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Effect of Seed and Pulp Aqueous Extracts of *Citrullus colocynthis* on Oxidative Stress Parameters of Albino Rats

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Abstract
The effect of different concentrations of Citrullus colocynthis seed and pulp aqueous extracts on some important oxidative stress parameters [Ascorbic acid (AA), Reduced glutathione (GSH), Malondialdehyde (MDA)] were determined. The activities of Superoxide dismutase (SOD), Catalase (CAT) and Lactate dehydrogenase (LDH) of albino rats were assayed. Protein status such as albumin, globulin and total protein were also determined using standard methods. Thirty-five albino rats weighing 156-186g were divided into seven groups of five rats each. Different doses (100, 200 and 400mg/kg body weight per day) of Citrullus colocynthis seed and pulp aqueous extracts were orally administered daily to Groups 2-4 and 5-7 respectively, while Group1 (Control) received only 0.5ml of distilled water for a total treatment period of 28 days. Results of the study indicate that there was a significant ($p<0.05$) reduction in the concentrations of AA and GSH as the concentrations of the extracts increased, in contrast to the concentration of MDA when compared to the control. There was a significant ($p<0.05$) increase in the activities of SOD and CAT when compared to the control. However, there was no significant ($p>0.05$) effect on the activity of LDH as well as the concentrations of protein status (albumin, globulin and total protein) when compared to the control. The results revealed that AA, GSH, MDA, SOD and CAT were responsive to the administration of aqueous seed and pulp extracts of Citrullus colocynthis, and the effects were concentration-dependent.

Key words: *Citrullus colocynthis*, seed, oxidative stress, antioxidants, rats.

Introduction
Traditional medicines support well over 80% of the population in developing countries especially in the rural areas [1]. Available evidence suggests that even in urban areas which are well served by modern healthcare facilities, a good number of people rely on herbal supplements to meet some of their health needs [2], Medicinal plants have been used for decades before the advent of orthodox medicine for the treatment of many illnesses. Various plant parts such as leaves, flowers, stem barks, roots, seeds, fiber, pulp and fruits have all been used as constituents of herbal medicines. The medicinal values of these plant parts lie in their phytochemical compositions, which produce definite physiological action on human body [3].

Citrullus colocynthis, a member of the *Cucurbitaceae* family is known as Bitter Apple in English language, Hindal in Arabic, and Abujahl melon in Persian. The extract of the fruit is called Colocynthine due to its extreme bitter taste. The known compounds found in *Citrullus colocynthis* include glycosides, alkaloids, and flavonoids [4]. A number of plant secondary metabolites including Cucurbitacins, flavonoids, caffeic acid derivatives, terpenoids and phenolic compounds have previously been reported from this plant [4]. However, unknown compounds may also contribute to its therapeutic reputation. The Curcurbitacins (highly oxygenated tetracyclic glycosides) have a broad range of applications due to their wide spectrum of biological activities [5]. They are found mainly in plants belonging to the *Cucurbitaceae* family, but have also been found in several other families of the plant kingdom [5].

In folk medicine, *Citrullus colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent [5]. Although, the whole fruit is often used for the treatment of the aforementioned diseases, but some particular parts of the fruit are also used for specific purposes. One of such example is the traditional application of the dried pulp and seed extract of *Citrullus colocynthis* for the treatment of constipation and diabetes [6];[7]. Despite the wide therapeutic potentials attributed to this fruit, the development of complications during treatment is not uncommon. Some adverse effects include bloody diarrhea and toxic colitis. They are responsible for *Citrullus colocynthis* classification as a toxic plant, where it is considered among the top ten [7]. Interestingly, most of the studies on the toxic effects of this medicinal plant were performed on the whole fruit extract (*colocynthine*). Since different parts of the fruit, such as the pulp or the seed, are claimed to exert different therapeutic effects, it is reasonable to suggest that a particular therapeutic benefit, or toxic side effect could be attributed to one part of the fruit, and not another. *Citrullus colocynthis* is known to increase peristaltic movement of the gut, and cause diarrhea. Given the varied ethno-medicinal uses of *Citrullus colocynthis* when used singly or in combination with other plants, the present study evaluated the effect of the seed and pulp aqueous extracts of the plant on oxidative stress parameters of albino rats.

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Materials and Methods

Collection, Identification and Preparation of Plant Extracts

The fruit of *Citrullus colocynthis* used for this work was bought at Ekeonuwa Market, in Owerri Municipal Council, Imo State Nigeria. The plant was identified by Mr. Francis Iwueze of the Department of Forestry and Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri. The seeds of *Citrullus colocynthis* were carefully removed from the fruit rinds, and air-dried. After drying to constant weight for few days, the seed and pulp were ground into fine powder using a mechanical homogenizer. The fruit pulps were carefully removed from the epicarp and air-dried. The extracts of the ground seed and pulp were made as follows: A 100g portion of the ground seed and pulp of *Citrullus colocynthis* was each soaked in 500ml of water for 24h, filtered and then exhaustively extracted with the aid of soxhlet extractor (Gallenkamp, England). The solvent from each extract was then distilled off in a distillatory and evaporated to dryness at 40 ^{0}C. The solid extract was each placed in a sterile container, labeled and stored at 4^{O}C in a refrigerator from where portions were taken and prepared for the study.

Slide 1: The fruit of *Citrillus colocynthis*

Slide 2: The seeds and pulp of *Citrillus colocynthis*..

Animal Handling: Thirty-five (35) male albino rats, weighing 156-186g used for the study were purchased from the Animal Unit, Abia State University, Uturu, Abia State, Nigeria. The animals were treated and handled humanely in accordance with the standard principle of the laboratory animal care of the National Institute of Health [8]. They were supplied with feed and water *ad libitum*.

Animal Grouping and Treatment: The animals were divided into seven groups of five (5) rats in each cage according to their relative body weights. The weights of the rats before the administration of feeds were recorded. The animals were allowed to acclimatize to the laboratory environment for one (1) week on a regular water and feed. After acclimatization, each group was administered with their respective concentrations. Rats in Group 1 (Control) were orally administered with 0.5ml distilled water (the vehicle) while those in Groups 2 to 4 and Groups 5 to 7 were administered the same volume of *Citrullus colocynthis* seed and pulp extracts, respectively, at 100, 200 and 400 mg/kg body weight/day, for a period of 28 days.

Collection of Blood Samples: After 28 days, the rats were anesthetized by exposure to dichloromethane vapor in covered transparent plastic container. Incisions were then made into the thoracic regions and blood collected by cardiac puncture using 5ml hypodermic syringe and needle. The blood samples were dispensed into sterile sample bottles, allowed to clot and centrifuged at 3000 rpm for 10 min. The serum was separated using micropipette and used for the various parameters

Estimation of Oxidative Stress parameters: Ascorbic acid concentration was determined by the method of [9]. Reduced glutathione (GSH) concentration was determined by the method of [10]. Lipid peroxidation was estimated by determining the concentration of malondialdehyde (MDA) produced spectrophotometrically using the method of [11]. Catalase (CAT. E.C.1.11.1.1.) activity was assayed for by measuring spectrophotometrically at 570 nm the rate of decomposition of hydrogen peroxide (H_2O_2) over a period of 30 min (at 1 min interval) as described by [12]. Superoxide dismutase (SOD, E.C.1.15.1.1.) activity was assayed according to the method of [13]. Lactate dehydrogenase (E.C.1.1.1.27.) activity was assayed using standard procedures as described in the assay kit of Randox laboratories Ltd United Kingdom.

Determination of Protein Status: Serum protein concentration was determined according to the method of [14]. The concentration of serum albumin and globulin were determined according to the method of [15].

Statistical Analysis: All data generated were expressed as mean ± standard deviation and analyzed for statistical significance by using one-way Analysis of Variance (ANOVA). Values were considered significance at $p < 0.05$ followed by Turkey post-Hoc test.

Results and Discussion

The concentration or activity of an antioxidant may be increased or reduced under chemical stress, depending on the intensity and duration of stress applied, as well as the susceptibility of exposed species [16]. The results obtained in this study reveal the extent to which the oxidative parameters measured were affected by the administration of seed and pulp aqueous extracts of *Citrullus colocynthis.*

The ascorbic acid concentrations of the treated rats are presented in Table 1. Ascorbic acid is a potent water-soluble antioxidant capable of scavenging/neutralizing an array of reactive oxygen species such as hydroxyl, alkoxyl, peroxyl, superoxide anion, hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide and peroxy nitrate at very low concentration [17]. In addition, ascorbic acid can regenerate other antioxidants such as α- tocophenoxyl, urate and β-carotene radical from their radical species [16]. Thus, ascorbic acid acts as a co-antioxidant for α-tocopherol by converting α-tocopheroxy radical to α-tocopherol and helps to prevent the α-tocopheroxy radical-mediated peroxidation reaction. Oral administration of aqueous seed and pulp extracts of *Citrullus colocynthis* significantly ($p<0.05$) reduced the mean concentration of ascorbic acid of the albino rats when compared to the control. This is in agreement with the findings of [18], who reported a substantial reduction in serum ascorbic acid concentration in albino rats fed different concentrations of ground seeds of *Picralima nitida* in contrast to that of the control rats. The observed significant ($p<0.05$) reduction in ascorbic acid concentration due to administration of aqueous seed and pulp extracts of *Citrullus colocynthis* could be attributed to the involvement of ascorbic acid in reactions with reactive species, thereby reducing its concentration [19].

Glutathione (GSH) is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. The thiol group is a potent reducing agent, rendering GSH the most abundant intracellular small molecule thiol, reaching millimolar concentrations in some tissues. As an important antioxidant, GSH plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by glutathione-S-transferases (GST) and glutathione peroxidases (GPx) [20]. The importance of GSH is evident by the widespread utility in plants, mammals, fungi and some prokaryotic organisms. In addition to detoxification, GSH plays a role in other cellular reactions, including the glyoxylate system, reduction of ribonucleotides to deoxyribonucleotides, regulation of protein and gene expression via thiol: disulfide exchange reactions [20].

In this study, the results obtained, as presented in Table 1, also revealed that serum GSH concentrations of albino rats administered with different concentrations of aqueous seed and pulp extracts of *Citrullus colocynthis* were responsive to the treatment. The effect of the treatment caused a significant ($p<0.05$) reduction in GSH concentration.

Malondialdehyde (MDA) occurs naturally and is a marker for oxidative stress [21]. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE) [22]. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism [21]; [23].

Table 1: Ascorbic acid, glutathione (GSH) and malondialdehyde (MDA) concentrations of rats administered different concentrations of *Citrullus colocynthis* seed and pulp extracts.

Groups	Ascorbic acid (mg/ml)	GSH (µmol/l)	MDA (x 10^{-7} mol/l)
1	1.60 ± 0.03^{a}	4.29 ± 0.02^{a}	3.03 ± 0.06^{ac}
2	1.50 ± 0.02^{a}	4.11 ± 0.03^{b}	3.03 ± 0.05^{a}
3	1.45 ± 0.04^{b}	4.00 ± 0.03^{c}	3.13 ± 0.05^{ac}
4	1.30 ± 0.02^{c}	3.71 ± 0.02^{d}	3.33 ± 0.05^{b}
5	1.50 ± 0.04^{a}	4.01 ± 0.02^{c}	3.17 ± 0.06^{c}
6	1.40 ± 0.03^{b}	3.82 ± 0.02^{e}	3.48 ± 0.05^{d}
7	1.35 ± 0.02^{c}	3.51 ± 0.03^{f}	3.68 ± 0.05^{e}

Values are mean ± standard deviation. Values with different superscript letters per column are significantly different at $p<0.05$

The result obtained for MDA as presented in Table 1 reveals that there was a significant ($p<0.05$) increase in the concentration of MDA in albino rats administered with different concentrations of aqueous seed and pulp extracts of *Citrullus colocynthis* when compared to the control.

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide [24]; [25]. SOD enzymes are present in almost all aerobic

cells and in extracellular fluids. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally the Ni type which binds nickel [26]. In higher plants, SOD isozymes have been localized in different cell compartments. Mn-SOD is present in mitochondria and peroxisomes, Fe-SOD has been found mainly in chloroplasts but has also been detected in peroxisomes, while Cu/Zn-SOD has been localized in cytosol, chloroplasts, peroxisomes and apoplast [26]; [27]; [28].

The results obtained in this study as presented in Table 2 reveals that superoxide dismutase activity increased significantly ($p<0.05$) only at high concentrations of the seed extract but increased with increasing concentration of pulp extract used. This corroborates the reports of [29] on the hepato-protective potentials of *Moringa oleifera* leaf extract on alcohol-induced hepato-toxicity in Wistar rats and [18] on the biochemical assessment of *Picralima nitida* seeds on oxidative stress parameters of albino rats.

Catalase is a common enzyme found in nearly all living organisms, which are exposed to oxygen where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen [30]. Hydrogen peroxide is a harmful by-product of many normal metabolic processes. To prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules [31]. All known animals use catalase in every organ, with particularly high concentrations occurring in the liver [32].

Table 2: Superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) activities of albino rats administered seed and pulp aqueous extracts of *Citrullus colocynthis*.

Groups	SOD (U/L)	Catalase (x 10^{-2} U/L)	LDH (U/L)
1	1.43 ± 0.10^{a}	2.23 ± 0.04^{a}	379.40 ± 9.26^{a}
2	1.49 ± 0.20^{a}	2.46 ± 0.13^{b}	392.70 ± 9.26^{a}
3	$1.50\pm.23^{a}$	2.79 ± 0.11^{c}	388.70 ± 15.35^{a}
4	1.63 ± 0.11^{ab}	2.57 ± 0.09^{b}	392.70 ± 16.03^{a}
5	1.49 ± 0.20^{a}	2.88 ± 0.04^{cd}	395.40 ± 9.26^{a}
6	1.55 ± 0.08^{a}	2.91 ± 0.07^{cd}	396.70 ± 8.02^{a}
7	1.87 ± 0.04^{b}	3.00 ± 0.06^{d}	392.70 ± 9.26^{a}

Values are mean ± standard deviation. Values with different superscript letters per column are significantly different at $p<0.05$

The results of the activity of catalase as presented in Table 2 also indicate that there was a significant ($p<0.05$) increase in the activity of catalase of rats treated with aqueous seed and pulp extracts of *Citrullus colocynthis* when compared to the control. The results also agree with the report of [18] who worked on the biochemical assessment of *Picralima nitida* seeds on oxidative stress parameters of albino rats. This increase in activity of catalase as a result of administration of these extracts needs further investigation.

Lactate Dehydrogenase (LDH) is an enzyme found in nearly all living cells (animals, plant and prokaryotes). LDH catalyses the conversion of pyruvate to lactate. LDH has been of medical significance because it is found extensively in body tissues such as blood cells and heart muscles. Because it is released during tissue damage, it is a marker of common injuries and disease such as heart failure. The results of LDH in Table 2, also reveal that oral administration of different concentrations of aqueous seed and pulp extracts of *Citrullus colocynthis* did not have any significant ($p>0.05$) effect on the activity of LDH. This indicates that *Citrullus colocynthis* aqueous seed and pulp extracts at these concentrations were not cardiotoxic. This observation needs further investigation since there were marked significant ($p< 0.05$) changes in all the other oxidative stress parameters investigated. However, the results could imply that there was no direct damage elicited by the extracts administered.

Table 3: Albumin, globulin and total protein concentrations of rats administered different concentrations of seed and pulp aqueous extracts of *Citrullus colocynthis*.

	Concentrations (mg/ml)			
	Albumin	Globulin	Total Protein	Albumin/globulin ratio
1	5.12 ± 1.02^{a}	1.64 ± 0.07^{a}	6.76 ± 1.50^{a}	3.12^{a}
2	5.08 ± 1.05^{a}	1.62 ± 0.08^{a}	6.70 ± 1.40^{a}	3.14^{a}
3	4.05 ± 1.03^{b}	1.60 ± 0.05^{a}	5.65 ± 1.60^{ab}	2.53^{b}
4	4.06 ± 1.04^{b}	1.62 ± 0.09^{a}	5.80 ± 1.30^{a}	2.51^{a}
5	4.05 ± 1.06^{b}	1.54 ± 0.04^{b}	5.60 ± 1.70^{ab}	2.63^{b}
6	3.98 ± 1.05^{c}	1.52 ± 0.02^{b}	5.51 ± 1.50^{b}	2.62^{c}
7	3.96 ± 1.03^{c}	1.50 ± 0.06^{b}	5.46 ± 1.40^{b}	2.64^{c}

Values are mean ± standard deviation. Values with different superscript letters per column are significantly different at $p<0.05$

The liver is the sole site for the synthesis of albumin, which makes up to approximately 60% of total serum protein concentration. Concentrations of albumin, globulin and total protein as presented in Table 3 indicate that the extracts slightly impaired the synthesis of albumin and subsequently serum total protein, hence causing no significant ($p>0.05$) difference in the concentrations of these parameters when compared with the control. The mean values of the results obtained for albumin, globulin and total protein with respect to protein status indicate a slight cellular toxicity of the aqueous extracts of *Citrullus colocynthis* on the protein status of the albino rats. This result agrees with the reports of [33] who worked on combined toxicity of *Cassia senna* and *Citrullus colocynthis* of albino rats. The damage is however not total as the extracts did not affect significantly ($p>0.05$) the concentrations of albumin, globulin and total protein.

This study revealed that oral administration of *Citrullus colocynthis* seed and pulp aqueous extracts at high concentrations have adverse effects on some important oxidative stress parameters but without direct tissue damage in the albino rats. We therefore conclude that *Citrullus colocynthis* seed and pulp aqueous extracts irrespective of its efficacy in the management of various diseases may not be completely "safe" and should not be used at high concentration and over a long period of time as patients taking the preparation might be predisposed to premature ageing due to stress imposed by the extracts.

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