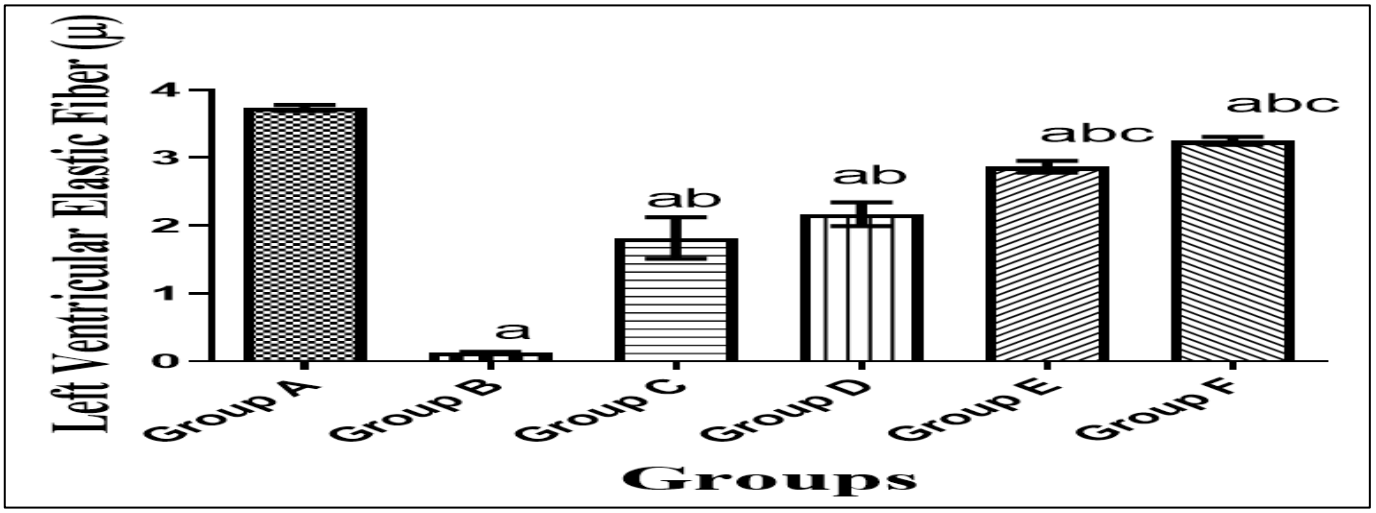

Fig 6: Effects of *Apium Graveolens* on Elastic Fiber Content of Left Ventricle in Ethanol-Induced Rats. Values are Expressed as Elastic Fiber Content (µ) ±SEM, a= Significant Difference when Compared with Control ($P < 0.05$), ab = C and D are not Significant Different from Each Other ($P>0.05$) but Different Compared with A, E and F ($P<0.05$), abc = E and F are not Significant from Each Other ($P>0.05$) but Different Compared with Control ($P<0.05$). MEAG- Methanol Extract of *Apium Graveolens*

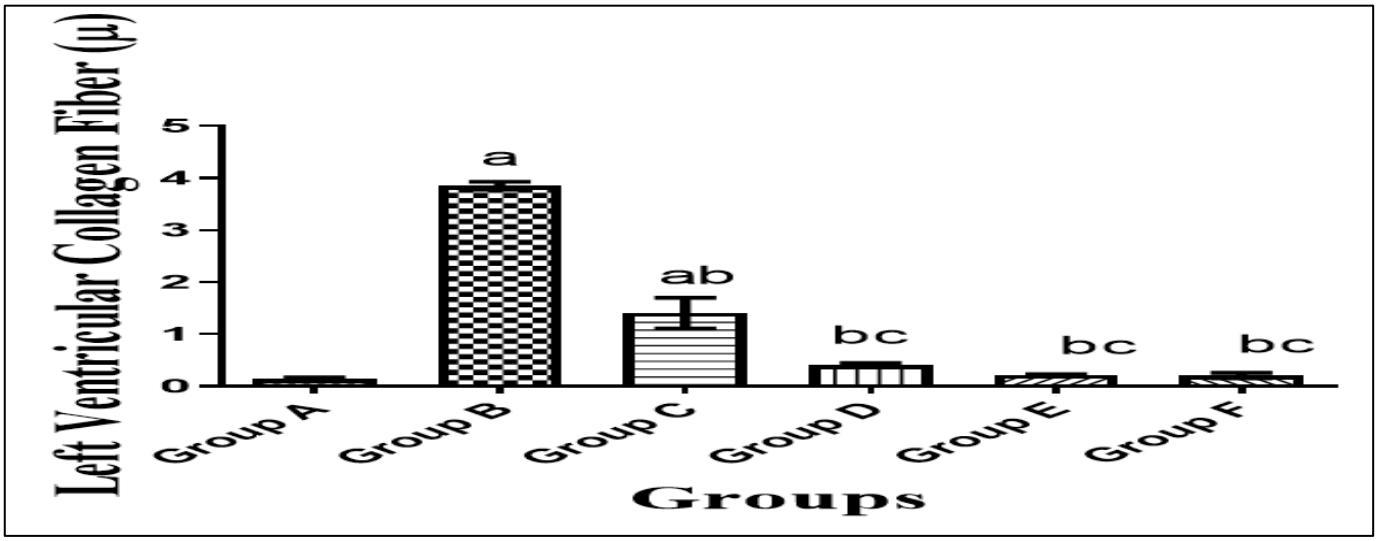

Fig 7: Effects of *Apium graveolens* on Collagen Fiber Content of Left Ventricle in Ethanol-Induced Rats. Values are Expressed as Elastic Fiber Content (µ) ±SEM, a= Significant Difference and Increased when Compared with Control and Treated Groups (C, D, E and F) at $P<0.05$, ab= Significant Difference and Mild Increase when Compared with Control and Treated Groups ($P<0.05$), bc= D, E and F are not Significantly different when Compared with Control at ($P>0.05$). MEAG- Methanol extract of *Apium graveolens.*

V. DISCUSSION

The highest relative heart weight was observed in ethanol-induced group (group B) while the lowest was observed in rats treated with *Apium graveolens* extract and propranolol, according to this study.

Troponin is a protein that appears in the blood only when the heart muscle is damaged, as in heart attack [11, 12]. Cardiac troponin detects damage to the heart muscle [13, 4]. Increase in the concentration of cardiac troponins in biological fluids, in particular in blood serum indicates damage to cardiomyocytes [14]. The result obtained from the assay of cardiac troponin I level in this research showed that the cardiac troponin I was significantly higher ($P< 0.05$) in group B when compared with control and all treated groups and indicate damage to cardiomyocytes which support the previous study stated above [15]. The results of this study showed that alcohol administration significantly raised serum concentration of cardiac troponin I level [16].

In this study, ethanol administration resulted in left ventricular hypertrophy and distorted striations in group B, while the control group showed normal myocardial histology. This present study was in accordance with the research of Komolafe etal., [9]and Saka et al., [10]. However, treatment with *Apium graveolens* extract in groups C, D, and E prevented these distortions, and propranolol prevented distortion in group F, resulting in a normal appearance, due to antioxidants properties it possesses [17, 18].

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VI. CONCLUSION

This study investigated the effects of ethanol-induced cardiac damage and the potential cardioprotective role of *Apium graveolens* leaves. Results showed that ethanol caused harmful histological changes in the left ventricle, as evidenced by changes in cardiac TRP I, LDH, NO, and elastic and collagen fiber deposits. However, the methanol extract of *Apium graveolens* leaves was found to have a cardioprotective effect due to its antioxidant properties with therapeutic potentials for preventing and treating cardiovascular disease. These findings suggest that *Apium graveolens* leaves may be a useful complementary treatment for cardiovascular diseases.

- **Author's Contribution:** Conceptualization and design: FRA; Experimentation, data collection, data analysis, and data interpretation: FRA and KOA, Draft manuscript: FRA; Vetting and approval of the manuscript for submission: KOA
- **Conflict of Interest:** The authors have no conflict of interest to declare.
- **Funding:** This work received no funding

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