THE EFFECTS OF AQUEOUS *VERNONIA amygdalina* (BITTER LEAF) EXTRACT ON THE LIPID PROFILE AND SOME HEMATOLOGICAL PARAMETERS IN RATS EXPOSED TO CYANIDE

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Accepted 15 November, 2017

Despite the fact that cyanide is known to be toxic, it is found in popular foods consumed by humans and animals, there is therefore the need to find an appropriate food that can help ameliorate its effect in both man and animals. The study aims to determine the effect of different concentrations of bitter leaf extract (BLE) on hematological parameters and blood lipid profile of rats exposed to cyanide. A total of twenty rats were used for this study and they were divided into five groups, each containing four rats. All groups were fed with growers mash and in addition Group 1 (control) was given water only, Group 2 was given cyanide alone, Groups 3, 4 and 5 were given 100,200 and 300 mg/kgb.wt of BLE and cyanide respectively. The experiment was carried out in 3 weeks. At the end of the experiment, the packed cell volume (PCV), hemoglobin level (Hb), Red blood cell count (RBC) and white blood cell count (WBC) was determined using known biochemical procedures. The results indicated a significant increase ($p < 0.05$) in PCV, Hb, RBC and WBC level of Groups 3, 4 and 5 when compared to the cyanide group (Group 2). The result from the experiment indicates that total cholesterol (TC), LDL-cholesterol and triglyceride (TG) were significantly lower in all the treated groups (Groups 3, 4 and 5) when compared with the untreated group given cyanide alone (Group 2). However, no significant difference in TC was indicated in all the groups given different concentration of bitter leaf extract (BLE) when compared with the control (Group 1) and no statistical difference in TG was also indicated in the groups given 200 and 300 mg BLE when compared with the control. A significantly higher HDL-cholesterol ($p<0.05$) was however recorded in all the groups given different concentration of BLE when compared to cyanide alone. The study reveals that bitter leaf in respective of the dose is able to improve the hematological parameters and lipid profile in cyanide exposed rats.

Key words: Cyanide, PCV, WBC, Hb, RBC, lipid profile.

INTRODUCTION

Plants usefulness to man is not only as a source of raw materials for industries, but also as source of food and medication. From earliest times, plants have provided man with diverse means of healing. They have been known to contain or possess abundant phytochemicals and pharmacologically active principles, which include; anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids *(*Sofowora, 2006; Erasto et al., 2006*).*

Herbal preparations from different parts of plants (leaves, roots, barks and twigs) have been known for the treatment of a variety of diseases such as diabetes mellitus, breast cancer, hypertension, etc. (Pinto and Rivlin, 1999; Li and Schellhorn, 2007). One of such plants suspected to have these medicinal values is the bitter leaf (*Vernonia amygdalina*). The leaves are green with a characteristic odor and bitter taste (Ijeh and Ejike, 2011). It is so unique that every part of it is economically important. *V. amygdalina* contain significant quantities of lipids (Ejoh *et al*., 2007), proteins with essential amino acids (Igile *et al*., 1994). It is also rich in carbohydrates (Eleyinmi *et al*., 2008) and little quantity of carotenoids (Udensi, *et al*., 2002). It also contains essential elements like calcium, iron, protein, potassium, manganese, copper and phosphorus) (Bonsi *et al*., 1995).

V. amygdalina has also been applied in the treatment of various ailments. It is a medicinal herb popularly used by traditional practitioners. The plant has been known to have

anti-helminths, blood purifying, anti-laxative and anti-malaria qualities. It is used by scientists in curing diabetes, malaria, toothache, fertility problems and gastro-intestinal (Ijeh and Ejike, 2011). It is also used as digestive tonic, appetizer and febrifuge, and for topical treatment of wounds as a substitute for iodine (Iwu, 1986).

Cyanide is a very common and potent cytotoxic agent known for its rapid destructive action and toxicity. The sources for cyanide poison are diverse, ranging from fruit pits, nuts, or seeds to industrial-based materials, such as those used in metal processing, electroplating, rubber and plastic production, insecticide and rodenticide production, chemical synthesis, and extraction of gold and silver ores. Also some drugs of medicinal importance, like Laetrile and Nitroprusside, can give out cyanide (Ellenhorn, 1997).

The toxicity of cyanide is as a result of its high potency as a respiratory poison in all aerobic forms of life. Acute cyanide intoxication and chronic toxicity have frequently been reported in recent years, and suggests that the most widespread problems arising from cyanide are from chronic dietary, industrial and environmental sources. Cyanide have been shown to induce oxidative stress and damage in a number of biological systems (Okolie and Iroanya, 2003; Okolie and Asonye, 2004). It has also been reported that prolonged sublethal cyanide exposure can cause biochemical and histo-pathological alterations in different species (Okolie and Osagie, 2000; Sousa et al., 2002; Soto-Blanco and Gorniak, 2003; Tulsawani *et al.*, 2005).

Limitations of commonly available cyanide antidotes (for example, sodium nitrite, 4-dimethyl aminophenol, sodium thiosulphate, dicobalt edetate, etc.) prompted research for better treatments by new mechanistic based antidotes (Baskin et al., 1999; Bhattacharya and Tulsawani, 2009). With respect to serious problems resulted from long-term ingestion of low amounts of cyanide, it is important to carefully assess the effects of sub lethal doses of cyanide as well as to identify suitable compounds with potential for protecting against resultant tissue damages.

So, the present study was undertaken to assess the blood and lipid profile of rats following the sub lethal cyanide exposure and also to evaluate the possible protective effect of bitter leaf (*V. amygdalina*) in cyanide exposed rats. These studies may be of value for further understanding the pathophysiology of cyanide poisoning and as an aid in diagnosis and supportive therapy of long-term exposure to cyanide.

MATERIALS AND METHODS

Preparation of bitter leaf extract

Fresh bitter leaves (*V. amygdalina*) were gotten from Ethiope East Local Government area of Abraka, Delta State. The leaves were washed to remove impurities, and were sun dried for 2 weeks. The dried leaves were pulverized to get to a coarse powder which was used to for the extraction. 300 g of the bitter leave powder was macerated in 2.4 L of distilled water for 24 h, after 24 h it was filtered first with handkerchief before using Whitman filter paper. The filtrate was dried to powder with the aid of a rotary evaporator at a temperature of 40 to 60ºC. The residue referred to as the crude extract was prepared by dissolving 1g of the extract in 9ml of aqueous Tween AT (Transporter) which was stored in an air-tight container under refrigerator until it was ready to use.

Management of experimental animals and experimental design

Twenty, wister rats weighing between 100 to 150g were used for this experiment. The animals were procured from the animal house of the Department of Animal and Environmental Biology, Delta State University. The rats were kept in a stainless wire rat cages, equipped with drinkers with wood carvings to keep them warm. The animals were fed (Chick growers mesh), and acclimatized for one week, during the course of acclimatization and experimentation. The rats were divided into five groups of four rats each. Group 1 served as the control group, group 2 cyanide alone, groups 3, 4, and 5 served as the treatment groups. Group 3 were treated with 100mg/kgbw of Bitter Leaf Extract (BLE), group 4, 200mg/kgbw of BLE and 5, 300mg/kgbw of BLE respectively with exposure to cyanide poisoning for 28 days, Groups 2, 3, 4, and 5, received the same concentration of Sodium cyanide (NaCN) (0.09mg/ml) in their

drinking water. Oral cannula was used to administer different concentration of the bitter leaf extract to experimental rats. The animals were handled in accordance with the principles of laboratory animal care as documented in National Institute of Health for laboratory animal welfare (National research council, 1996)

Determination of hematological parameters

Hemoglobin (Hb) concentration was determined using cyanomethaemoglobin method as described by Hewitt (1984). Packed cell volume (PCV) was determined by microhaematocrit technique using capillary tube as described by Schalm *et al* (1975). Red blood cell counts (RBC) and White blood cells count (WBC) was determined by the method of Brown (1976)

Determination of lipid profile

Total cholesterol was determined according to the method of Allain et al. (1974), triglyceride was also determined according to the method of Bucolo and David (1973). HDL-cholesterol was determined by the method of Warnick and Wood (1995). While LDL-cholesterol was calculated by the method of Puavilai and Laoragpongse, (2004)

Statistical analysis

All the data obtained were subjected to statistical analysis using analysis of variance (ANOVA) and Least Significance Test. The level of significance used was $p< 0.05$.

RESULTS

The result in Table 1 showed a significant increase ($p<0.05$) in the PCV levels of the treated groups, group 3 (36.00±4.94), group 4 (30.00±4.95), group 5 (31.00±2.53), when compared with the group given cyanine without treatment (group 2) (23.43±1.57).

A significant increase ($p<0.05$) was also indicated in Hb levels of the treated groups, group 3 (12.31±4.18), group 4 (11.10±1.35), group 5 (9.35±0.92), when compared with the group given cyanine without treatment (group 2) (5.42±0.37). WBC of the treated groups group 3 (3.54±0.26), group 4 (9.76±0.13), group 5 (2.08±0.39) were also significantly higher ($p<0.05$) than the group rats (3.77±0.19) (Table 1). A similar trend was also recorded for the RBC levels. All the treated group 3 (5.25±0.20), group 4(4.95±0.12), group 5 (5.05±0.04), were significantly higher ($p<0.05$) than the untreated group 2 (2.65±0.10).

Table 1. The effect of bitter leaf on some hematological parameters of rats exposed to cyanide.

Parameter	Control	cyanide	100mg bitter leaf	200 mg bitter leaf	300 mg bitter leaf
PCV (%)	$41.50±4.69^a$	$23.43±1.57^b$	$36.00±4.94^a$	$30.00±4.95^c$	$31.00±2.53^c$
HB (g/dl)	$16.17±0.68^a$	$5.42±0.37^b$	$12.31±4.18^c$	$11.10±1.35^a$	$9.35±0.92^c$
WBC (10^3/µL)	$10.67±0.35^a$	$3.77±0.19^b$	$8.52±0.26^a$	$9.76±0.13^a$	$8.08±0.39^a$
RBC (10^6/L)	$8.10±0.06^a$	$2.65±0.10^b$	$5.25±0.20^c$	$4.95±0.12^c$	$5.05±0.04^c$

Values are given as Mean ± Standard deviation, n=6. Values not sharing a common superscript letter differ significantly at ($p < 0.05$).

The result from Table 2 indicates that total cholesterol (TC), LDL-cholesterol and triglyceride (TG) was significantly lower in all the treated groups (groups 3, 4 and 5) when compared with the untreated group given cyanide alone (group 2). However, no significant difference in TC was indicated in all the groups given different concentration of bitter leaf extract (BLE) when compared with the control (group 1) also no statistical difference in TG was also indicated in the groups given 200 and 300mg BLE when compared with the control. A significantly higher HDL-Cholesterol ($p<0.05$) was however recorded in all the groups given different concentration of BLE when compared to cyanide alone.

DISCUSSION

This study is designed to determine the effect of

Table 2. The effect of bitter leaf on the lipid profile of rats exposed to cyanide.

Parameter	Control	Cyanide	100 mg bitter leaf	200 mg bitter leaf	300 mg bitter leaf
Total cholesterol	160.38±3.49	180.28±7.31[b]	158.27±6.23	155.48±8.40	154.45±9.96
HDL-cholesterol	85.23±8.84	40.30± 5.30	61.34± 2.69	62.96±2.12	65.34±5.01
LDL-cholesterol	33.08±4.85	81.87±7.20	50.59±2.15	49.07±11.57	46.42±4.29
Triglyceride	210.38± 10.79	290.57±16.46[a]	232.17±7.13[b]	217.23± 11.92	213.45± 7.14

The values are given as Mean ± Standard deviation, n=6. Values with letter superscript differs significantly at ($p < 0.05$) from the control.

bitter leaf on the hematological parameters (Packed cell volume, hemoglobin, red blood count and white blood cell count) and lipid profile in rats exposed to cyanide poisoning. In this study (Table 1) a significant decrease was indicated in the PCV level of the cyanide alone group (23.45±1.57), when compared with the control group (30.50±4.69). While a significant high PCV was recorded in all groups treated with the aqueous bitter leaf extract [group 3 (42±4.94) group 4 (36±4.95) and group 5 (31±2.53)], when compared to the group given cyanide alone without treatment. PCV is the percentage of red blood cells in blood (Purves et al., 2003). It is involved in the transport of oxygen and absorbed nutrients, and increased PCV indicate a better transportation (Isaac et al., 2013).

The result of the hemoglobin (Table 1) showed a significant rise in the hemoglobin level of the treated groups. Group 3 cyanide + 100mg/kg of BLE (16.31±4.18), group 4 cyanide + 200mg/kg of BLE (12.10±1.35) and group 5 (9.35±0.92), when compared with the cyanide group (5.42±0.37). Hemoglobin is the iron containing oxygen transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates. It carries oxygen from the respiratory organs (lungs) to the rest of the body where it releases the oxygen to oxidize nutrients to provide energy to control the functions of the organism (Biagioli et al., 2009). Aqueous bitter leaf extract was able to improve the hemoglobin levels in rats due to the presence of iron (Bonsi et al., 1995; Ejoh et al., 2007; Eleyinmi et al., 2008).

White blood cell count is a test that measures the number of white blood cells in the body and an increase in WBC indicates an immune disorder or an infection. The WBC result (Table 1) indicated a significant decrease in the cyanide alone group (1.77±0.19) when compared with the control group (10.67±0.35). However, there is a significant increase in the level of white blood cell in the treated groups, group 3 Cyanide +100mg/kg of BLE (3.57±0.26), group 4 cyanide + 200mg/kg of BLE (9.76±0.13) and group 5 cyanide + 300mg/kg (2.08±0.39) when compared to the cyanide alone group (1.77±0.19), indicating the ability of bitter leaf to increase the White blood cell count.

Results from this study (Table 1) indicates a significant decrease in the level of the RBC in all groups exposed to cyanide, both the treated group 3 cyanide + 100mg/kg of BLE (4.25±0.20), group 4 cyanide + 200mg/kg of BLE (1.95±0.12) and group 5 cyanide + 300mg/kg of BLE (1.95±0.12) and the untreated group 2 (2.65±0.10) when compared to the control group (8.10±0.06). RBC count is a test employed to determine the number of red blood cells in the blood.

A high red blood count indicates an increase in oxygen carrying cells in the blood. If the level of red blood cell depreciates, it may indicate anemia or other causes. RBC are continuously exposed to both endogenous and exogenous source reactive oxygen species that can damage and impair its function of which cyanide is one. Studies indicate that binding to RBCs is primarily due to cyanide reacting with ferric iron (Fe^{3+}) in methemoglobin to form the nontoxic complex cyano methemoglobin (Farooqui and Ahmed, 1982). The aqueous bitter leaf extract 100mg/kg bw of BLE (4.25±0.20) as indicated in Group 3 was however able to increase the red blood count when compared with the untreated group (2.65±0.10). Aqueous bitter leaf extract is probably able to improve the hematological parameters due to the presence of iron and due to its antioxidant property (Iwalokun *et al*., 2006; Adaramoye et al., 2008).

The result from the lipid profile (Table 2) indicated that the bitter leaf extract greatly improved the lipid profile in the rats by the

significant increase in the HDL-Cholesterol and significant decrease in the LDL-Cholesterol and triglyceride level when compared with the untreated group. High levels of triglyceride and LDL-C has been associated with heart disease, diabetes and insulin resistance.

This work is in agreement with work of Ajuru et al. (2013) who reported that bitter leaf extract greatly improved the lipid profile of rats given BLE. It is also consistent with the work of Akah et al. (2009) and Nwanjo and Umukoro, (2004) who also reported that methanolic and aqueous extract of bitter leaf improved both lipid profile and hematological parameters in diabetic rats.

CONCLUSION

The study reveals that bitter leaf irrespective of the dose is able to improve the hematological parameters and lipid profile in cyanide exposed rats.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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