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An Evaluation of Sodium Flouride Toxicity on the Liver Function Parameters of Albino Rats and the Ameliorating Potentials of Extract of *Phyllantus Amarus*

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Abstract
This study investigated the toxicity of sodium fluoride on apartate transaminase, alanine transferase, alkaline phosphatase, serum total protein, serum albumin, and the ameliorative effects of extract of Phyllantus amarus. The study consisted of six equal groups of five albino rats that were administered with sodium fluoride and the Phyllantus amarus over a period of two weeks. The result indicated toxicity of sodium fluoride on AST and ALT, indicating liver damage and the probable capacity of the extract of Phyllantus amarus to ameliorate this toxicity.

Keywords: *Toxicity; Sodium fluoride; alkaline phosphatase; aspartate transaminase; alanine transferase; Phyllantus amarus*

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I. Introduction

Nature has provided many things for human kind over the years and ancient civilization depended on plant extract for the treatment of various ailments. Plants are very useful source of various bioactive compounds which have a direct or indirect use in the treatment of various human ailments (Unuofin and Lebelo, 2020)). According to many health experts and other related government sectors, people started to pay attention towards their healthier intake (Gundgaard *et al*., 2003).

Medicinal plant is used to maintain health and to prevent, diagnose and treat physical and mental illness differently from allopathic medicine based on theories, belief and experiments (Che, 2017). According to Soforowa (1982), about 60 – 85% of the population in every country of developing world has to rely on medicinal plant. Globally, there are evidence-based studies to verify the efficacy of medicinal plants, and some of these shreds of evidence have provided insights into synthesis of plant-based compounds with therapeutic application (Dhama *et al.,* 2014) which made the annual global market value of medicinal plant products extended (Singh and Ogunbodede, 2013). The availability of synthetic drugs used in the treatment of specific disease is common but because of the high cost and side effects associated with the use (Philomena, 2011) attention is currently being focused on the use of medicinal plant products in the prevention or management of various disease or ailments.

Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries like Nigeria. It is calculated that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States (Demissew and Dagne, 2001). In recent times, more Nigerians are coming down with different diseases like hepatitis B virus and for several other biological activities such as kidney and gallbladder stones, cold flu, tuberculosis, and other viral infections like liver diseases, jaundice and liver cancer (Unander *et al*., 1995).

Fluoride is a well-determined non-biodegradable and moderate pollutant, which at high levels causes serious health problems (Basha, 2014). The liver is the target organ of sodium fluoride sodium fluoride toxicity (Guo-Ving *et al.,* 2003). It was assumed that NaF would induce both pathomorphological and metabolic changes in the liver (Dabrowaska *et al.,* 2006). Fluoride toxicity is a condition in which there are elevated levels of fluoride ion in the body, the excessive intake of the fluorine indices pathological changes and disturbs the function of many tissues and cells (Zhao *et al., 2014*). Sodium fluoride is the most commonly used compound in oral caries prevention in the form of fluorinated drinking water, salts or milk, toothpaste, mouthwashes and fluoride tablets that adversely affect liver functions parameters (Al-Harbi *et al.,* 2014).

The liver is the largest organ in the body (Adam Felman, 2018). The gallbladder sits under the liver, along with parts of pancreas and intestines. The liver and these organs work together to digest, absorb and process food. The liver's main job is to filter the blood coming from digestive tract, before passing it to the rest of the body (Adam Felman, 2018). The liver also detoxifies chemical and metabolizes drugs. As it does so, the liver secretes bile that ends up back in the intestines. The liver also makes proteins important for blood clotting and other functions.

Phylantus amarus is a genus of flowering plant used in herbal medicine and it is gaining momentum because of its novel antiviral activity against these diseases. It also acts against liver cell toxicity and improves the immune system of patients and has been found effective against hepatitis A (Jayaram *et al.*, 1997). The whole plant is used for the treating migraine, jaundice (Samy *et al.*, 2008), gonorrhea and syphilis, skin disease and malaria (Upadhyay *et al.*, 2010). Paste of leaves or its decoction (Shanmugam *et al.*, 2009) and juice of roots (Rajakumar *et al.*, 2009) are used for treating jaundice. Chronic dysentery, menstrual problems, anorexia, urinary tract infection, and diabetes are also treated by leaf extract taken orally (Samy *et al.*, 2008).The aim of this study is to evaluate the sodium fluoride toxicity on liver function parameters and ameliorating potential of phylanthus amarus on the liver parameters of male albino rats.

II. Method

Animals

Male albino rats were obtained from Nnamdi Azikiwe University, Awka. These animals were housed in steel cages within the laboratory of Applied Biochemistry of Enugu State University of Science and Technology. They were maintained, given standard feed and clean drinking water ad lbitum. The animals were acclimatized for a period of one week prior to the experiment. All experiments were in compliance with the National Institute of Health Guide for care and use of laboratory animals.

Collection and preparation of plant materials

Phyllantus amarus leaves were collected from Agbani, in Nkanu of Enugu State and identified by Prof. Nwamba of Applied Biology, Enugu State University of Science and Technology. The plucked leaves were air dried for seven days and then were ground into powder using manual grinding machine.

Extraction of samples

A quantity, 800 g of the ground sample was weighed using analytical weighing balance and soaked in 4000 ml of petroleum ether, it was shaken vigorously and left inside the bottle for 24 hours. The ratio of mixing the sample and solvent is 100 g : 500 ml. The percentage yield of the extract is calculated by the formula:

$$\frac{\text{Weight of concentrate (in grams)}}{\text{Weight of grounded sample (in grams)}}$$

The mixture was decanted and filtered into a beaker using separating funnel and a filter paper. The weight of the residue was taken after it is dried and the extract was concentrated.

Feeding of rat

The weights of the rats were taken immediately they were purchased and were subjected to one week acclimatization. During this period of acclimatization, the rats were all fed with the grower's mash feed. The feeding method was adlibitum.

Experimental design

Forty-eight sexually mature male adult albino rats were obtained from the animal house of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. These animals were housed in steel cages within the laboratory of Applied Biochemistry of Enugu State University of Science and Technology. They were maintained, given standard feed and clean drinking water and libitum. The animals were acclimatized for a period of one week prior to the experiment. After acclimatization, the rats were grouped in seven equal groups of five rats each as follow:

Group I: received daily oral dose of normal feed (positive control)
Group II: received daily oral of NaF (10 mg/kg) (negative control)
Group III: received daily oral dose of NaF (10 mg/kg) + extract (200 mg/kg)
Group IV: received daily oral dose of NaF (10 mg/kg) + extract (300 mg/kg)
Group V: received daily oral dose of NaF (10 mg/kg) + extract (800 mg/kg)
Group VI: received daily oral dose of NaF (10 mg/kg) + Viagra (2 mg)

Sacrifice and sample collections

The rats were at the time of first weighted and then anaesthetized with chloroform and cardiac puncture as performed to obtain blood samples for AST, ALP, albumin and total protein analysis. Then cervical dislocation was carried out. The testes were collected, cleared from the surrounding fat and weighed using analytical weighing balance, body weights were assessed relative to animal testes weight.

Assay for liver function

Liver Function Test

The liver function test was conducted using enzymatic colourimetric method and Reitman and Frankel (1957).

Aspartate transaminase (AST)

The serum AST concentration was determined using enzymatic colourimetric method which is based on the principle that Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. The set-up consisted of test tubes labeled reagent blank and sample placed in a test tube rack. The reagent blank test tube contained 0.5 ml of solution R1 and 0.1 ml of distilled water, while the sample test tube contained 0.5 ml of solution R1 and 0.1 ml of serum. They were properly mixed and incubated for 30 min at 37 ^{o}C before aliquot of (0.5 ml of solution R2) was added to both the reagent blank and sample respectively. All the test tubes were properly mixed and incubated for 20 min at 25 ^{o}C, after which (5.0 ml) of sodium hydroxide was added to all the test tubes mixed properly and the absorbance of the sample (sample) was read against the sample blank at 546 nm.

Alanine Aminotransferase (ALT)

The serum ALT concentration was determined using enzymatic colourimetric method which is based on the principle that Alanin aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. L-alanine and α-oxoglutarate are converted by Alanin aminotransferase to L-glutamate and pyruvate. The reagent blank test tube contained 0.5 ml of solution R1 and 0.1 ml of distilled water and sample test tube contained 0.5 ml of solution R1 and 0.1 ml of serum. They were properly mixed and incubated for 30 min at 37 ^{o}C before aliquot of (0.5 ml of solution R2) was added to both the reagent blank and sample respectively. All the test tubes were properly mixed and incubated for 20 min at 25 ^{o}C, after which (5.0 ml) of sodium hydroxide was added to all the test tubes mixed properly and the absorbance of the sample (sample) was read against the sample blank at 546 nm.

Alkaline Phosphate

The Alkaline phosphate concentration using Randox kit manual method, which is based on the principle that serum Alkaline phosphate quantitatively determination of p-nitriphenol librated from the catalytic activity of ALP on p-nitrophenylphosphate. The set-up consists of test tubes labeled sample placed in a test tube rack. The sample test tube contained 0.05 ml of serum. An aliquot of 3 ml of reagent (mixture of one bottle of R1a and R1b) were added and the content was mixed thoroughly and the first absorbance A1 was read 1 minute after mixing. Exactly 2 minutes later the second absorbance A2 was read, while the third absorbance was read 3 minutes at a wavelength of 405 nm.

ALP concentration U/l = 3300 * ΔA 405nm/min

Serum Total Protein

The serum total protein was determined using the Biuret method (Randox kit), which is based on the principle that cupric ions in an alkaline medium, interact with peptide bonds of proteins resulting in the formation of a coloured complex that absorbs monochromatic light between 530 – 570 nm. Provided are Biuret reagent and CAL-standard. The composition of biuret reagent (mmol/l) was NaOH (100), Na-K-tartrate (16), potassium iodide (15), and cupric sulphate (6). The CAL-standard contains protein (5.95g/dL). The set-up consisted of test tubes labeled reagent blank, standard and sample placed in a test tube rack. The reagent blank test tube contained 0.02 ml of distilled water, standard test tube contained 0.02 ml of protein and sample test tube contained 0.02 ml of serum. After adding 1 ml of biuret reagent to all the test tubes, they were properly mixed and incubated for 30 min at 25 ^{o}C and the absorbance of the sample (sample) and of the standard (A standard) read against the reagent blank at 546 nm. The total protein concentration was calculated using the relation:

Total Protein concentration = [sample/standard x 5.95] g/Dl

Serum Albumin Concentration

The serum albumin concentration using Randox kit manual method, which is based on the principle that serum albumin quantitatively bind to the indicator 3.3',5,5'-tetrabromo-m Cresol sulphonephthalein (Bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being

directly proportional to the concentration of albumin in the sample. Provided are BCG concentrate and CAL-standard. The composition of BCG concentrate (mmol/l) at pH 4.2 was succinate buffer (75), Bromocresol green (0.15), Brij 35, and preservative, while CAL- standard contains albumin (4.58 g/dL). Before carryingout the analysis 13.5 ml of BCG concentrate was diluted with 87 ml of distilled water. The set-up consists of test tubes labeled reagent blank, standard and sample placed in a test tube rack. The reagent blank test tube contained 0.01 ml of distilled water; standard test tube contained 0.01 ml of albumin and sample test tube contained 0.01 ml of serum. An aliquot of 3 ml of diluted BCG concentrate were added to all the test tubes and were properly mixed and incubated for 5 min at 25^{o}C. The absorbance of the sample (A sample) and of the standard (A standard) was read against the reagent blank at 578 nm. The albumin concentration was calculated using the relation: Albumin concentration = [sample/standard x 4.58] g/dL

III. Results

Table 1 Shows the level of AST, ALT, Albumin and total protein of the albino rats on the evaluation of sodium fluoride toxicity and the ameliorating potential of phylanthus amarus on the liver of the rats.

Table 1: Levels of AST, ALT, Albumin and total protein in liver parameters of albino rats

Groups	AST (U/L)	ALT (U/L)	Albumin (g/dl) /L)	Total Protein (g/dl)
1	45.191 ± 40.987	68.000 ± 45.255	3.637 ± 1.071	4.768 ± 2.630
2	30.978 ± 21.512	24.500 ± 14.849	4.343 ± 0.304	7.086 ± 0.784
3	6.662 ± 0.000	17.000 ± 0.000	2.065 ± 0.000	3.029 ± 0.000
4	7.217 ± 4.868	63.500 ±40.305	3.856 ±0.304	4.851 ± 1.604
5	9.549 ± 2.512	47.000 ± 38.184	3.131 ± 1.157	3.651 ± 1.699
6	19.875 ± 0.000	29.000 ±0.000	2.334 ± 0.000	3.753 ± 0.000

IV. Discussion

Aspatate aminotransferase (AST), Alanine amino transferase (ALT), alkaline phosphate (ALP) and Albumin are liver enzyme that play important role in metabolism (Rentman and Franke, 2005). Aspatate aminotransferase (AST) catalyses the reductive transfer of amino group from aspatate to α-ketoglutarate to yield oxaloacetate and glutamate. Alanine aminotransferase (ALT) plays an important role in gluconeogenesis and amino acid metabolism (Sookoian and Pirola, 2012)). It catalyses the reductive transfer of an amino group from alkaline to α-ketoglutarate to yield glutamate and pyruvate. Alanine aminotransferase activity is the most frequently relied biomarker of hepatoxicity. Elevated serum activity of this enzyme is usually during liver damage. The ratio of serum AST and ALT can be used to differentiate liver damage from other organ damaged (Nathwani *et al*., 2005), it is particularly present in cell which lines the bilianary ducts of the liver.

In this study, there was a decrease in AST level ALT upon compared to the control and an increase upon administration of the extract. ALT and AST are two well-known diagnostic indication of liver damage. In liver damage with hepatocellular lesion's, these marker enzymes are released from damaged tissue and their levels increased in blood flow (Nkosi *et al*., 2005). There was ameliorating potential of *Phylanthus amarus* extract on the albino rats at 200 mg/kg, 300 mg/kg and 800 mg/kg body. Thus the extract would also prevent the release of hepatic enzyme at the level of the blood stream by a reduction of the tissue lesions. It also implies that the liver's synthetic activity and ability to maintain nutrient homeostasis was enhanced as a result of the administration of the *Phylanthus amarus* extract. The administration of the extract caused an increase in the total protein when compared to the other groups which indicate stabilization of plasma membrane and protection of liver cell membrane. It also implies that the liver's synthetic activity and ability to maintain nutrient homeostasis was enhanced as a result of the administration of *Phylanthus amarus* extract.

V. Conclusion

In general, it can be concluded that ethanol extract of *Phylanthus amarus* ameliorates liver pathology in the experimental animals after the evaluation of sodium fluoride toxicity on the liver function parameters.

References

[1]. Al-Harbi, M.S., Hamza, R.Z. and Dwari, A.A. (2014) Ameliorative Effect of Selenium and Curcumin on Sodium Fluoride Induced Hepatotoxicity and Oxidative Stress in Male Mice. Journal of Chemical and Pharmaceutical Research, **6**:984-998.

[2]. Basha, S.K. and Rao, K.J. (2014) Sodium Fluoride Induced Histopathalogical Changes in Liver and Kidney of Albino Mice. Acta Chimica and Pharmaceutical Indica, **4**:58-62.

[3]. Che, C.-T. George, VIjinu, T.P. Pushpangadan, P. and Andrae-Marobela, K. (2017). Traditional Medicine. Fundamentals, Applications and Strategies. Pharmacognosy, 15-30

[4]. Dabrowaska, E., Letko, R. and Balunowska, M. (2006) Effect of Sodium Fluoride on the Morphological Picture of the Rat Liver Exposed to NaF in Drinking Water. Advances in Medical Sciences, **51**:91-95.

[5]. Demissew, S. and Dagne, Ermias (2001). Proceedings of National Workshop on Conservation and Sustainable Use of Medicinal Plants in Ethiopia. Basic and applied research on medicinal plants of Ethiopia, 29-33.
[6]. Dhama, K., Tiwari, R., Chakrabort S. (2014). Evidence Based Antibacterial Potentials of Medicinal Plants And Herbs Countering Bacterial Pathogens Especially in the Era of Emerging Drug Resistance: An Integrated Update. International Journal of Pharmacology, **10**(1):1–43.
[7]. Gundgaard, J., Nielsen, J.N., Olsen, J. and Sorensen, J.(2003). Increased Intake of Fruit and Vegetables: Estimation of impact in terms of life expectancy and healthcare costs. Public Health Nutrition, 6: 25-30.
[8]. Guo-Ving, X., Sun, G. and Shun, Y. (2003) Oxidative Stress from Fluoride Induce Hepatotoxicity in Rats. Fluoride, **36:**25-29.
[9]. Gundgaard, J. Nielsen, J.N. Olsen, J. and , J. (2002). Increased intake of fruit and vegetables: estimation of impact in terms of life expectancy and healthcare costs. Public Health Nutrition, **6**(1), 25–30
[10]. Hari Kumar, K.B. and Kuttan, R. (2006) "Inhibition of drug metabolizing enzymes (cytochrome P450) in vitro as well as in vivo by Phylanthus amarus Schum & Thonn," Biological and Pharmaceutical Vulletin,1310-1313.
[11]. Joseph, B. and S.J. Raj, (2010). Phytopharmacological and phytochemical properties of three Ficus species - An overview. International Journal of Pharmacological Bioscience, **1**: 246-253.
[12]. Liu H., Zeng Q., Cui Y., Zhang L., Fu G., Hou C., Zhao L. and Wang A. (2014). Fluoride-induced thyroid cell apoptosis. Fluoride, **47**:161–169.
[13]. Lui, R-LH and Huang, YL. (2003). Genus Phyllanthus for chronic hepatitis B virus infection: A systemic review. Viral Hepatitis, **8**:358–366.
[14]. Nathwani, R.A., Pais S, Reynolds, T.B. and Kaplowitz, N. (2005) Serum alanine aminotransferase in skeletal muscle diseases. Hepatology, (2):380-2. doi: 10.1002/hep.20548. PMID: 15660433.
[15]. Nkosi, C.Z., Opoku, A.R and Terblanche, S.E. (2005). Effect of pumpkin seed (Cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in ccl_4 induced liver injury in low protein fed rats. Physiology Research, **19**;341-345
[16]. Philomena, G. (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. Journal of Applied Pharmaceutical Sciience,**1**(6):40–4.
[17]. Rajakumar, N. and Schivanna, M.B, (2009) "Ethno-medicinal application of plants in the eastern region of Shimoga district, Karnaraka, India," Journal of Ethnopharmacology, pp. 64-73.
[18]. Rakhee, R.N and Regin S.A. (2008). Mini- Review: Integrating the science of pharmacology and Bioformatics. Phyllanthus "The wonder plant". Adr Biotechnology, **10**:28-30.
[19]. Rentman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, **28**:56-62.
[20]. Row, L.R, Satyanarayana P., and Rao G.S. (1967). Crystalline constituents of euphorbiaceae VI: The synthesis and absolute configuration of phyllanthin. Tetrahedron, **23**(4):1915-8.
[21]. Samuel, J.K. and Andrews, B., (2010) "Traditional medicinal plant wealth of Pachalur and Periyur hamlet Dindigul district, Tamil Nadu," Indian Journal of Traditional Knowledge, pp. 264 -270.
[22]. Samy R.P., P.N. Pushparaj, and P.A. (2008). Gopikrishnakone, "Compilation of bioactive compounds from Ayurveda," Bioinformation, pp. 100 – 110.
[23]. Samy, R.P., Pushparaj, P.N., and P.A. (2008). Gopikrishnakone, "Compilation of bioactive compounds from Ayurveda," Bioinformation, pp: 100-110,.
[24]. Santos, ARS, Ailho, VC, Yunes, RA. and Calixto, JB. (1995). Analysis of the mechanism underlying the Anti-nociceptive Effect of the Extracts of plants from the Genus Phyllanthus. General Pharmacoogy, **26**:1499–1506.
[25]. Singh A., Ogunbodede E., Onayade A. The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines. 2013;10(5):210–229.
[26]. Sofowora, E.A. (1982) Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd., Hoboken, 64-79.
[27]. Sookoian S, Pirola CJ (2012). Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. World Journal of Gastroenterology, 7;18(29):3775-81.
[28]. Unander DW, Webster GL, Blumberg BS (1995). Usage and bioassays in Phyllanthus (Euphorbiaceae): IV. Clustering of antiviral uses andother effect. Journal of Ethnopharmacology, 45: 1-18
[29]. Unuofin JO, Lebelo SL (2020). Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. Oxidative Medicine Cellular Longevity, 1356893.
[30]. Upadhyay, B., Parveen, P., Dhaker, A.K., and Kumar, A., (2010) "Ethnomedicinal and ethnoparmaco-statistical studies of Eastern Rajasthan, India, "Journal of Ethnoparmacology, 64-86.
[31]. Zhao, J., Wang H., Tian E., Dong F. and Zhou B. (2014). Toxic effects of fluoride on primary lymphoid organs and white blood cells in female mice. Fluoride, **47**:227–234.

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