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Influence of Crude and Degummed Seed Oil of *Citrullus lanatus* on Some Serum Minerals and Oxidative Stress Markers in Wistar Albino Rats

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Abstract: The present study was carried out to evaluate and examine the effect of *Citrullus lanatus* (CL) crude and degummed seed oil on some antioxidant minerals (Mg^{2+}, Zn^{2+} and Ca^{2+}), vitamins C and Eand some antioxidant enzymes, catalase(CAT), malondialdehyde(MDA) and glutathione peroxidase (GPx). Fifteen (15) wistar albino rats were distributed into 3 different groups of 5 rats each with groups 1 and 2 being administered CL crude and degummed seed oil, respectively at a dose of 6 ml/kg body weight (BW).Group 3 whhich served as the control group was administered distilled water. The administration lasted for a period of 21 days after which blood was drawn for analysis of the parameters using standard methods. The result showed a significant ($p<0.05$) decrease in Zn^{2+} concentration across the test groups (administered *Citrullus lanatus* crude seed oil and degummed seed oil), while concentration of Mg^{2+} was reduced non-significantly ($p>0.05$) in both treated groups as well. Ca^{2+} was non-significantly ($p>0.05$) increased in the group administered the crude seed oil. There was no significant ($p>0.05$) difference in the group treated with the degummed seed oil.Vitamin C showed a significant decrease in both treated groups while vitamin E showed a significant($p<0.05$)decrease and increase in the crude seed oil and degummed seed oil treated groups, respectively. CAT and MDA showed significant ($p<0.05$) decreases in both CL crude seed oil groups while a non-significant ($p>0.05$) difference was seen in the group administered degummed seed oil. This study suggests that *Citrullus lanatus* seed oil is not a good source of antioxidant compounds.

Key words: *Citrullus lanatus* · Antioxidants minerals · Antioxidant enzyme and Degummed seed oil

INTRODUCTION

Antioxidants are nutrients in food that protect the cells from damage arising from free radicals. It is believed that antioxidants exert their protective effect by acting as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists and metal-chelating agents and these (both enzymatic and nonenzymatic) antioxidants exist in the intracellular and extracellular environment to detoxify ROS. Two principle mechanisms of action have been proposed for antioxidants [1]. The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation [2]. Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk [3] and increased oxidative stress at the cellular level cancome about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Protection against all these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet [4].

*Citrullus lanatus*commonly called watermelon is a popular fruit in many parts of the world which is notable for its high water content and attractive look. The fruit comes in various shapes, sizes and pattern. Although the seed of watermelon is often discarded as waste, it contains various amounts of carbohydrate, proteins, fibre

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and minerals such as, magnesium, iron, calcium and phosphorus. According to Borguini and Da Silva Torces, (2009), *Citrullus lanatus*seed oil contains a multitude of health-supporting vitamins and minerals such as vitamins C, E, B6, folic acid, niacin, potassium and trace elements, which include, copper, magnesium, iron, zinc and calcium. Researchershaveshown that the seed of *Citrullus lanatus* fruit have a much higher food value than the fles and possesses nice fruity taste significant amounts of vitamin C, mineral fat, starch and riboflavin have been obtained from them [5]. They can be dried, roasted and eaten as such or ground into flour to make bread [6]. The flour is said to contain saponin and is also used as a detergent and it has also been shown that the seed contains a high percentage of oil which is similar to pumpkin seed oil and can be used in cooking [6]. *Citrullus lanatus*seeds are increasingly used for their oil in semi-arid regions and also the use of the oil in are in prospect for the improvement of infant nutrition in view of their high protein and fat content [7]. Further studies have also shown the activities *Citrullus lanatus*seed oil which include anti-aging and gastroprotective effect [8], diuretic activity [9], anti-inflammatory potential [10] and a good source of vitamin E [9].

MATERIALS AND METHODS

Plant Material: Water melon (*Citrullus lanatus*) seeds were purchased from Ubani market, UmuahiaAbia state, Nigeria. They were washed severally with distilled water, air-dried for a week and screened to remove the bad ones. Samples of water melon seeds were crushed using a commercial blender, put in air tight container and store in a desiccators for further analysis.

Animals: Albino wistar rats of age 6-8 weeks were purchased from the Department of Zoology University of Nigeria Nsukka. They were acclimatized for two weeks and given feed and water *ad libitum*.

Oil Extraction: Crudeoil from the seeds of water melon (*Citrullus lanatus*) was extracted according to the method [11].

Degumming of Oil: This was done according to the method described by [12].

Experimental Design: Fifteen (15) albino rats were divided into three (3) equal groups to represent. Groups 1 and 2 were administered crude seed oil and degummed seed oil at 6 ml/kg body weight, respectively while group 3 was administered normal water and this served as the control.

ASSAYS

Serum Calcium Determination: The determination of calcium ion was done by the method of Faulker and Meister (1982).

Serum Zinc Determination: Estimation of serum zinc level was done by the method of [13].

Serum Magnesium Determination: This is done according to the method of Ferrel (1984) as contained in Biotech assay kit.

Serum Calcium Determination: The determination of calcium ion was done by the method of Faulker and Meister (1962).

Estimation of Vitamin E: Vitamin E content was estimated by method of [14].

Estimation of Catalase: The activity of catalase was assayed by the method of Sinha (1972).

Estimation of Glutathione Peroxidase: This was done according to the method of [17].

Total Antioxidant Capacity (TAC): TAC was evaluated using the method of [18], with slight modifications.

Estimation of Extent of Lipid Peroxidation (Malondialdehyde): Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by [19].

Statistical Analysis: Data was treated with one way ANOVA (Analysis of variance) and Tukey's post hoc test was used for the multiple comparism of mean at the level of 5% of the probability.

RESULTS AND DISCUSSIONS

The seed oil of *Citrullus lanatus* as reported by, contains a multitude of vitamins (B_6, C, E, folic acid, niacin, as well as minerals; potassium and trace elements, i.e Cu, Mg, Fe, Zn and Ca) and these play significant roles in protection mechanisms by scavenging free radicals.

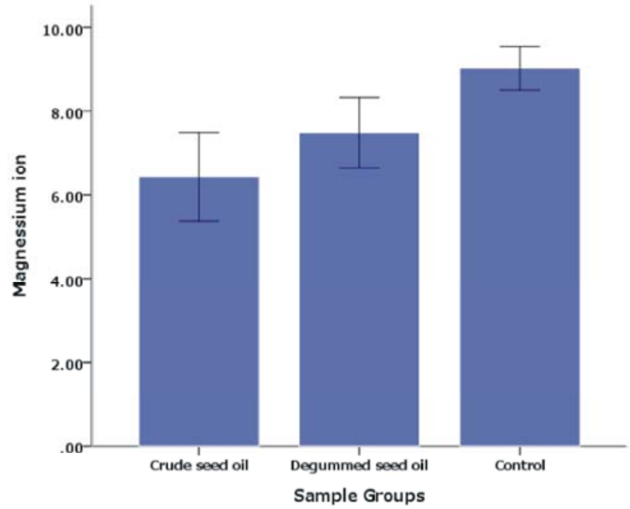

Fig. 1: The figure above shows a significant ($p<0.05$) decrease in Mg^{2+} concentration in the rats administered degummedand crude seed oil

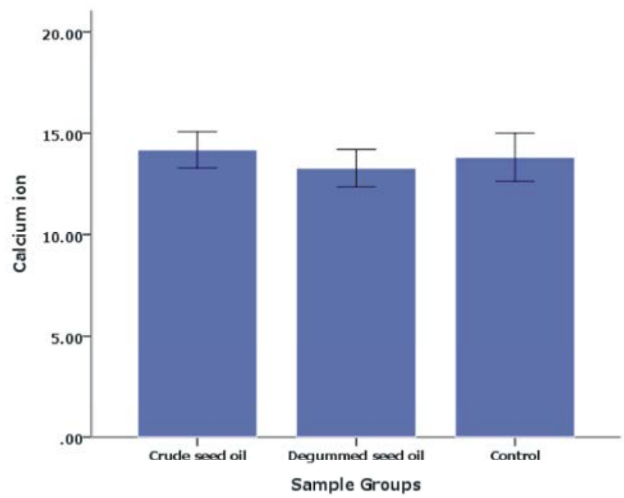

Fig. 2: The Figure above shows a non-significant ($p>0.05$) difference in calcium ion concration across all groups

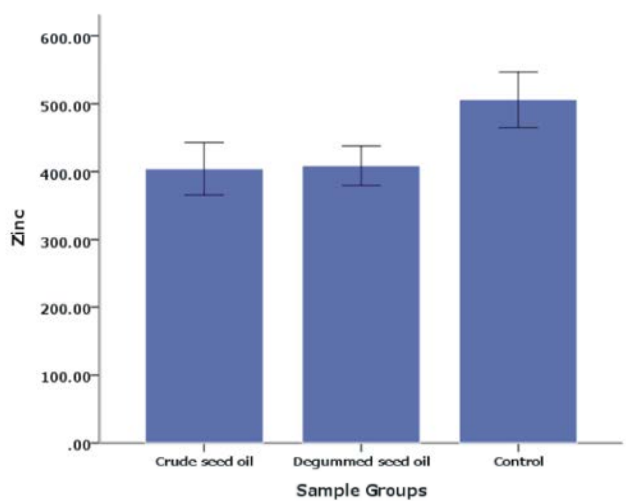

Fig. 3: From the figure above, it is observed that the Zn^{2+} concentration of the treated groups are significantly ($p<0.05$) lowered than the control

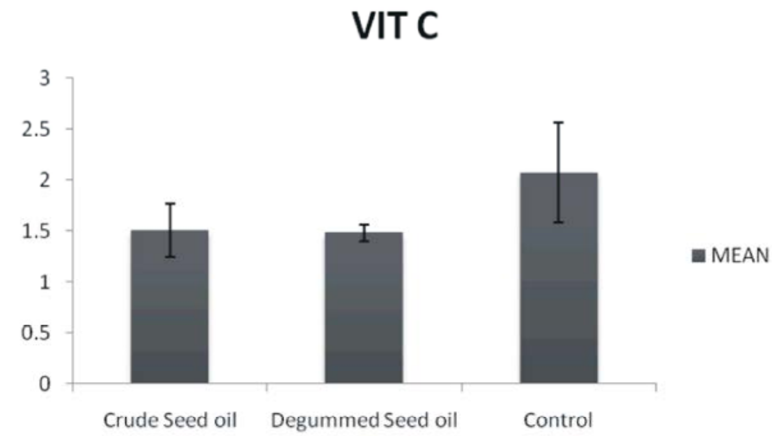

Fig. 4: The figure above shows a significant ($p<0.05$) decrease in vitamin C levels of the rats administered the extracts when compared against the control group

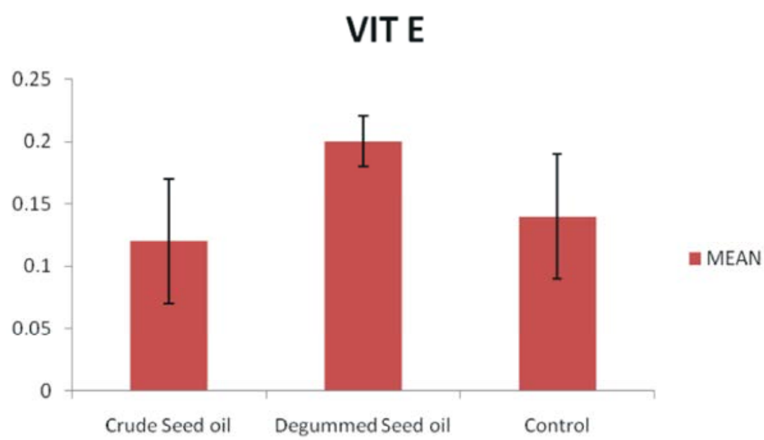

Fig. 5: From fig. 5 above, it was seen that the degummed seed oil and crude seed oil showed a significant ($p<0.05$) increase and non-significant ($p>0.05$) decrease, respectively when compared against the control group

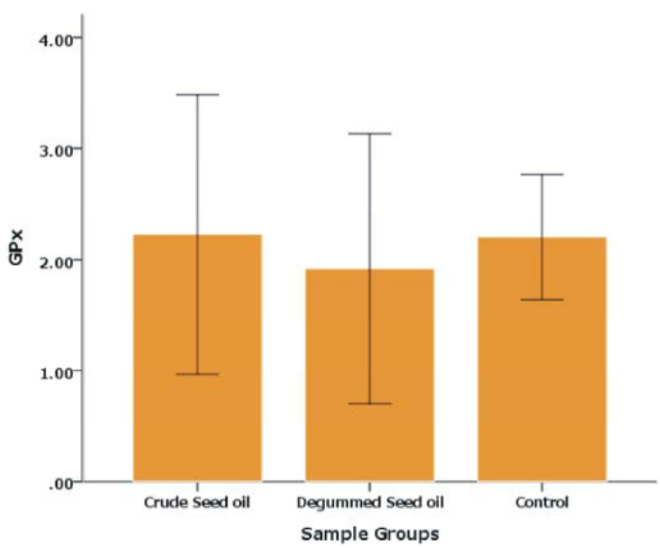

Fig. 6: Figure 6 above shows a non-significant ($p>0.05$) decrease and difference in the treated groups administered degummed and crude seed oil, respectively when compared against the control group

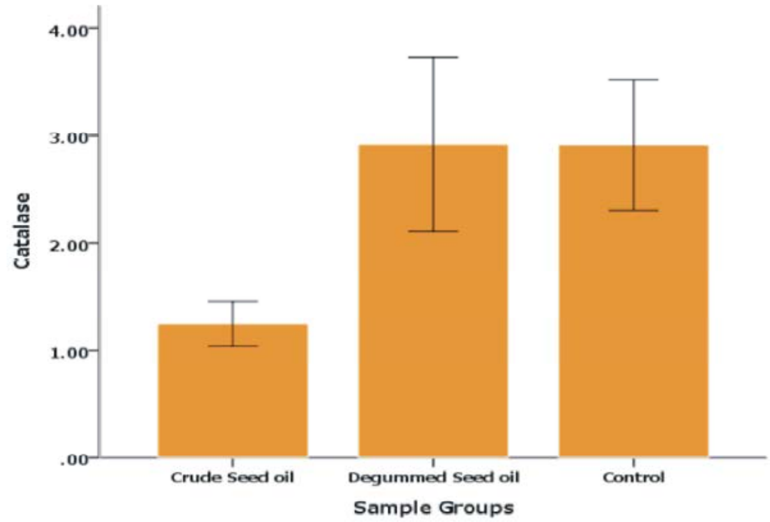

Fig. 7: The above figure (Fig. 7) shows a significant ($p<0.05$) reduction in catalase activity in the group administered crude seed oil and a non-significant ($p>0.05$) difference in the group administered degummed seed oil, when compared against the control group

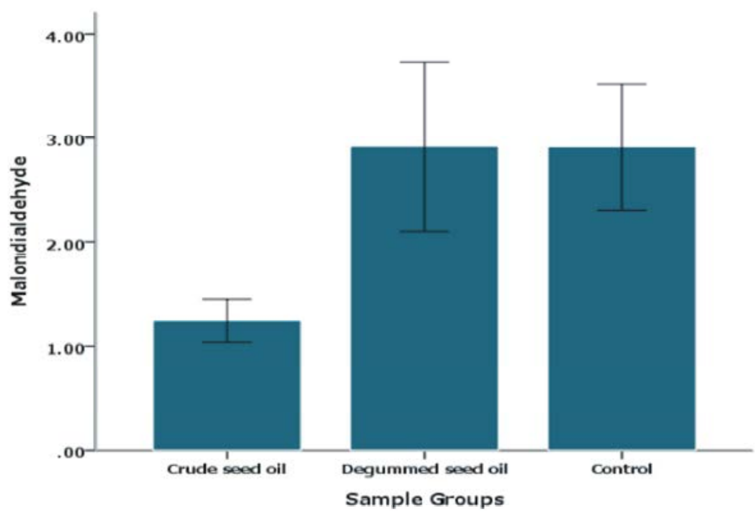

Fig. 8: From fig. 8 above, the group administered the degummed seed oil and crude seed oil showed non-significant ($p>0.05$) difference and significant ($p<0.05$) decrease, respectively when compared against the control group.

From the result above, there was a significant ($p<0.05$) in magnesium ion levels of the treated groups as compared against the control. Reports have shown that a significant decrease in either plasma or serum magnesium levels has been reported in patients with liver steatosis indicating the toxicological potential of the crude extract and degummed seed oil extract of *C. lanatus.* Magnesium is one of the most important micronutrients which play a vital role in the immune system both in innate and acquired immune response [15]. The work also shows a non-significant ($p>0.05$) difference in the calcium ion levels of the treated groups compared to the control group. The zinc and vitamin C levels of the treated animals were significantly ($p<0.05$) decreased when compared against the control group; while vitamin E showed a significant ($p<0.05$) decrease and increase in the rats administered *C. lanatus* crude seed oil and degummed seed oil, respectively. Zinc is an element needed in its oxidized form (Zn^{2+}) for proper functioning of enzymes in biological systems. These enzymes include carbonic anhydrase as well as bovine pancreatic carboxypeptidase [20]. It also serves as signaling molecule and perhaps, this is the the most exciting area in contemporary zinc biology due to its role in information transfer within and between cells [21]. Vitamins C and E are antioxidant vitamins and protect the cells or tissues of the diabetic against degenerative changes associated with the syndrome [22, 23, 24]. This is in agreement with the study by Erhirhie and Ekene, (2013), [10], who reported the antioxidant properties of *Citrullus lanatus* methanolic seedextract.

This study also shows a near-close non-significant ($p>0.05$) difference in glutathione peroxidase (GPx) activity in both treated groups as compared to the control. This could be attributed to equal glutathione present in the entire sample groups since they are synthesized in the body and not from the diet.The enzyme GPxcatalyses the reduction of hydrogen peroxide using GSH, thereby protecting mammalian cells against oxidative damaged. In fact, metabolism of GPx is one of the most essential antioxidant defense mechanism utilized by the cells [25]. Catalse (CAT) activity and malondialdehyde (MDA) were significantly ($p<0.05$) reduced in the group administered the crude seed oil. CAT is an antioxidant enzyme widely distributed in animal tissues which decomposes hydrogen peroxide and protects the tissues from high reactive hydroxyl radicals [26], while MDA is the end product of lipid peroxidation and a measure of free radical generation [27].

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