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Combined Effects of Aqueous Extracts of *Xylopia aethiopica* and *Monodora myristica* Seeds on Lipid Profile and Haematological Indices in Rats Exposed to Cyanide

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ABSTRACT: The purpose of this study was to determine combined effect of Xylopia aethiopica seed (Xa) and Monodora myristica seed extracts (Mm) on haematological parameters {pack cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC) and white blood count (WBC) and lipid profile {Total cholesterol (TC), triglycerides (TG), highdensity lipoproteins (HDL) and low-density lipoproteins (LDL) } in cyanide exposed rats. The study was conducted using 32 male rats weighing between 120 to 150 g. The rats were divided into 8 groups of 4 rats in each group as follows; Group 1: (control), Group 2: cyanide control (exposed to cyanide only), Group 3: given Xa only, Group 4: given Mm only, Group 5: Xa+ Mm mixture only, Group 6: cyanide + Xa, Group 7: cyanide + Mm and Group 8: cyanide plus (Xa+Mm). The rats in Groups 3-8, were given the spice extracts 60mg (Xa, Mm and Xa+Mm mixture) at 1ml/kg three times per week, and Rats in Groups 2, 6, 7 and 8 received CN in form of NaCN solution at concentration of 9.0 mg/kg in the drinking tap water every day for 4 weeks. The results showed that the haematological parameters (PCV, HB, RBC and WBC) was significantly (p>0.05) increased in all the Groups treated with the Xa and Mm extracts (Groups 6, 7 and 8) when compared with the untreated Group 2. However there was no significant difference in the haematological parameters between the groups given the mixture of the two plant extracts Group 8 (Xa +Mm) and those given the two extracts separately Group 6 and 7). Results from the lipid profile indicated a significant decrease TC, LDL and TG in all the cyanide exposed rats treated with the extracts (Group 6, 7 and 8) when compared with the untreated Group 2, However a significantly lower TC, LDL and TG was indicated in Group 8 rats given the mixture when compared with Group 6 and 7 given Xa and Mm separately. In conclusion, the result indicates that although both extracts are able to improve the haematological and lipid profile parameters in cyanide exposed rats, the combined extracts (Xa+ Mm) gave better results in the lipid profile compared with the individual plant extracts in cyanide exposed rats.

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Cyanide is among the most potent cytotoxic poisons known to humans and animals (Mathangi andNamasivayam, 2000). It occurs naturally and has been detected in surface water (WHO, 2004). Naturally cyanide is found in some plant species such as cassava, sorghum, bamboo etc. as cyanogenic glycosides (ATSDR, 2006). It is also found in air (atmosphere) as hydrogen cyanide where it is released into the atmosphere from burning, volcanoes, natural biogenic processes from plant, bacteria and fungi (Okafor, 2000). A rise in blood cyanide level has also been observed in smoke inhalation injury and death. Studies has shown that chronic cyanide ingestion can cause impaired body growth, neurological and thyroid disturbances as well as some pathologic effects on different tissues (Tulsawani et al., 2005).Several studies have shown that cyanide exposure causes an increase in the production of free radicals thereby increasing oxidative stress. (Okolie and Osagie, 2000). Monodora myristica (Africa nutmeg) belong to the family Annonaceae. The common names are calabash nutmeg, ehuru, ariwo, Jamaica nutmeg etc (Burkill 1985). *M. myristica* Gaertn is perennial tree growing in the tropical rainforest in Liberia Angola, Nigeria, Cameroon, Ghana, Uganda and West Kenya (Weiss, 2002). It is a wild plant among the most used as food and drug. Studies on *M.myristica* have reported the leaves contain  $\beta$ - caryophyllene,  $\alpha$ -humulene and  $\alpha$ -pinene, however studies have also shown that  $\alpha$ -phellandrene,  $\alpha$ -pinene, myrcene, limonene and pinene are the major compound found as the essential oil in the seed (Fournier *et al.*, 1999).

*M.myristica* spice has been shown to possess good antioxidant properties due to the presence of some flavonoids and phenols in its seeds. (Akinwunmi and Oyedapo 2013;Uheghu *et al.*, 2011; Ekeanyanwu and Etienajirhevwe, 2012).

Fruits of *Xylopia aethiopica* popularly called African pepper in West Africa, has different biological

activities including analgesia, vasorelaxant, and antiinflammatory effects in rodents Ameyaw, *et al*, (2014). The fruit of *X. aethiopica* contains kaurenoic and xylopic acid (XA) which is kauranes, a class of diterpenes *X.aethiopica* extract contains an antioxidant activity and it also increases the antioxidant defense in rats subjected to oxidative stress radiation (Karioti *et al.*, 2004).

Studies indicate that when drugs or plants are ingested orally they alter hematological indices of an organism either positively or negative. (Owoyele *et al.*, 2011, Ajagbonna *et al.*, 1999) Studies on the haematological parameters will supply information on inflammation, necrosis as well as the presence of stress. (Melillo, 2007; Ekeanyanwu and Etienajirhevwen, 2012).

Lipid distribution in the body is an indication of one's health status. Cholesterol, triglycerides, and highdensity lipoproteins are important constituents of the lipid profile in the human body. Cholesterol is essential for the normal functioning of all animal cells and is a fundamental constituent of cell membranes. It also functions, as precursors of different substances in the body, such as adrenal, gonadal steroid hormones and bile acids used in the emulsification of fats. Triglycerides are fatty acid esters of glycerol and they represent the main lipid component of dietary fat and fat deposits in animals. While high density lipoprotein, mostly referred to as "the good cholesterol", functions in the amelioration of excess of fatty acids in the blood, by carrying them to the liver to be metabolized. (Mard-Soltani, et al., 2012).

In most part of Nigeria were *M. myristica* and *X. aethiopica* are consumed as food, the seed from both plants extracts are usually used together hence the objective of this research is to investigate the combined effects of the aqueous extracts of these two plant on the haematological indices and lipid profile in albino rats.

## **MATERIALS AND METHODS**

Sample Collection: The spices X. aethiopica and M.myristica were purchased from Abraka main market, Delta State. The plants spices were identified by a taxonomist at the Department of Botany, Delta State University, Abraka.

*Experimental Animals*: 32 albino rats weighing between 150g and 180 g were obtained from the animal house Delta State University, Abraka. The rats were fed on growers mash and were given water ad libitum. The rats were housed in cages constructed of aluminum sheet and wire gauze under control condition of 12h light/ 12 dark cycle.



Fig 1: Picture of X. aethiopica whole pods



Fig 2: Picture of *M. myristica* seeds

*Experimental Design*: A total of thirty two (32) rats were used for the study. The rats were divided into 8 groups of 4 rats per group as follows: Group 1: normal control: rats in this group received tap water daily throughout the experiment. Group 2: cyanide control; Groups 3 and 6; Rats in this group received 60mg/kg /0.1mlXaE three times per week for 4 weeks. Group 4 and7: Rats in this group received 60mg/kg/0.1ml Mm three times per week for 4 weeks.

Group 5: Rats received 30mg/kg /0.05ml Xa and 30mg/kg/0.05Mm

Group 6: Rats in this group received CN +Xa mixture three times per week for 4 weeks. Group 7: Rats in this group received CN + Mm mixture three times per week for 4 weeks

The oral administrations of the *X.aethiopica* and *M.myristica* extract were carried by gavage, three times per week for 4 weeks. Rats in Groups 2, 6, 7 and 8 received CN in form of NaCN solution at concentration of 9.0 mg/kg in the drinking tap water every day for 4 weeks. On the last day the rats were allowed to fast overnight and sacrificed by cervical decapitation

#### **RESULTS AND DISCUSSION**

The purpose of this study was to determine combined effect of Xylopia aethiopica seed (Xa) Monodora myristica seed extracts (Mm) on haematological parameters {pack cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC) and white blood count (WBC)} and lipid profile (Total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) in cyanide exposed rats. In the Blood, haemoglobin carries oxygen from the respiratory organs to the rest of the body, where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism (Biagioli et al., 2009; Maton et al., 1993). A deficiency can therefore result from a decrease in he amount of hemoglobin molecules, as in anemia, or by a decrease in the ability of each molecule to bind oxygen at the same partial pressure of oxygen. The results in this present study indicates that the PCV and Hb level were significantly (p < 0.05) decreased in all rats exposed to cyanide (Groups 2,6,7 and 8) when compared to control (Group1). Treatment of the rats with X. aethiopica, M. myristica and X. aethiopica and M. myristica mixture (group 3 and 4) indicated

significant increase when compared with the control (Group1). This is in agreement with the work of Onyebuagu et al (2014) and Agomuo et al., (2014) that worked on Xa and Mm respectively. Nworah et al., (2012) also, reported that the content of iron in X. aethiopica is high and this could have accounted for the rise in Hb since the main component in the Heme synthesis (a precursor in the synthesis of Haemoglobin). However, among the rats exposed to cyanide a significant increase was indicated in both the rats treated with the individual extracts (Groups 6 and 7) and the combined extract (Group 8) when compared with Group 2 not treated. The decreased in haematocrit value, in cyanide exposed animals indicates destruction of erythrocytes. The treatment of the rats with the extracts both singularly and combined form was however able to improve the haematocrit values in the rats confirming their antioxidant properties. (Fariss, 1991). However, although there was no significant difference between the Groups given the combined extract (X. aethiopica+M.myristica) when to those treated with the individual compared extracts(Xa and Mm), the PCV and the Hb values were higher in those given the combined extract.

 Table 1: The combined effects of aqueous extract of Xylopia aethiopica (Xa) and Monodora myristica (Mm) on haemalogical parameters in rats exposed to cyanide toxicity

Groups	PCV (%)	RBC (x10 <sup>-12</sup> /L)	WBC (x10 <sup>-12</sup> /L)	Hb (mg/dl)
1 control	30.00±9.83 <sup>a</sup>	8.10±0.12 <sup>a</sup>	10.67±0.42 <sup>a</sup>	11.10±4.13 <sup>a</sup>
2 CN control	23.90±2.42 <sup>b</sup>	2.65 ± 0.02 <sup>b</sup>	1.77±0.08 <sup>b</sup>	$5.42 \pm 1.98^{b}$
3 Xa	35.56±1.94°	10.50±1.73 °	11.40±0.65 <sup>a</sup>	12.21±1.53 <sup>a</sup>
4 Mm	37.83±4.23°	12.15±0.17 °	11.92±0.27 <sup>a</sup>	13.33±4.99 <sup>a</sup>
5 Xa+Mm	38.17±4.73 <sup>d</sup>	14.00±1.15 °	12.52±0.37 <sup>a</sup>	13.77±1.99 <sup>a</sup>
6 CN +Xa	$29.20 \pm 4.73^{a}$	6.61±0.46 <sup>a</sup>	6.10±0.12°	7.29± 1.44°
7 CN + Mm	32.72±4.31 <sup>a</sup>	8.75±0.29 <sup>a</sup>	6.88±1.02°	8.23±5.64°
8 CN +(Xa+Mm)	33.00±3.55 <sup>a,c</sup>	9.75±0.29 <sup>a</sup>	7.66±0.62 <sup>a,c</sup>	10.48±2.88 <sup>a,c</sup>

Each value represents mean  $\pm$  SD. n = 4 in each group. Values not sharing a common superscript letter in the same column differ significantly at (p < 0.05).

 Table 2: The combined effects of Aqueous extract of Xylopia aethiopica (Xa) and Monodora myristica (Mm) on the lipid profile of rats exposed to cvanide toxicity

<b>Groups/ Parameter</b>	Total cholesterol	HDL	LDL	Triglycerol
1 control	162.38 ±3.49 <sup>a</sup>	86.23± 8.94 <sup>a</sup>	$33.87 \pm 2.80^{a}$	211.38± 12.79 <sup>a</sup>
2 CN control	181.28±7.21 <sup>b</sup>	$41.30 \pm 4.20^{b}$	$81.39 \pm 2.52^{b}$	292.57 ± 17.36 <sup>b</sup>
3 Xa	$167.61 \pm 18.54^{a}$	91.38± 1.10°	36.93± 5.29 <sup>a</sup>	196.48± 8.09°
4 Mm	156.47± 14.47 <sup>a</sup>	89.23± 1.49 <sup>a</sup>	26.93± 3.55°	201.56±10.82 <sup>a</sup>
5 Xa+Mm	149.54±10.02°	$92.32 \pm 1.49^{a}$	17.85±11.17c	$198.34 \pm 10.84^{a}$
6 CN +Xa	152.35± 17.02°	68.95± 2.60°	$38.33 \pm 1.26^{d}$	225.37± 22.73°
7 CN + Mm	154.39±18.39°	70.53± 3.52°	39.76± 1.79 <sup>d</sup>	220.50± 10.45°
8CN+(Xa+Mm)	159.29± 15.89 <sup>a</sup>	79.52±4.86°	$35.20 \pm 3.74^{a}$	207.88± 7.11 <sup>a</sup>

Each value represents mean  $\pm$  SD. n = 4 in each group. Values not sharing a common superscript letter in the same column differ significantly at (p < 0.05).

The two major functions of the Red and white blood cells are carriage of oxygen and defense against microbial attack respectively. The present study indicates that the RBC and WBC level were significantly (p < 0.05) decreased in all rats exposed to cyanide as compared to the groups not exposed to cyanide. This indicates that cyanide decreases the oxygen carrying capacity of the red blood cells and

reduces the ability of the white blood cells to protect the organism against microbial attack. (Okolie and Asonye, 2004). However when comparing the results amongst the cyanide exposed rats, those treated with the extracts in singular forms (Xa and Mm) and combined forms (Xa + Mm) (group 6, 7, and 8) showed a significant decrease in RBC and WBC when compared with Group 2not treated (cyanide control) In addition there was no significant difference when comparing those given the combined extracts Group 8 (Xa+Mm) with Group 6 (XA) and Group 7 (Mm). Phytochemical screening indicates that *M.myristica* and *X. aethiopica* extract are good antioxidant (Nwozo *et al.*, 2015;Nguefacket al., 2004). This indicates that these plants are able to reduce the damage resulting from oxidative stress induced by cyanide (Nwoke *et al.*, 2015; Karioti *et al.*, 2004).

Lipid distribution in the body is an indication of its health status. Cholesterol, triglycerides, and highdensity lipoproteins are important constituents of the lipid profile in the human body. Cholesterol is an unsaturated alcohol belonging to the sterane family. It is essential for the normal functioning of all animal cells and is a fundamental constituent of cell membranes. It also functions, as precursors of different substances in the body, such as adrenal, gonadal steroid hormones and bile acids used in the emulsification of fats. Cholesterol serves as a component of the cell membrane. (Hanukoglu, 1992) and is known, for its association in cardiovascular risk factors with lipoprotein in the blood. Determination of total cholesterol is important in the evaluation of metabolic conditions, such as hypercholesterolemia.

In this present study there was a significant increase (P>0.05) in TC, LDL and TG levels in the rats exposed to cyanide alone (Group 2) when compared with the control not exposed to cyanide. However a concomitant decrease was indicated in the TC, LDL and TG in rats exposed to cyanide and treated with Xa (group 6) and Mn (Group 7). This is in agreement with the works of Nwozo, *et al.*, (2011) which they reported that Mn extract is able to improve the lipid profile in hypercholesteromic rats and Nwafor, 2013 who observed that X. aethiopica had a dose dependent effect on the TC, LDL and TG levels in rats. However a significantly lower TC, LDL and TG was indicated in Group 8 rats given the mixture when compared with Group 6 and 7 given Xa and Mn separately.

*Conclusion*: In conclusion, the result indicates that although the individual extracts are able to improve the haematological and lipid profile parameters in cyanide exposed rats, the combined extracts (*X.aetiopica* and *M.myristica*) gave better results particularlyin the lipid profile of rats exposed to cyanide compared with the individual plant extracts given separately. Therefore a combined intake of *X.aetiopica* and *M.myristica* is better able to prevent anemia, coronary heart diseases and other diseases associated with high total cholesterol and triglycerides.

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