

PAS Exfoliation in Watermelon and Aloe Vera Recuperate Glycogen in Heart Tissue Damaged by Cadmium in 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 major concern of World Health Organization for public health and is a highly toxic element which is naturally present in all parts of the environment. Watermelon contributes nutritional agents as antioxidants and some specific amino acid. Aloe vera plants contain cell wall carbohydrates such as cellulose and hemicellulose and storage carbohydrates such as acetylated mannans. The aim of this study was to observe the antioxidant property of watermelon and aloe vera against cadmium effect on glycogen deposition in the heart using Periodic Acid Schiff stain

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(PAS). Thirty five Wistar rats were obtained and acclimatized for two weeks. They were divided into 7 groups, five 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 group3 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 4weeks. The result obtained showed normal glycogen deposition in group 1, reduced glycogen deposition in group 2, decreased glycogen deposition in group 5, 6 and 7 but better than animal in group 2, presence of glycogen deposition in groups 3, 4 even after Cd administration. The result from the study shows that cadmium is capable of greatly reducing glycogen deposition in the heart. Watermelon and aloe vera extracts show potency in ameliorating the effect of cadmium in induced heart as in the case of therapeutic drugs, vitamin C and Vitamin E.

Keywords: Cadmium; aloe vera; watermelon; glycogen deposition; PAS.

1. INTRODUCTION

Cadmium (Cd) is a major concern of World Health Organization for public health and is a highly toxic element which is naturally present in all parts of the environment, which includes; food, water, and soil [1]. It is a non-essential element and has a half-life which is extremely persistent in the environment [2,3]. Cadmium's concentration in ocean seawater ranges from approximately 0.01 to 42 $\mu\text{g/L}$ [4,5]. Cadmium is sourced naturally from volcanic activities, forest fires and generation of sea-salt aerosols, thus increasing cadmium levels in the environment [6]. 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 [7,8]. Cadmium accumulates in the organ it enters, affecting the cell physiology and growth [9,10] induces disorders in the humoral and cellular immune responses [11-13]. Cadmium and its associated metals lead, copper, zinc, mercury, arsenate and vanadium, have been found out to have the ability to produce reactive oxygen species (ROS).

Several authors have shown that antioxidants should be one of the important components of an effective treatment of cadmium poisoning [14,15,16]. Antioxidant defense is used to prevent undesired oxidative damage induced by reactive species. Vitamin C could serve as an effective antioxidant against restraint stress induced pro-oxidant status and increase the antioxidant enzyme activity in rat brain [17] and liver [18].

Watermelon (*Citrullus lanatus*) fruit 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 [19-21]. Watermelon contributes nutritional agents as antioxidants (e.g. lycopene, beta-carotene) and some specific amino acids (e.g. arginine, citrulline). 100 g of watermelon consumed provides 30 Kcal to the body as it contains almost 92% water and 7.55% of carbohydrates, out of which 6.20% are sugars and 0.40% dietary fiber [22].

Aloe recently is members of the family *Asphodelaceae* but formerly considered part of the lily family (*Liliaceae*) [23]. The name, aloe, derived from the Arabic "alloeh" or Hebrew "halal" which means bitter shiny substance [24]. The thick fleshy leaves of aloe plants contain cell wall carbohydrates such as cellulose and hemicellulose and storage carbohydrates such as acetylated mannans [25].

The fresh leave of Aloe vera contains two components; bitter yellow latex from peripheral bundle sheath of aloe, called Aloe vera sap, Aloe vera juice or aloes and a mucilaginous gel from the parenchymatous tissue in the leaf pulp of Aloe vera. Aloes contains anthraquinone derivatives (aloe emodin) and their glycosides (aloin) which have cathartic/purifying effect [26]. Aloes also contains amino acids, auxins, gibberellins, minerals, vitamins, aspirin like compound, magnesium lactate and various enzymes like superoxide dismutase (SOD) and catalase [27]. The mucilaginous gel can be used to treat burns, wounds [28], skin diseases, constipation, inflammation, cancer, ulcer, diabetes and as a free radical scavenger [29,30].

The aim of this study is to observe the antioxidant property of watermelon and aloe vera against cadmium effect on glycogen deposition in the heart using Periodic Acid Schiff stain (PAS).

2. MATERIALS AND METHODS

2.1 Plant Material

Three 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 COTECH electronic weighing balance manufacture by indiaMART limited and then dissolved in 5ml of phosphate buffer.

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) [31].

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 [32].

2.4 Experimental Animal

Thirty five Wistar rats (50g-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.

Group no	Group name	Administration
1	Control group	Phosphate buffer (3 mg/kg/bw) intra-peritoneally
2	Induced group	$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ 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 + $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ intra-peritoneally (3 mg/kg/bw)
5	Treatment group	$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ intra-peritoneally (3 mg/kg/bw) + Watermelon extract (80 mg/kg/bw) orally, twice daily
6	Treatment group	$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ intra-peritoneally (3 mg/kg/bw) + Aloe vera extract (80 mg/kg/bw) orally, twice daily
7	Treatment group	$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ intra-peritoneally (3 mg/kg/bw) + Watermelon (40 mg/kg/bw) and Aloe vera extract (40 mg/kg/bw) orally, twice daily

At the termination of the experiment, the rats were fasted overnight and then sacrificed by cervical dislocation. The hearts were collected and placed in 10% formaline for histological investigation using PAS stain.

3. RESULTS AND DISCUSSION

PAS stain is carried out to observe the polysaccharide (e.g. glycogen) deposition in tissues [33]. Some experiments have stated the importance of antioxidants against effects of toxic environmental chemicals [34]. Watermelon and aloe vera plant have been identified with antioxidant properties capable of scavenging reactive oxygen species [35-37].

The normal control group demonstrated normal deposition of glycogen. The glycogen deposition in Cd induced group was greatly reduced as compared to the normal control group, which indicates the deleterious ability of Cd in breaking

down the antioxidant defense system of the rats [38]. Experiment carried out by Javed and Usmani, [39] showed that heavy metal decreases glycogen in liver and muscles in order to enhance glycolytic capacity. The watermelon group 5, aloe vera group 6 and watermelon and aloe vera treatment group 7 had more sites for depositions of glycogen as compared to the Cd induced group 2, which shows the antioxidant properties of the extracts to prevent and scavenge the generated free radicals by Cd [29,35]. Vitamin C and Vitamin E (groups 3 and 4) showed protective properties which are in line with ability of the vitamins to strengthen the antioxidant defense system of the experimental animals [40].

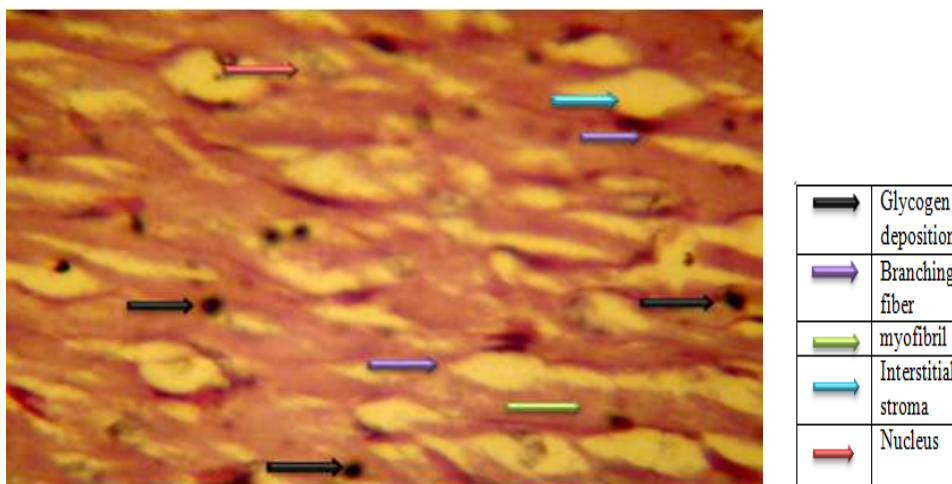


Fig. a. Normal control group demonstrated numerous glycogen depositions in the cardiac muscle. (X400 PAS stain)

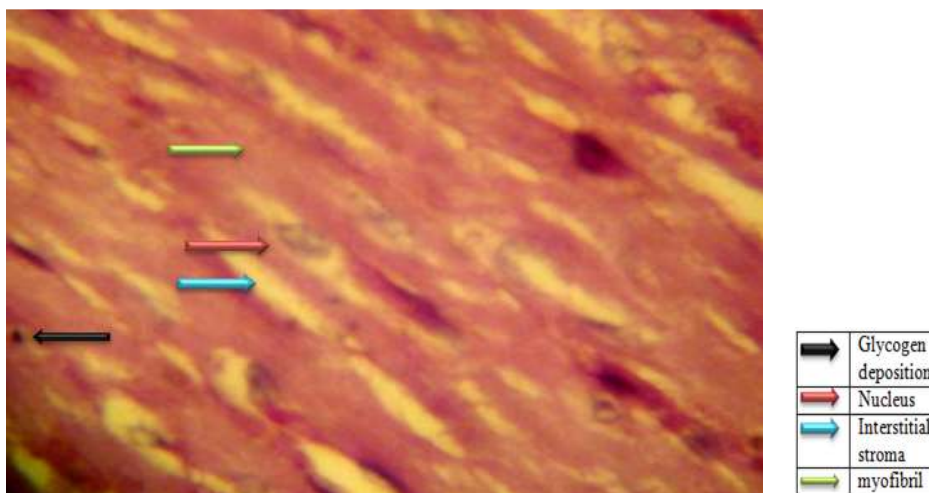


Fig. b. Cadmium induction caused decrease in glycogen deposition. (X400 PAS stain)

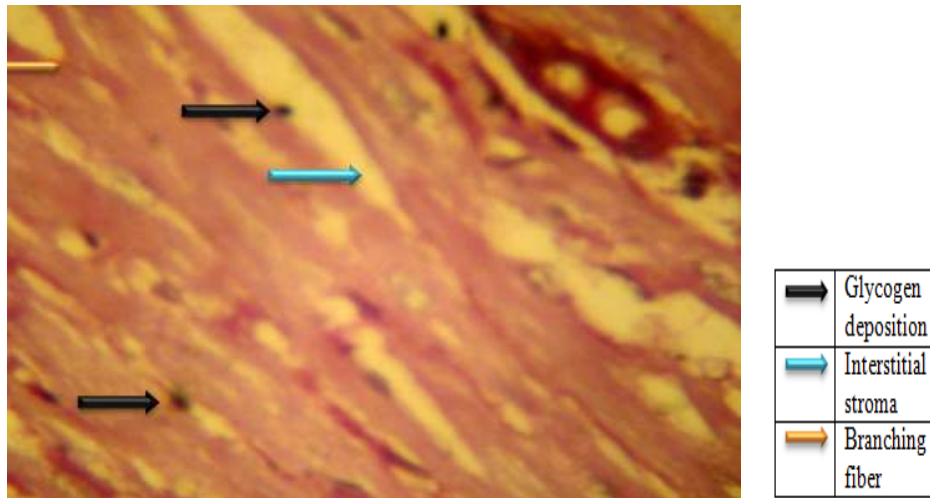


Fig. c. Vitamin C and vitamin E showed deposition of glycogen better cadmium induced group. (x400 PAS stain)

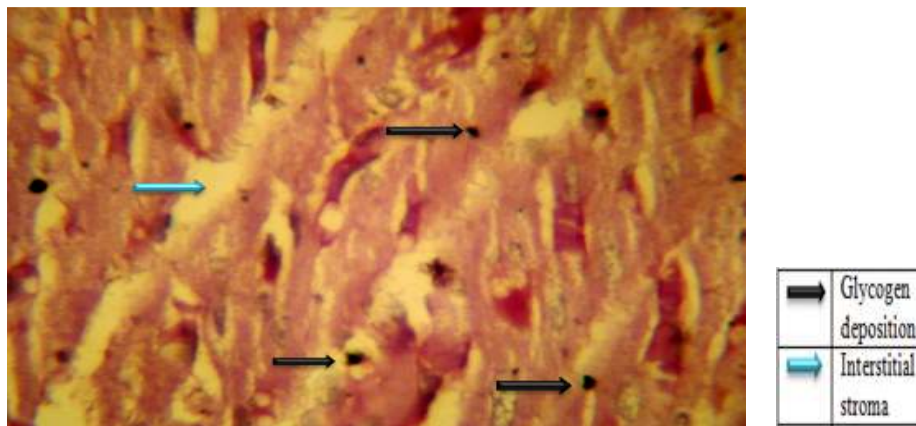


Fig. d. Vitamin C, E and Cadmium shows presence glycogen deposition even after administration of cadmium. (x400 PAS stain)

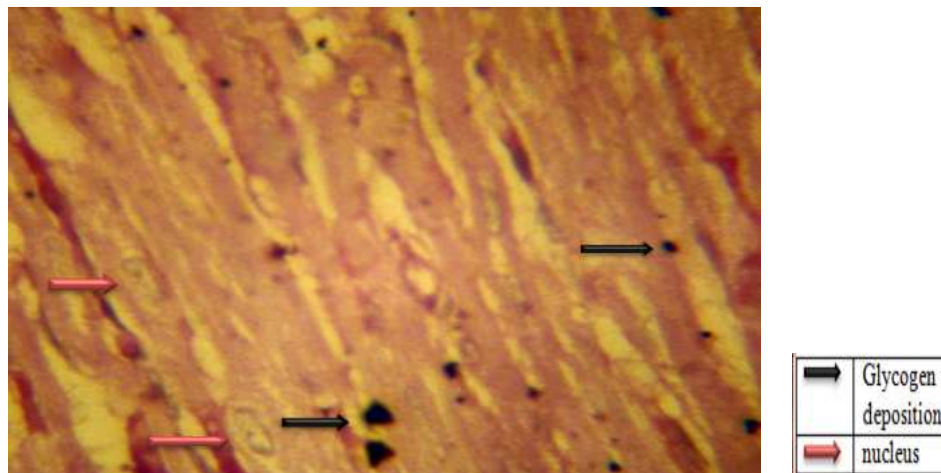


Fig. e. Watermelon treatment counteracts cadmium reduction as glycogen deposition is present. (x400 PAS stain)

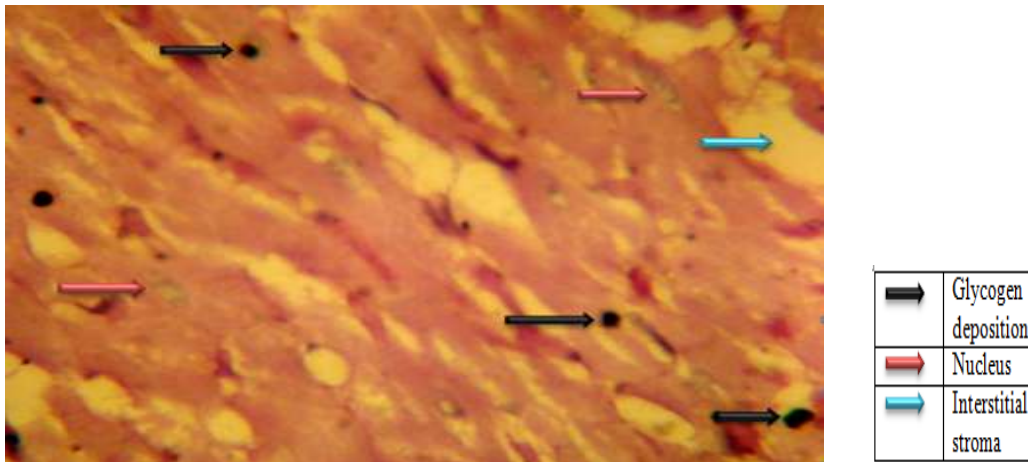


Fig. f. Glycogen deposition is numerous than in cadmium only in aloe vera treated group. (x400 PAS stain)

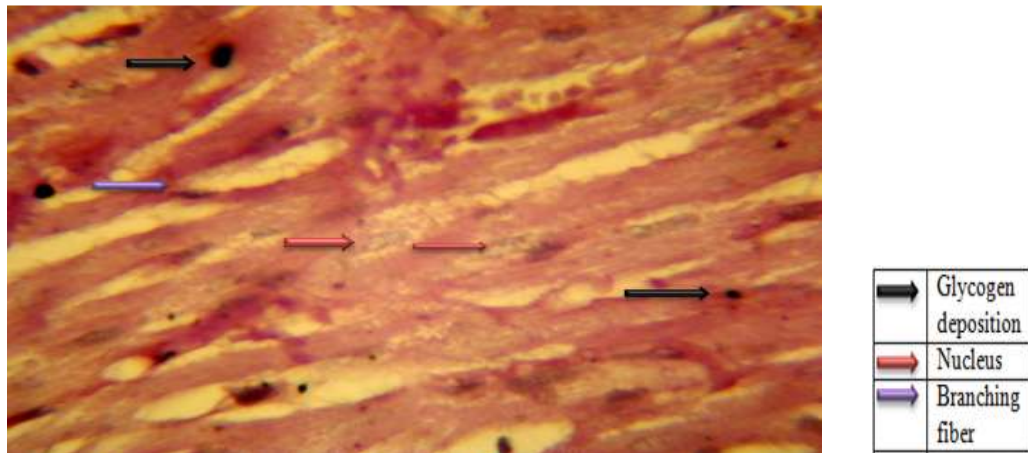


Fig. g. Watermelon and aloe vera combined treatment showed better glycogen deposition compared to cadmium induced group. (X400 PAS stain)

4. CONCLUSION

The result from the study showed that cadmium is capable of greatly reducing glycogen deposition in the heart. Watermelon and aloe vera extracts show potency in ameliorating the effect of cadmium in the heart as in the case of therapeutic drugs, vitamin C and E.

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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