



RESEARCH ARTICLE

THERAPEUTIC APPROACH OF ANTIOXIDANTS ON PHENYTOIN INDUCED TERATOGENIC EFFECT IN ALBINO RATS

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ABSTRACT

A considerable amount of clinical and experimental evidence suggests that many biochemical pathways are strictly associated with Phenytoin. It increases the production of Reactive Oxygen Species (ROS) which could be counteracted by the antioxidants such as Vitamin C and E supplementation. In this study, Phenytoin was administered at the dose of 80 mg/kg/day in the pregnant rats during fetal organogenesis period. Whereas, Vitamin C and E were supplemented throughout the pregnancy at the doses of 1.60 gm/kg/day and 0.8 gm/kg/day respectively. Phenytoin treatment induced the morphological changes in fetus with flexure defect and unequal sized limbs. It also decreased the fetal body and brain weight. However, Phenytoin plus Vitamin C and E treated group gave birth to normal fetuses with significantly increased body as well as brain weight. Interestingly, Malondialdehyde (MDA) level in the maternal rats was also significantly reduced, one of the possible causes of reduced fetal-tissue damage. So, it strongly suggests that Vitamin C and E as antioxidants possess the protective effect over Phenytoin induced teratogenesis.

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INTRODUCTION

Although human embryo is well protected in the uterus, environmental factors may cause developmental disruptions following maternal exposure to them and hence act as teratogen. Teratogen is an agent that can produce congenital anomalies; include not only gross structural defects but also microscopic and molecular anomalies resulting in malformations and hence physiological disturbances (Drew and Noden, 2003). Phenytoin, one of the most commonly used antiepileptic drug also acts as a potent teratogen. It acts by maintaining the deactivation of voltage sensitive sodium channels thereby blocking the repetitive firing of neurons (Goodman et al., 1996, Tripathi, 1999). During the metabolism of Phenytoin, the formation of Reactive Oxygen Species (ROS) has been implicated in the induction of fetal malformation (Winn and Wells, 2002). The ROS attack upon the membrane to convert polyunsaturated fatty acids into lipid hydroperoxides. In the presence of iron and copper ions, it forms a wide range of cytotoxic aldehydes such as malondialdehyde and hydroxynonenal which are capable of chemically modifying proteins and DNA.

These hydroperoxides tend to migrate away from the hydrophobic interior of the membrane to the surface, thus disrupting membrane organization. Peroxidation of biological membranes increases their leakage to ions and cause damage to transmembrane proteins such as receptors and enzymes (Marshall and Bangert, 1995). Brain is the most susceptible target of free radical damage since it has large content of myelin sheaths, more easily peroxidizable fatty acids, consumes an inordinate fraction (20%) of the total oxygen consumption for its relatively small weight (2%) and is not particularly enriched in antioxidant defenses (Frei et al., 1988). The pathology of oxidative stress can be prevented by antioxidants known to be effective in treating conditions associated with oxidative damage (Brogaard and Clausen, 1997). In this study we have targeted the combination of Vitamin C (scavenging the oxygen radicals in the aqueous phase) and Vitamin E (scavenging the oxygen radicals within the membranes) to investigate whether its synergistic effect could protect the cells against the phenytoin induced damage in albino fetal rats.

MATERIALS AND METHODS

Twenty virgin female Wistar rats weighing about 150–200 g were taken to carry out the present study.

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They were housed under normal environmental conditions (light/dark cycle of 12 h /12 h at room temperature) to acclimatize and given standard pellet diet and water *ad libitum*. They were subjected to get pregnant which were then divided into four groups, five rats in each. For Group 1, tap water was given as control. For Group 2, Vitamin C and E at the doses of 1.60 gm/kg/day and 0.8 gm/kg/day respectively were supplemented mixing with their food pellets throughout the pregnancy. For Group 3, Phenytoin at the dose of 80 mg/kg/day was administered intraperitoneally at gestation days 9-12. And for Group 4, Phenytoin was administered on days 9-12 of gestation in those rats which were receiving Vitamin C and E at the doses mentioned above. On gestation day 20, all the experimented pregnant rats were sacrificed using ether induced-deep anesthesia and immediately blood was collected by direct cardiac puncture for the measurement of Malondialdehyde level. Gross observations were noted for all the groups. This study has included only the live experimented rats for morphological and biochemical examinations. MDA level which determines the degree of lipid peroxidation, was measured by Thiobarbituric Acid Reactive Substances (TBARS) assay. The intensity of the pink pigment formed by MDA-TBA reaction indicates the extent of lipid peroxidation as mentioned by Yagi K (1984).

RESULTS

Morphology of all fetuses were found normal except in the Phenytoin treated group. In latter, 25.0% and 39.0% cases were found with flexure defect (Fig.1) and unequal sized limbs (Fig. 2) respectively as shown in Table-1.

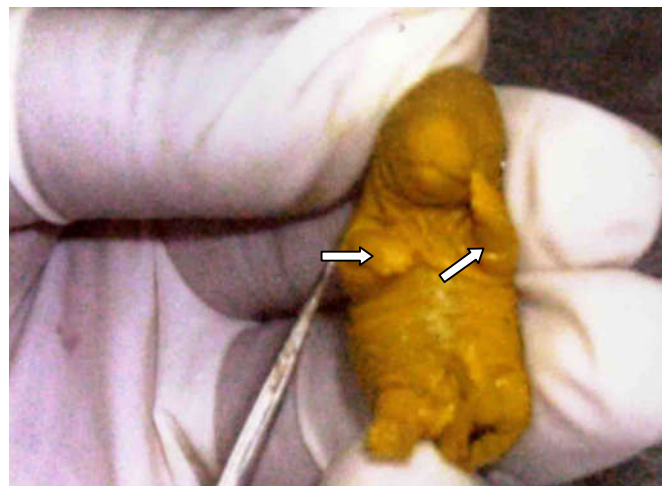


Fig 2. Fetal rats with unequal sized limbs

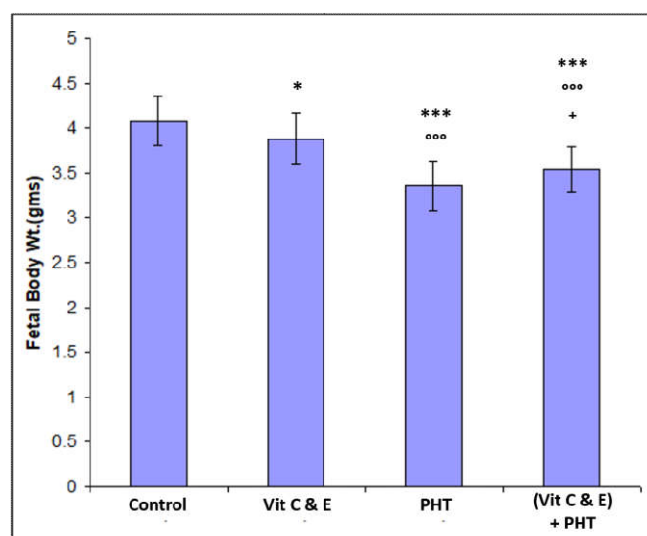
The fetal body and brain weight were found to be significantly less ($P < 0.001$) in Phenytoin treated group (Group 3). However, Phenytoin plus antioxidants Vitamin C and E (Group 4) treated group showed a significant increase ($P < 0.05$) in the weight of both parameters (Fig. 3-4), strongly suggests the protective role of antioxidants over the oxidative stress caused by Phenytoin. Moreover, the fact was supported by the measurement of Malondialdehyde level which was found to be decreased ($P < 0.001$) in Phenytoin plus antioxidants Vitamin C and E group (Group 4) as compared to the group which was treated with Phenytoin only (Fig. 5).

Table 1. Morphological observation of fetal rats in Phenytoin treated group

Phenytoin given on the gestation days	Serial no. of rats in Group	No. of fetuses	No. of fetuses with flexure defect	No. of fetuses with unequal sized limbs
9-12	3			
	1.	7	3	3
	2.	4	2	2
	3.	4	0	3
	4.	3	0	1
	5.	5	1	0
	Total	23	6	9

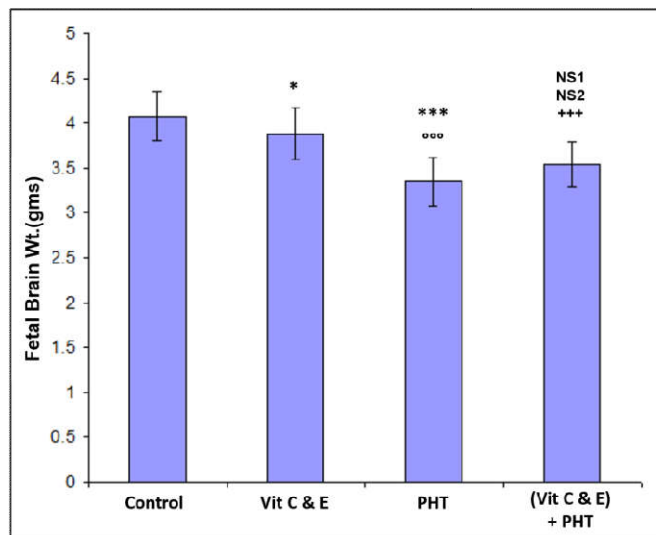


Fig 1. Fetal rats with flexure defect on limbs



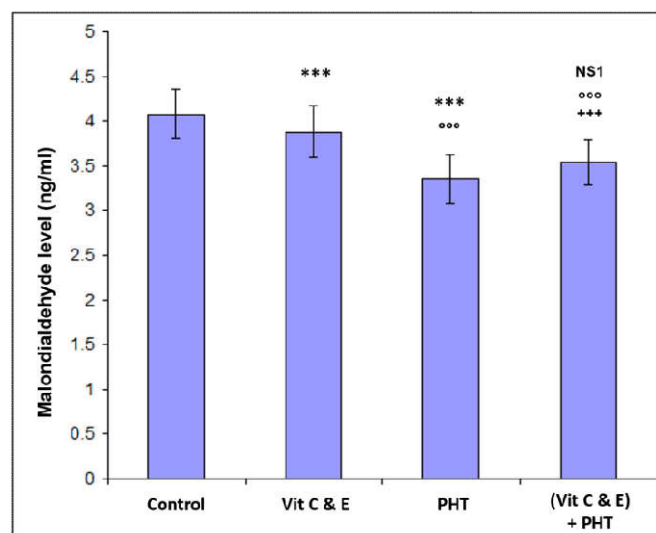
*** $P \leq 0.001$ vs. Control, °°° $P \leq 0.001$ vs. Vit. C & E, * $P \leq 0.05$ vs. PHT.

Fig 3. Vitamin C and E decreased the influence of Phenytoin on fetal body weight. * $P \leq 0.05$ vs. Control,



(***P \leq 0.001 Vs. Control, $^{\circ\circ}$ P \leq 0.001 Vs. Vit. C & E, NS1 P $>$ 0.05 vs. Control, NS2 P $>$ 0.05 Vs. Vit. C & E, $^{+++}$ P \leq 0.001 Vs. PHT.

Fig. 4. Vitamin C and E decreased the influence of Phenytoin on fetal brain weight



***P \leq 0.001 Vs. Control, $^{\circ\circ}$ P \leq 0.001 Vs. Vit. C & E, NS1 P $>$ 0.05 vs. Control, $^{\circ\circ}$ P \leq 0.001 Vs. Vit. C & E, $^{+++}$ P \leq 0.001 Vs. PHT.

Fig. 5. Vitamin C and E decreased the influence of Phenytoin on Malondialdehyde level in the blood serum of maternal rats

Whereas, the group treated with Vitamins only (Group 2) decreased all the parameters while comparing with control group. It suggests that the given doses of Vitamins have positive influence only under the oxidative stressed-condition i.e. Phenytoin treated group.

DISCUSSION

Phenytoin although being a widely used antiepileptic drug, also is a potent teratogen (England and Seifter, 1986 and Tiboni *et al.*, 2003). The prenatal exposure to Phenytoin results in a spectrum of structural, developmental and behavioral changes known as the fetal hydantoin syndrome (Scheinfeld, 2003). But the teratogenic effect also depends on the dose and gestation days on which the drug is administered. Several studies have shown its teratogenic effect on different gestation day at different doses. The present study involved administration of Phenytoin at dose of 80 mg/kg/day on gestation day 9-12 (which is supposed to be the organogenesis period of fetal rats). The fetuses of these groups were found to

have flexure defects and unequal sized limbs. It may be because of the fact that Phenytoin can reduce the Bone Mineral density (BMD) as well as the Bone Mineral Content (BMC) (England and Seifter, 1986). Lyon H.M. (2003) has also shown the malformations of the limbs and the talipes deformity which are the potential effects of vascular disruption, a postulated fetal effect of both Phenytoin and cigarette smoking (Lyon *et al.*, 2003). Since the brain has large content of myelin sheath and not particularly enriched in antioxidant defenses, therefore it is supposed to be the most susceptible target of free radical damage (Gupta *et al.*, 2004; D'Souza and D'Souza, 2003; Floyd and Carney, 1992). Supporting the fact, Phenytoin treated group of the present study found to have reduced fetal brain weight as well as whole body weight which was highly significant (P $<$ 0.001) as compared to that of control group. One of the mechanisms of Phenytoin induced teratogenicity is supposed to induce the fetal hypoxia, leading to vascular disruption and necrosis of existing and developing structures (Danielson *et al.*, 1992). Another mechanism is due to inducing the Reactive Oxygen Species (ROS) which can react with lipid (membrane lipids, lipids in circulating lipoproteins such as low density protein lipid) resulting in lipid Peroxidation shown by measuring the increased level of Malondialdehyde level (MDA) in blood (Winn and Wells, 2002; Danielsson *et al.*, 1997; Kim *et al.*, 1997). The present study selected the antioxidants; Vitamin C and Vitamin E which function as water soluble and lipid soluble chain breaking antioxidants respectively and protect lipids, proteins and membranes from oxidative damage. The interaction between Vitamin C and E can take place not only in homogenous solutions but also in liposomal membrane systems where they both reside separately outside and inside the membrane respectively acting as synergist (Niki, 1978). The present study evidenced the highly significant decrease in MDA level (P $<$ 0.001) of maternal rats in Phenytoin plus Vitamins treated group (Group 4) while comparing with the group treated with Phenytoin only (Group 3). This result was concomitant with the normal gross morphology and significantly higher body (P $<$ 0.05) and brain weight (P $<$ 0.001) of the fetuses derived from the rats in Group 4. Altogether, it can be concluded that Vitamin C and E as antioxidant attenuates the influence of Phenytoin in the MDA level of maternal rats, leading to improvement of fetal body and brain weight. Hence, this result has hinted the necessity of antioxidant nutrient balance for those pregnant women who are basically compelled to have exposed with various oxidants.

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