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INVIVO ANTIOXIDANT POTENTIALS OF VERNONIA AMYGDALINA AND GONGRONEMA LATIFOLIUM LEAF EXTRACTS ON DIABETIC INDUCED RATS

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ABSTRACT

Vernonia amygdalina (VA) *and Gongronema latifolium* (GL) are known to have antioxidant activities. The extent to which heat treatment will affect the antioxidant activities of these green vegetable leaves is not well known this work is aimed at comparing the activities 300mg/Kg bodyweight of raw and boiled extracts of VA and GL on the Superoxide dismutase (SOD) and Glutathione peroxidase (GSH) enzymes of rat liver. The control groups were given 5ml of saline for the 14days. The result showed that normal treated rats (NTR) had no significant difference ($p<0.05$) from normal treated control group. The alloxan diabetic induced rats showed a significant difference ($p<0.05$) in SOD activity between normal, the diabetic treated and control group. Glutathione had no significant difference ($p<0.05$) between the treated and control groups. The raw and boiled extracts showed no significant activities of Glutathione between them except is in the NTR where boiled GL had significant lower SOD activity. The result encourage short time heat treatment to reduce the anti-nutrient compounds and yet not affect the antioxidant activity of the leaves.

KEYWORDS: Glutathione Peroxidase, Gongronema Latifolium, Superoxide Dismutase and Vernonia Amygdalina

INTRODUCTION

Superoxide Dismutase (SOD) is an antioxidant naturally found in the body cells and is a part of a class of enzyme that catalyzed the dismutation (partitioning) of superoxide into oxygen and hydrogen peroxide. SOD is one of the major antioxidant enzymes that prevent the biological macromolecules from oxidative damage (Zhang *et al*., 2003). During normal metabolic processes, reactive oxygen species (ROS) such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen (O_2) are produced (Ananimous, 2015) and can cause cellular damage through oxidative stress (Alscher *et al.,* 2002). Superoxide Dismutase as an antioxidant defence metalloenzyme in the body overpowers the damaging reaction of superoxide and protects the cell from its toxicity; this is brought about by alternately adding or removing an electron from the superoxide molecules (Beyer *et al.,* 1991; John *et al.,* 2013). The reaction equation for the action of superoxide dismutase is shown as follows:

$$CU^{2+}\text{-}SOD + O^{2-} \_ CU + SOD + O_2$$

$$CU^{+} + SOD + O_2 + 2H^{+} \_ CU^{2+}\text{-}SOD + H_2O_2$$

Glutathione peroxidase also catalyzes the breakdown of hydrogen peroxides (HOOH) and lipid peroxide (ROOH) which easily become radicals (Ward law & Hampl 2007). Glutathione peroxidase (GSH) is often referred to as the body master antioxidant. It is composed of three areas cysteine, glycine and glutamate and can be found in virtually every cell as essential component of the body's natural defence system. The highest concentration is in the liver making it critical in the body's detoxification process. Glutathione depletion has been correlated with lower immune function and increased

venerability to infection due to the liver's reduced ability to detoxify. As the generation of free radicals exceeds the body's ability to neutralize and eliminate them, oxidative stress occurs. A primary function of glutathione is to alleviate this oxidative stress by recycling, which is catalyzed by glutathione disulfide reductase using NADPH to reconvert GSSG to GSH (Deponte, 2013). So these enzymes act as antioxidants with mechanism involving both free radical scavenging and metal chelating activities. The body is normally under a dynamic equilibrium between free radical generation and quenching. The physiological defence systems to counteract free radicals encompass endogenous enzyme systems, such as catalase, glutathione reductase and superoxide dismutase, as well as glutathione peroxidase are vital (Valko *et al.,* 2006). All these molecules have an antioxidant effect due to their ability to transform reactive oxygen species (ROS) into stable and harmless compounds or by scavenging both reactive oxygen species (ROS) and reactive nitrogen species (RNS) with a redox-based mechanism (Valko *et al.,*2006). The imbalances between ROS/RNS and antioxidants turns in favour of the former, (oxidative/nitrosative stress) may lead to such diseases like diabetes.

Diabetes mellitus was discovered to be caused by metabolic disorder, which refers to the way the human bodies use digested food for energy and growth (Ugochukwu and Babady 2003). When glucose is not made available to the cells it can cause death because cells and tissues will be starved. Physical injury or emotional disturbances that predispose the body to stress frequently appear as the initial cause of the disease.

The medicinal value of plants has assumed an important dimension because plants have been shown to contain not only minerals and primary metabolites but also diverse secondary metabolites many of which are antioxidants (Nwanjo, 2005). These antioxidants are free radical scavengers, exhibit antibacterial and antifungal properties and are proven preventives to cancer and metabolic diseases (Ho *et al*., 2012). Antioxidants have been shown to have beneficial effects on arthritis, diabetes, cardiovascular diseases including Ischemic heart disease, atherosclerosis, hypertension, thrombosis fibromyalgia and immune disorder. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis ischemic heart disease, cancer and Alzheimer's disease and in aging process (Eleyinmi, 2007; Jensen *et al*., 2008). The therapeutic effects of several plants and vegetables which are used in traditional medicine are usually attributed to the presence of antioxidant compounds (Alscher *et al*., 2002). Preference for plant based antioxidants relative to the synthetic ones due to safety concerns has inspired the widespread screening of plants for possible antioxidant properties. This has led to the isolation and characterization of diverse phytochemicals, the development and utilization of antioxidants of natural origin (Eleyinmi, 2007). Lotito and Frei (2006) noted that a profile of the chemical composition of a plant and knowledge of its antioxidant activity will give a fair estimate of its therapeutic potentials. The prevalence of degenerative diseases such as cancer, cataracts and heart disease is lower in Asians due to their predominant vegetarian habits with ample use of fresh fruits and vegetables. Green leafy vegetables (GLV) may therefore be considered as a cheap natural fortificants for both iron, nutrients, phytochemicals and antioxidants. While the body produces antioxidant, nutrients from food also keep free radicals in check especially when the body is subjected to environmental stress (Uchimura 1999). These antioxidant compounds can be extracted from vegetables and taken as a supplement twice daily with normal meals and this increases plasma antioxidant levels and improve oxidative defence mechanism. Fresh vegetable drink or extract in a diet helps to accelerate and enhance the process of restoring chemically starved body tissues by maintaining a balance in the potassium/sodium imbalance in the body. Potassium is an important nerve conductor and it acts as a catalyst for many enzymes. It is an important alkalizing agent that maintains the acid/alkaline balance in the blood and tissues. In this way good health is restored, ageing process is deferred and the onset of diabetes prevented.

Among the vegetables popularly consumed in Southern Nigeria are *Vernonia amygdalina* and *Gongronema latifolium. Vernonia amygdalina* (VA) (*Onugbu*---Igbo;*Ewuro ejijij*---Yoruba; *Shiwaka* or *Chusa-diki* Hausa) is a perennial plant that belongs to the Compositae family (Akpaso *et al*., 2011) and is planted in homes in villages as a fence post and pot herb. It grows in many zones in Africa and is drought tolerant (Bonsi *et* al*.,* 1995; Akpaso *et al.,* 2011). It contains bitter pigment necessitating much squeezing, washing, several soakings in water or boiling before cooking and consumption (Oboh *et al.,* 2005; Obeta and Ani, 2015). It is cherished in Nigeria for its distinctive flavour on the dishes in which it is a component. All parts of *Vernonia amygdalina* (VA) have been used as folk medicine for treatment of wounds, malaria and diabetes. *Gongronema latifolium* (*Utazi* –Igbo *Arokeke*--Yoruba) is a tropical rain forest plant from Ascelepindaceae family. *Gongronema latifolium* (GL) is also known to have antioxidant, hypoglycemic, hypolipidemic and anti-inflammatory properties (Morebise *et al.,* 2002, Ugochukwu and Babady 2002). *Vernonia amygdalina* and *Gongronema latifolium* have been established to possess antioxidative potentials but traditionally undergo some processing to reduce the anti-nutrients, improve palatability and keeping quality. The extents to which the *in-vivo* antioxidant properties of VA and GL vegetables are affected by such heat processing methods have not been investigated.

MATERIALS AND METHODS

Sources of Material

Fresh leaves of *V. amygdalina* and *G. latifolium* were bought from Nsukka main market and were authenticated at the Department of Botany, University of Nigeria, Nsukka. Albino rats were procured from Department of Veterinary Medicine, University of Nigeria, Nsukka. Chemicals which were purchased were of analytical grade.

Sample Preparation

Vernonia amygdalina and *Gongronema latifolium* twigs were destalked, washed and drained. The washed leaves were each divided into two (2) portions and subjected to different processing treatments.

Treatment 1: Fresh leaves of *V. amygdalina* (VA) (bitter leaf) and *G. latifolium* (GL) (Utazi) were prepared into aqueous extracts, using the method of Ugochukwu and Cobourne (2003) with modifications. Fresh leaves (1000g) VA and GL (1000g) were separately homogenized with de-ionized water (2000ml) using a kitchen blender (Philips, England / China HR 1727,) for 15mins in each batch and extracted for 6 hours. Each homogenized sample was first filtered with cheese cloth and the filtrate was again filtered using Whatman No.1 filter paper using a pressure vacuum pump (locally made). Each filtrate was centrifuged at 10,000×g for 10mins at 4^0C (Falcon 6/300R, England, CEK- 243-010J) and concentrated to half the volume (1000ml) using rotary evaporator (Diagonal coil, China, RE 300 P) at 80^0C. Each sample concentrate was freeze dried (Edward freeze dryer, England, Modulyo) to a yield of 6.50g and 6.25g for VA and GL respectively and designated as raw aqueous extract (V. amygdalina raw (VAR) and G. latifolium raw (GLR)).

Treatment 2: Fresh leaves of VA (1000g) and GL (1000g) was each boiled in 2000ml of de-ionized water for 5mins. After boiling each sample was homogenised, extracted as described in treatment 1 and freeze dried (Edward freeze dryer, England, Modulyo). Each sample gave a yield of 5.60g and 5.50g for VA and GL respectively and designated as boiled aqueous extract. Treatment 1 and 2 were used for the animal study only.

CLASSIFICATION OF RATS

The rats were divided into 2 classes as shown in Table 1. Each class was divided into five groups and each group

had five rats. Each group (1 to 4) received 300mg of extract per kilogram body weight while the control (group 5) received 1ml of saline daily for 14 days. Animals in all the groups had free access to water and feed throughout the 14 days of study.

Class 1: Normal treated rats (NTR).

Class 2: Diabetic induced rats (DIR)

Table 1: Classification of Rats according to Treatments

	CLASS 1		CLASS 2	
	Normal Treated Rats (NTR)		Diabetes Induced Rats (DIR)	
Groups	No. of Rats	Extract Given	No. of Rats	Extract Given
1	5	VAR	5	VAR
2	5	VAB	5	VAB
3	5	GLR	5	GLR
4	5	GLB	5	GLB
5	5	No extract	5	No extract
Total	25		25	

VAR – *V. amygdalina* raw, VAB – *V. amygdalina* boiled

GLR – *G latifolium* raw, GLB – *G. latifolium* boiled

Induction of Diabetes on the Rats

After 7 days of acclimatization the 30 rats for diabetic class were subjected to 14 hours fast. Diabetes was induced with freshly prepared alloxan (sigma St Louis) at a dose of 140mg per kg body weight intra-peritorially. They were confirmed diabetic after three days by testing their blood glucose. Twenty five rats each were randomly selected from normal fed class and diabetes induced class and used for animal study.

ANTIOXIDANT ACTIVITIES OF THE TISSUE ENZYMES

Superoxide Dismutase

The quantitative *in vivo* determination of superoxide dismutase in the liver tissue serum was done using the method of Ukeda *et al.,* (1997). Xanthine and Xanthine Oxidase (XOD) were used to generate superoxide radicals that reacted with 2 (-4-iodophenyl 1-3 -(4 – Nitrophenol) -5 phenyltetrazolium chloride (INT) to form a red formazen dye. The SOD activity measured the degree of inhibition of the reaction using RanSOD superoxide dismutase assay kit (Randox laboratories Ltd. (UK). One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Xanthine ___XOD_____Uric acid + O_2

INT _____O_2^-_____Formazan dye

The standard solution was used for calibration after dilution and read at 505nm after 30sec for initial absorbance (A_1) and after 3min for final absorbance.

$A_2 - A_1 = \Delta A$/min of standard or sample.

3

$100 - \frac{(\Delta A \text{ std/min} \times 100)}{} = \%$ inhibition.

(ΔA SI/min

100 - (ΔA sample/min × 100) = % inhibition.

(ΔA SI/min

(SI = rate of uninhibited reaction = 100%)

Glutathione Peroxidase

The quantitative *in-vivo* determination of Glutathione Peroxidase in the liver tissue was done using the method of Paglia and Valentine (1967). Ransel Glutathione Peroxidase assay kit (Randox laboratories Ltd UK) was used.

The Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydro peroxide The oxidised Glutathione (GSSG) is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was measured at 340nm. The Glutathione peroxidase concentration was calculated thus:

Glutathione peroxidase concentration (U/L) = 8412 × ΔA 340nm/min.

RESULT AND DISCUSSIONS

Effect of Extracts on Superoxide Dismutase (SOD) Activity of Rats

Figure 1 shows the effect of extracts on the liver tissue superoxide dismutase (SOD) in normal treated rats (NTR) and diabetic induced rats (DIR) groups. The SOD activity in the CTRL group of NTR was higher but not significantly different ($p>0.05$) from the treated groups. This minor disparity in activity may be suggesting that the extract administered to NTR have created initial oxidative stress which lowered the enzyme activity. This observation did not agree with Ho *et al*., (2012) who reported increase in SOD activity in the different organs of mice when compared with control. The SOD activity in the diabetic rats treated with VAR, VAB, GLR and GLB were lower than the SOD activity in NTR groups but higher ($P<0.05$) than the activity of the CTRL group of diabetic rats. This observation agrees with the report of Nwanjo *et al*., (2006) who noted that untreated diabetic rats were subjected to oxidative stress and this was exhibited by a significantly low SOD activity.

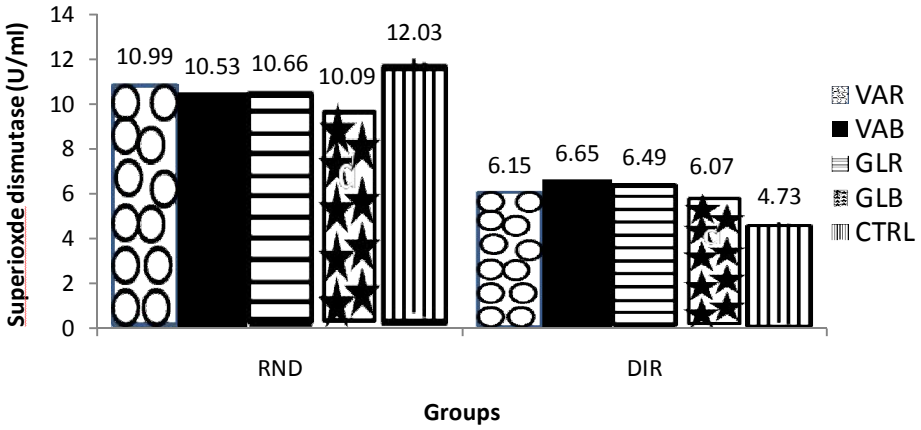

Figure 1: Effect of Extracts on Superoxide Dismutase (SOD) Activity of Rats

Values are means of SOD value for 5 rats in each group ± standard deviation

VAR =rats fed raw VA, VAB = rats fed boiled VA, GLR =rats fed raw GL, GLB =rats fed boiled GL and CTRL =rat used as control

NTR = normal treated rats, DIR = Diabetic induced rats,

VA *Vernonia amygdalina and* GL *Gongronema latifolium.*

The body respond to the diabetic stress by increasing the hormone production which will lead to release of more glucose to the bloodstream. The rise in blood glucose will create more stress thereby lowering the SOD activity. This response of the body leads to initial increase in SOD activity at the onset of diabetes due to the production of superoxide anions but the administration of extracts reduced further production of ROS with resulting decrease in SOD activity (Cho *et al*., 2002). There was no significant difference ($p>0.05$) between the activities of raw and boiled VA and GL extracts in both NTR and DIR. This suggest that boiling for 5min did not drastically affect the antioxidant properties of the leaves rather it reduced the anti-nutrients in the vegetables and aid bioavailability of other nutrients such as minerals (Cu, Zn, Mn and Ca) which are co-enzymes of SOD (Obeta and Ani, 2015)

Effect of Extract Treatment on Glutathione Peroxidase Enzyme on Normal Treated Rats (NTR) and Diabetes Induced Rats (DIR)

Figure 2 showed the effect of extracts on Glutathione peroxidase on normal treated rats (NRT) and diabetes induced rats (DIR). The CTRL (without extract) group of NTR had the highest enzyme activity (4518.3 U/L) which differed ($p<0.05$) from the activity of extract treated rats (2803.7 U/L, 2608.3 U/L, 3435.7 U/L and 3119.7 U/L respectively).

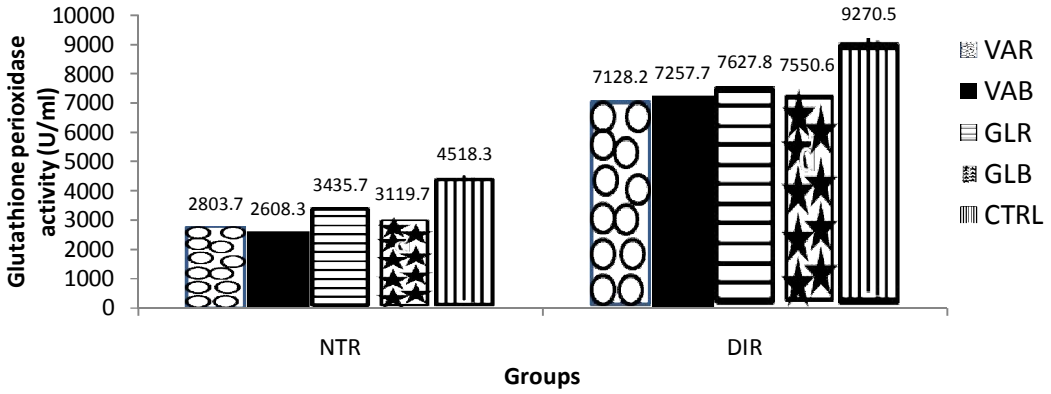

Figure 2: Effect of Extracts Treatment on Glutathione Peroxidase Activity

Values are means of glutathione peroxidase value for 5 rats in each group ± standard deviation VAR =rats fed raw VA, VAB = rats fed boiled VA, GLR =rats fed raw GL, GLB =rats fed boiled GL and CTRL =rat used as control NTR = Normal treated rats; DIR = Diabetes induced rats, VA = *Vernonia amygdalina and* GL = *Gongronema latifolium.*

This could mean that administration of extract created an initial stress on non-diseased rats which lowered the activity of the enzyme. The diabetic had a very high enzyme activity in all the groups. Glutathione peroxidase activity in the CTRL group of DIR was higher but did not differ significantly ($p>0.05$) from the glutathione peroxidase activity of the DIR treated groups. This observation conforms with the report of Ugochukwu and Cobourne (2003) that showed a non-

significant higher glutathione peroxidase enzyme activity in the diabetic control group than in the rats treated with *Gongronema latifolium*. The disease condition increased the production of glutathione peroxidase to enable the animals cope with the stress.

From the result the blood glucose was controlled within the fourteen days of extract administration showing the rate at which the VA and GL extracts can be metabolized in the blood plasma and liver organ. This rate of absorption according to Ho *et al.,* 2012 was much higher than the absorption of vitamin C indicating the antioxidant capacity of *V. amygdalina over Vitamin C.*

CONCLUSIONS

From the study *V. amygdalina* and *G. latifolium* showed higher Superoxide Dismutase activity in diabetic treated groups than the control. Glutathionone peroxidase did not differ significantly between the treated and untreated DIR indicating a higher production of glutathione peroxidase during disease condition for the body to cope with the diabetic stress. A short time heat treatment of these vegetables to reduce the anti-nutrients and improve the nutritive value did not affect the antioxidant activities of the liver enzymes. Further study on the effect of longer time heat treatment on the vegetables for managing diabetes in rat is necessary.

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