

### Original Research Article

## **Effects of sub-chronic Administration of Ethanolic Extract of *C. odorata* Leaf on Biomarkers of Cardiovascular System of Wistar Rats**

### **ABSTRACT**

**Aim:** The effect of chronic administration of ethanolic extract of *C. odorata* (*C. odorata*) on biomarkers of the cardiovascular system in Wistar rats was investigated in this study. **Methods:** Twenty male Wistar rats were randomly assigned to four groups of five animals each. Group 1 (control group) received only normal rat feed and distilled water, whereas groups 2, 3, and 4 received 250mg/kg, 500mg/kg, and 1000mg/kg body weight (b. w) of the extract, respectively. The treatment lasted 14 days. **Result:** The results showed a dose-dependent increase in the serum activity of Creatinine-kinase-MB (CK-MB) and Cardiac Troponin T (cTn-T) in the treated groups, but these increases were not statistically significant ( $p>0.05$ ) compared to the control group. There were no significant ( $p>0.05$ ) dose-related changes in the serum activities of Lactate dehydrogenase (LDH) and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) in all treatment groups. However, serum Interleukin-1Beta (IL-1 $\beta$ ) levels were significantly ( $p<0.05$ ) lower in the higher dose groups. Histopathological examination of the heart did not reveal any significant morphological alterations in all treatment groups. **Conclusion:** According to the findings of this study, the administration of ethanolic leaf extract of *C. odorata* extract may be safe for the cardiovascular system at the dosages tested, but its continuous usage at high concentrations may adversely affect cardiac functions and consequently predispose to cardiovascular disease.

**Keywords:** *Chromolaena odorata*, cardiovascular disease, cardiovascular biomarker, histopathology.

### **INTRODUCTION**

[Plants have been used by humans](#)[Humans have used plants](#) for centuries as a source of food, medicine, and raw materials for various industries [1]. Throughout history, different plant parts, such as fruits, seeds, barks, roots, and flowers, have been utilized for their medicinal properties in treating various diseases in humans and animals [2-4]. Traditional medicine, which often includes the use of plant extracts, is particularly prevalent in countries like China and India, where it is integrated into the primary healthcare system [5]. Nigeria is one such country where herbal medicines are commonly used to treat ailments like asthma, tuberculosis, ulcers, diarrhea,

and dysentery, especially in communities located in the South-South, South-East, and South-West geopolitical zones [5-7].

Plants are rich sources of phytochemicals, antimicrobials, and bioactive compounds such as anthraquinones, flavonoids, saponins, polyphenols, tannins, and alkaloids, which contribute to their therapeutic properties [5,8]. While orthodox medicine is widely accepted globally, traditional medicine is seen as an acceptable alternative in many regions, particularly in developing countries where access to conventional medical care is limited [9]. According to the World Health Organization, approximately 80% of people in developing countries use traditional medicine, with the majority relying on plant extracts [9].

Certain medicinal herbs have been found to possess antioxidant effects and can help reduce blood lipids. Various herbal preparations derived from different plant parts have gained popularity for treating conditions such as diabetes mellitus, breast cancer, hypertension, atherosclerosis, and diarrhea [10]. *Chromolaena odorata* (*C. odorata*), also known as Siam weed, is a perennial scrambling shrub native to Central and South America and the Caribbean. It is used as a medicinal herb in the southeastern region of Nigeria [11]. The leaf extracts of *C. odorata* are known for their astringent and antimicrobial properties, making them effective in preventing blood loss from wounds and treating open wounds [12,13]. The plant is also widely recognized in Asia and Africa for its anthelmintic properties and has been used to treat conditions such as diarrhea, malaria fever, toothache, diabetes, skin diseases, dysentery, and colitis [13]. The aqueous extract and the decoction from the leaves of this plant have been used throughout Vietnam for the treatment of soft tissue wounds, wounds, and skin infections [14,15]. Additionally, the leaf extracts of *C. odorata* have demonstrated anti-cancer activity in both human and mouse cell lines [16].

Despite the widespread use of medicinal plants, there is often little concern for their safety [17]. The phytochemical components of herbal medicines play a crucial role in their therapeutic applications but can also be responsible for their toxicity if not properly managed [18]. Therefore, scientific research is necessary to determine the safe dosage and therapeutic index of plant extracts containing various phytochemicals [19]. Studies evaluating the effects of *C. odorata* on animal models have shown adverse effects on kidney function and intestinal

histology [20], secondary medical effects on the liver [21], and potential induction of spermatogenic arrest in male rats [22].

However, there is limited information available regarding the effects of *C. odorata* on the cardiovascular system when used in therapy. Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide, and the identification and diagnosis of CVDs rely on biomarkers [23]. Biomarkers, including serum cardiac function biomarkers and inflammatory cytokines, play a crucial role in assessing cardiovascular health [23]. Therefore, this study aims to evaluate the effects of sub-chronic administration of ethanolic *C. odorata* leaf extract on cardiovascular biomarkers in Wistar rats. By assessing the extract's systemic health impact, this research will provide valuable information on its safety when used in therapy and contribute to the understanding of the plant's medicinal potential.

**Comment [PM1]:** Why just ethanolic extract ?

## **MATERIALS AND METHOD**

### **Collection of Plant Material**

*C. odorata* leaves were collected from around the Rivers State University campus and authenticated by the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt.

### **Preparation of Plant Extract**

The leaves of the plant were thoroughly washed under running water to remove contaminants and were air dried at room temperature in the laboratory for 7 days. The dried leaves were then grinded into a fine powder using an electric grinder and were dissolved in 1L of absolute ethanol and allowed to stand for 48 hours. The extract was filtered into a clean beaker using Whitman no 1 filter paper, the filtrate was concentrated by heating at 60°C using a water bath to get the

extract that was used for the study. Then the extract was reconstituted in distilled water at different concentrations using Tween 80 as an emulsifier [24].

**Comment [PM2]:** Can you explain more?

### **Preparation of Sample Stock**

To make a concentration of 300mg/ml, 3g of the extract was dissolved in 10ml of distilled water with a few drops of tween 80. Throughout the administration period, the stock solution was prepared on a daily basis. The extract was given to the experimental rats in dosages based on their body weight.

### **Experimental Animals**

Twenty (20) appreciably healthy adult male albino rats weighing 120-130g were obtained and housed in the Department of Biochemistry, Rivers State University, Port Harcourt, for this study. The rats were housed in ventilated cages with wire mesh tops and sawdust (for water absorption). They were allowed to acclimate to the animal house for one week and had free access to standard pellet feed and water ad libitum. The study received ethical approval from the Interfaculty Ethics Committee of Rivers State University, Port Harcourt, and all experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

### **Experimental design**

The rats were randomly divided into four groups of five (5) rats each after acclimatization. The control group received distilled water, while the treatment groups received 250, 500, and 1000

mg/kg body weight of *C. odorata* orally via gastric gavage for 14 days. These doses were chosen based on previous research [22].

### **Collection of Samples for Biochemical Analysis**

On the final day of the dosage administration, the animals were fasted overnight and weighed, and three (3) animals from each group were sacrificed via cervical dislocation, with blood samples collected via cardiac puncture using sterile needles into plain bottles. The blood was allowed to clot before being centrifuged and the serum was collected for biochemical analysis to determine the levels of heart function and pro-inflammatory biomarkers in the serum. Heart tissues were also collected in culture bottles containing 10% formaldehyde for histological examination.

### **Determination of heart function markers**

Serum activities of creatinine-kinase- MB and lactate dehydrogenase as well as serum level of cardiac troponin-T were determined using an enzyme immunosorbent assay (ELISA) kit (Bioassay tech lab UK) according to the manufacturer's instructions.

### **Determination of pro-inflammatory biomarkers**

Interleukin-1Beta (IL-1 $\beta$ ) and Tumour Necrosis Factor-alpha (TNF- $\alpha$ ) were measured by enzyme-linked immunosorbent assay ELISA kits (Calbiotech Inc. and Elab Science, USA) according to the manufacturer's instructions.

### **Histological Examination of the Heart Tissues**

For Histological examination, the cardiac tissues were dissected out, blotted free of blood and weighed with the help of a digital weighing balance and were fixed in alcoholic fixative and embedded in paraffin. Transverse sections of the organs were cut at 5  $\mu$ m and stained with hematoxylin and eosin and were studied under a light microscope (Nikon) at 100 and 400 magnifications. Slides of all the groups were studied and photographed with Cannon digital camera.

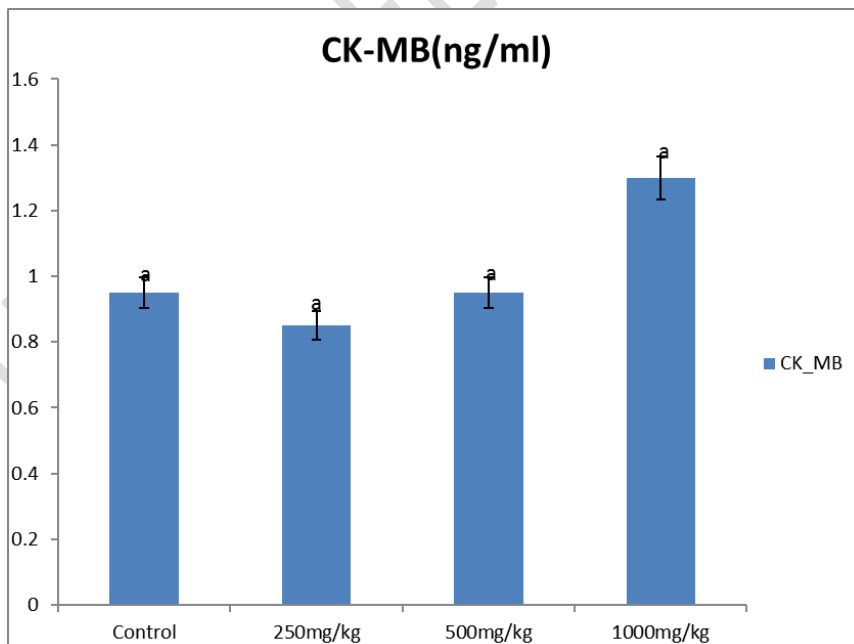
### **Statistical analysis**

All values were normally distributed and were expressed as the mean  $\pm$  standard error of the mean (SEM). Differences between the groups were determined by one-way analysis of variance (ANOVA) and post hoc testing was performed for intergroup comparisons using Tukey's test using SPSS software version 20. Values were regarded as significantly different at  $p < 0.05$ .

## **RESULTS**

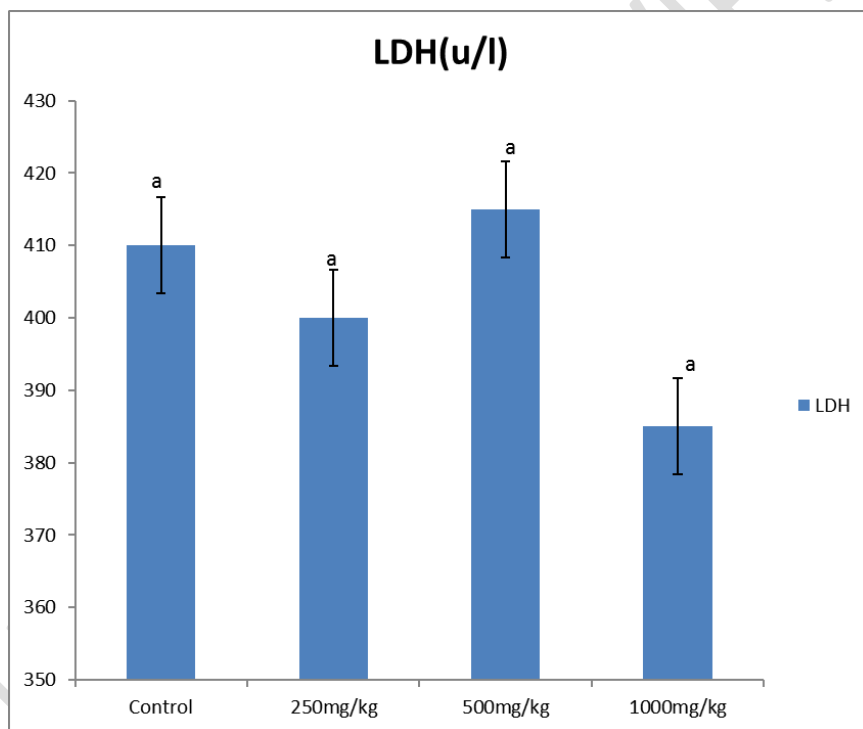
### Effect of *C. odorata* leaf extract on heart function biomarkers

The result for serum activities of Creatine kinase, lactate dehydrogenase, and cardiac troponin-T blood are presented in Figures 1, 2, and 3. The result obtained shows that after 14-days of administration of *C. odorata* leaf extract, there were no significant ( $p>0.05$ ) differences in the serum activities of Creatine kinase- MB and lactate dehydrogenase as well as serum levels of cardiac troponin-T of all treatment groups when compared to the control group. However, we observed a dose-dependent insignificant ( $p>0.05$ ) increase in serum activities of Creatine kinase- MB and cardiac troponin-T when compared to the control group. The mean serum Creatine kinase-MB activity in control, groups 2, 3, and 4 are  $0.95\pm 0.14$ ,  $0.85\pm 0.03$ ,  $0.95\pm 0.09$ , and  $1.30\pm 0.06$  ng/ml respectively while the mean serum cardiac troponin-T levels in control, groups 2, 3 and 4 are  $0.53\pm 0.03$ ,  $0.55\pm 0.09$ ,  $0.60\pm 0.06$  and  $0.60\pm 0.06$  ng/ml respectively.



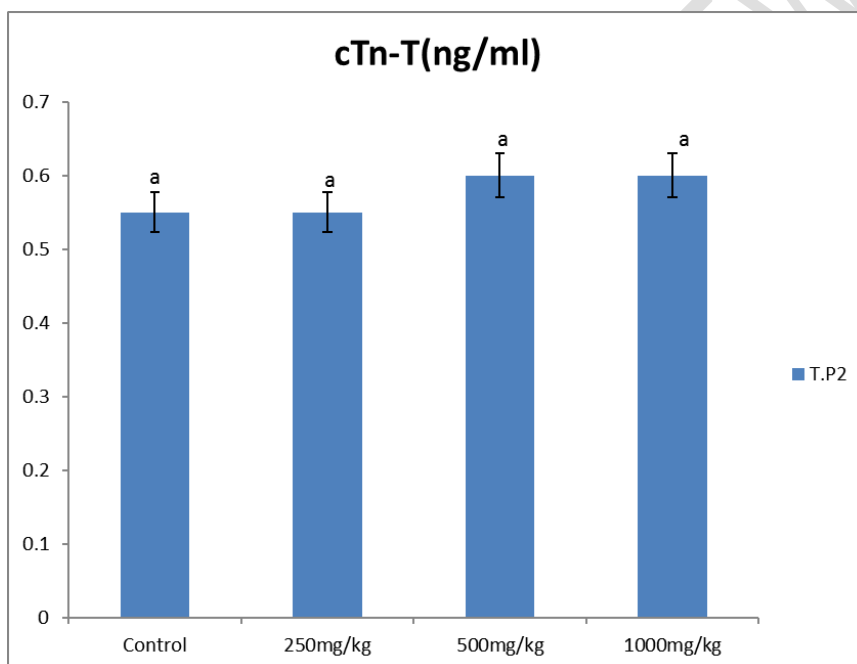
**Figure 1: The effect of administration of ethanolic extract of *C. odorata* on the serum Creatine kinase- MB activity of male Wistar albino rats.**

Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscripts are statistically different at ( $p < 0.05$ ). Superscript (a, b) compares groups two to four (250mg/kg, 500mg/kg, and 1000mg/kg) to group one (control) down the group.



**Figure 2: The effect of administration of ethanolic extract of *C. odorata* on the serum lactate dehydrogenase activity of male Wistar albino rats.**

Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscripts are statistically different at ( $p < 0.05$ ). Superscript (a, b) compares groups two to four (250mg/kg, 500mg/kg, and 1000mg/kg) to group one (control) down the group.



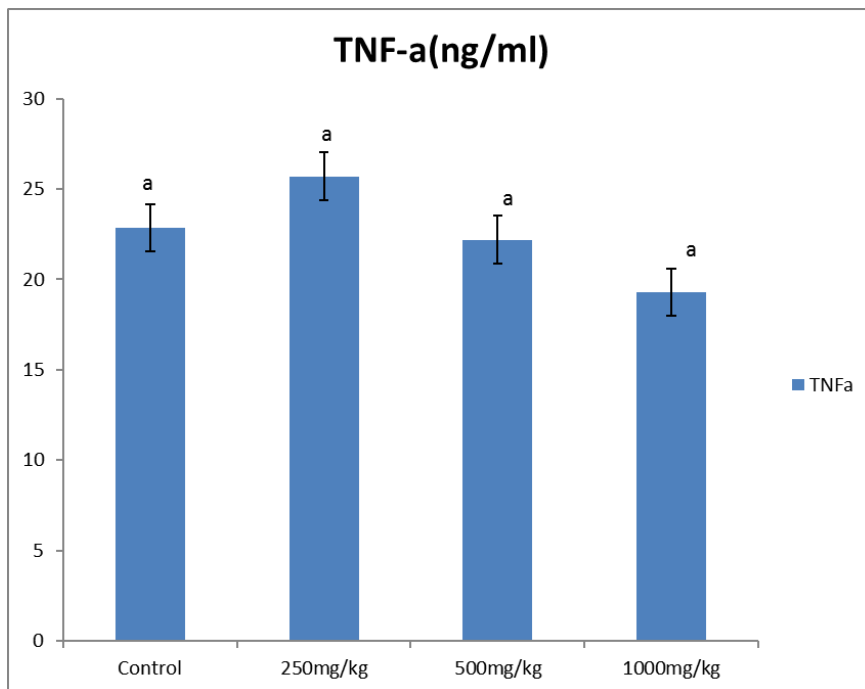
**Figure 3: The effect of administration of ethanolic extract of *C. odorata* on the serum level of cardiac troponin-T of male Wistar albino rats.**

Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscripts are statistically different at ( $p < 0.05$ ). Superscript (a, b) compares groups two to four (250mg/kg, 500mg/kg, and 1000mg/kg) to group one (control) down the group.

### **Effect of *C. odorata* leaf extract on serum level of pro-inflammatory biomarkers**

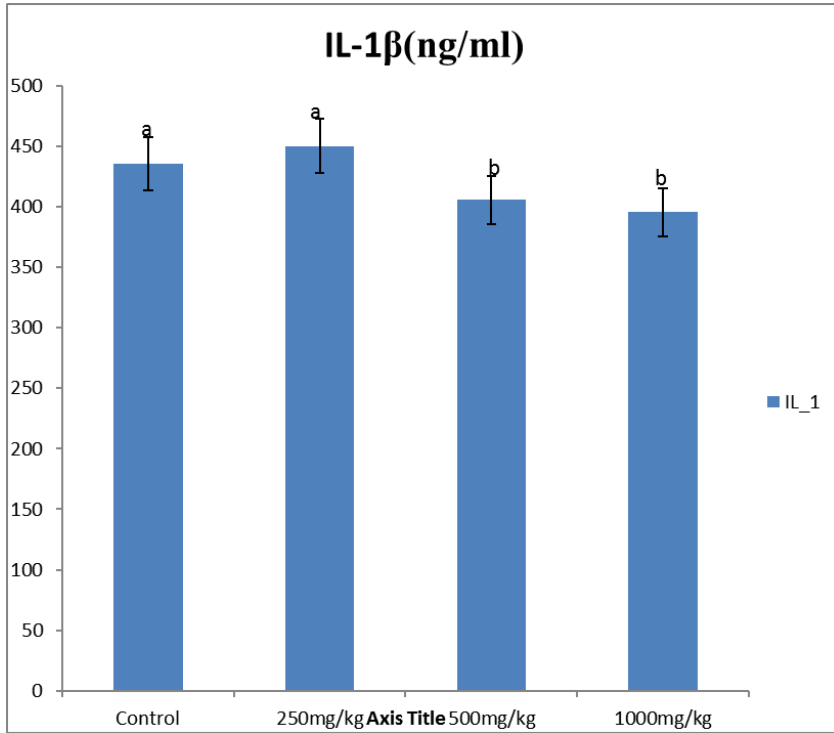
Figure 4 shows that after 14 days of administration of *C. odorata* leaf extract, there were no significant ( $p>0.05$ ) differences in the serum level of tumor necrotic factor- $\alpha$  (TNF- $\alpha$ ) of all treatment groups when compared to the control group.

The result for serum interleukin-1 $\beta$  level is presented in Figure 5. The mean serum interleukin-1 $\beta$  level in control, groups 2, 3, and 4 are  $435.55\pm 8.63$ ,  $450.30\pm 5.77$ ,  $405.50\pm 2.71$  and  $395.60\pm 2.89$  ng/ml respectively. The result showed no significant ( $p>0.05$ ) differences in the serum level of interleukin-1 $\beta$  in group 2 animals who received 250mg/kg body weight of the extract when compared to the control group while administration of the extract at higher doses of 500mg/kg (group 4) and 1000mg/kg (group 5) resulted in a significant ( $p<0.05$ ) decrease in serum level of interleukin-1 $\beta$  when compared to the control (group 1).



**Figure 4: The effect of administration of ethanolic extract of *C. odorata* on the serum level of tumor necrotic factor- $\alpha$  of male Wistar albino rats.**

Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscripts are statistically different at ( $p < 0.05$ ). Superscript (a, b) compares groups two to four (250mg/kg, 500mg/kg, and 1000mg/kg) to group one (control) down the group.

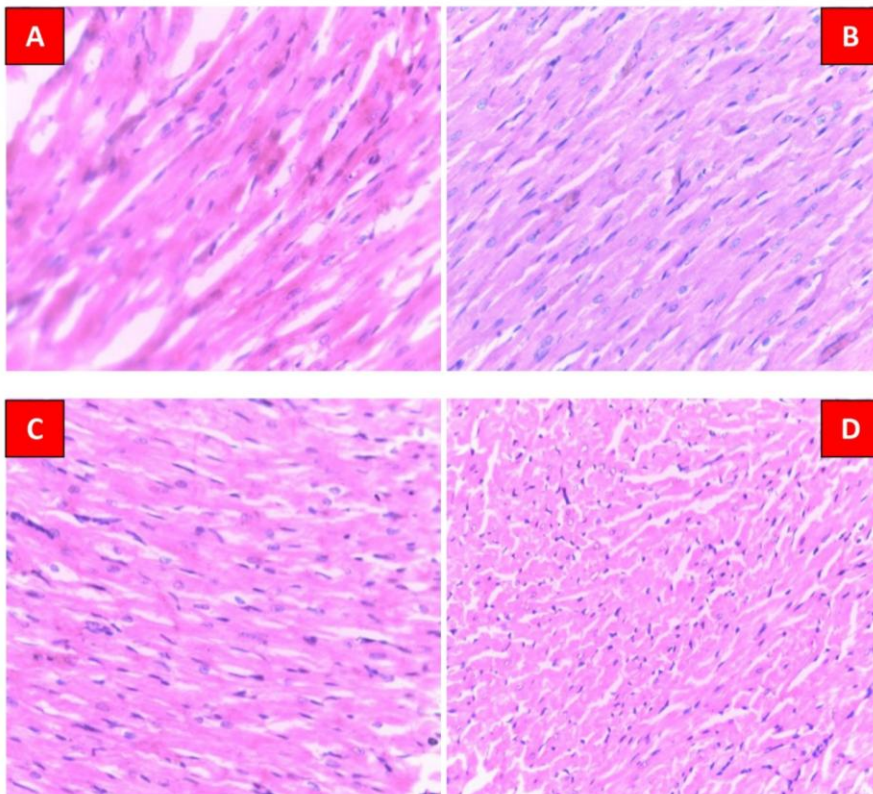


**Figure 5: The effect of administration of ethanolic extract of *C. odorata* on the serum level of interleukin-1 $\beta$  of male Wistar albino rats.**

Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscripts are statistically different at ( $p < 0.05$ ). Superscript (a, b) compares groups two to four (250mg/kg, 500mg/kg, and 1000mg/kg) to group one (control) down the group.

### Histopathology Examination of the Heart

Figure 6 shows photomicrographs of the hearts of rats in groups 1 (control rats), group 2 animals who received 250mg/kg body weight of the extract, group 3 animals who received 500 mg/kg body weight of the extract, and group 4 animals who received 1000 mg/kg body weight of the extract. The result shows sections of heart tissue with unremarkable myocardium with central nuclei and striation of the eosinophilic cytoplasm in all treatment groups.



**Figure 6:** Photomicrographs of the hearts of rats. (A) groups 1 (control rats), (B) group 2 animals who received 250mg/kg body weight of the extract, (C) group 3 animals who received 500 mg/kg body weight of the extract, (D) group 4 animals who received 1000 mg/kg body weight of the extract.

## DISCUSSION

The causes of 71 percent of deaths today worldwide are diseases like cardiovascular conditions. Cardiovascular diseases are the most common cause of disease-related death and account for 17.9 million annual deaths. In addition to behavioral factors like unhealthy diets, inactivity, tobacco use, and excessive alcohol consumption, unrestrained drug, and herbal product use may also raise the risk of death from cardiovascular diseases [25].

This study reports the effect of sub-chronic administration of ethanolic *C. odorata* Leaf Extract on some biomarkers of Cardiovascular. Creatine kinase, formerly known as creatinine phosphokinase, is an intracellular enzyme found in high concentrations in skeletal muscle, myocardium, and brain, with lower concentrations found in other visceral tissues. In the heart muscle, Creatine kinase-MB predominates. When cell membranes are disrupted due to hypoxia or another injury, CK-MB is released from the cytosol into the systemic circulation. As a result, elevated serum CK-MB levels have been used as a sensitive and primary marker of myocardial damage. Al-Hassan [26] and Okwakpam et al. [27]. The result for serum activity of Creatine kinase-MB shows that after 14-days of administration of *C. odorata* leaf extract, there was a dose-dependent none significant ( $p > 0.05$ ) increase in the serum activity of Creatine kinase-MB in all treatment groups when compared to the control group. This observation may be due to the phytochemicals such as cardiac glycosides and flavonoids present in the herb of *C. odorata* [18,28]. Cardiac glycosides have been reported to have an inverse relationship with cardiovascular disease [18]. Flavonoids have been reported to possess vasodilatory as well inhibitory effects on platelets, and coronary heart disease [29,30]. However, caution should be taken in the prolonged use of the extract at a higher dose of 1000mg/kg. Asomugha et al. [30] reported that the administration of aqueous leaf extract of *C. odorata* three times per week for 90

days significantly increased Creatine kinase activity at a high dose of 587 and 1097 mg/kg body weight.

LDH is an enzyme found in all body cells, with the highest concentrations found in the heart, liver, muscles, kidneys, lungs, and erythrocytes. It has a tetrameric isoform and can form five different tetramers, the most common of which is LDH-1 [31]. LDH-2 concentration is greater than LDH-1 in normal conditions, but in myocardial infarction, the ratio flips, resulting in LDH: LDH-2 greater than I [32]. An elevated level of LDH is a marker for blood flow deficiency, cerebrovascular accident (stroke), heart attack, muscle injury, and muscular dystrophy. The result obtained from this study revealed that there was no significant difference in LDH level in all treatment groups following 14 days of administration of ethanolic leaf extract of *C. odorata*. The plant's abundant flavonoids and cardiac glycosides, which are phytochemicals, may be the cause of the extract's observed effect on LDH [18]. According to reports, cardiac glycosides are cardioactive substances with an innate property on the aglycone portion of the sugar moiety that exert a number of effects on neural tissue and subsequently indirectly affect the mechanical and electrical activities of the heart by modifying vascular resistance and capacitance [28].

Cardiac troponins (cTn), an inhibitory protein complex in all striated muscles consists of three subunits (I, T, and C). Troponins do not exist in the blood of healthy individuals or are suggested to exist in very small amounts (Jaffe et al., 2000). Cardiac troponins (cTnI and cTnT) are the "gold standard" for myocardial injury due to their great sensitivity, specificity, and better efficacy [34]. Cardiac troponins have been highlighted by their roles in cardio-specific diagnosis, prognostic risk assessment, detection of myocardial re-infarction, and detection of coronary reperfusion and renal failure [35]. Elevated cTn levels indicate cardiac injury including acute peri-myocarditis, acute coronary injury including acute pulmonary embolism, acute heart failure,

and tachycardia [36]. The result of this present study showed that the administration of *C. odorata* leaf extract induced a dose-dependent none significant increase in serum cTn-T activity in all the treated groups when compared to the control (group 1). The flavonoids and tannins found in the plant may be responsible for this effect. Tannins have been shown to inhibit LDL cholesterol oxidation, reduce body fat, and thus reduce the risk of heart disease [34]. The non-significant dose-dependent increase observed suggests that prolonged use of the extract may result in cardiac injury.

Tumor necrosis factor alpha (TNF- $\alpha$ ) plays an important role in cardiac ~~inflammation~~inflammation; it activates NF-KB which becomes the driving force of inflammation. The result of the effect of ethanolic leaf extract of *C. odorata*. on TNF- $\alpha$  indicate that the TNF- $\alpha$  level showed no significant changes in all treatment groups when compared to the control (group 1).

The result for serum interleukin-1 $\beta$  level is presented in Figure 5. The result showed no significant ( $p>0.05$ ) differences in the serum level of interleukin-1 $\beta$  in group 2 animals that received 250mg/kg body weight of the extract when compared to the control group while administration of the extract at higher doses of 500mg/kg (group 4) and 1000mg/kg (group 5) resulted in a significant ( $p<0.05$ ) decrease in serum level of interleukin-1 $\beta$  when compared to the control (group 1). These cytokines are crucial for starting the inflammatory process that causes tissue damage. By promoting the synthesis of more mediators, they can increase the severity of inflammation, cause tissue damage, and decrease the ability to repair injured tissue [25]. A wounded surrounding tissue triggers inflammation as a subsequent tissue reaction. It is a reaction to an irritant by the body's immunological system (Ujong et al., 2022). -Therefore, the decrease in interleukin-1 levels may represent the anti-inflammatory effects of flavonoid-rich medicinal

plant extracts [38]. –A possible mechanism could be because flavonoids block the enzymes cyclo-oxygenase and 5-lipoxygenase, which have been linked to cardiovascular disease [30]. Though sources of pharmacologically active chemicals from medicinal plants have been important, it is doubtful that this will result in the development of new drugs unless the mechanisms of action are understood.

The histological findings on the heart showed that there was no significant difference in the test groups when compared to the control (unremarkable, myocardium). *Chromeleana Odorata* extract may coat flavonoid compounds with anti-atherogenic properties [39]. These flavonoid compounds may lower the risk of developing coronary heart disease by decreasing the LDL, oxidation and inflammation [40]. This is consistent with the findings of Ilegbedion et al. [41], who discovered that treating Wistar rats with 800 mg/kg aqueous leaf extract of *Chromeleana Odorata* for fourteen days did not change the morphology of the cardiac muscle. Asomugha et al [30] reported that administration of 587 and 1097 mg/kg body weight aqueous leaf extract of *C. odorata* three times per week for 90 days significantly increased serum Creatine kinase levels with no histopathological changes in cardiac muscle and that this could be an early indicator of the toxic lesion.

## CONCLUSIONS

According to the present findings, it was proven that administration of ethanolic leaf extract of *C. odorata* extract may be safe for the cardiovascular system at the dosages tested in this study but its continuous usage at high concentration may adversely affect cardiac functions and consequently predispose to cardiovascular disease due to the dose-dependent increase observed

**Comment [PM3]:** It would be good for the authors to try to finely analyze this extract to perfectly define its composition both in terms of the nature of biomolecules and the quantity of these.

in the activities of Creatine kinase-MB and cardiac troponins (cTn) which are primary markers of the myocardial damage.

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