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An Evaluation of the Antioxidant and Blood Lead Lowering Effect of Vernonia Amygdalina Aqueous Extract on Lead Acetate Induced Oxidative Stress in Wistar Rats

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Abstract

The research was set to assess the antioxidant effect of Vernonia amygdalina aqueous extract on lead acetate poisoned Wistar rats. Blood lead determination and assessment of antioxidant status was carried out on five groups of rats, each group consisting of eight rats. Group I were unchallenged and unsupplemented while groups II-V were poisoned with a 150ppm lead acetate in water for 14 days. Thereafter, group II remained unsupplemented with V.Amygdalina extract, while groups III, IV and V were supplemented with 25, 50 and 75mg/kg body weight of extract for 10days. A statistically significant difference was observed when the SOD and CAT activities in groups II and III were compared to that of the control group. Similarly, significant variations were observed when the serum TBARS and blood lead levels in groups II, III and IV were compared with that of control. These values reveal a fairly proportional increase in antioxidant but reduced blood lead levels in response to increasing dosage of extract. Thus, V. amygdalina aqueous extract was able to restore the antioxidant parameters and blood lead levels back to normal. It appears to possess a dose dependent antioxidant and toxic lead excreting property. These activities may be due to the presence of phytochemical antioxidant and lead removing factor(s)/principle(s) which, if properly harnessed, can be used in the treatment of oxidant challenges and lead contamination.

Keywords: Antioxidant effect, Lead, V.amygdalina

1. Introduction

Physiologically, the rate of reactive oxygen species (ROS) generation is counterbalanced by the rate of elimination. In ideal conditions, ROS generation is tightly regulated by endogenous cellular antioxidants, which include superoxide dismutase (SOD), catalase, thioredoxin, glutathione, and antioxidant vitamins [1]. In contrast, under pathological conditions, ROS are produced in concentrations that cannot be matched by protective antioxidant mechanisms in the cells. When this happens, a state of oxidative stress ensues [2]. Vernonia amygdalina is a Nigerian plant, the extract of which have been reported to possess hepatoprotective properties[3], chemotherapeutic effects against breast cancer cells [4], antibacterial effect [5]. Oyagbemi and Adejimi [6] and Adedapo *et al*.[7] reported its anticoccicidal and antihelminthic activities respectively, While Odewusi and Tope-ajayi [8] explained its diuretic property. It should be noted that the presence of phytochemicals is the rationale underlying the inclusion of plant parts in various native medicinal preparations [9]. This research work is therefore designed to assess and ascertain the effect of aqueous extract of Vernonia amygdalina on lead acetate induced oxidant challenge

2. Materials and Methods

2.1 Sources of materials

Freshly harvested V. Amygdalina leaves and young shoots were collected from forest lands adjoining Achievers University, Owo, Ondo State, Nigeria. The botanists in the Plant Science Unit of the Biological Sciences Department identified the plant.

2.2 Bitter leaf extraction

The sample leaves were washed with water, dried at room temperature for two and a half weeks and grounded into powder using a blender. 400g of the ground leaf was dissolved in 1litre of water and placed in an oven at 40^{o}C for 3 hours. The extract solution was then obtained by filtering. The extract obtained was condensed and later dried at 45^{o}C for 1 week in an oven.

2.3 Preparation of standard *V.amygdalina* leaf extracts solution

About 200mg of the bitter leaf extract was dissolved in 5ml of normal saline at a temperature of 50oC.This was then made up to 10ml with distilled water to make a 200mg/10ml(20mg/ml) bitter leaf extract stock solution. 2.5mg/ml, 5mg/ml and 7.5mg/ml bitter leaf extract solution was prepared by appropriate dilution of stock extract solution with sterile normal saline.

2.4 Laboratory animals

Forty (40) Wistar rats (age 10 to 12 weeks, mean weight 105 grams) were used in this research. These animals were reared under standard animal management practices throughout the duration of research. They were quarantined in deep litter cages with absorbent materials to absorb moisture (8 rats per cage) 7 days before the commencement of lead acetate administration, VA supplementation, and also throughout the period of the research. The animals were fed with chow pellets from CAPS feeds mill and water ad libitum in standard quantity all through.

2.5 Grouping of experimental animals and dosage of extract

Wistar rats were divided into five treatment groups (I-V) of eight (8) rats each. All rats in group I was unchallenged with VA extract as well as unsupplemented with lead acetate, these served as control. Each rat in groups II, 111 and V were challenged orally with 150ppm lead acetate in drinking water for 2weeks. Groups II, III and IV and V were thereafter supplemented through oral administration of 25, 50 and 75mg/kg body weight of V amygdalina extract in saline respectively, for 10 days, so that the effect observed, if due to treatment, should likely show a multiplying effect.

2.6 Blood samples

Before blood collection, rats were anaesthetized by cervical dislocation. Blood samples were collected by cardiac puncture. Blood samples were placed in heparinized tubes for superoxide dismutase (SOD) and catalase (CAT) and blood Lead estimations. About 1.0 ml was dispensed into a plain tube for serum Thiobarbituric acid reactive substances (TBARS) determination. It

was allowed to clot. Samples were centrifuged as soon as possible at 12000 rpm for 10 min at 4 °C. Serum samples for TBARS were stored at -70 °C until the time of analysis. Erythrocytes were washed three times in normal saline and were heamolysed by dilution in water and stored at −20 °C until used for measurement of SOD and CAT activities

2.7 Determination of Biochemical parameters .

TBARS as a marker for lipid peroxidation and therefore oxidative stress, was determined using the Thiobarbituric acid (TBA) method of Okhawa et al. [10] .

Erythrocyte SOD activity was determined according to the method of Marklund and Marklund[11] which is based on the ability of SOD to inhibit auto-oxidation of pyrogallol. One unit of SOD being the activity of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in the assay mixture. The results are expressed in units/gHb.

Erythrocyte CAT activity was determined according to the method of Aebi [12], which is based on the decomposition of H202 by CAT. Enzyme activity was expressed as the first order kinetic constant (K) of the rate of disappearance of H202 for 15s as measured by decrease in absorbance at 240nm. Results were expressed as K/gHb.

Blood Lead level was determined by atomic absorption spectrometry (AAS) as explained by Robinson (1960) [13] .

2.8 Statistical analysis

All values are expressed as mean ± standard deviation. Statistical analysis was performed using the student's t test. Differences were considered to be significant or otherwise at $p<0.05$.

3. Results and discussions

Antioxidant parameters were measured to assess the relationship between toxic lead contamination and selected antioxidant parameters in lead acetate poisoned rats, and the effect of V.Amygdalina supplementation on the resultant oxidant stress. V amygdalina has been known to contain carbohydrates, saponins, alkaloids, tannins, proteins and steroids, flavonoids and glycosides [14]. The presence of phytochemicals in plant parts have been known to confer antioxidant and other properties on them as stated by heborn [15], hence their inclusion in folk medicine. TBARS was measured to reflect the extent of lipid peroxidation and was found to be significantly higher when group II, III and IV were compared with the unchallenged unsupplemented group (control) but was found to be insignificantly increased in group IV giving an indication that V. amygdalina supplementation at a dose of 75mg/kg body weight was able to scavenge reactive oxygen species, hence lipid peroxidation (Table 1, Fig.3). It also implies that as much as lead contamination exacerbates lipid peroxidation, its effects could be reversed upon the administration of V amygdalina extract. Superoxide dismutase (SOD) is an antioxidant enzyme. Among the known antioxidant proteins, (SOD) is thought to play a central role because of its ability to scavenge superoxide anions, the primary ROS generated from molecular oxygen in cells[16], that it has been thought of as the body's first line of defense against oxidative stress [17]. In this research, there is a significant reduction in SOD activity when both groups ii and iii were compared with control (Tables 1, Fig. 1), making it evident that the administration of VA extract at doses of 50 and 75mg/kg body weight for 10 days was effective enough in restoring SOD activity in lead poisoned rats. This implies that the administered VA extract was instrumental to the restoration of the antioxidant SOD activity. The reason for a diminished SOD activity consequent to lead contamination could not be farfetched; SOD is a metalloenzyme, upon contamination with any toxic heavy metal, they are likely to replace Cu, Mn and Zn as the metal constituents of SOD [18]. When this happens, the enzyme activity is diminished, therefore the level of the superoxide which should have been scavenged increases, leading to, among other conditions, accelerated lipid and protein peroxidation. Catalase (CAT) is the enzyme that breaks down the hydrogen peroxide produced as a result of superoxide dismutation. In this research the pattern of erythrocyte CAT activity follows that of SOD, giving the hint that the activity of both enzyme in blood correlate (Tables 1, Fig. 2).

Table 1.showing significance testing of all groups versus control

Variables	students t (P value)			
	Group II	Group III	Group IV	Group V
SOD (U/gHb)	7.3955(.000) **	5.2467(.000) **	1.9233(.075)	0.3368(.741)
CAT (U/gHb)	5.8831(.000) **	3.5706(.003)*	1.5924(.134)	0.7184(.484)
TBARS (nMol/L)	7.5388(.000) **	6.3798(.000) **	2.2724(.039)*	1.4932(.158)
Blood Pb(µg/dl)	26.6792(.000)**	21.0678(.000)**	10.6976(.000)**	0.2593(.799)

**= significant at P<0.001 *=significant at P<0.05

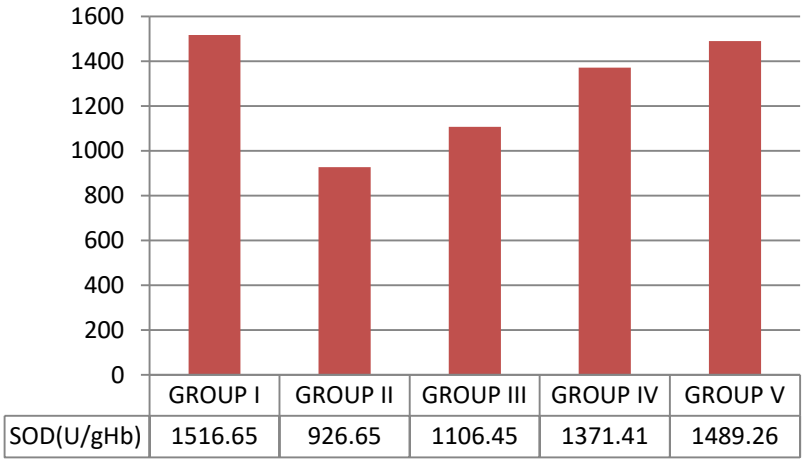

Figure 1. Erythrocyte SOD activity in all groups

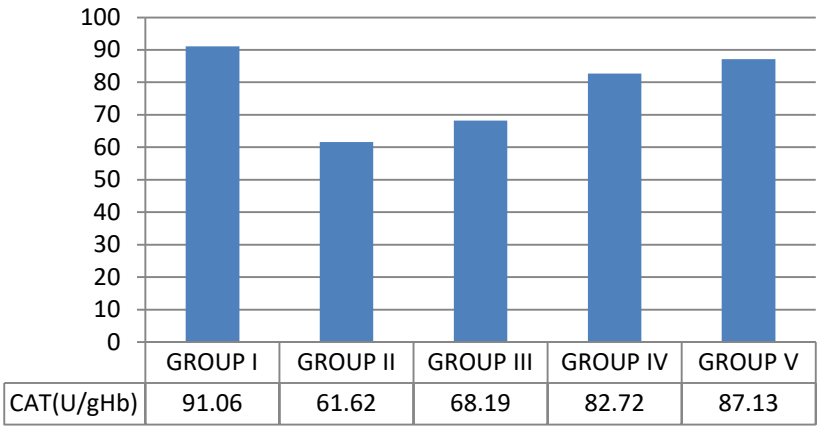

Figure 2. Erythrocyte CAT activity in all groups

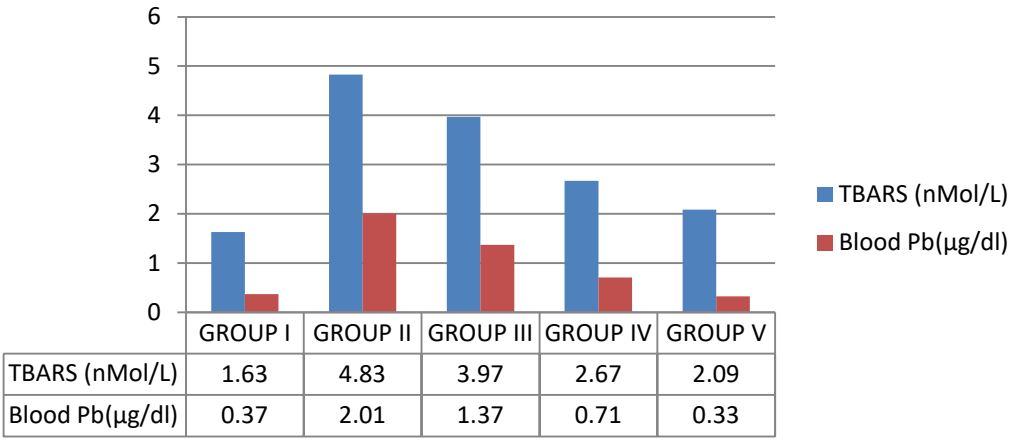

Figure 3. Serum TBARS and blood Lead levels in all groups

Blood lead levels was also determined in all groups under examination to assess the extent of lead contamination in relation to its attendant effects on antioxidant status parameters. Blood lead levels was found to be extremely higher in groups II, III and IV but insignificantly lower in group V rats, implying that V. amygdalina extract at a dose of 75mg/kg body weight was potent enough to bring down the blood lead content from a 2.01µg/dl high to a .33µg/dl low in ten days (tables 1 and 2, Fig.3). It is the thought of the investigator in this research that the mode of blood lead lowering effects could be similar that of chelating agents in clinical use. It is also the thought of the investigators in this research that when Pb chelation has being achieved, and the clinical cause of oxidant challenge removed, the antioxidant parameter levels would tend towards normalcy with time. This research found out that a state of oxidant challenge follows lead contamination. Lead contamination and its oxidative end point may, as a matter of fact, aggravate an already existing state of oxidative stress related condition such as diabetes and atherosclerosis hence, hypertension [19, 20]. Various works have linked an enhanced oxidative stress condition as observed in Lead poisoned rats to increased free radical production, lipid peroxidation and diminished antioxidant status [19]. This research ventured into and found that administration of vernonia amygdalina crude extract has a dose dependent antioxidant and blood lead lowering effect in rats. The phytochemicals responsible for

these properties could be isolated purified, characterized and may be administered in the treatment of disorders associated with oxidative stress and or lead contamination. An advantage of using herbal mixes in clinical practice is that they produce very little acute toxicity and in general, they can be considered as mild and good drugs, in comparison to conventional drugs used nowadays in the therapeutics [21, 22].

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