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Protective effect of ethanolic extract of *Grewia carpinifolia* leaves on vanadium induced toxicity

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ABSTRACT:

Pentavalent vanadium (metavanadate salt) toxicity is a challenging environmental hazard that affects living organisms. Studies have shown that plants play important roles in protecting against heavy metal toxicity. This study was designed to evaluate the protective activity of ethanolic extracts of *Grewia carpinifolia* leaves following vanadium toxicity. Twenty-five male Swiss mice were randomly divided into five groups (A–E) of five rats each. Group A rats served as control and were given distilled water, Group B was administered with sodium metavanadate and a known antioxidant agent; α-tocopherol, Groups C and D were administered with sodium metavanadate and ethanolic extract of *Grewiacarpinifolia* leaves orally at 100 and 200 mg/kg body weight respectively while Group E was administered with only sodium metavanadate. After a daily single oral dosing for seven days, changes in behaviour, haematology and serum biochemistry parameters were analysed. Sodium metavanadate caused a significant decrease ($p \leq 0.05$) in haematocrit levels, haemoglobin (Hb) concentration, white blood cell count, neutrophil count and serum cholesterol level. A significant ($p \leq 0.05$) lymphocytosis was also observed in the group administered with sodium metavanadate alone. *G.carpinifolia* extract given concomitantly with sodium metavanadate was able to restore PCV, Hb concentrations and serum total protein to levels comparable with the control and standard groups. *Grewia carpinifolia* also significantly reduced the elevated serum levels of AST and ALT after vanadium induced hepatotoxicity. Our findings suggest that *G. carpinifolia* extract protected against the toxicity induced by vanadium; the plant extract at 200 mg/kg however appear to offer a better protection.

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1. INTRODUCTION

Vanadium is a heavy transition metal (Habib and Ibrahim, 2011), which has been recognized to be acutely toxic by most routes of introduction following environmental exposure in large doses. Disposition of vanadium in specific tissues may be involved in the pathogenesis of certain neurological disorders and cardiovascular diseases (Venkataraman and Sudha, 2005). Human activities such as combustion of fossil fuels (Aragón and Altamirano-Lozano, 2001), inhalation in the vicinity of metallurgical plants in addition to oil exploratory activities as seen in the Niger Delta region of Nigeria have further led to environment exposure to vanadium. Consequently, this has impacted negatively on the aquatic and terrestrial habitats in this region which represents about 12% of Nigeria's total surface area with over 28 million inhabitants (Federal Government of Nigeria, 1991). Vanadium compounds are acutely toxic by most routes of introduction following environmental exposure in most species. In general, the toxicity of vanadium compounds is linked to its oxidation state or valency, which increases with the oxidation state **(**Erdmann *et al.* 1984; Venkataraman and Sudha, 2005) Vanadium within the cells has predominantly an oxidation state of +4 as a result of reactions with intracellular antioxidants (Aureliano and Gândara, 2005; Kordowiak and Holko, 2009). Furthermore, vanadium compounds in the +4 oxidation state are oxidized by atmospheric oxygen to the +5 oxidation state with accompanying emission of a superoxide anion radical and generation of a hydroxyl radical via a Fenton-like reaction (Cuesta *et al.,* 2011). Following

reduction with NADPH, the reaction may proceed with generation of hydrogen peroxide (Cuesta *et al.,* 2011). Vanadium has been shown to induce various toxicities via these effects of reactive oxygen species (ROS) generation (Obianime*et al.,* 2009; Olopade and Connor, 2011). For example, it has been investigated that vanadium compounds induce ROS generation in the brain thus contributing to degeneration of dopaminergic neuronal cells of the substantianigra, which in turn may lead to Parkinson's disease (AfesehNgwa*et al.,* 2009; Cuesta *et al.,* 2011). Vanadium could also damage genetic material; induce hematotoxicity, immune toxicity, hepatotoxicity, lung toxicity, neurotoxicity and reproductive toxicity (Shi *et al.,* 1996; Yang *et al.,* 2004; Soares*et al.,* 2008; Hosseini*et al.,* 2012; Altamirano-Lozano *et al,* 2014). Therefore, reduction of +5 forms to +4 forms represents an effective means of reducing the impact of vanadium on a living system (Galli*et al.,* 1991). Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, nuts, seeds, leaves, roots and barks (Narayanaswamy and Balakrishna, 2011; Sharma *et al.,* 2013) Several herbal products had been reported to mitigate oxidative stress due to ROS and ameliorate the effects of heavy metals (Dailiah and Padmalatha, 2012; Ola-Davies *et al.,* 2013). Several members of the genus *Grewia* have been documented to possess antibacterial, analgesics, antioxidant properties (Ahamed*et al*., 2007; Goyal, 2012). *Grewia carpinifolia* is well distributed in the warmer parts of the world. *G. carpinifolia* is used in washing hair to remove and prevent lice as well as in treatment of skin lesions amongst others (Goyal, 2012.) In view of the fact that antioxidants plants have been known to protect against the deleterious effects of heavy metals in living organism, this study was therefore embarked upon to ascertain this claim. This study was carried out to investigate the protective effects of *Grewia carpinifolia* following hematotoxicity and hepatotoxicity induced by vanadium in the mice. The findings from this work may add to the overall value of the medicinal potential of this plant.

2. MATERIALS AND METHODS

2.1. Plant Material and Authentication

Leaves of *Grewia carpinifolia*were collected from the Botanical Garden of the University of Ibadan. The identification and authentication was carried out at the Forestry Research Institute of Nigeria (FRIN) where herbarium specimen (voucher number FHI 109693) was deposited.

2.2. Experimental Animals

Twenty five male mice were acquired and kept at the Laboratory Animal House, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. The animals were housed under standard conditions of temperature (25 ± 2°C) and light, (approximately 12/12 h light-dark cycle), fed on standard diet and given water *ad libitum.* The animals were acclimatized to laboratory conditions for two weeks before the commencement of the experiment.

2.3. Extract Preparation and Experimental Protocol

The dried plant material was grinded to a coarse powdered form and kept for defatting with petroleum ether and extraction was carried out by cold maceration of 1500 g of the coarse powder with 7.5L of 100% v/v ethanol for 72 h, with constant shaking using the GFL shaker (no. 3017GBh, Germany). The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated to dryness *in vacuo*at 40°C using rotary evaporator. Aliquot portions of the extract were weighed and dissolved in 2% tween 20 for use in this study.

Mice weighing between 40-60 g were randomly divided into 5 groups (A-E) of 5 mice each and the treatment protocol was for seven consecutive days in a single dose as follows;

Group A- Control rats; given distilled water throughout the study period

Group B-Standard group; received sodium metavanadate at 3mg/kg (i/p) and vitamin E at 50mg orally

Group C-sodium metavanadate at 3mg/kg (i/p) and ethanolic extract of *Grewiacarpinifolia* leaf orally at 100 mg/kg

Group D- sodium metavanadate at 3mg/kg (i/p) and ethanolic extract of *Grewiacarpinifolia* leaf orally at 200 mg/kg

Group E-sodium metavanadate at 3mg/kg (i/p) only

Blood collection

On the eight day of the study, blood was drawn from each of the animal by cardiac puncture for hematology and serum biochemistry.

Hematology

Hematological parameters including Packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by an automatic analyzer (BC-3000 Plus Auto

Hematology Analyzer, Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China). Leukocyte differential counts were also determined.

Serum biochemistry

The blood collected was allowed to clot and was centrifuged at a speed of 3500 rpm for 10 minutes to obtain serum. The serum was stored at –20 °C until tested. Serum biochemical parameters were analyzed: Aspartate aminotransferase (AST) (Reitman and Frankel, 1957), alanine aminotransferase (ALT) (Reitman and Frankel, 1957). Total protein, albumin, blood urea nitrogen (BUN) using RANDOX® laboratory reagent kits (RANDOX® Laboratories Ltd., Ardmore, United Kingdom), globulin was obtained from the difference between total protein and albumin. Cholesterol (TC) and triglycerides (TG) were determined according to the method of Meiathnin*et al.* (1978). Creatinine levels were determined using spectrophotometric methods described by Coles (1986). Electrolytes (Na^+, K^+), were determined using the method described by AOAC (1970).

2.4. Data analysis

Obtained data were expressed as mean ± standard error of mean (SEM), comparison was performed by the student t-test using Graphpad Prism version 5.0 for Windows, Graphpad Software. A value of $p \leq 0.05$ was considered statistically significant.

3. RESULTS

3.1. Mortality and clinical signs

During the study period, all the animals were observed daily for clinical signs and mortality patterns before dosing, immediately after dosing and up to 4 hours after dosing. Animals in Groups C and E were observed to develop diarrhea from the fourth to the 7th day of the experiment. Reduction in feed consumption and activity were observed in Groups B, C, D and E. In addition, body scratching, restlessness, shivering and lethargy were observed in group E.

3.2. Effect of ethanolic extract of *Grewiacarpinifolia* leaves following Vanadium Intoxication on hematological parameters in mice

The PCV and Hb concentrations of groups C and E were significantly lower ($p \leq 0.05$) when compared to the other groups. A significant reduction ($p \leq 0.05$) in WBC and neutrophils were also observed in group E. The lymphocytes count in group C was significantly ($p \leq 0.05$) higher than those of other groups (Table 1). The values of the hematological parameters of group B and D were not statistically different from the control group.

3.3 Effect of ethanolic extract of *Grewiacarpinifolia* leaves following Vanadium Intoxication on serum biochemical parameters in mice

Significant reductions ($p \leq 0.05$) in total serum protein and cholesterol levels were observed in groups C and E. Therewas a significant increase in the levels of ALT and AST in group E; in addition a significant decrease ($p \leq 0.05$) in potassium levels was observed in groups C, D and E (Table 2).

TABLE 1: Mean hematological parameters of experimental animals.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
PCV (%)	44.50±6.36	41.50±9.19	33.75±3.2016*	40.50±3.54	32.40±4.10*
Hb (gm/dL)	15.05±1.63	13.80±2.97	11.13±1.08*	11.80±1.27	12.38±1.61*
RBC ($X10^{12}$/L)	11.50±1.95	9.85±1.17	8.07±2.32	8.96±1.36	11.16±3.21
MCV (fl)	39.00±1.41	42.00±4.24	43.75±9.00	39.50±2.12	35.00±11.42
MCH (pg)	13.50±0.50	14.00±1.41	14.25±2.87	13.50±0.71	12.20±4.15
MCHC (g/dL)	33.00±0.00	33.00±0.00	33.00±0.00	33.00±0.00	33.00±0.00
PLATELETS ($X10^9$/L)	13.00±1.41	12.00±2.83	10.00±0.00	10.00±0.00	10.80±1.10*
WBC ($X10^9$/L)	28.10±8.06	18.40±3.39	21.20±4.36	18.40±11.31	12.80±4.40*
LYMPHOCYTES (%)	67.00±4.24 (18.83)	67.00±7.07 (12.33)	74.75±3.69* (15.85)	70.50±10.61 (12.97)	70.60±7.50 (9.04)
NEUTROPHILS (%)	32.50±4.95 (9.13)	32.00±7.07 (5.88)	28.00±3.23 (5.09)	30.00±11.31 (5.15)	28.20±7.22* (3.61)
MONOCYTES (%)	1.00±0.02 (0.281)	1.00±0.01 (0.18)	1.25±0.50 (0.27)	1.50±0.71 (0.30)	1.20±0.45 (0.15)
EOSNOPHILS (%)	0.00	0.00	0.00	0.00	0.00

Values are expressed as mean ± SEM (n= 5 mice/ group); *statistically different from the control

at p≤0.05; PCV: Packed cell volume; Hb: haemoglobin; WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobinconcenrtration, absolute values of differential wbc are indicated in parenthesis

TABLE 2: Mean serum biochemical parameters of experimental animals.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
TOTAL PROTEIN (g/dL)	4.50±0.42426	3.85±0.91924	3.18±0.21*	4.35±0.21	3.46±0.43*
ALBUMIN (g/dL)	1.35±0.25	1.25±0.15	1.13±0.75	1.20±0.100	1.36±0.15
GLOBULIN (g/dL)	3.15±0.71	2.60±0.71	2.05±0.58*	2.15±0.71*	2.18±0.16
CREATININE (mg/dL)	2.25±0.21	1.13±0.14	1.50±0.58	1.25±0.21	1.65±0.63
BUN (mg/dL)	29.97±1.95	26.3±0.91	26.7±2.07	22.7±1.81	29.8±2.64
SODIUM (mmol/L)	59.00±7.07	36.00±11.31	31.00±1.155*	34.50±0.71*	34.20±8.79*
POTASSIUM (mmol/L)	47.00±7.07	32.00±11.31	23.25±1.50*	39.00±1.41	26.00±5.10*
ALT (IU/L)	43.00±4.24	32.00±14.14	47.50±1.91	45.00±5.82	75.40±9.07*
AST (IU/L)	49.50±6.36	39.00±18.40	41.50±2.52	455.00±7.07	81.20±10.06*
CHOLESTEROL (mg/dL)	30.00±2.83	26.00±5.66	22.00±1.63*	25.50±0.71	21.40±4.10*
TRIGLYCERIDE (mg/dL)	40.00±7.07	26.00±2.83	24.50±3.00	25.00±4.24	21.80±8.90

Values are expressed as mean ± SEM (n= 5 mice/ group); *statistically different from the control at p≤0.05; BUN-blood urea nitrogen; ALT- alanine aminotransferase; AST- aspartate aminotransferase

4. DISCUSSION

It is well known that heavy metals are widely distributed in the environment and some of them do cause physiological and biochemical disorders. Living organisms are exposed to these metals from contaminated air, water, soil and food. Therefore, we attempted to investigate the effect of ethanolic extract of *Grewia carpinifolia* leaves against vanadium toxicity in this study.

It was observed in the present study that vanadium administration resulted in diarrhea. Although the plant extract at 200 mg/kg was found to reduce vanadium induced diarrheal episodes but the mechanism of its anti-diarrheal activity is still uncertain. This result is in agreement with studies by Fatum*et al.*, 2002 and NIEHS (2008) who reported diarrhea in rats following acute sodium metavanadate administration, our search through literature however revealed not so much work have been done as it relates to the mechanism of diarrhea induction by vanadium.

In the present study we found normochromic normocytic anaemia, with a decrease in haemoglobin and packed cell volume in the group administered with sodium metavanadate only. This anaemia could be a consequence of haemolysis, as other researchers have proposed (Zaporowska and Slotwinska 1996; Yang *et al.*, 2003) or might be a kind of suicidal erythrocyte death identified as eryptosis; specifically described for vanadium toxicity (Föller*et al.*, 2008) as a result of oxidative stress (Bracci*et al.*, 2002; Barvitenko*et al.*, 2005), an impairment of erythropoiesis could also be assumed. Our results are in consonance with earlier observations of anaemia, changes in haemoglobin and reduction in haematocrit concentrations as previously reported (MarkkuKiviluoto*et al.*, 1981; Zaporowska and Wasilewski, 1992) but in contrast with other reports of Dai and McNeill (1994) and Fawce*et al*, (1997) who observed no changes in haematological indices following acute vanadium intoxication. The variance in reports may be due to difference in duration and routes of the vanadium exposure as well as difference in vanadium compounds used. Our plant extract at 200mg/kg given concomitantly with sodium matavanadate was able to restore PCV and Hb concentrations to levels comparable with the control and standard groups.

In the present study, sodium metavanadate vanadium significantly decreased total WBC count and absolute neutrophil counts. Earlier reports have indicated that vanadium induces myelosuppression and this property has been employed in cancer therapy (Crawford *et al.*, 2004). Seven days simultaneous treatment with *G. carpinifolia* significantly reversed vanadium induced

neutropenia and changes in total WBC count. Though the exact mechanism of action of *G. carpinifolia* is still unknown, this indicates that it is a potential agent to be evaluated clinically and may be useful in improving antitumour treatment induced myelosuppression (Manju*et al.,* 2011)**.** The presently used agents like GM-CSF and G-CSF though effective, have a major limitation due to their exorbitant cost. *G. carpinifolia* being an indigenous plant could be a more economical alternative immunostimulant to reduce side effects of anticancer drugs. Our result is similar to that of Aglal (2012) who reported that *G. tenax*also decreased white blood cells in mice.

Sodium metavanadate resulted in a decrease in cholesterol levels. This finding in this study is in line with that of Cooper (2007) that vanadium pentoxide inhibited cholesterol biosynthesis and lower plasma cholesterol levels being a potent inhibitor of many enzymes.

A number of soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. The increase in the activities of these enzymes in plasma is indicative for liver damage and thus causes alteration in liver function (Adedapo et al, 2004). In our study, the increased activities of ALT and AST in serum obviously indicate that liver is susceptible to vanadium induced toxicity. These increases could be attributed to the hepatic damage resulting in increased release of functional enzymes from biomembranes or their increased synthesis (Chang, 2009). This elevation of serum liver enzymes is similar to that reported by Sidhu*et al.,* (2004), Adedara*et al*., (2011) Elshaari*et al.,* (2011) and Hoda*et al*., (2012). The ability of the extract to lower the levels of these enzymes to values comparable with the control group and that of the standard antioxidant, may suggest that the plant extracts did not have necrotic effect on the liver. This may also be due to the fact that the extracts offer protection and maintain functional integrity of hepatic cells. The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by vanadium (Murugaian*et al,* 2008). The extract could be said to protect against vanadium induced hepatotoxicity in this regard. The liver is also an important site for the synthesis of many serum proteins (Ahsan*et al*, 2009). The reduction in serum total protein observed in the sodium metavanadate group may also be associated with decrease in the number of hepatocytes which consequently results in decreased hepatic capacity to synthesize protein. Simultaneous treatment by α-tocopherol and ethanolic extracts of *Grewia carpinifolia* leaves significantly increased serum total protein indicating the hepatoprotective activity of the extracts most probably through hepatic cell regeneration (Olorunnisola*et al,* 2011). The significant decrease in potassium levels observed in groups C and E may be due to diarrhea (Zychlinski and Byczkowski, 1990) as reported that hypokalemia may result from excess renal or gastrointestinal loss (Huang and Kuo, 2007).

It is evident from our study that sodium metavanadate result in negative alteration in hematological and serum biochemical parameters of experimental mice. This study has also demonstrated that administration of α-tocopherol (vitamin E) and *Grewia carpinifolia* concomitantly with vanadiumameliorates this vanadium induced damage, however the *G. carpinifolia* extract could be more effective at 200mg/kg. The results of this study are in congruence with previous report of Haider and El-Fakhri (1991) and Fatum*et al.* (2002) where α-tocopherol (vitamin E) protected the experimental animals (rats) against vanadium-initiated damage.

The various phytoconstituents of *G. carpinifolia* extract might be responsible for its ameliorative effects following vanadium induced toxicity. The genus *Grewia* has been found to possess phytochemicals such as triterpenoids, fatty components, flavonoids, steroids, saponins and tanins (Goyal, 2012). Several of these compounds have been described to have various medicinal properties such as hepatoprotective, heamatinic, antioxidant, antibiotic (Ajayi*et al.,* 2011; Bhumi and Savithramma, 2014) and can consequently serve as sources of new drugs.

Although *G. carpinifolia* reduced the toxicity of vanadium, much remains to be studied regarding its mechanism of actions.

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