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Serum Heavy Metal Study In Female Wistar Rats Administered With Counterfeit Neurobion

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ABSTRACT

Purpose: While trace elements are known for the very important role they play as cofactors of diverse biochemical reactions, heavy metal on the other hand have been recognized for their toxic effects and the fact they do not carry out any meaningful role that is beneficial to a living mammalian system. Yet exposure has been reported through different sources, exposure through fake drug administration being one of them. The aim of the present study is to determine the serum levels of Cd, Pb, Al, Si, Hg, Ni, and As in 12-14 weeks old female Wistar rats administered with fake neurobion tablets at 30 mg/kg level.

Method: Twelve of the eighteen rats used for the study were divided equally into 2 groups and administered with either 30 mg/kg BW fake neurobion or genuine products. Six other rats served as the control. Route of administration was by gastric gavage and blood was obtained through retro-orbital bleeding. Serum levels of arsenic, cadmium, aluminum, silicon, lead, mercury, and nickel were estimated by the atomic absorption spectrometric method. Statistical analysis was carried out on data obtained using analysis of variance (ANOVA). Differences were considered significant at $p \leq 0.05$.

Results: Of all the heavy metals assessed, none was significantly different.

Conclusion: The results of the study suggest that the absence of the heavy metals in the fake neurobion used for the present study.

Key-words: Heavy metal; fake drug; neurobion; female rat.

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INTRODUCTION

Neurobion® tablets are known to contain thiamine mononitrate, pyridoxal HCl, and cyanocobalamin; important components of vitamin B complex[1]. Vitamins generally are considered dietary supplements that do not require doctor's prescription before being purchased[2].Yet they are sometimes combined with other therapeutic agents in standard regimen for the treatment of a number of clinical conditions. For example, while thiamine or its derivatives is beneficial in the management of inflammatory condition, polyneuropathy, and Alzheimer's disease[3]; pyridoxal has been found to be of great benefit in the treatment of neuroleptic-induced tardive dyskinesia in individuals with schizophrenia and pyshcotic disorders. Cyanocobalamin on the other hand has been used extensively in the management of a variety of conditions.

Aside their use in management of clinical conditions, vitamins are known to carry out important functions in many physiologic processes. Vitamin B1 (thiamine) in its pyrophosphate form participates in carbohydrate, and protein metabolism, as well as in synthesis of nucleic acids as a co-enzyme. It also influences the neural impulse conduction in the synapse. Vitamin B6 (pyridoxal) on the other hand, is a part of the following enzymes: decarboxylase and transaminase, thereby participating in histamine, free fatty acid, and amino acid metabolism. It also plays a vital role in the normal functioning of the central and peripheral nervous system, skin, and gastrointestinal tract.

A wide range of items, substances or chemicals have been identified as contaminants in fake drugs e.g. diethylene glycol, wheat flour, starch[4,5]and a number of physiological and biochemical abnormalities have also been associated with exposure to fake or counterfeit agents. The aim of this study is to determine the serum levels of Cd, Pb, Al, Si, Hg, Ni, and As in female Wistar rats dosed with 30 mg/kg of fake neurobion tablet.

MATERIALS AND METHODS

Experimental Animals: Experimental animals consisted of eighteen adult female albino rats of between twelve and fourteen weeks of age. They were supplied by the Experimental Animal Unit of the Faculty of Veterinary Medicine of the University of Ibadan, Nigeria. The animals were left in their respective cages to acclimatize for about a period of two weeks prior to commencement of the experiment. Animals were kept in cages at ambient temperature of 25±2°C and a 12 h light; 12 h dark cycle and were fed standard laboratory chow and given water ad libitum. The rats were divided into 3 groups; each group comprised of 6 rats. For each of the rats, oral route of administration was employed. The first group of rats was dosed with 30 mg/kg BW of Fake neurobion; rats in the second group were administered with genuine neurobion while the third group served as the control and were administered with distilled water. Genuine neurobion that was used for the study was produced by Merck Marker (Pvt) Ltd (7, jail road Quetta), under license of Merck GaADamstadt Germany. The drug administered to the rats in the first group was obtained from National Agency for Food and Drug Administration and Control (NAFDAC); Western region office in Ibadan, the original product was purchased from a reputable Pharmacy. The duration of the experiment was for a period of 21 days. This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

Preparation of serum samples & heavy metal estimation: The day after the last dose was given to the rats; blood was drawn from all the rats through the method of retro-orbital bleeding and dispensed into an anticoagulant free bottle. Subsequent to this, blood was centrifuged at 3000 g after which serum was separated and stored at -20 ºC. Levels of arsenic, cadmium, aluminum, silicon, lead, and nickel in serum were estimated by the atomic absorption spectrometric method (Buck Scientific 205 Atomic Absorption (East Norwalk, Connecticut, USA).

Statistical analysis: Data were subjected to statistical analysis using SPSS package (version 15) to obtain mean ± SEM (standard error of mean). The level of significant difference among the three groups was determined by using analysis of variance (ANOVA). P≤ 0 .05 was considered significant.

RESULTS

Results presented in **Table 1** revealed that administration of fake neurobion did not result in significant differences in the serum levels of heavy metals such as aluminum, cadmium, silicon, nickel, lead and arsenic. Moreover, administration of genuine Neurobion also did not cause changes in the serum levels of heavy metals estimated. Therefore using analysis of variance all the results were not significantly different at p ≤ 0 .05.

Table 1:Serum levels of select heavy metals in fake and genuineneurobion-administered rats.

	Pb (µg/L)	As	Cd (µg/dl)	Si (µg/L)	Al (µg/L)	Ni (µg/dl)
Controls	0.14±0.02	0.08±0.01	6.14±1.06	0.10±0.02	0.16±0.02	0.11±0.02
Fake neurobion	0.14±0.02	0.07±0.01	5.39±0.80	0.09±0.03	0.17±0.03	0.12±0.20
Original neurobion	0.15±0.02	0.07±0.01	5.86±1.23	0.10±0.02	0.17±0.02	0.12±0.10
F-value	1.140	1.488	0.824	2.380	1.128	1.304
P-value	0.342	0.252	0.455	0.121	0.346	0.296

Results are expressed as mean ± standard deviation. *p <0.05 is significant using ANOVA, n=6.

DISCUSSION/CONCLUSION

A variety of drugs have been reported to be counterfeited all over the world. Even vaccines and other lifesaving medications, such as heart medication, diabetes drugs, and antimalarials have been reported to be frequently counterfeited in China. The devastating effects of counterfeit drug are not limited to the developing world. In 2007, Marcia Bergeron died from cardiac arrhythmia after taking counterfeit drug that was purchased in Canada from an online pharmacy. The drug originated from Czech Republic and was found to contain high levels of metals[6]. In some cases these fakes are put in packaging where only a letter or two is altered on the name. An example of this is a type of counterfeit product frequently found in East-African markets, a drug called “Vegra,” a play off of the brand Viagra. Also during the height of the H1N1 influenza outbreak in October 2009, all five products represented online as Tamiflu that were purchased and analyzed by the US FDA; were found to be fakes. With one particular order from India in an unmarked envelope found to contain only talc and acetaminophen[7].

A number of items have been identified in counterfeit drugs. In 2008, several tons of expired, unregistered, and counterfeit medicines including antibiotics, pain killers, antimalarials and vitamins were seized in Tanzania[8]. The counterfeit antimalarials especially were found to contain only wheat flour[4]. Counterfeit rabies vaccines, made with a combination of distilled water and starch have also been seized in China). Most “fillers” in drugs such as impotence medicines and anabolic supplements are safe placebos like sugar, but some pills contained anti-freeze, wood polish, plaster, amphetamine, lead and other dangerous compounds[9, 10]. This means that very harmful substances have also been reported to be incorporated into fake drugs.

In 2006, Chinese government shut down a factory producing both fake Viagra and birth control pills which contained starch, glucose, and toxic substances[11]. Rat poison, boric acid and lead paint have also been reported as contaminants of therapeutic drugs[12]. This practice is more common in the developing world (e.g. the Gambia) where there are no systems that monitor either drug quality or regulate pharmacy operations. In these countries counterfeit drugs are as prevalent as authentic products. Chalk has been reportedly sold as aspirin, paracetamol, or as anti-malarials. Many of these deceptive drugs being linked to nations like China, India or Nigeria.

Metal contamination of therapeutic drugs has been reported and unlike essential trace elements even at relatively low levels, heavy metals can still trigger a variety of processes that will invariably result in a number of pathological conditions. Heavy metals though not essential to life are known to be found in minute quantities in the body of even healthy individuals. They are introduced into the body through water, air or food products. Since the level of an element in the serum usually is a reflection of its general metabolism i.e. absorption, excretion, utilization and tissue distribution, the results of this study will help to address whether fake neurobion administered to Wistar rats altered the general metabolism of heavy metals.

Aluminum salt in its phosphate form is known to cause elevation in lipid peroxidation rate, leading to an increase in the levels of malondialdehyde and a decrease in GSH[13]. Arsenic on the other hand, inhibits the mitochondrial enzymes, impairs the cellular respiration, and induces cellular toxicity. Since it can substitute phosphate intermediates, theoretically this means it slows down the rate of metabolism and interrupt the production of energy[14]. In addition, Ramos et al.[15] have indicated that there is a positive correlation between arsenic concentration and lipid peroxidation levels in liver, kidney, and heart. Another toxic metal, cadmium inhibits liver metabolic enzyme systems containing sulphydryl groups and uncouples oxidative phosphorylation in mitochondria[16]It also causes elevated lipid peroxidation, hepatic congestion, ischemia, hypoxia[17], and a distortion of defense enzymes are reported[18]. Avidly binding of Al to proteins such as transferrin causes its rapid distribution through the body with higher accumulation in the brain and bone[19,20]. In the bone, it disturbs normal calcium exchange; such that Al replaces calcium at the point of mineralization thereby impairing normal osteoid formation[21]. In the brain it accumulates in the neurofibrillary tangle and focal accumulation of Al had an association with neurofibrillary degeneration in the hippocampal neurons. Aside from its impact on the central nervous and skeletal systems, it also causes abnormality of hematopoietic and skeletal systems as well as cognitive deficits in young children[22,23].

These are some of the likely events that may accompany heavy metal contamination of fake drug. The results of the study though seem to suggest that heavy metal contamination is lacking in the neurobion formulation used for this study. This is because all heavy metals investigated in the serum of fake drug administered rats were not significantly different compared with control. This suggests that while adulteration of drugs and dietary supplements has been reported in the past, it seems the agent under investigation is not heavy metal-contaminated.

While heavy metal contamination of therapeutic drug is a known possibility and has been identified to be the cause of some counterfeit drug- related pathologiesin some past studies, the results of this study of no significant differences in the serum levels of all heavy metals estimated suggest absence of any of the following Cd, Pb, Al, Si, Hg, Ni, and As in the fake neurobion used for the present study.

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