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An Investigation of the Effects of Counterfeit Neurobion on Serum Micronutrient Levels in Female Rats

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Abstract – Micronutrients such as vitamins and minerals are essential to life, health and development. While their deficiencies have been widely linked with various pathological presentations, their serum elevated levels have also been recognized in many disease states. Many agents even those with therapeutic usefulness have been known to alter serum micronutrients levels. This raises the stake with fake drug administration. The aim of the study therefore is to identify if administration of fake neurobion is capable of causing serum micronutrient alterations in female Wistar rats of average age of thirteen weeks. Eighteen rats were divided into 3 groups of 6 rats each. The first, second and third groups of rats were dosed with 30 mg/kg BW of fake Neurobion, genuine neurobion®, and distilled water respectively. The route of administration was by gastric gavage. Determination of serum mineral and vitamin levels were carried out using Atomic Absorption Spectrometric method and high performance liquid chromatographic technique (HPLC) respectively. Of all the minerals (Zn, Cu, Se, Mn, Co, Fe, Mo, and Cr), none was significantly different with their p value being $p>0.05$ except Mn. Serum levels of riboflavin, niacin, folic acid, pantothenic acid, and vitamins A, C, D and E were also not significantly different but those of thiamine, pyridoxal and cyanocobalamin were significantly different, with significantly higher levels observed only in genuine neurobion administered rats. It seems evident from results obtained that the fake neurobion used for the study is deficient in significant amount of thiamine, pyridoxal and cyanocobalamin.
Keywords – Neurobion, Counterfeit Drug, Mineral, Vitamin

1. Introduction

Micronutrients (vitamins and trace elements) are known to play important roles in the body [1] and a number of foreign chemicals or substances have been reported to modulate their metabolism [2], [3]. Fake drugs especially are known to contain very harmful chemicals [4], [5]. Exposure to these counterfeit agents has been reported to be a common cause of death in the developing world. Even when they do not cause instant tissue damage or death, the possibility of micronutrient alteration occurring in fake drug-exposed animals cannot be ruled out. Whereas, it is a common knowledge that in most cases, deficiency of these micronutrients occurs as a result of inadequate intake but their abnormal low levels in the body have also been reported when adequate intake co-exists with conditions of increased oxidative stress [6], [7].

Both vitamin and mineral supplements or preparations are found in wide range of products. An example of which is neurobion that is made up of thiamine, pyridoxal and cyanocobalamin, although food sources are the common means by which vitamins and minerals are obtained in humans. It was not until the mid-1930s that the first commercial yeast-extract and semi-synthetic vitamin C supplement tablets were sold. Prior to this, vitamins were obtained mainly through food intake. Since the middle of the 20th century, vitamins have been produced and made widely available as inexpensive semisynthetic and synthetic chemicals- a source of multivitamin dietary supplements [8], [9]. As a result of this wide availability and non-requirement of a doctor's prescription before being purchased as well as its usefulness even in healthy subjects, there is great demand for vitamins thereby increasing the possibility of consuming fake preparations. This is especially more likely in the developing world where a high percentage of therapeutic agents or nutritional supplements are fake products [10], [11]. Therefore, the objective of this study is to assess the levels of thiamine, pyridoxal and cyanocobalamin and other micronutrients in the serum of rats treated with 30 mg/kg BW of fake neurobion formulation. Micronutrients are known for their antioxidant activities and the vital roles they play in a number of metabolic processes in mammals.

2. Materials and Methods

2.1 Experimental Animals and Animal Treatment

Female Wistar rats of average age of thirteen weeks were employed for the study, which was carried out in accordance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). They were purchased and kept at the Experimental Animal Unit of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were kept in cages at ambient temperature of 25±2°C and a 12 h light, 12 h dark cycle and were supplied with feed and water without any form of restriction. The study was for a period of 21 days. Eighteen rats were divided into 3 groups comprising of 6 rats per group. The first group of rats was dosed with 30 mg/kg BW of fake neurobion while those in the second group received genuine

neurobion®. Rats in the third group served as the control and were administered with distilled water. The route of administration was by gastric gavage for all the rats in the 3 groups. The fake neurobion used for the study was obtained from National Agency for Food and Drug Administration and Control (NAFDAC), Western region office in Ibadan. On the other hand, rats in the second group received genuine neurobion®, which was obtained from a reputable Pharmacy and produced by Merck Marker (Pvt) Ltd (7, jail road Quetta), under license of Merck GaA Damstadt Germany. The blood was collected through retro-orbital bleeding and discharged into anti-coagulant free bottle, centrifuged at 3000 g for ten minutes. The serum obtained was stored at - 20ºC until required for analysis.

2.2 Determination of serum mineral and vitamin levels

Serum levels of thiamine, riboflavin, niacin, folic acid, pantothenic acid, and vitamins A, B_6, $B_{12,}$ C, D and E were determined using High Performance Liquid Chromatographic technique (HPLC). The serum concentrations of Zn, Cu, Se, Mn, Co, Fe, Mo, and Cr, were quantified using the Atomic Absorption Spectrometric method. The HPLC equipment supplied by Waters® Corporation Milford, Massachusetts USA and Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) were employed for these estimations.

2.3 Statistical analysis

The degree of significant difference among the serum levels of micronutrients for the three groups was determined, by using analysis of variance (ANOVA). A p value of ≤ 0.05 was considered significant. SPSS package version 15 was used for this purpose.

3. Results

Results of the study presented in **Table 1** show that treatment of female Wistar rats with either fake or genuine neurobion did not cause significant changes in the serum levels of all the minerals (Zn, Cu, Se, Mn, Co, Fe, Mo, and Cr) estimated, except Mn. Serum concentrations of riboflavin, niacin, folic acid, pantothenic acid, and vitamins A, C, D and E were also not significantly different whereas the levels of thiamine, pyridoxal and cyanocobalamin were significantly different, with significantly higher levels observed only in genuine neurobion administered rats as presented in **Table 2**.

Table 1: Levels of vitamins in serum of rats administered with fake and genuine neurobion

	Control	Fake drug	Genuine drug	F-value	P-value
Vitamin E (µmol/L)	27.98±4.05	30.22±2.80	27.78±1.35	1.480	0.254
Vitamin C (mmol/L)	65.67±3.26	68.65±4.85	67.71±7.90	0.504	0.612
Vitamin A (µmol/L)	1.91±0.03	1.88±0.06	1.92±0.03	1.766	0.199
Riboflavin (nmol/L)	405.71±15.39	403.44±7.91	410.90±16.05	0.551	0.586
Folic (nmol/L)	20.11±2.14	20.48±1.70	19.70±1.14	0.362	0.701
Pyridoxine (nmol/L)	81.88±6.25	80.98±4.09	94.98±6.98	12.331	0.005*
Niacin (nmol/L)	117.41±6.43	115.98±7.75	112.31±5.77	1.082	0.360
Thiamine (nmol/L)	131.83±14.39	136.31±13.24	165.19±16.22	23.506	0.002*
Vitamin D (nmol/L)	61.22±7.56	61.81±2.57	58.62±2.67	0.851	0.443
Pantothenic acid (µmol/L)	2.06±0.06	2.07±0.07	2.00±1.17	0.977	0.396
Cyanocobalamin (ng/L)	364.41±11.08	349.51±18.94	481.80±42.25	28.290	0.009*

- Significant difference at ≤ 0.05

Table 2: Levels of minerals in serum of rats administered with fake and genuine neurobion

	Control	Fake genuine	Original	F-value	P-value
Se (µmol/L)	1.40±0.05	1.44±0.05	1.42±0.06	0.699	0.510
Cu (µmol/L)	25.26±2.75	26.17±2.57	24.01±1.25	1.586	0.232
Zn (µmol/L)	18.72±2.33	20.99±2.19	20.55±1.86	2.206	0.139
Mn (nmol/L)	190.34±6.20	186.06±12.41	206.52±18.03	9.525	0.002*
Fe (µg/dl)	150.67±8.47	155.66±4.31	153.83±9.11	1.103	0.353
Mo (nmol/L)	23.36±2.37	25.23±1.40	22.87±2.87	2.057	0.157
Cr (nmol/L)	1.65±0.05	1.60±0.04	1.62±0.04	2.245	0.135
Co (nmol/L)	3.96±0.27	4.12±0.06	4.02±0.04	1.496	0.251

- Significant difference at ≤ 0.05

4. Discussion

Micronutrients (i.e. vitamins and trace elements), though found in the body in small quantities are very essential for health, growth and reproduction. The fact that trace elements occur in small amount but have profound effects on a number of physiologic processes in the body is the basis of one of their characteristics i.e. amplification of action. Some of these important functions include the following: L-ascorbic acid is a known antioxidant that also serves as a co-factor for enzymes. Specifically, it plays this role for hydroxylases and monooxygenases; these are enzymes that take part in the formation of carnitine and neurotransmitters [12]. In addition, it has the potential to prevent the oxidation of low density lipoprotein (LDL) primarily by scavenging the free radicals and other reactive oxygen species in the aqueous state [13]. The role of ascorbic acid in wound repair and healing/regeneration process as it stimulates collagen synthesis has also been documented.

Many other components of the micronutrients such as the metals (e.g. Zn, Cu) that play a role in the antioxidant property of superoxide dismutase were not significantly different except Mn, when the 3 groups were compared using ANOVA. Vitamin A is important in vision, growth, reproduction, and embryonic development as well as immune response[1] while vitamin E plays an essential role in reproduction[1], normal neurological function and prevention of red cell hemolysis. In addition, vitamin E inhibits free-radical chain reactions of lipid peroxidation particularly within the polyunsaturated fatty acids of membrane phospholipids. These two vitamins (vitamins A & E) with antioxidant potential were also not significantly different when the three groups were compared. Interestingly, pyridoxal, thiamine and cyanocobalamin were also not significantly different when the fake group was compared with control, rather a significantly higher serum levels of the three vitamins were recorded in rats administered with genuine neurobion. This was expected neurobion is made up of pyridoxal, thiamine and cyanocobalamin.

World Health Organization (WHO) has defined fake drug as agents that may contain excessive amount of active ingredients, sub-optimal level or even adulterants that are toxic, others agents without any active ingredient whatsoever are also classified as fake drug. contain no active ingredients at all. The results of this study seems to suggest that this may be an instance of a complete lack of active ingredients i.e. pyridoxal, thiamine and cyanocobalamin in the neurobion preparation. This is not surprising, because items such as plaster of Paris, baking soda or other inexpensive ingredients have been found as components of some pills. Of the various types of fake drugs seized by NAFDAC some of them have been found to contain lactose or even chalk especially those in tablet form and olive oil in capsules. While induction of oxidative stress has been well documented in relation to fake drugs, that seems to be absent in this case since antioxidant vitamins like vitamins A, C and E were not significant different when the three groups were compared.

Folate known for its role as a coenzyme in several single carbon transfers involved in biosynthesis of purine nucleotides and deoxythymidylic acid, essential for DNA and RNA synthesis as well as provision of one-carbon unit for methylation of a wide variety of biological substances including DNA, proteins, phospholipids, and neurotransmitters [14] was also not significantly different. Other micronutrients like Cr, Co, Se, Fe and Mo were not significant different when the three groups were compared as well as riboflavin, niacin, vitamin D, and pantothenic acid.

This may be one of the reasons why most times the effects of fake drug go unnoticed as Erhum et al. [15] have rightly pointed out. They highlighted that in most cases, effects of consuming fake drugs go unnoticed except when mass deaths result from their exposure. This may also be one of the reasons why there is no reliable data on the mortality or morbidity arising from the consumption of counterfeit drugs in Nigeria. This is because many of them contain neither active ingredients nor toxin. Unfortunately, the kind of results obtained through this study, may not provoke serious government involvement in combating the harmful effects of fake drugs. Since the effects are not dramatic, rather it is incidence like that of 1990, in which 109 children died after being administered with fake paracetamol[5] that usually stimulate governments in the developing world into taking positive steps in addressing issues related to drug counterfeiting.

That the serum levels of pyridoxal, thiamine and cyanocobalamin were significantly higher and not significantly different in genuine and fake drug administered rats respectively compared with control suggests absence of these three vitamins in fake neurobion used for this study. This is in agreement with many observations and submissions in the past. According to the World Health Organisation (WHO) about 25 percent of medicines consumed in some developing countries, including Nigeria are counterfeit or substandard. Instances like this will likely compound many of the problems being encountered by many of these countries. For example, it is known that drug counterfeiting exposes consumers to harmful and ineffective drugs and retailers to reduced consumer demand and profits.

Inadequate legislation, weak enforcement and a poor distribution system seem to be some of the reasons why drug counterfeiting has remained a thriving business in Nigeria. In other parts of the world, regulations are in place to protect consumers from harmful effects of counterfeit drugs. In 1994, the Dietary Supplement Health and Education Act (DSHEA) amended the Federal Food, Drug, and Cosmetic Act (FDC Act) to set up a distinct regulatory framework for what are now commonly known as dietary supplements. This was to provide consumers access to safe dietary supplements to help maintain or improve their health.

The drug distribution network that is in a pathetic state must be contributing to the fake drug problems in Nigeria. A drug distribution network that consists of open markets, patent medicine stores, community pharmacies, private and public hospitals, wholesalers/importers and pharmaceutical manufacturers. In Nigeria it is common to see petty traders who hawk kola-nuts, cigarettes, and oranges, among other items, in market kiosks, motor parks, and road sides hawking drugs ranging from over-the-counter medications to antibiotics (popularly called “capsules”) [16] without any regard for their ideal storage. For instance, exposure of some types of medications to sunlight has been known to facilitate deterioration of the active ingredients. A good example of this being multivitamin supplements; vitamins like riboflavin are light sensitive.

Moreover, patent medicine stores are owned by the holders of patent and proprietary medicine vendors licenses; these are stores where over-the-counter medicines are the only drugs authorized to be sold. Unfortunately, because of financial benefits, all types of drugs are sold by vendors. And since the basic knowledge of these vendors is minimal- (the minimum academic requirement to obtain a license is the first school-leaving certificate)- this means they are not well equipped to differentiate between fake and genuine products [17]. Other reasons that have been adduced for availability of counterfeit drugs are inadequate law, ineffective enforcement of existing laws, non-health professionals in drug business, loose control systems, high cost of drugs and corruption [15], [18] – [20].

4. Conclusion

Irrespective of the cause of drug counterfeiting, one thing this study has brought to the fore is that even when the usage of fake drug does not produce instant death (since 0% mortality was recorded), serious health consequences may result. In addition, the non-significant difference in the serum levels of pyridoxal, thiamine and cyanocobalamin of rats dosed with fake neurobion tablets suggests that little or no active ingredient was incorporated in the formulation used for the study.

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