

## **Ameliorative Effects of Watermelon and *Aloe vera* against Cadmium Damage on the Histomorphology of the Heart Tissue of Adult Wistar Rats (*Rattus norvegicus*)**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors ODO, SAA, NOA, UPI and HBA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SAA, ODO, NOA, UPI and ISO managed the literature searches and analyses of the study performed the spectroscopy analysis. Authors SAA, ODO and ISO managed the experimental process. Authors SAA and ODO identified the species of plant. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Cadmium (Cd) is a toxic element that can produce reactive oxygen species. Fruits and vegetables are natural sources of various bioactive compounds. Watermelon contains nutritional agents like lycopene, citrulline and arginine and *Aloe vera* is used for therapeutic purposes. This study was carried out to investigate the antioxidant property of watermelon and *Aloe vera* against cadmium

damaging effect on the heart tissues using Haematoxylin and Eosin (H/E) stain. Thirty five (35) Wistar rats were obtained and acclimatized for two weeks. They were divided into seven (7) groups, five (5) rats each. Group 1 normal control received 3 mg/kg/bw of phosphate buffer (pH 7.4) intraperitoneally. Group 2, received 3 mg/kg/bw of Cd ( $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) intraperitoneally. Group 3 (therapeutic control) received 100 mg/kg/bw of vitamin C and 300 mg/kg/bw of vitamin E orally once daily. Group 4 (therapeutic control and induced) received same as group 3 with Cd induction intraperitoneally two days before sacrifice. Group 5, 6 and 7 treatment groups were induced with 3 mg/kg/bw of Cd intraperitoneally before treatment with 80 mg/kg/bw of watermelon, 80 mg/kg/bw of *Aloe vera* and 40 mg/kg/bw of both extract orally and twice daily respectively. The experiment lasted for 4 weeks. The histomorphological result obtained showed normal cardiac features in group 1, damage in myofibril, displaced nuclei in group 2, displaced striation in group 5 and 6 but, improvement was observed as compared to group 2 rats, little displacement of nuclei in group 4, normal morphology in groups 3 and 7 animals were evident. The result from this experiment demonstrated the high degree of potency in the action of *Aloe vera* and watermelon combined in preventing oxidative tissues damage to the heart due to cadmium interaction.

**Keywords:** Cadmium; histomorphology; watermelon; *Aloe vera*.

## 1. INTRODUCTION

Cadmium (Cd) is a highly toxic element and is naturally present in all parts of the environment, which includes; food, water, and soil [1] and by the World Health Organization is major concern for public health [2]. It is a non-essential element and have half-life which is extremely persistent in the environment [3,4]. Through non-ferrous mining and refining processes, manufacturing and application of phosphate fertilizers, fossil fuel combustion, production and use of nickel-cadmium batteries, cadmium can enter the environment [5,6]. Humans are at risk to cadmium exposure through the food chain because cadmium is not degraded in the environment [7]. Cadmium accumulates in the organ it enters, affecting the cell physiology and growth [8,9], induces disorders in the hormonal and cellular immune responses [10-12]. Cadmium have been found to have ability to produce reactive oxygen species (ROS), which results in lipid preoxidation and antioxidant enzymes alterations, leading to oxidative stress [13].

Antioxidants (like vitamin C and E) have been suggested to be one of the important components of an effective treatment of cadmium poisoning [6,14,15]. Fruits and vegetables are natural sources of various bioactive compounds [16]. Watermelon (*Citrullus lanatus*) botanically belongs to the family Cucurbitaceae(s) and has deep green or yellow coloured smooth thick exterior rind with gray or light green vertical stripes. Generally, watermelon flesh is the main consumable portion; however, outer rind is also used in some parts of the world [17-19]. Watermelon

contributes nutritional agents as antioxidants (e.g. lycopene, beta-carotene) and some specific amino acids (e.g. arginine, citrulline).

*Aloe vera* (*Aloe barbadensis* Miller) is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities [20]. *Aloe barbadensis* Miller (Ab), one of the *Aloe vera* types, is the most commonly used form for commercial and also therapeutic purposes in North America, Europe, and Asia [21]. Aloes contains anthraquinone derivatives (aloe emodin) and their glycosides (aloin) which have cathartic/purifying effect [16,22]. It also contains amino acids, auxins, gibberellins, minerals, vitamins, aspirin like compound, magnesium lactate and various enzymes like superoxide dismutase (SOD) and catalase [20].

This study was carried out to investigate the antioxidant property of watermelon and *Aloe vera* against cadmium damaging effect on the heart using Haematoxylin and Eosin (H&E).

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Watermelon fruits and *Aloe vera* plant were obtained and authenticated in the botany Department in Bingham University Clinic, Karu, Nigeria.

### 2.2 Preparation of Cadmium Solution

0.008 g (8 mg) of Cadmium ( $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) was weighed using a sensitive weighing balance and then dissolved in 5 ml of 0.9% w/v phosphate buffer.

**Table 1. Group number, group name and dosage of treatment given**

Group no	Group name	Administration
1	Control group	Phosphate buffer (3 mg/kg/bw) intra-peritoneally
2	Induced group	3CdSO <sub>4</sub> .8H <sub>2</sub> O intra-peritoneally (3 mg/kg/bw)
3	Therapeutic group	Vitamin C (100 mg/kg/bw) and vitamin E (300 mg/kg/bw) orally, once daily
4	Therapeutic and induced group	Vitamin C (100 mg/kg/bw) and vitamin E (300 mg/kg/bw) orally, once daily + 3CdSO <sub>4</sub> .8H <sub>2</sub> O intra-peritoneally (3 mg/kg/bw)
5	Treatment group	3CdSO <sub>4</sub> .8H <sub>2</sub> O intra-peritoneally (3 mg/kg/bw) + Watermelon extract (80 mg/kg/bw) orally, twice daily
6	Treatment group	3CdSO <sub>4</sub> .8H <sub>2</sub> O intra-peritoneally (3 mg/kg/bw) + <i>Aloe vera</i> extract (80 mg/kg/bw) orally, twice daily
7	Treatment group	3CdSO <sub>4</sub> .8H <sub>2</sub> O intra-peritoneally (3 mg/kg/bw) + Watermelon (40 mg/kg/bw) and <i>Aloe vera</i> extract (40 mg/kg/bw) orally, twice daily

## 2.3 Preparation of Plant Extracts

### 2.3.1 Aqueous watermelon extract

The watermelon fruits were washed and weighed. They were peeled and cut into slices. The seeds were removed. The slices were blended and then filtered using a sieve. The aqueous extract was placed in a water bath and allowed to evaporate to paste form. The paste was weighed and diluted with phosphate buffer (1:2) [23].

### 2.3.2 Aqueous *Aloe vera* extract

*Aloe vera* plant was washed and cut open to remove the gel. The gel was homogenized using an electric blender and turned into a container and refrigerated. The residue and the gel obtained were weighed. The extract was diluted with water (1:2) and then placed in the water bath for 15 minutes. It was allowed to cool and then filtered. The aqueous extract was turned into a container and stored in a cool place [24].

### 2.3.3 Phytochemical analysis of plant extracts

Phytochemical analysis of the watermelon and *Aloe vera* plant extract for the following phytochemicals: alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides were carried out in pharmacognosy laboratory, pharmacology department, university of Jos, Nigeria.

## 2.4 Experimental Animal

Thirty five (35) Wistar rats (50 g-176 g) were obtained from National Veterinary Research

Institute, Vom, Jos, Nigeria. They were housed in the Bingham University animal house in 12 hour of light and 12 hour of dark. The rats were allowed to acclimatize for 2 weeks. They were provided with feed and water (Vital Feed Growers (Pelletised) produced by Grand Cereals Ltd, Nigeria) *ad libitum*.

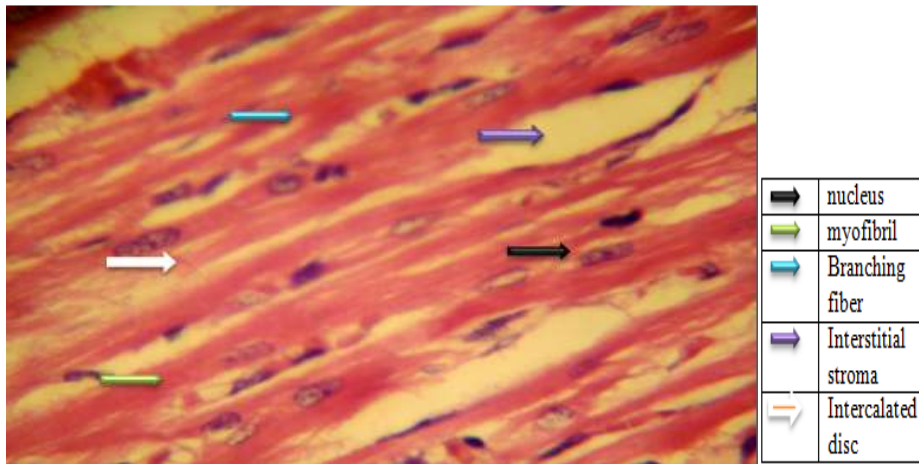
## 2.5 Experimental Design

The experiment lasted for 4 weeks. The rats were divided into seven groups, 5 rats in each group (see Table 1 above).

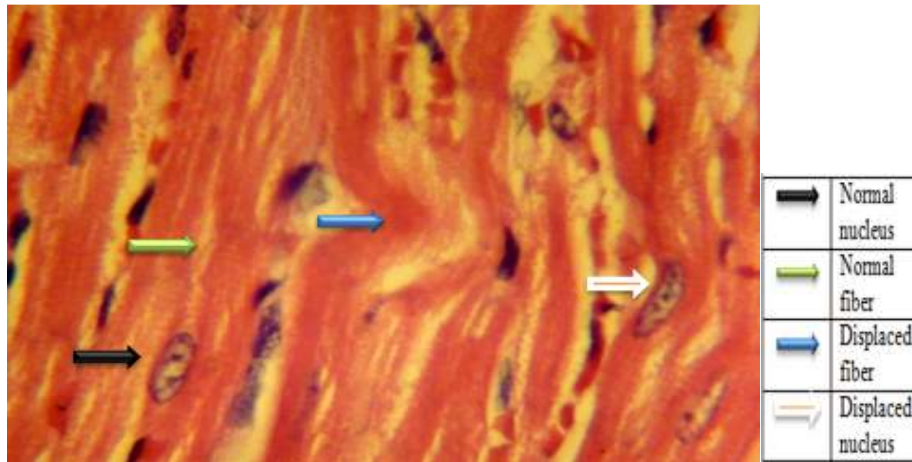
At the termination of the experiment, the rats were fasted overnight and then sacrificed by cervical dislocation. The hearts were harvested and placed in 10% formal saline for processing through histological techniques and stained with H&E following the procedure of Kieman [25].

## 3. RESULTS AND DISCUSSION

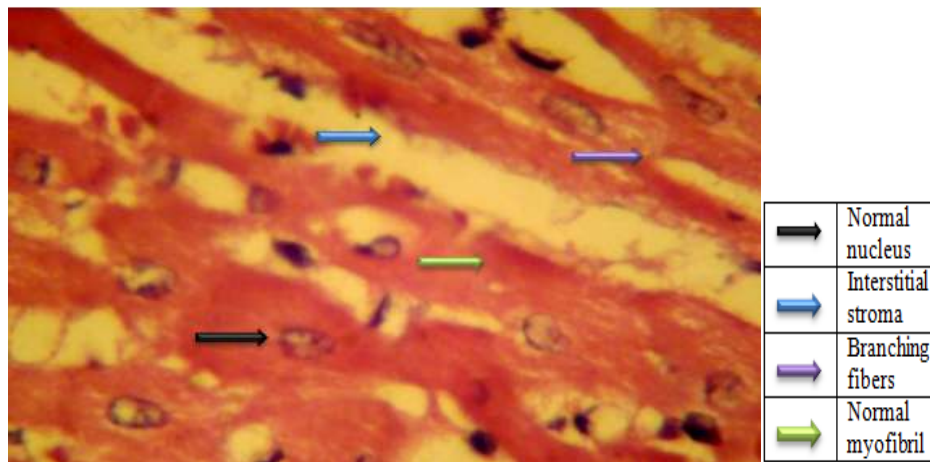
This study examined histologically the ability of cadmium (Cd) to cause damage to the heart tissue and to determine the ameliorative effects of watermelon and *Aloe vera* against Cd damage to the heart. Cd is capable of inducing oxidative stress in body tissues [26-28] by enhancing peroxidation of membrane lipids through inhibiting the antioxidant enzymes [29]. On the other hand, some experiments have stated the importance of antioxidants against effects of toxic environmental chemicals [30]. Watermelon and *Aloe vera* plant have been identified with antioxidant properties capable of scavenging reactive oxygen species [31-35].



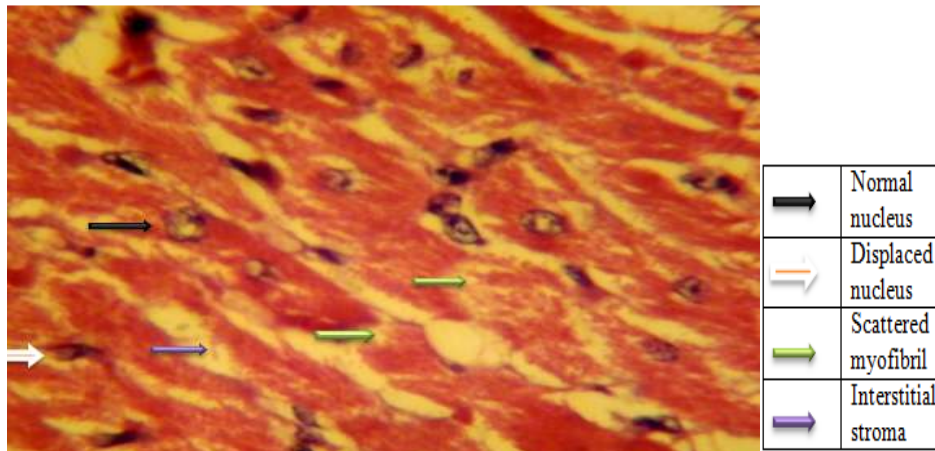
**Fig. 1. Control showing normal cardiac muscle. The nucleus and myofibril have normal arrangement. (X400 H&E stain)**



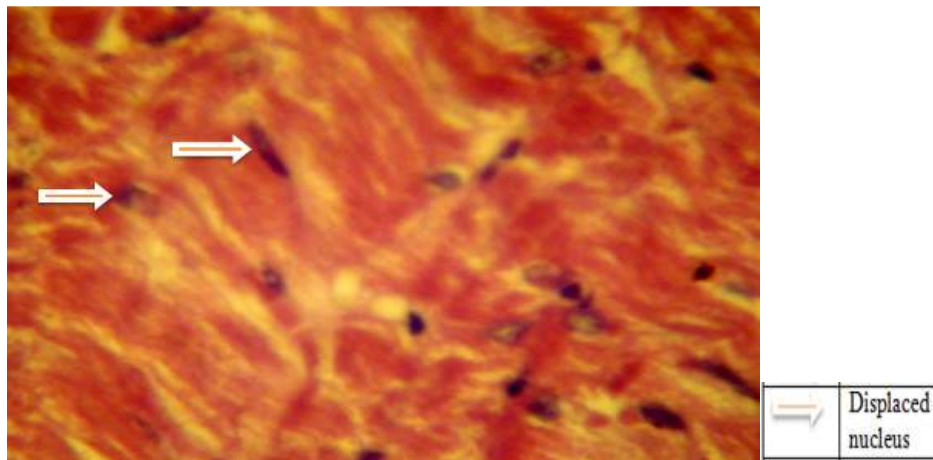
**Fig. 2. Cadmium induction caused displacement of nucleus and myofibril in some part of the heart. (X400 H&E stain)**



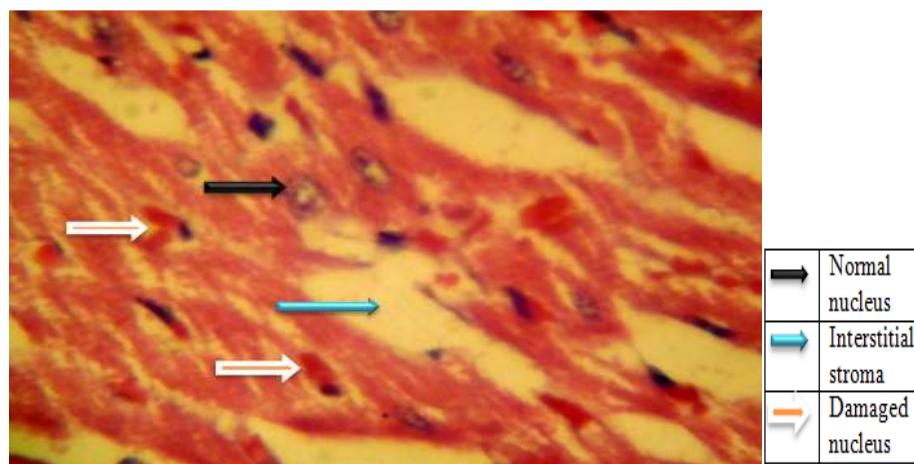
**Fig. 3. Vitamin C and Vitamin E administration caused normal morphology of cardiac muscle with is better than cadmium induced group. (x400 H&E stain)**



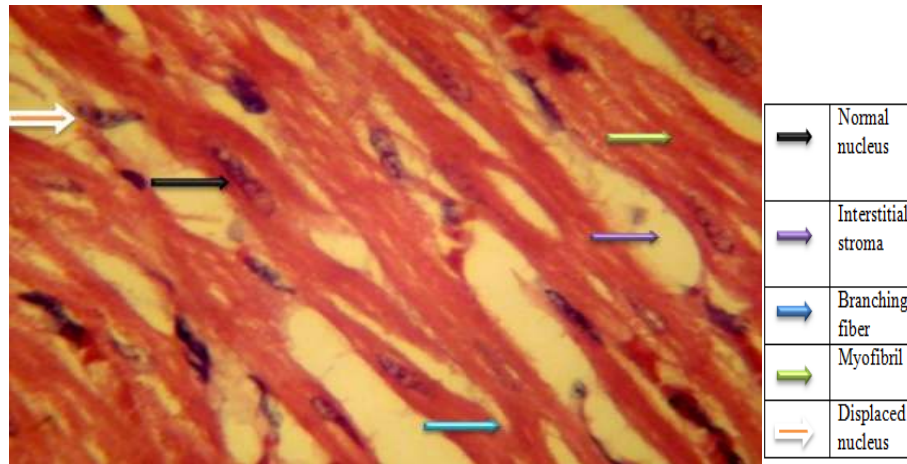
**Fig. 4.** Majority of the nuclei intact, but there is slight displacement of nuclei better and scattered myofibril due to the induction of cadmium before the termination even after pre-treatment with vitamin C and vitamin E. (x400 H&E stain)



**Fig. 5.** Treatment with watermelon after cadmium induction showed displacement of nucleus and the myofibril appears scattered. (X400 H&E stain)



**Fig. 6.** *Aloe vera* treatment after cadmium induction showed scanty nuclei, damaged nuclei and scattered striation. (x400 H&E stain)



**Fig. 7. *Aloe vera* and watermelon treatment after cadmium induction showed better striation and less displaced nuclei. (x400 H/E stain)**

Haematoxylin and Eosin (H&E) stains are essential for recognizing various tissue types and the morphologic changes that form the basis of contemporary cancer diagnosis [36]. The histomorphology of the heart of the normal control group 1 demonstrates normal morphology. Cd caused damage to heart tissue in the Cd induced group 2, by displacing the nucleus and myofibril in some areas of the heart. Studies have shown Cd is capable of causing damage to the heart and muscle [29,37]. Groups 5 and 6 treated with watermelon and *Aloe vera* respectively after Cd induction demonstrated damage to the muscle with less displacement of nuclei as compared with the group 2 induced with only Cd. This suggests the possible ameliorative effect of watermelon and *Aloe vera* against Cd damage to the heart due to the presence of antioxidant properties against reactive oxygen species [31,32]. The combination of watermelon and *Aloe vera* in treating Cd in group 7 demonstrated a far better histomorphology than the Cd induced group 2, watermelon group 5 and *Aloe vera* group 6. Group 3 administered with only vitamin C and E demonstrated better morphology when compared with the normal control group 2 while the group 4 treated with Vitamin C and E before induction with Cd two days before sacrifice presented displacement of myofibril. Experiment carried out on the effects of vitamin C or vitamin E against Cd production of free radicals, have established the protective effect of these vitamins [38,39]. The result thus, suggests that Vitamin C and E might not be able to fortify the heart against the effect of Cd effectively if administered for longer period of time before the Cd administration.

#### 4. CONCLUSION

The results from this experiment demonstrated the high degree of potency in the *Aloe vera* and watermelon combined over *Aloe vera* treated only and watermelon treated only in preventing oxidative tissues damage to the heart due to cadmium interaction.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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