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Research Article

CHANGES IN LEVELS OF ANTIOXIDANT MINERALS AND VITAMINS IN WISTAR MALE RATS EXPOSED TO METHIONINE CONTAINING ACETAMINOPHEN FORMULATION

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ABSTRACT
This study was carried out to determine the effect of toxic and subtoxic doses of acetaminophen on antioxidant vitamins and minerals in male Wistar rats. Five groups served as the test groups and received different doses of the acetaminophen\methionine formulation in ratio 9:1. The sixth group served as the control and received only the vehicle: physiologic saline. At the end of 24 hours of exposure, blood was obtained from each rat through retro-orbital bleeding, the levels of antioxidant vitamins and minerals were determined using high-performance liquid chromatography and atomic absorption spectrophotometric technique. Results showed that at 100 mg/kg BW level of exposure, niacin, vitamin A, vitamin E, zinc, copper, manganese, selenium were significantly increased ($p< 0.05$) whereas riboflavin was not significantly different ($p>0.05$) compared with controls. At both 350 and 1000 mg\kg BW levels of exposure, all these antioxidant indices were significantly decreased ($p<0.05$) except niacin which was not significantly altered ($p>0.05$) at 350 mg\kg BW (body weight) level of exposure compared with the control group. The animals in 3000 mg\kg BW and 5000 mg\kg groups suffered 40% and 100% mortality respectively by the end of the 24th hour. This study therefore showed that acetaminophen\methionine formulation in the ratio of 9:1 may cause alteration of vital elements and biomolecules not only at toxic levels but also at tolerable level of 100 mg\kg BW.
KEYWORDS: acetaminophen, methionine, vitamin, mineral, Wistar rat.

INTRODUCTION

Acetaminophen (paracetamol) is the standard analgesic and antipyretic agent. It is used for the treatment of mild to moderate pain states[1,2]. It was first synthesized in 1878 by Harman Northrop Morse, but was not used for the treatment of pyrexia until 1893[3]. The discovery that acetanilide could cause methemoglobinemia led to the replacement of acetanilide with acetaminophen. It gained wide appeal as a therapeutic agent especially in children in 1980's when aspirin was linked to Reye's syndrome[4,5]. At toxic level of exposure, it causes depletion in mitochondrial and cytosol glutathione levels in susceptible tissues especially the liver. The major metabolite generated at toxic level of exposure; the N-acetyl p- quinoneimine is responsible for many of the clinical manifestations of toxicity especially centrilobular necrosis for which acetaminophen is noted[5,6].

To prevent toxicity as a result of accidental and intentional overdose, McLean[7] suggested the inclusion of an antidote in paracetamol tablets. The use of acetaminophen-methionine has been on for some time. Methionine in vivo is converted to cysteine; cysteine bears the –SH group responsible for the detoxification process. Its administration has also been reported to replete or increase glutathione level in experimental animals[8]. Although acetaminophen has high safety profile, the inclusion of an antidote especially methionine to forestall cases of overdose may call for caution. Especially as it has been reported that at high doses it causes nausea, vomiting, drowsiness, spleno-hepatomegaly. Its association with increase homocysteine level may increase risk of cardiovascular diseases[9]. Specifically a raised plasma homocysteine has been linked to peripheral vascular disease, stroke and ischemia heart disease[10]. There are also study reports to suggest that methionine may alter some vital elements and biomolecules e.g. B_6, B_{12} and folate[10, 11].

Moreover, acetaminophen itself has been linked to oxidative stress-induced diseases e.g cancer, and although an increase in risk of cancer due acetaminophen exposure has not been demonstrated epidemiologically in vitro and animal studies have shown that acetaminophen binds covalently to DNA, inhibits its repair system thereby causing chromosomal aberration in somatic

cells[12]. In addition, two studies have revealed a higher level of chromosomal aberration in the lymphocytes of human volunteer subjects[12].

This study is embarked on to establish a relationship between exposure to methionine-containing acetaminophen and some antioxidants at both tolerable and toxic levels of exposure. This is important as long time depletion of these antioxidants may result in a number of oxidative-stress induced diseases.

MATERIALS AND METHODS

Animal preparation and treatment design

Thirty male Wistar rats (200-240) g were obtained from the animal house the Department of Veterinary Physiology, Faculty of Veterinary Medicine, the University of Ibadan, Nigeria. All the animals were kept in cages in a well-ventilated room. They were fed with standard diet obtained by Ladokun Feeds, Ibadan and supplied with adequate clean drinking water with any form of restriction. All the experimental animals were handled in compliance with internationally accepted principles for laboratory animals' use and care as found in US guidelines (NIH publication\85-23, revised in 1985).

The paracetamol formulation containing paracetamol: methionine in ratio 9:1 were dissolved in physiologic saline produced by Unique pharmaceutical (Nigeria). The preparation was administered through intraperitoneal route. The control group received only the vehicle while the treatment groups were divided into five groups, namely groups-1, 2, 3, 4 & 5. Groups 1, 2, 3, 4 & 5 received 100 mg\kg BW (body weight), 350 mg\kg BW, 1000 mg\kg BW, 3000 mg\kg BW & 5000 mg\kg BW of the formulation respectively. At the end of the 24th hour of exposure, the study was terminated and the animals in all the six groups were sacrificed to obtain blood for biochemical estimations.

Sample preparation and vitamin and mineral estimation

Blood sample was obtained from each animal through retro-orbital bleeding. The blood that was left to clot for two hours was centrifuged at 3000 r.p.m. and the serum aspirated into plain bottles and stored at -20°C until they were required for analysis. The level of trace elements in the serum samples were estimated using the atomic absorption spectrometry technique. Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was utilized for this purpose at wavelength suitable for each element; zinc (213nm), copper (324nm), selenium (196nm) and manganese (279nm). Both fat and water soluble vitamins; vitamin A, vitamin E, riboflavin and niacin were estimated through the high performance liquid chromatographic techniques (HPLC), Waters 626 LC SYSTEM (Waters Corporation Milford, Massachusetts, USA) was used for this purpose.

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS. Results were expressed as mean ±SD (standard deviation). Student t' test was used to test the level of significance in the test groups compared to the controls. Analysis of variance (ANOVA) was used to determine differences between various groups. Values of $P<0.05$ was considered as significant.

RESULTS

Estimation of the concentrations of antioxidants e.g. vitamins and minerals is an important way to assess the degree of oxidative stress in an individual. Administration of this formulation resulted in a significant increase at 100 mg\kg BW and significant decreases at 1000 & 3000 mg\kg BW in the levels of niacin compared to the control group ($p<0.05$). Only rats in the 350 mg\kg BW remained significantly unchanged ($p>0.05$). For riboflavin significant decreases were recorded at 350 mg\kg and 1000 mg\kg BW levels of exposure as well as a significant increase at 3000 mg\kg BW of exposure compared to controls ($p<0.05$). No significant difference was recorded at 100 mg\kg BW level of exposure (Table 1).

Also in table 1, both fat soluble vitamins; vitamin A and vitamin E showed only slight changes in their trend of significant alterations. At 100 mg\kg BW both were significantly higher in exposed rats compared with controls ($p<0.05$). Both were equally significantly decreased at 350 mg\kg and 1000 mg\kg BW levels of exposure compared to the controls ($p<0.05$), but at 3000 mg\kg BW level of exposure there was no significant difference and a significant decrease for vitamin A and Vitamin E respectively compared with controls.

In table 2, zinc and copper important cofactors for the antioxidant enzyme superoxide dismutase showed the same trend of alteration at 100, 350 and 1000 mg\kg BW levels of exposure. At 100 mg\kg BW of exposure both were significantly increased whereas at both 350 and1000 mg\kg levels of both were significantly decreased compared to the control group ($p<0.05$). It was only at 3000 mg\kg BW that a significant decrease ($p<0.05$) and a non-significant difference were recorded for zinc and copper respectively compared with the control group.

Manganese and selenium two other cofactors for important antioxidant enzymes also showed the same pattern of alteration, at 100, 350, 1000 mg\kg levels of exposure. At 100 mg\kg level of exposure both recorded significant increases compared with control whereas, at both 350 and 1000 mg\kg levels of exposure, manganese

and selenium recorded decreases compared to controls ($p<0.05$). At 3000 mg\kg BW level of exposure manganese was statistically increased ($p<0.05$) whereas Se remained statistically unchanged compared to controls ($p>0.05$).

Vitamin A, copper and selenium showed the same pattern of presentation of statistical increases at 100 mg\kg level of exposure, statistical decreases ($p<0.05$)at 350 and 1000 mg\kg levels of exposure and no statistical change ($p>0.05$) at 3000 mg\kg level of exposure compared to the controls. Zinc on the other hand was continuously depleted with increasing level of exposure compared to controls. Animals in 3000 and 5000 mg\kg exposure groups suffered 40% and 100% mortality respectively.

DISCUSSION

The addition of an antidote to a therapeutic drug is not a new occurrence. Prior to the call for the inclusion of an antidote (methionine) in paracetamol tablet in 1970's by MacLean, naloxone had been incorporated into pentazocin[1, 10]. Pentazocin has the tendency to cause addiction. The addition of naloxone was to forestall this. Methionine in vivo is converted to cysteine [a component of glutathione] which bears the -SH group responsible for the detoxification of xenobiotics or their reactive metabolites example of which is N- acetyl –P-benzoquinoneimine[1].

Essential trace elements-zinc, copper, manganese and selenium as well as fat and water soluble vitamins; vitamins A and E & niacin were statistically increased at 100 mg\kg BW level of exposure. This level of exposure is considered to be within the tolerable level for both man and many strains of rats since the therapeutic dose is 94.4 mg\kg BW without methionine and 94.1 mg\kg BW with the antidote methionine in man[13]. This kind of observation is not rare since a number of therapeutic drugs have also been reported to affect the metabolism of trace elements and alter their levels in serum or plasma[14]. Although a number of studies point to an association between methionine ingestion and folic acid deficiency[10] and both selenium and vitamin C have been reported to counteract the side effects of large doses of methionine[10]. That some of the essential trace elements served as backup in ameliorating the toxic effect of acetaminophen at very high doses is evident by continued depletion of these elements with increases in dosage especially at 350 and 1000 mg\kg BW exposure levels. For example after the high plasma level at tolerable dose (100mg\kg BW) Zn, Cu & Mn-important cofactors for the antioxidant enzyme cytosol and mitochondrial superoxide dismutase, recorded significant decreases at 100mg\kg and 350mg\kg BW level of exposure. Moreover, at 1000mg\kg the plasma levels of Zn, Cu, Mn showed a continued depletion with increasing acetaminophen dosage. This same trend was also observed for both fat and water-soluble vitamins; vitamin A, vitamin E, niacin and riboflavin. This therefore may be an indication of antioxidant role of these agents especially as with increasing level of exposure, there was increase in degree of oxidative stress with a consequent decrease in their plasma levels, which may be an indication of increasing utilization due to increasing demand. Paracetamol is considered not only as a glutathione depletor but is also capable of generating reactive species[4, 5]. This 24-hour study is a better reflection of the interaction between trace elements and paracetamol effect since the peak of toxicity has been reported to be between the 16th and 24th hour of exposure[15].

Moreover, to buttress that the essential trace elements played a role in preventing hepatotoxicity, at 3000mg\kg BW the levels of some of antioxidant indices – manganese was statistically raised compared to control group when tissue necrosis had set in and the number of functioning hepatocytes to ultilize this element was diminished. This may also show that at high level of exposure, acetaminophen may possess the ability to increase the body contents of some elements. This is in agreement with reports of earlier studies. Ikegwuonu[16] reported that exposure of rats to high dose of another xenobiotic, aflatoxin B1 resulted in increase tissue contents of zinc in the kidney and brain; of manganese in testis, kidney and the cerebellum as well as increase copper contents in the kidney. This may also be an indication of tissue damage especially as 40% of the animals in this group died by the end of 24th hour. Cell damage is characterized by high plasma levels of some intracellular proteins, the most prominent of these which can easily be assessed are the enzymes.

Zinc and vitamin E which have been used in combination therapy to ameliorate side effect\ oxidative effect of therapeutic drugs still remained depleted compared not only to control group but other exposure groups as well.

The role of an antioxidant by manganese had earlier been observed by Atessahin et al.[17] where low doses of $Mn2+$ had abolished the nephrotoxic effect of gentamicin. The statistical difference in a number of this essential elements and vitamin may point to their involvement in preventing toxicity. For example, zinc metabolism has been reported to be affected by acetaminophen toxicity as well as the use of the antidote –N- acetyl-L-cysteine[18]. Also its role in correcting the metabolic disturbances caused by paracetamol toxicity has also been reported. By using leucine and zinc sulfate in a ratio of 4:1 (100 mg\kg BW), this mixture conferred

hepatoprotective effect by preventing ultrastructural injury of the liver and disturbances of free amino acids usually associated with a toxic dose of acetaminophen[18]. That zinc does this through its antioxidant is evident by the results of the study carried out by Woo et al.[19] where malondialdehyde an index of lipid peroxidation was reduced when zinc sulfate was used as an antidote in the treatment of acetaminophen-induced hepatotoxicity in experimental animals. With all these evidence for the involvement of essential trace elements in acetaminophen toxicity Lei et al.[20] & Zhu and Lei.[21] reported that double null of selenium-glutathione peroxidase-1 and copper, zinc-superoxide dismutase enhanced resistance of mouse primary hepatocytes to acetaminophen toxicity.

In conclusion, a statistical decrease in the levels of niacin observed in this study may further support the need to identify niacin as an antioxidant biomolecule. Especially as Kroger et al.[22] has reported that nicotinic acid amide inhibited acetaminophen-induced injury in mice kept on standard laboratory diet and even suggested the addition of nicotinic acid amide in combinational use in pharmaceutical preparation of acetaminophen, so as to avoid hepatic injury in patients who have higher sensitivity to this agent or to minimize hepatic injury as a result of an overdose.

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Table 1: Antioxidant vitamins in acetaminophen exposed and control male Wistar rats.

EXPO. TYPES	NIACIN ng\ml	RIBO. nmol\L	VIT. A µmol\L	VIT. E µmol\L
(control)	8.99±0.20	502.74±10.10	0.90±0.02	25.71±0.93
100mg\kg	10.52±0.3 *	518.70±23.41	1.01±0.04 *	30.83±1.0 *
350mg\kg	8.62±0.71	370.54±7.45 *	0.67±0.04 *	21.94±0.76 *
1000mg\kg	7.05±0.14 *	352.98±12.77 *	0.62±0.04 *	19.40±0.79 *
3000mg\kg	6.94±0.69 *	708.36±116.51 *	0.93±0.15	17.62±1.43 *

Abbreviations: RIBO., riboflavin; VIT.A, vitamin A; VIT.E, vitamin E. Results are expressed as mean ±standard deviation (SD); *- significant difference at <0.05.

Table 2: Antioxidant minerals in acetaminophen exposed and control male Wistar rats.

EXPO. TYPES	ZINC µmol\L	COPPER µmol\L	Mn µmol\L	Se µmol\L
(control)	13.85±0.23	16.64±0.79	1.27±0.02	0.57±0.02
100mg\kg	16.68±0.51 *	19.28±0.22 *	1.58±0.02 *	0.81±0.05 *
350mg\kg	12.17±0.39 *	14.55±0.19 *	1.12±0.02 *	0.51±0.03 *
1000mg\kg	11.98±0.39 *	13.53±0.58 *	1.05±0.04 *	0.50±0.02 *
3000mg\kg	12.10±0.18 *	18.33±1.09	1.61±0.15 *	0.68±0.11

Abbreviations: Mn, manganese; Se, selenium.
Results are expressed as mean ±standard deviation (SD); *- significant difference at <0.05.

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