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Research Article

SERUM VITAMIN LEVELS IN FEMALE WISTAR RATS ADMINISTERED WITH DIFFERENT DOSES OF PARACETAMOL AND PARACETAMOL/METHIONINE – AN ACUTE STUDY

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ABSTRACT
Testosterone has been identified to play a role in the metabolism of paracetamol in the CD 1 mouse, resulting in differences in renal presentations of male and female mice. Moreover, alterations have been observed in the serum levels of vitamins in male Wistar rats administered with paracetamol/methionine. The aim of this study is to determine if the sex of an animal plays a role in serum vitamin presentation in the Wistar strain after paracetamol/methionine administration. This will be achieved by comparing observation made from this study with an earlier one on male Wistar rats. Moreover, comparison of presentations at the 4th & 16th hours will be carried out, so as to establish how earlier in the course of exposure to paracetamol/methionine vitamin alteration takes place. Female Wistar Rats consisting of eight rats per group were administered with different doses of paracetamol & paracetamol/methionine (5:1) ranging from 350-5000 mg/kg. Results indicate that significant alterations ($p<0.05$) in the levels of all the vitamins commenced as early as the 4th hour in both paracetamol & paracetamol/methionine administered groups. Moreover, significant alterations in the female rats parallel those of male rats obtained from an earlier study. Evidence from this study when compared with an earlier one indicates that sex probable plays no role on the impact of paracetamol on serum vitamins in Wistar rats.
Key words: paracetamol; methionine; vitamin; female Wistar rat.

INTRODUCTION

Paracetamol (acetaminophen), the principal active metabolite of phenacetin is a very well-known analgesic and antipyretic drug[1]. Its potency is comparable with that of aspirin especially in the central nervous system, but it lacks many of side effects of aspirin. At therapeutic level, both epidemiologic studies and studies in many animal species have proved that this agent is well tolerated and interactions with other drugs are not observed. Whereas at overdose level, Mitchell et al.[2] as well as Zhang et al.[3] have reported that this agent causes acute centrilobular and renal necrosis in both human and experimental animals.

Although symptoms of APAP toxicity in individuals taking it on prescription are not common except in alcoholics, but its easy acquisition because of its over-the-counter status has contributed to reported cases of toxicity. Moreover, this agent has been reported to have a narrow therapeutic index making it a relatively dangerous drug for individuals who abuse it[4]. At therapeutic dosage, glucuronic and sulfate pathways are the major pathways by which APAP is metabolized, a third pathway, the oxidative pathway plays a role minor but it becomes a major pathway at toxic level of exposure. The monooxygenases (CYP) are essentially the main enzymes which catalyze the oxidative pathway. Differences in the activities of individual CYP have been reported in many animal species which have been adduced to be the basis of differences in response to acetaminophen and other xenobiotic exposure by these animals. Other factors such as age, sex, alcohol consumption, co-existing pathological conditions etc. have also been identified to cause this phenomenon- the intra & extra species differences to xenobiotic metabolism.

Hoivik et al[5] have suggested that differences in sex of animals may play a role in metabolic presentation of APAP at overdose level, by utilizing male and female CD1 mice, they demonstrated that only the males exhibited cytochrome P450 dependent nephrotoxicity and selective protein covalent binding, but did not observe renal toxicity in female mice. And by pre-treating females CD1 mice with testosterone propionate for 6 days, these rats had lesions that were similar to those of male mice. An indication that testosterone may be capable of playing a modulating role in tissue presentation after cases of overdose. Furthermore, pre-treatment of female animals with testosterone, caused enhanced activation of APAP in vitro in kidney microsomes. Their study showed that the male sex hormone is capable of altering the effect of CYP in pathological presentation at overdose level.

Although there are studies to prove that APAP at overdose level (independently or in association with an antidote e.g. methionine) is capable of altering the levels of serum vitamins, weather this may show sex bias has not been fully determined. Differences in tissue presentations of female and male rats necessitate a study of this nature especially as one of our earlier studies has recorded significant differences in the levels of niacin, riboflavin, vitamins A & E in male Wistar rats, many of which have been reported to play a role in APAP metabolism[6-8]. Therefore, by using female rats in the present study, we hope to establish if such characteristic changes in vitamin levels observed in male rats is not sex dependent.

MATERIALS & METHODS

Animals

Adult female Wistar rats weighing averagely 300 g, bred by the Department of Veterinary Physiology University of Ibadan, animal house were procured and utilized for the study. The animals were given access to feed pellets and water *ad libitum*. The study was in conformity with accepted principles for the use and care of laboratory animals as found in US guidelines (NIH publication\85-23, revised in 1985). The animals were divided into groups; consisting of eight rats per group. Group 1 received physiologic saline, Groups 2 & 3 received 350 mg/kg APAP & 350 mg/kg APAP:70 mg/kg methionine; Groups 4 & 5 received 1000 mg/kg APAP & 1000 mg/kg APAP:200 mg/kg methionine; Groups 6 & 7 received 3000 mg/kg APAP & 3000 mg/kg APAP:600 mg/kg methionine; Groups 8 & 9 received 5000 mg/kg APAP & 5000 mg/kg APAP:1000 mg/kg methionine, Groups 10 & 11 received 350 mg/kg APAP & 350 mg/kg APAP:70 mg/kg methionine; Groups 12 & 13 received 1000 mg/kg APAP & 1000 mg/kg APAP:200 mg/kg methionine; Groups 14 & 15 received 3000 mg/kg APAP & 3000 mg/kg APAP:600 mg/kg methionine; Groups 16 & 17 received 5000 mg/kg APAP & 5000 mg/kg APAP:1000 mg/kg methionine respectively. These were dissolved in 5 ml of physiologic saline per rats.

The route of administration was by gastric gavage. Study in groups 2-9 was terminated at the end of the 4th hour while that of groups 10-17 was at the end of the 16th hour. The 4th and the 16th hours chosen as periods of study are considered as the peak of absorption and peak of toxicity respectively[9, 10] while 350 mg/kg and 1000 mg/kg are considered subtoxic & toxic doses respectively[10, 11]. At the end of each study period, blood was obtained from each animal through retro-orbital bleeding. The blood was allowed to clot and was centrifuged at 3000g for 10 minutes. The serum obtained was used for the estimations of vitamins (folic acid, niacin, riboflavin, vitamins A, C & E) while High Performance Liquid Chromatographic technique was used for these estimations. The HPLC equipment was supplied by Waters® Corporation Milford, Massachusetts USA. Methionine and acetaminophen were supplied by Sigma-Aldrich Chemicals® (St. Louis, MO).

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 15 was utilized for the analysis of data obtained. Student t test was employed to establish the level of significant difference between of each of the treated group and the control group. Results are reported in Mean ± SD. $P \leq 0.05$ was considered significant.

RESULTS

The results of this study are presented in **Tables 1 & 2** below. The serum vitamin levels of rats exposed to paracetamol & paracetamol/methionine for duration of 4 hours **(Table 1)** show that riboflavin, niacin, vitamin C and vitamin A were significantly decreased ($p<0.05$) at all levels of exposure (350, 1000, 3000, 5000 mg/kg) in not only the paracetamol-exposed group but in rats in the other group co-administered with methionine compared with controls. On the other hand, although significant decreases were recorded for folic acid and vitamin E, it was not at all levels of exposure, specifically, non significant differences ($p>0.05$) were observed in the folic acid levels in rats administered with paracetamol/methionine at both the 350 & 3000 mg/kg levels of exposure, whereas, vitamin E was not significantly different ($p>0.05$) at all levels of exposure in paracetamol/methionine administered groups compared with controls. The serum vitamin levels of rats administered with paracetamol & paracetamol/methionine for a period of 16 hours **(Table 2)** show significant decreases ($p<0.05$) in the levels of all vitamins studied at all levels of exposure in both paracetamol-administered and paracetamol/methionine administered groups compared with controls.

DISCUSSION

Administration of a number of therapeutic drugs have been identified to induce vitamin depletion, examples include sodium valproate which depletes folic acid and furosemide which depletes vitamin C[12-13]. Although these depletions have not been identified to be affected by the sex of subjects but cimetidine a histamine H_2-receptor antagonist which is used in the treatment of gastric and duodenal ulcers have been recognized to have sex-specific response to copper and zinc; higher plasma copper level was observed in male rats while higher zinc level was observed in the female rats administered with the same dosage of the drug when compared to their respective controls. This is an indication that therapeutic agent is capable of inducing sex specific changes in micronutrient levels in animals.

Results of an earlier study[6] carried out on male rats administered with acetaminophen/methionine revealed that niacin, vitamins A & E showed significant decreases ($p<0.05$) at most levels of exposure. Likewise, this study in female rats of the same strain showed significant decrease in the levels of these vitamins. This may be an indication that administration with acetaminophen/methionine may not induce sex specific changes in the serum vitamin level. Moreover, this study also revealed that such decreases had commenced by the 4th hour pointing to the fact that vitamin alteration is an early evident in post-paracetamol administration i.e. it precedes tissue necrosis which does not commence before the 8th hour.

According to Laval[14] oxidative stress takes place in a cell when the equilibrium between prooxidant and antioxidant species is broken in favor of the prooxidant state. These prooxidants are reactive oxygen species (ROS) generated either by the cellular metabolism such as phagocytosis, mitochondrial respiration, xenobiotic detoxification, or through exogenous factors (e.g. ionizing radiation or chemical compounds). Some ROS are highly reactive and binds with macromolecules such as lipids, nucleic acids and proteins to cause cellular damage. Although some of the cell defense systems used to counteract the deleterious effects of ROS includes proteins (e.g. superoxide dismutase, catalase and glutathione peroxidase), small molecules (e.g. glutathione, alpha-tocopherol, vitamins A and C) also help in eliminating generated ROS. Moreover, most disease conditions have also been linked to the generation of Reactive Oxygen Species[15] and antioxidants have been reported to play prominent roles in the prevention of ROS generation[16]. Specifically, antioxidant vitamins A, C and E have been reported to play a role in the protection against cardiovascular and malignant diseases[17]. The significant decreases recorded for these vitamins points to the fact that acute abuse of acetaminophen even when it is incorporated with methionine may predispose an individual to some of the above named conditions.

The possible causes of alterations in the levels of these vitamins are diverse, according to Merrik et al[18] after 24 hr, 68 serum proteins were significantly altered out of which 23 proteins were increased by >5 fold and 20 proteins were newly present compared to controls. Many of these are proteins with enzymatic activities which may require vitamins as coenzymes. Some of these altered proteins include SUMO1 (small ubiquitin-like modifier-1), activating enzyme E1B, complement c5, cyclooxygenase-1, peroxiredoxin 1, and regucalcin, and other upregulated proteins with reparative roles were also found to be increased in acetaminophen intoxicated SJL mice. Vitamin C is essential for the activity of cyclooxygenase. In addition, vitamin C is a co-factor for hydroxylases and monooxygenase enzymes taking part in the metabolism of acetaminophen and in synthesis of a number of molecules. They are also capable of acting as antioxidants in some other metabolic pathways independent of glutathione action, especially lipid peroxidation which has also been identified as one of the minor metabolic complications of APAP toxicity.

Zinc and vitamin E which have been used in combination therapy to ameliorate side effect\oxidative effect of therapeutic drugs were significantly ($p<0.05$) decreased at both the 4th and the 16th hours as well as at all levels of exposure in both paracetamol and paracetamol\methionine combination treated rats compared with controls, the only exception was the non-significant difference ($p>0.05$) in the level of vitamin E observed in paracetamol\methionine treated rats by the end of the 4th hour (at all levels of exposure). The results of our study also showed that vitamins A and C were significantly decreased at all levels of exposure. All these further suggest that these vitamins may be involved in metabolic processing of APAP.

Moreover, the significant decreases might also have arisen because of the roles of these vitamins as hepatoprotective agents. A possible hepatoprotective role of both vitamins A & E has been put forward by Ekam and Ebong[19], the results of their study showed that vitamins A & E have some hepatoprotective effects on APAP

toxicity, administration of vitamins A and E to Wistar albino rats caused significant decreases ($P < 0.05$) in AST and ALP activities in APAP treated groups. The possibility of vitamin C playing a hepatoprotective role has also been reported by El-Ridi & Rahmy[20]. They indicated that administration of APAP at overdose level resulted in a high mortality rate and hepatorenal toxicity as indicated by significantly higher levels of hepatorenal indices. It also caused cellular alterations and necrosis of hepatocytes and of some renal cortical cells. Vitamin C administered at a dose level of 320 mg\kg normalized the levels of liver glutathione and serum hepatorenal indices except bilirubin.

CONCLUSION

The results of this study of alteration in the levels of vitamins in rats administered with acetaminophen/methionine suggest that use of this antidote is not without its side effects. Furthermore, by comparing the results of this study with an earlier one in male Wistar rats, there is the possibility that sex differences do not play a role in serum vitamin presentations in paracetamol/methionine dosed rats, especially in the Wistar strain.

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TABLE 1: SERUM LEVELS OF VITAMINS IN PARACETAMOL-EXPOSED AND CONTROL WISTAR RATS- 4 HOURS POST-DOSING.

	RIBOFLAVIN (nmol/L)	FOLIC (nmol/L)	NIACIN (nmol/L)	VITAMIN C (mmol/L)	VITAMINA(µmol/L)	VITAMIN E(µmol\L)
X ± SD (controls)	1157.00±39.9	18.35±0.23	68.77±3.07	43.72±2.84	2.58±0.09	21.58±0.93
350mg\kg						
X ± SD (P)	340.21±40.96*	6.46±0.32*	37.38±8.61*	11.92±1.14*	2.46±0.04*	13.69±0.70*
X ± SD (P&M)	546.36±43.62*	20.20±7.36	37.67±0.95*	26.69±2.84*	2.33±0.08*	20.14±1.62
1000mg\kg						
X ± SD (P)	583.87±44.42*	8.09±1.00*	42.78±7.74*	11.92±1.14*	2.43±0.02*	14.38±0.46*
X ± SD (P&M)	537.05±31.92*	10.55±0.36*	45.92±2.77*	26.12±1.14*	2.37±0.07*	20.88±0.93
3000mg\kg						
X ± SD (P)	751.45±40.96*	11.80±045*	45.261.46*	11.92±1.14*	2.06±0.04*	14.89±0.93*
X ± SD (P&M)	532.27±40.17*	24.30±14.81	43.44±0.51*	27.25±1.70*	2.37±0.05*	27.10±14.08
5000mg\kg						
X ± SD (P)	739.48±217.32*	14.36±0.72*	38.84±1.39*	22.14±1.14*	1.79±0.06*	14.62±0.70*
X ± SD (P&M)	842.95±73.15*	13.50±0.63*	55.70±3.29*	27.82±2.28*	2.25±0.14*	20.88±0.48

*Results are expressed as mean ± standard deviation; $p < 0.05$ is significant.

TABLE 2: SERUM LEVELS OF VITAMINS IN PARACETAMOL-EXPOSED AND CONTROL WISTAR RATS-16 HOURS POST-DOSING.

	RIBOFLAVIN (nmol\L)	FOLIC (nmol\L)	NIACIN (nmol/L)	VITAMIN C (mmol/L)	VITAMIN A (µmol/L)	VITAMIN E (µmol/L)
X ± SD (controls)	1157.00±39.9	18.35±0.23	68.77±3.07	43.72±2.84	2.58±0.09	21.58±0.93
350mg\kg						
X ± SD (P)	489.44±35.11*	11.53±034*	42.41±5.40*	34.07±1.70*	2.43±0.11*	13.46±2.09*
X ± SD (P&M)	816.62±43.36*	14.63±0.88*	34.16±2.42*	23.85±1.13*	1.71±0.05*	15.31±1.19*
1000mg\kg						
X ± SD (P)	489.97±28.99*	11.80±0.52*	49.28±3.65*	34.07±2.24*	2.24±0.18*	15.54±1.16*
X ± SD (P&M)	814.49±40.43*	15.92±0.50*	41.83±15.18*	26.69±1.65*	2.29±0.15*	12.76±0.93*
3000mg\kg						
X ± SD (P)	491.57±40.96*	11.30±0.57*	50.44±1.24*	33.50±3.97*	2.26±0.14*	16.47±1.40*
X ± SD (P&M)	809.44±47.61*	16.31±0.43	47.23±1.75*	34.07±0.57*	2.30±0.23*	11.37±0.70*
5000mg\kg						
X ± SD (P)	651.17±51.07*	13.73±0.93*	43.72±0.66*	32.93±0.54*	1.69±0.11*	14.15±0.46*
X ± SD (P&M)	824.6±28.99*	16.17±0.52*	50.74±1.68*	23.28±1.18*	2.30±0.19*	15.78±1.16*

*Results are expressed as mean ± standard deviation; $p < 0.05$ is significant.