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Oral administration of ascorbic acid attenuates renal and hepatic toxicity of phenobarbitone in experimental rats

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ABSTRACT

Phenobarbitone, is a barbiturate with soporific, sedative and hypnotic properties and most widely used as anticonvulsant worldwide. This study investigated the effect of administration of ascorbic acid on phenobarbitone induced renal and hepatic toxicity. For the study, twenty four adult Wister rats were randomized into three groups of 8 rats each. The rats in first group (PV) were given phenobarbitone (15mg/kg orally) along with vitamin C (12.5mg/kg); the second group (P) were given phenobarbitone (15mg/kg orally) while the third group (C) were made the control and was given distilled water (1ml/200g). The treatments were given at 12hrs, 24hrs, 48hrs, 72hrs and 96hrs. At the end of the treatment period, the rats were sacrificed and biochemical assays were carried out on the plasma, kidney and liver tissues. The result showed that there was significant increase in the level of thiobarbituric acid reactive substances (TBARS), uric acid, catalase and aspartate aminotransferase (AST) in the plasma; there was increase in the level of TBARS and catalase in liver and kidney while Liver AST and kidney uric acid were reduced. All these changes were restored in animal treated with phenobarbitone and ascorbic acid. Hence, ascorbic acid may help to attenuate the hepatic and renal toxicity effect of phenobarbitone.

Key words: Phenobarbitone, ascorbic acid, hepatotoxicity, renal toxicity, antioxidant

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INTRODUCTION

Phenobarbitone is an important sedative as well as hypnotic agent [1]. It is also one of the oldest drugs used for the treatment of epilepsy [2]. It helps reduces seizure frequency and severity and in a lot of cases also stops seizures altogether [3]. It exerts its anti-epileptic action by facilitating γ-aminobutyric acid (GABA), a chief inhibitory neurotransmitter in the mammalian central nervous system by reducing neuronal excitability throughout the nervous system and also directly responsible for the regulation of muscle tone [4,5].

Phenobarbitone has also been used for prevention and treatment of unconjugated hyperbilirubinemia in preterm neonates [6]. Side effects of phenobarbitone include neurotoxicity, withdrawal symptoms (e.g. sweating, insomnia, hallucination and hypertension), vitamin deficiency, connective tissue disorder, hyper sensitivity reaction and teratogenesis [1]. There is concern of both abuse and withdrawal following long term use as these may also increase the risk of suicide.

Ascorbic acid otherwise called vitamin C, a water soluble vitamin, is necessary for the synthesis of collagen, a protein that has many connective functions in the body [7]. Ascorbic acid is also required for hormones and neurotransmitters synthesis and in certain amino acids and vitamins metabolism [8]. Ascorbic acid takes part in detoxification of toxic substances in the liver and also contributes to immunity in blood level [9]. Ascorbic acid can also act as an antioxidant to react with histamine and peroxide for reducing inflammatory symptoms.

Hence, from the forgoing side effects that is associated with phenobarbitone continuous use, and the prospect of ascorbic acid as a good antioxidant, this research work intend to investigate the antioxidant effect of ascorbic acid on phenobarbitone toxicity in experimental animal examination of some parameters in the plasma, liver and kidney.

EXPERIMENTAL SECTION

Reagents and Chemicals

Phenorbarbitone was purchased from Aldrich Limited, London, England. Ascorbic acid, thiobarbituric acid (TBA), 5, 5'-dithio 2-nitrobenzoic acid (DNTB), copper sulphate ($CuSO_4$), sodium hydroxide (NaOH), potassium iodide (KI) and sulphuric acid (H_2SO_4) were obtained from BDH Chemical Limited, Poole, England. All other chemicals were of analytical grade.

Experimental Animal

Twenty four (24) Wister rats of weight range 150-160 grams were obtained from the Biological Science Department, University of Ibadan, Nigeria. The rats were fed with standard rat pellets (Top Feed Nigeria Ltd., Ibadan, Nigeria) and water *ad libitum*. They were housed in individual wire cages in a temperature and humidity controlled room, having a 12 hours light and dark cycle.

The animals were acclimatized for 2 weeks, during which the rats were given their standard pellet and were allowed to get adapted with the new environment after which they were weighed and randomized into three groups: The rats in the first group (PV) were given phenobarbitone(15mg/kg) orally along with vitamin C (12.5mg/kg); the second group (P) was given phenobarbitone(15mg/kg) orally; and the third group (C) was made the control and was given distilled water (1ml/200g). The treatments were given at 12hrs, 24hrs, 48hrs, 72hrs and 96hrs in the three groups.

After 4 days of drug administration, the animals were sacrificed by cervical decapitation and the blood was collected using heparin as anticoagulant. The plasma sample, the liver and kidney homogenates were prepared as described by Alabi *et al*. [10].

Biochemical Assays

Total cholesterol (TC) level was determined by the method of De Hoof *et al*. [11] and triglyceride (TG) level was determined by the method of Mochin and Leyva [12]. Catalase (CAT) activity was measured spectrophotometrically at 240nm by determining the rate of decomposition of hydrogen peroxide as described by Aebi in Bergmeyer [13]. Thiobarbituric acid reactive substances (TBARS) is a measure of lipid peroxidation and was estimated spectrophotometrically at 535 nm by the method of Buege and Aust [14] which involved the reaction between malondialdehyde and thiobarbituric acid to give a pink precipitate. Creatinine was determined by Jaffe's reaction as described by Toora and Rajagopali [15] and uric acid was determined by the method of Burtis and Ashwood [16]. Alkaline phosphates (ALP) activity was measured spectrophotometrically at 405 nm by the method of Williamson [17] where the concentration of p-nitrophenol formed when ALP reacts with p-nitrophenyl phosphate was monitored. Aspartate aminotransferase (AST) was determined by the method of Bergmeyer *et al*. [18].

Animal Care

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Use of Animals [19].

Statistics

Values are expressed as Mean of 5 replicates ± SEM (Standard Error of Mean). Data were subjected to one way analysis of Variance (ANOVA) and level of significance was done using Duncan Multiple Range Test (DMRT) at P = 0.05 of SPSS version 15 software.

RESULTS AND DISCUSSION

There was a progressive increase in the body weight of all the animals but this rate of increase was lower in phenobarbitone treated animal and the weight was restored to pre-treatment level when phenobarbitone was co-

administered with ascorbic acid (Figure 1). This was in agreement with the result of Chawla and Parmar [6], Patil *et al*. [3] and Verna *et al*. [20].

Table 1. Effect of co-administration of ascorbic acid and phenobarbitone on some plasma parameters

Group	TG (mg/dl)	TC (mg/dl)	TBARS (mg/dl)	Catalase (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)	ALP (U/l)	AST (U/l)
P	14.32±3.82	5.06±1.17	16.60±1.07	1.44±0.17	4.40±2.19	12.40±2.31	386.20±17.82	45.80±5.89
PV	10.68±2.19	4.78±0.38	8.20±1.02	1.40±0.18	2.00±0.00	4.88±1.32	404.20±0.97	18.20±5.89
C	8.88±1.14	4.23±1.24	7.00±1.14	1.38±0.21	2.00±0.00	4.40±1.56	404.20±0.67	14.80±3.42

All values were expressed as mean ±SEM of 5 determinations. Values in the same column with different superscript indicate significant difference at P<0.05.

Table 2. Effect of co-administration of ascorbic acid and phenobarbitone on some liver parameters

Group	ALP (U/l)	AST (U/l)	Catalase (U/l)	TBARS (mg/dl)
P	4.80±0.50	2.40±0.63	30.40±1.16	5.90±4.40
PV	8.40±0.58	4.18±0.77	23.80±0.58	1.29±0.35
C	8.58±1.78	8.58±1.78	23.00±0.32	1.08±0.31

All values were expressed as mean ±SEM of 5 determinations. Values in the same column with different superscript indicate significant difference at P<0.05.

Table 3. Effect of co-administration of ascorbic acid and phenobarbitone on some kidney parameters

Group	Creatinine (mg/dl)	Uric acid (mg/dl)	Catalase (U/l)	TBARS (mg/dl)
P	1.40±0.24	5.10±0.88	33.00±0.54	2.71±1.18
PV	1.98±0.54	6.10±0.19	23.40±0.57	1.27±0.33
C	2.20±0.48	6.20±0.80	24.00±0.01	1.15±0.53

All values were expressed as mean ±SEM of 5 determinations. Values in the same column with different superscript indicate significant difference at P<0.05.

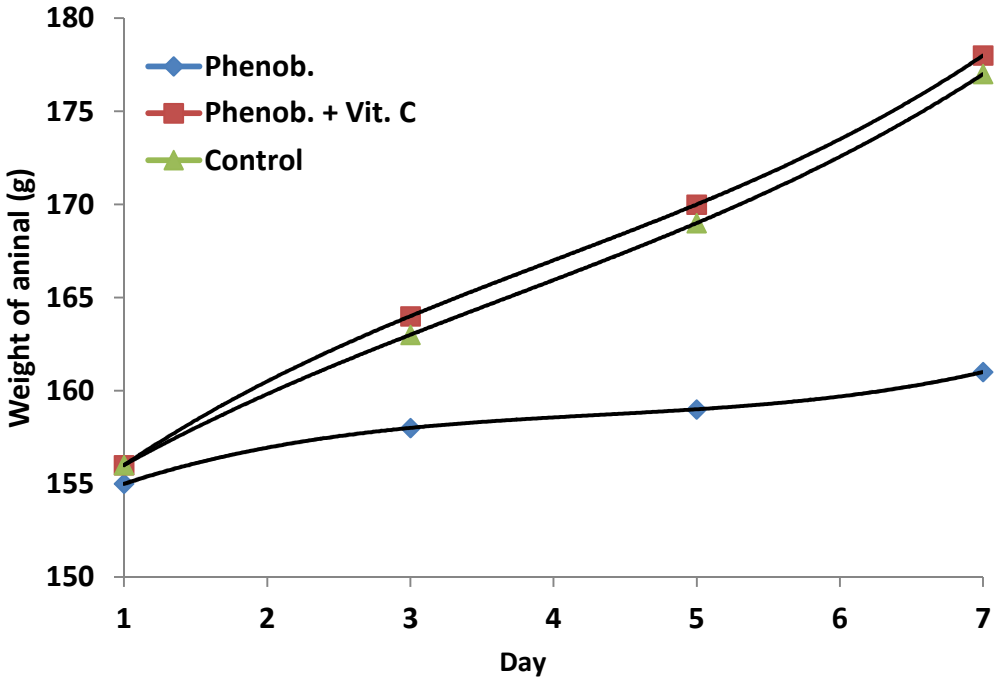

Figure 1. Effect of co-administration of ascorbic acid and phenobarbitone on the body weight

Phenobarbitone was observed to significantly increase the level of uric acid and TBARS as well as the activity of catalase and AST of the plasma (Table 1). These alterations were prevented the animal treated with ascorbic acid. On the other hand, there was no significant difference in the level of TG, TC, creatinine and ALP activity of the plasma in all the treated groups (Table 1).

The result showed that administration of phenobarbitone caused significant increase in the level of TBARS and catalase activity and decrease in AST activity of the liver (Table 2) which was restored on treatment with ascorbic acid. But there was no difference in activity of ALP of the liver in all the treated groups (Table 2).

The result also showed that phenobarbitone treatment significantly increase the level of TBARS, uric acid and catalase activity of the kidney (Table 3) but these changes were reversed in the PV group. Phenobarbitone does not significantly change the level of creatinine Table 3).

This showed a percentage increase of 3.87, 14.10 and 13.46 in phenobarbitone, phenobabitone plus vitamin C and control groups respectively.

In this study, there was increase in oxidative stress marked by increase plasma, liver and kidney TBARS and catalase activity with the administration of phenobarbitone. This is in agreement with previous studies on the oxidative stress associated with phenobarbitone therapy [21,22]. TBARS (i.e. malonyl aldehyde, MDA) is produced as a result of lipid peroxidation and higher values of TBARS imply an increase in free radical production, possibly by phenobarbitone. The significantly higher level of antioxidant enzyme (catalase) in the phenobarbitone group was possibly due to a compensatory increase in response to the higher levels of lipid peroxidation and free radical damage and this was restored when ascorbic acid was co-administered with phenobarbitone.

The study has reported increase plasma AST and uric acid with corresponding decrease in liver AST and kidney uric acid but this was resolved in phenobarbitone combined with ascorbic acid. This increase in plasma AST could be attributed to phenobarbitone which produced free radical that could have led to hepatic and renal dysfunction [23,24]. Mendis *et al*. [25] reported that oral administration ofphenobarbitone may lead to hepatocyte hypertrophy. Prolong administration of phenobarbitone has also been reported to induce hepatocellular adenomas and carcinoma in mice [26]. Increase in plasma uric acid may be as a result of renal impairment caused by phenobarbitone as it has been shown to produce lipid peroxidation in both the kidney and liver thereby releasing free radicals that will ultimately attack the renal and hepatic tissues.

However, there were no significant changes in the level of TG, TC, creatinine and ALP activity when rats treated with phenobarbitone was compared with the ones treated with and phenobarbitone and ascorbic acid.

CONCLUSION

This finding corroborates other previous work that phenobarbitone administration induces renal and hepatic toxicity. It is also indicated that co-administration of phenobarbitone with ascorbic acid may offer hepatic, renal and plasma protection. Hence, we recommend that more work should be done by co-administration of phenobarbitone with antioxidant vitamins to attenuate the toxic effect of the drug on hepatic and renal tissue.

REFERENCES

[1] Z Yasiry; SD Shorvon. *Epilepsia,* **2012**, 53(Suppl. 8), 26-39.
[2] P Kwan; M Brodie. *Epilepsia*, **2004**, 45(9), 1141-1149.
[3] SJ Patil; SR Banagar; RJ Banagar; SB Patil. *IJPAES*, **2012**, 2(1), 76-81.
[4] M Watanabe; K Maemura; K Kanbara; T Tamayama; H Hayasaki. *Int. Rev. Cytol.*, **2002**, 231, 1-47.
[5] AC Foster; JA Kemp. *Curr. Opin. Pharmacol.*, **2006**, 6(1), 7-17.
[6] D Chawla; V Parmar. *Indian Pediatr.*, **2010**, 47, 401-407.
[7] SJ Padayatty; A Katz; Y Wang; P Eck; O Kwon; JH Lee; S Chen; C Corpe; A Dutta; SK Dutta; M Levine. *J. Am. Coll. Nutr.*, **2003**, 22, 18-35.
[8] M Walingo. *AJFAND*, **2013**, 5(1), 1-13.
[9] MA Alabi; A Ajibola; NA Bisuga. *J. Med. Pharm. Sci.*, **2007**, 3(4), 5-8.
[10] MA Alabi; RM Sunday; T Olowokere; FA Kareem; F Osanaiye. *J. Med. Sci.*, **2013**, 13(1), 62-66.
[11] JL De Hoff; LM Davidson; D Kritchevsky. *Clin. Chem.*, **1978**, 24, 433-435.
[12] MC Mochin; JA Leyva. *Clin. Chim. Acta*,**1984**, 142, 281-285.
[13] HU Bergmeyer. Methods of Enzymatic Analysis, Verlag Chemie Weinhein, Academic Press, New York, **1974**, 673-684.
[14] JA Beuge; SD Aust. *Methods Enzymol*., **1978**, 30, 302-310.
[15] BD Toora; G Rajagopal. *Indian J. Exp. Biol.*, **2002**, 40, 352-354.
[16] CA Burtis, ER Ashwood. Tietz Textbook on Clinical Chemisty, 3rd ed, **2001**, WB Sanders Company, Philadelphia, PA.
[17] TA Williamson. *Med. Lab. Technol.*, 29, 182-187.
[18] HU Bergmeyer; P Scheibe; AW Wahlefeld. *Clin. Chem.*, **1978**, 24(1), 58-73.
[19] American Physiology Society. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **2002**, 283, 281-283.
[20] RS Verma; A Mehta; N Srivastava. *Indian J. Biochem. Biophys.*, **2005**, 42, 254-257.
[21] G Gathwala; A Marwah; V Gahlaut; P Marwah. *Indian Pediatr.*,**2011**, 48, 613-617.

[22] KH Reeta; J Mehla; YK Gupta. *Eur. J. Pharmacol.*, **2010**, 644(1-3), 106-112.
[23] SN Ahmad; ZA Siddiqi. *Seizure*, **2006,** 15, 156-164.
[24] SVSRS Gupta. *Tox. Subst. Mech.*, **1999**, 18(1), 3-10.
[25] GP Mendis; FB Gibberd; HA Hunt. *Seizure*, **1993**, 2, 319-323.
[26] D Williams. PSD Guidance Document: Interpretation of Liver Enlargement in Regulatory Toxicity Studies, **2005**, 1-20.