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# Effect of Methanolic Extract of *Vernonia amygdalina* (Common Bitter Leaf) on Lipid Peroxidation and Antioxidant Enzymes in Rats Exposed to Cycasin

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**Abstract:** This study investigated the effect of a methanolic extract of *Vernonia amygdalina* (VA) on lipid peroxidation and antioxidant status of the colon of rats maintained on a normal diet containing 5% *Cycas revoluta* (cycads). Fifty male Wistar albino rats were randomly assigned into five groups of ten experimental animals in a study that lasted for six weeks. One control group was maintained on a normal diet only while another group was fed a normal diet containing 5% cycads. The other three groups were maintained on the normal diet and 5% cycads and orally fed 200 mg VA/kg body weight for 1, 5 or 6 weeks. The results obtained revealed that the level of malondialdehyde (an index of lipid peroxidation) was significantly elevated ($p<0.05$) in rats exposed to cycads only compared with the control. However, oral administration of VA in conjunction with exposure to cycads appeared to reduce the extent of lipid peroxidation to values that are not significantly ($p>0.05$) different from those of the control. The activity of Superoxide Dismutase (SOD) was significantly reduced ($p<0.05$) in the experimental animals fed cycads compared with the controls. Oral administration of VA seemed to counteract the effect of cycads on SOD in the colon as no significant difference ($p>0.05$) was observed in rats fed VA compared with the controls. The results of this study suggest that methanolic extract of VA may mitigate the biochemical consequences of cycasin-induced lipid peroxidation in the colon of rats.

**Key words:** *Vernonia amygdalina*, malondialdehyde, superoxide dismutase, cycasin, normal diet

## INTRODUCTION

Colorectal cancer which is also known as colon cancer or large bowel cancer, includes cancerous growth in the colon, rectum and appendix of the gastrointestinal tract (Fang and Richardson, 2005). This cancer has been reported to be the second most common cause of cancer deaths among men in the US in 2010 and it is said to be on the increase in every country of the world (Das *et al*., 2009; Jemal *et al*., 2010). Socioeconomic and dietary factors have been suggested to account for the rising cases of colorectal cancers in developing countries (Park and Conteas, 2010).

It has been strongly suggested that disruption of oxidative balance is one of the major biochemical features that occur during colorectal carcinogenesis (Skrzydlewska *et al*., 2005). It is also well documented that oral exposure of rodents to cycasin can lead to colorectal cancer (Eriyamremu *et al*., 1995, 2007). Cycasin is the primary procarcinogen in the nuts and leaves of *Cycas revoluta* and is metabolised to the consummate carcinogen by the microbial flora of the gastrointestinal tract (Rosenberg *et al*., 2009).

Several species of plants are routinely applied in traditional medicine as prophylactics or curative agents for many diseases in Africa (Farombi, 2003; Iwu, 1993). The chemotherapeutic value of traditional plants is partly predicated on the fact that numerous cancer chemotherapies, such as vincristine and vinblastin can be traced to botanicals as well as the reciprocal relationship between the consumption of plant products and different cancer risks (Kobayashi *et al*., 2002; Maoka *et al*., 2001; Opata and Izevbigie, 2006).

One of such plants with proven anti-cancer activity is the aqueous extract of *Vernonia amygdalina* (VA) which was first described and reported in 2003 (Izevbigie, 2003). *Vernonia amygdalina* (VA), commonly called bitterleaf, is widely cultivated in Africa and is reported to have a wide spectrum of medicinal applications including anti-diabetic, antimalarial and antihelmintic (Abosi and Raseroke, 2003; Farombi and Owoeye, 2011). The leaves of the plant are usually consumed in the diet as vegetables and it is also common practice for the roots to be chopped into bits, along with the roots of other plants such as *Azadirachta indica* and immersed in bottles of alcohol which is drunk for medical reasons in most homes in Africa.

There is very scanty information on the chemotherapeutic potential of extracts of VA on colorectal cancer. The present study is designed to examine the probable effects of a methanolic extract of VA on the oxidative-antioxidative balance in the colon of rats orally exposed to cycasin.

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## MATERIALS AND METHOD

**Experimental design:** Fifty male albino Wistar rats (150-180 g) were purchased from the Department of Pharmacology of the University of Benin and subjected to a two-week acclimatization period. They were subsequently assigned to five groups of ten animals each; the distribution was such that the variation in weight was not more than 0.5 g. The animals were housed in clear cages with mesh floor to prevent coprophagy. They were all maintained on a normal diet and deionized water *ad libitum*.

The first group served as the control and was maintained exclusively on the normal diet while the second group was maintained on a normal diet that was tainted with 5% *Cycas revoluta* for the entire duration of the study (six weeks). The third group was fed a normal diet and cycads for five weeks and then a daily dose of 200 mg $kg^{-1}$ body wt of the methanolic extract of *Vernonia amygdalina* (VA) for the remaining one week. The fourth group was exposed to the normal diet and a daily dose of 200 mg VA/kg body wt for five weeks before 5% cycads was introduced in the diet for one week. The fifth group was maintained on the normal diet containing 5% cycads and a daily dose of 200 mg VA/kg body wt for the entire six weeks that the experiment lasted.

At the end of the study period, the rats were fasted overnight and sacrificed by cervical dislocation. The colon (about 10 cm of the proximal end of the large intestine was excised and flushed repeatedly with ice-cold 0.9% sodium chloride solution. Thereafter, it was inverted and the mucosa scraped off with a glass slide and the resultant colonic tissue was homogenized and centrifuged at 5000 g for 10 min. The supernatant was subsequently used for the analysis of the lipid peroxidation, superoxide dismutase and catalase.

**Preparation of methanolic extract of *Vernonia amygdalina* (VA):** Eight hundred twenty gram of sun-dried and pulverized roots of VA was immersed in 5 L of methanol for five days to ensure sufficient extraction of the active components. The suspension was filtered and the solvent evaporated to dryness using a rotary evaporator. Five grams % of the residue was reconstituted in deionized water and used as stock VA solution from which volumes corresponding to 200 mg $kg^{-1}$ body were calculated and administered to the experimental animals as required.

**Biochemical assays:** Colonic lipid peroxidation was estimated by measuring the concentration of malondialdehyde according to the method of Hunter *et al.* (1963) as modified by Gutteridge and Wilkins (1980). The assay involved the determination of Thiobarbituric Acid Reactive Substances (TBARS). Values for TBARS are reported as Malondialdehyde (MDA), quantitated using a molar extinction coefficient of $1.56\times10^5$ M/cm and expressed as μmole MDA $g^{-1}$. Superoxide Dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) and the results expressed as Units/g tissue. The activity of catalase was estimated by the method described by Cohen *et al.* (1970) and the result expressed as μmoles/min/mg tissue.

**Statistical analysis:** The results were expressed as Mean±SEM. Differences between the groups were determined by one-way ANOVA. Statistically significant differences between means were determined by the Duncan's multiple range test (Sokal and Rohlf, 1969).

## RESULTS

Colonic lipid peroxidation, represented by the level of maondialdehyde, was reported to be significantly elevated ($p<0.05$) in rats maintained on diet containing cycads compared with the control (Fig. 1). Prolonged administration of the extract of *Vernonia amygdalina* (VA) seemed to reversed this increase as there was no significant difference ($p>0.05$) in the colonic malondialdehyde levels of rats exposed to VA for a minimum of 5 weeks compared with the control (Fig. 1).

Exposure of the experimental animals to 5% cycads without VA intervention appeared to significantly reduce ($p<0.05$) the activity of colonic Superoxide Dismutase (SOD) compared with the controls (Fig. 2). Administration of VA appeared to have been effective in restoring the activity of colonic SOD to levels that are not significantly different ($p>0.05$) from the controls (Fig. 2). Priming of the

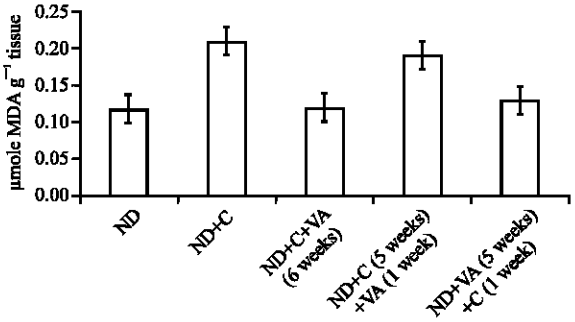


Fig. 1: Concentration of malondialdehyde (MDA) expressed as μmole MDA $g^{-1}$ colonic tissue (n = 10). ND is normal diet; C is 5% *Cycas revoluta*; VA is *Vernonia amygdalina* (200 mg $kg^{-1}$ b.wt.)

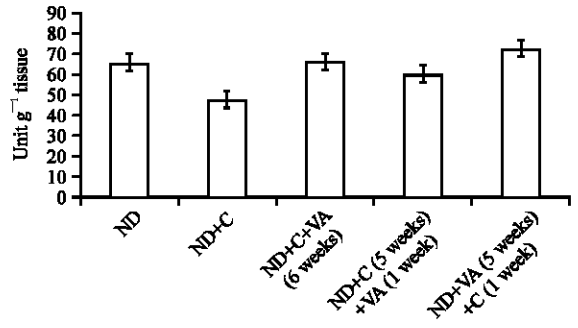


Fig. 2: Activity of superoxide dismutase (SOD) expressed as Units/g colonic tissue (n = 10). ND is normal diet; C is 5% *Cycas revoluta*; VA is *Vernonia amygdalina* (200 mg kg$^{-1}$ b.wt.). (1 Unit of enzyme activity is the amount of enzyme that is required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome)

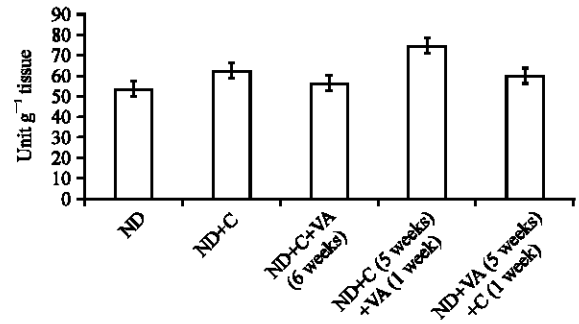


Fig. 3: Activity of catalase expressed as μmoles/min/mg colonic tissue (n = 10). ND is normal diet; C is 5% *Cycas revoluta*; VA is *Vernonia amygdalina* (200 mg kg$^{-1}$ b.wt.)

animals with VA for 5 weeks before exposure to cycads seemed to have elicited the most statistically significant increase ($p<0.05$) in the activity of colonic SOD compared with the controls (Fig. 2).

Treatment of the rats with cycads and extracts of VA did not result in any significant change ($p>0.05$) in the activity of colonic catalase (Fig. 3).

## DISCUSSION

It has been strongly suggested that colorectal carcinogenesis is associated with intense oxidative stress and the progression of the cancer is certain in the face of increased imbalance in the oxidative-antioxidative disorder (Skrzydlewska *et al*., 2005). The use of synthetic chemotherapeutic drugs has a major limitation of toxicity considering the damage that is done to several organs, especially the liver in the course of detoxifying the drugs. An attractive approach to mitigate this side effect involves the increasing application of natural plant products which are currently being investigated in several laboratories for their chemotherapeutic potentials. In this regard, the presence of beneficial phytochemicals in both the aqueous and organic extracts of *Vernonia amygdalina* (VA) has been reported to be effective in the management of some biochemical alterations in leukemic and breast cancers (Izevbigie, 2003).

Lipid peroxidation is mainly caused by the action of reactive oxygen species on polyunsaturated fatty acids which are integral structural components of biological membranes. In addition, the products of lipid peroxidation have been shown to interfere with the functional capabilities of cells considering their propensity to cause DNA cross-linkage, DNA breakage and DNA adducts (Mattagajasingh *et al*., 2008). *Cycas revoluta* and other members of the *Cycas* family have been shown to contain cycasin which has the ability to cause the formation of agents like azoxymethane which have free radical activity (Rosenberg *et al*., 2009). This may account for the elevated amount of malondialdehyde-an index of lipid peroxidation in the experimental animals exposed to cycads only compared with the controls.

Among the measures that are employed by cells to counteract the deleterious effects of lipid peroxidation is the alteration of the activity of antioxidant enzymes such as catalase, peroxidases and superoxide dismutase (Bhor *et al*., 2004). Non-enzymatic antioxidants such as flavoinoids and other secondary plant metabolites may also be administered to animal cells to augment the activities of the antioxidant enzymes (Eriyamremu *et al*., 2008). This may partly explain the observed amelioration of the effects of lipid peroxidation following oral exposure to the methanolic extract of VA (Fig. 1). This result is in consonance with the findings of similar works where carbon tetrachloride-induced hepatic lipid peroxidation was ameliorated by the methanolic extract of VA (Adesanoye and Farombi, 2010; Arhoghro *et al*., 2009). As VA contains high amounts of luteolin, luteolin 7-O-β-glucoside, luteolin 7-O-β-glucuronoside and phenolic antioxidants (Igile *et al*., 1994), it may in part account for the ability of VA to counteract lipid peroxidation.

Superoxide dismutase is a metalloenzyme that scavenges superoxide anions and exists as isoforms classified on the basis of their relative requirement for manganese, copper and zinc to maintain structural and functional integrity (Sasaki *et al*., 2000). The observed restoration of the activity of superoxide dismutase to

values within the normal range by the VA extract may be as a result of the established presence of flavonoids as well as micronutrients like zinc, manganese and copper in VA (Fig. 2) (Eyong *et al.* 2011). Factors that could impair the availability of these micronutrients such as dietary restrictions or chelators present in diets have long been reported to adversely affect the activity of the different isozymes of SOD (Harris, 1992).

Although the scope of this work did not include estimation of the activity of the different isozymes of SOD, it might not be out of place to speculate that the observed overall reduction of SOD activity in the absence of VA extract, as shown in Fig. 2, might be related to the presence of antinutrients in the cycad plant. This finding is consistent with the work of Eriyamremu *et al.* (2007) who, among other things, investigated the effect of different diet formulations containing cycads on the activity of SOD. Further studies will be required to ascertain the SOD isozymes that are most susceptible to the effects of cycads as well as their relative interaction with identified micronutrients in VA.

Another antioxidant enzyme that is known to be recruited by cells to check oxidative damage is catalase which catalyses the decomposition of hydrogen peroxide produced during the harmful oxidation process (Shinde *et al.*, 2012). This study did not record any change in catalase activity suggesting that this enzyme may not be among the first line of enzymatic antioxidant defence during the first weeks of exposure to cycasin (Fig. 3). This is in agreement with the earlier report from a similar work by Eriyamremu *et al.* (2007). The results presented in this study strongly suggest that the methanolic extract of *Vernonia amygdalina* could mitigate cycasin-induced oxidative damage in colonic tissues.

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