Scientific Research and Essays Vol. 5(9), pp. 999-1004, 4 May, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2010 Academic Journals

Full Length Research Paper

Chronic use of phenytoin reversibly suppresses fertility in male Sprague-Dawley rats

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Accepted 1 April, 2010

This study investigates the effect of chronic use of Phenytoin on male reproductive functions. Sixty male Sprague-Dawley rats were used. They were divided into five groups of 12 rats each (A, B, C, D and E). Group A served as the control and was given 0.1 ml of normal saline. Group B and C received 50 mg/Kg of Phenytoin. Group D and E were given 100 mg/Kg of Phenytoin. Administration of the drug was orally and twice daily for four weeks. After the first two weeks of administration, twenty-four mature untreated female rats with proven fertility were cohabited with animals in Group B and with those in Group D, in ratio 1:1. The administration continued for another two weeks during the co-habilitation. At the end of the fourth weeks, the animals in Group B and D were sacrificed. While the animals in Group C and E were allowed to recover for another four weeks, during which they were also tested for fertility, and eventually sacrificed. The results obtained were analyzed using t-test. The results show that Phenytoin significantly (*p*-value < 0.05) reduced fertility in male rats. Its effects were, however, reversible upon withdrawal.

Key words: Phenytoin, chronic use, reversibly, male fertility, testosterone, Sprague-Dawley rats.

INTRODUCTION

Anticonvulsant or antiepileptic drugs are used to treat epilepsy and seizures disorders. Phenytoin has been widely and effectively used in the treatment of epilepsy and arrhythmias (Tamura et al., 2000). Most of the antiepileptic drugs are central nervous system influencing drugs that act by exerting a modulatory effect on bioelectric activity of cell membrane. The commonly used anticonvulsants are barbiturates, carbamazepine, ethosuximide and sodium valproate. Clinical and experimental studies have shown that these centrally acting drugs have adverse effects on male reproductive functions (Balwin and Andrew, 2001).

There is increased in the metabolism of sex hormones and decrease in the free androgen index as a conesquence of increased serum sex hormone-binding globulin (SHBG) concentrations (Dana-Haeri et al., 1984; Macphee et al., 1988; Murialdo et al., 1994; Isojarvi et al., 1995) and/or enzyme induction in the liver (Ghosal et al., 1996) resulting from Phenytoin admi-nistration. Additionally, Phenytoin has been reported to inhibit testosterone production in rat Leydig cells *in vitro*, implying direct inhibitory effects of Phenytoin on steroidogenesis (Kuhn-Velten et al., 1990).

Sulfasalzine produced a significant decrease on male fertility in rats and there are associated reduction in sperm motility, sperm velocity and amplitude of lateral head displacement in treated rat (Horimotol et al., 1998).

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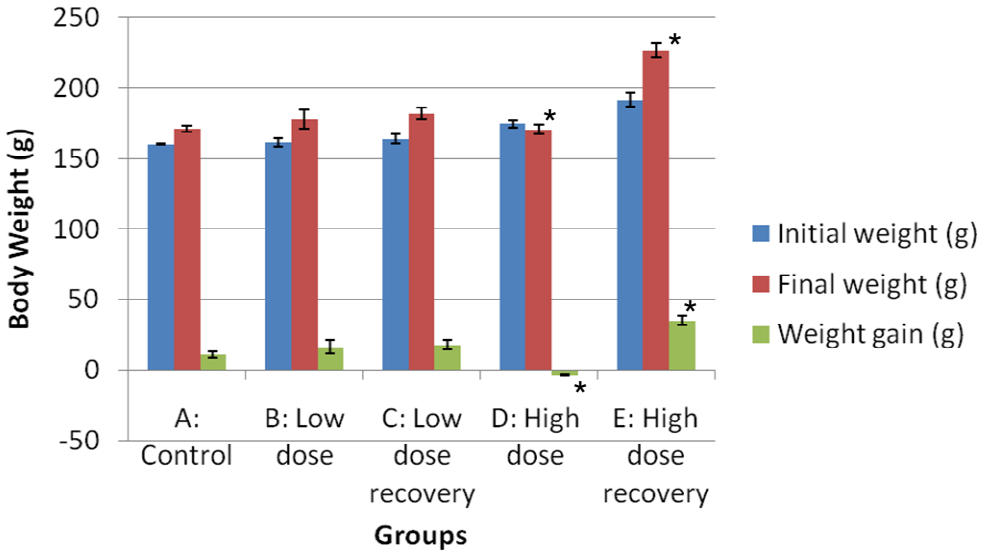

Figure 1. Effect of phenytoin on body weight (g).
* = (p-value < 0.05 when compared with the control). High dose of phenytoin reduces body weight. This reduction in body weight is, however, reversible resulting in higher weight gain in the recovery group.

Reduction in sperm parameters have been associated with some antiepileptic drugs such as Carboxiamides, (Teneja et al., 1994), Phenobarbitone, (Chen et al., 1992), bromide (Isojarvi et al., 2004), and Carbamazepine and Sodium Valporate (Baptista et al., 1999), thus, reducing male fertility and sex hormone. However, the effects of Phenytoin on male fertility have not been extensively explored. This study was designed to investigate the effect of chronic use of Phenytoin on male reproductive functions of male Sprague-Dawley rats.

MATERIALS AND METHODS

Sixty male Sprague-Dawley rats weighing between 180 - 220g were used for the study. They were inbred at the Animal House section of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso and were acclimatized over a period of two weeks. They were allowed to access food regularly and water *ad libitum*. The rats were randomly grouped into five (A, B, C, D and E) groups with each group having twelve rats. Group A served as the control and received 0.1 ml of normal saline orally. Group B and Group C served as the low dose groups and received 50 mg/Kg of Phenytoin. Group D and Group E served as the high dose groups and received 100 mg/Kg of Phenytoin.

The drug was administered orally and twice (8.00 am and 6.00 pm) daily for four weeks. After first two weeks of drug administration, twenty-four mature untreated female rats with proven fertility were cohabited with animals in Group B and Group D, in ratio 1:1. The administration of Phenytoin continued for the male rats for another two weeks during the cohabitation period. At the end of the four weeks, the animals in Group B and D were sacrificed. The animals in Group C and E were allowed to recover for another four weeks without administration of Phenytoin, thereafter, they were then tested for their fertility by introducing females of proven fertility to them at the second week of recovery.

Collection of data and statistical analysis

The animals' body weights and testes weights were measured and recorded. Semen parameters were taken and Serum Testosterone level was determined. Percentage fertility success was calculated taking into account the number of cohabitated female rats that were mated, the numbers that became pregnant, and the number of their litters. The control and test groups were compared using t-test. The results were presented as mean ± SEM and the level of significance was taken as p-value < 0.05.

RESULTS

Effect of phenytoin on body weight (g), testicular weight (g) and testosterone level

While low dose of phenytoin had no significant effect on weight gain, chronic high dose significantly (p-value < 0.05) reduced weight gain (Figure 1). There was, however, a significantly (p-value < 0.05) higher weight gain in the recovery group (Group E) of high does (Figure 1). On the other hand, there was a dose dependent significant (p-value < 0.05) reduction in testicular weight and serum testosterone concentration (Figure 2). But these were normalized after recovery in Groups C and E. The correlation (relationship) between testicular weight and serum testosterone concentration in all the groups (control and treated groups) are shown in Figure 3.

Effect of phenytoin on sperm indices

Phenytoin significantly (p-value < 0.05) reduced sperm count in all the phenytoin treated groups. This reduction

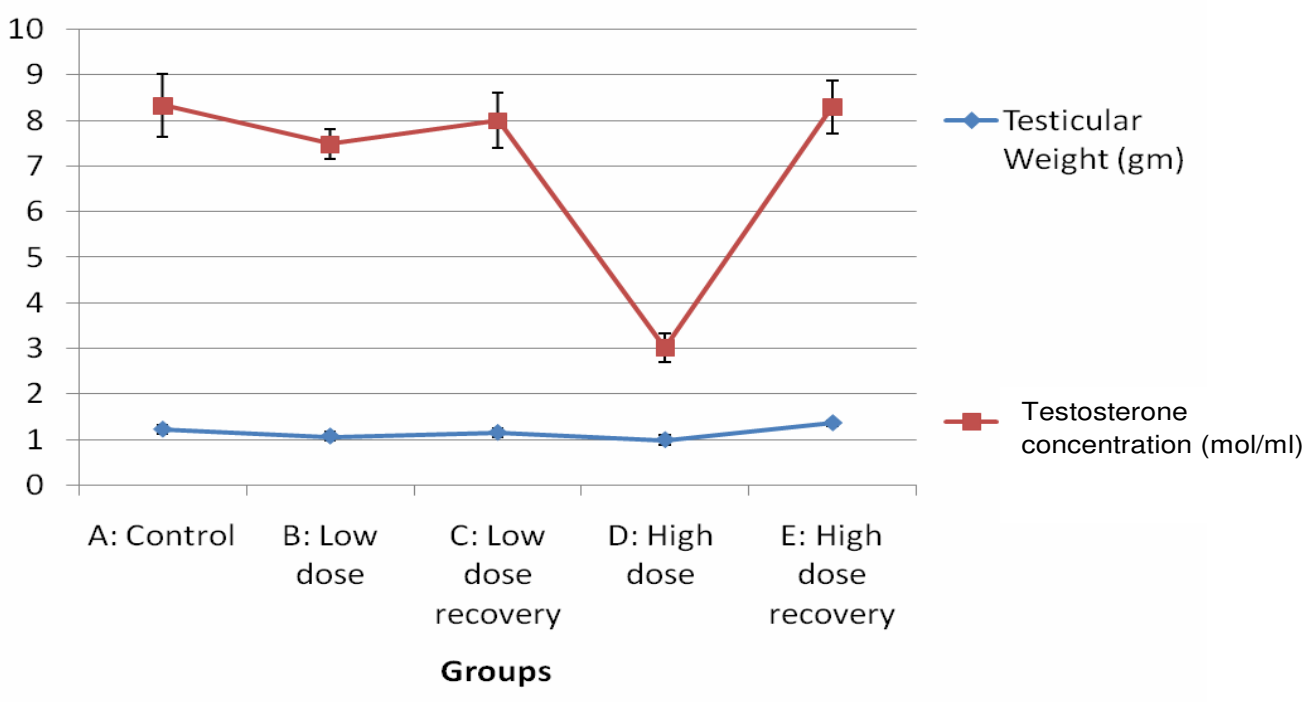

Figure 2. Testicular weight (gm) and Serum Testosterone concentration (mol/ml).
* = (*P*-value < 0.05 when compared with the control). Phenytoin significantly (p-value < 0.05) reduced Testicular weight (gm) and Serum Testosterone concentration in a dose dependent manner. These reductions were reversible in the recovery groups.

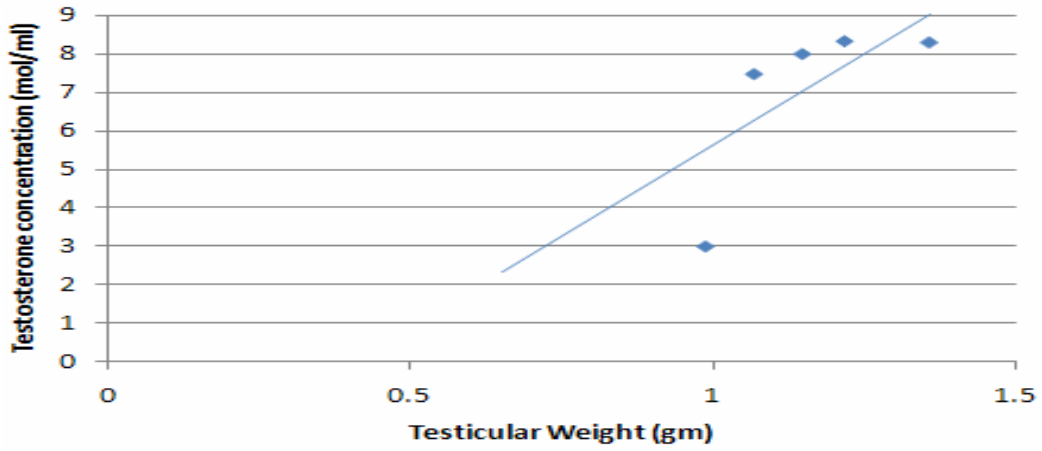

Figure 3. Correlation (relationship) between testicular weight (gm) and serum testosterone concentration in the control and treated groups.
Note: Positive correlation (relationship) was noticed between testicular weight and serum testosterone concentration.

in sperm count was lower in the recovery groups upon withdrawal of the drug. Low dose of Phenytoin had no significant (p-value > 0.05) effect on sperm motility (as seen in Groups B and C), but at high dose Phenytoin significantly (p-value < 0.05) reduced sperm motility. There was, however, a significant (p-value < 0.05) improvement in sperm motility from 28.37 ± 4.75% in Groups D to 53.11 ± 10.00% in Group E upon withdrawal of phenytoin. Phenytoin significantly (p-value < 0.05) reduced percentage of normal sperm cells (sperm morphology) from 81.25 ± 2.39% (in the control) to 68.75 ± 2.39% (in group B), 71.25 ± 2.40% (in group C), 56.25 ± 2.39% (in group D) and 61.25 ± 1.25 (in group E).

This trend as well shows significant (p-value < 0.05) improvement in sperm morphology (percentage of normal sperm cells). Sperm viability was found to be significantly

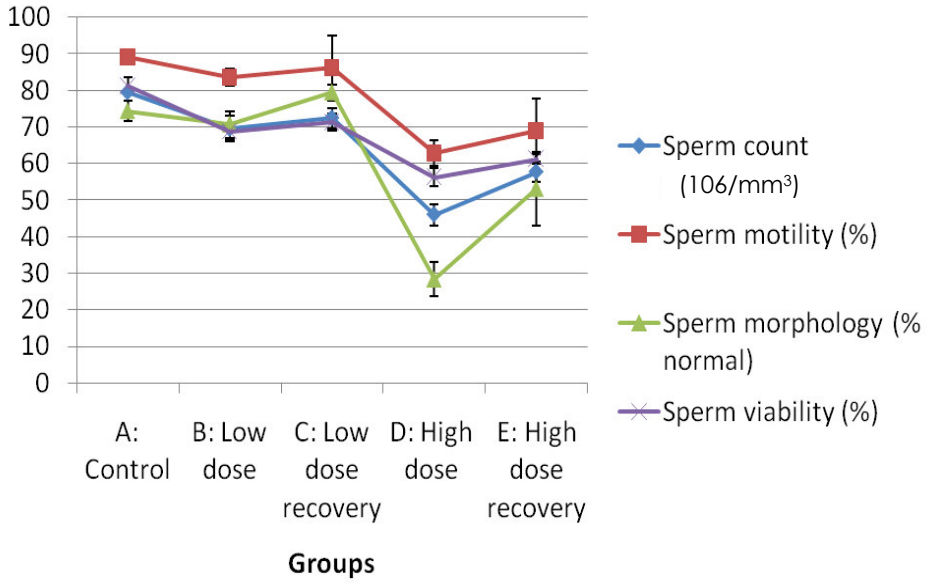

Figure 4. Effect of phenytoin on sperm indices.
* = (*P*-value < 0.05 when compared with the control). Sperm Indices were significantly (p-value < 0.05) reduced in all the phenytoin treated groups (Groups B, C, D and E). Significantly (p-value < 0.05) improvement was, however, noticed in the recovery groups (Group C and E) compared with the respective non recovery groups (Groups B and D respectively).

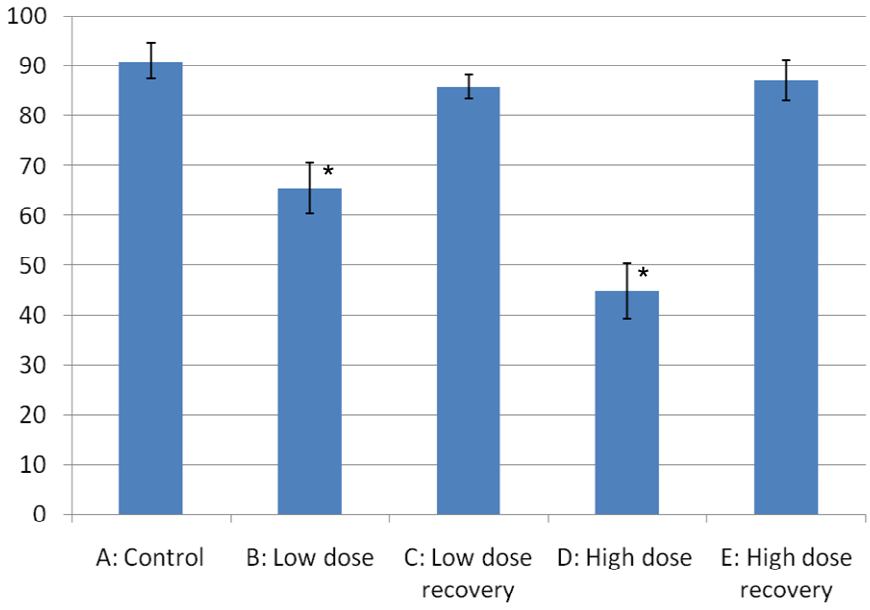

Figure 5. Percentage fertility success.
* = (p-value < 0.05 when compared with the control). Percentage Fertility Success was significantly reduced in the phenytoin-treated-no-recovery groups (Groups B and D). There was no significant reduction in the Percentage Fertility Success of the groups (C and E) that were allowed to recover from the drug.

(p-value < 0.05) lower in all the Phenytoin treated rats but was significantly (p-value < 0.05) improved in the recovery groups (Group C and E) compared with the respective non recovery groups (Groups B and D,

respectively) (Figure 4).

Effect of phenytoin on percentage fertility success

The percentage fertility success was significantly (p-value < 0.05) reduced in both the low dose and high dose phenytoin treated animals (Groups B and D). However, there was no significant reduction in the percentage fertility success of the groups (C and E) that were allowed to recover from the drug (Figure 5).

DISCUSSION

The result of this study shows that at high doses, Phenytoin reduces body weight. This reduction in body weight is, however, reversible resulting in higher weight gain in the recovery group (Figure 1: Group E). Similar observation was reported by Raji et al. (2005); Teneja et al. (1994) following the antipsychotic and anticonvulsive agents, respectively, where there are significance weight gains when the medication are either withdrawn or changed. The decrease in body weight observed in Group D may be an indication of the toxic effect of the high dose of Phenytoin on the rats. The dose dependent significant (p-value < 0.05) reduction in testicular weight noticed in Groups B and D suggests that chronic administration of phenytoin has toxic effects on the testis. This was in agreement with the conclusion of Simmons et al. (2002) that reduction in weight or a change in either absolute or relative organ weight after administration of a chemical is an indication of the toxic effect of such chemical or agent, more so that, this was found to be dependent on the doses of Phenytoin administered.

The reduction in sperm counts, motility, morphology, and viability were doses are dependent and this may be due to direct effect of Phenytoin on spermatogenesis because of it interference with acetyl-cholinesterase and glucose that are required in the process of spermatogenesis. This is because sperm motility, in addition to being under the control of acetyl cholinesterase (Nelson, 1972), depends also on glucose availability. This finding is in line with that of Chen et al. (1992) that antiepileptic drugs cause reduction in sperm motility and stated that, the lowering effect of antiepileptic drugs on sperm motility is because of their ability to interfere with sperm membrane functions. Isojavrii et al. (2004); Kuhn-Velter et al. (1990); Conn et al. (1978) observed in there respective studies that antiepileptic/anticonvulsant drugs significantly reduce male reproductive parameters (sperm count, sperm motility, sperm morphology (% normal), and sperm viability). They concluded that Phenytoin most likely affects male reproductive system at the testicular level.

The plasma testosterone concentration of animals treated with Phenytoin was found to be significantly reduced in a dose dependent manner (Figure 2). This was similar to the findings of Raji et al. (2005), in which the plasma testosterone concentration is reduced in rats treated with thioridazine and chloropromazine; and to the findings of Baptista et al. (1999) in which a significant reduction in plasma testosterone, FSH and LH concentrations was found in rats treated with antiepileptic drugs. In this study, phenytoin would cause the noticed significant (p-value < 0.05) reduction in serum testosterone concentration by either of these two ways or the two together: (1.) the dose dependent significant (p-value < 0.05) reduction in testicular weight noticed in phenytoin treated groups. Because a positive correlation (relationship) was noticed between testicular weight and serum testosterone concentration (Figure 3) and Testosterone is produced in the testes, it becomes reasonable to link the dose dependent significantly (p-value < 0.05) low serum testosterone concentration in the phenytoin treated groups with the corresponding reduction in testicular weight; (2.) the fact that, antiepileptic drugs induced the production of the enzyme aromatase in the liver and this enzyme converts testosterone to estradiol. This thus, reduces the availability of free testosterone (Herzog et al., 1982). Also, phenytoin is excreted in human semen in some small quantities and this may possibly affect the level of testosterone. A similar observation has been documented by Meng et al. (2001) that plasma concentration of free testosterone is reduced especially in male epileptic patient. This suggests that Phenytoin suppresses fertility in rats not only by interfering with testicular functions, but also by affecting the hypothalamo-pituitary-testicular-axis and thus, reducing the amount of testosterone in circulation (Baptista et al., 1999). This alone can even reasonably account for the ability of Phenytoin to suppress fertility. This is because testicular functions and male reproductive system are almost entirely controlled by testosterone.

The recovery groups (C and E), however, showed no significant (p-value > 0.05) reduction in serum testosterone concentration when compared to the control. This further establishes that the antifertility effect of chronic administration of phenytoin is reversible upon withdrawal of the drug. This contradicts the speculation of Vijay et al. (2009) that “the gonadotoxic effect of Phenytoin may not be reversible”. Furthermore, Bauer et al. (2004) observed that generally, epileptic patients have impaired central nervous system response to low testosterone production and this low circulating testosterone triggers increase LH secretion from the pituitary, but the feedback mechanism appears impaired in men with temporal lobe epilepsy, and thus, low the level of testosterone.

Finally, the fact that the Percentage Fertility Success was found to be significantly lower in the “phenytoin-treated-no-recovery” groups (Groups B and D), but not significantly reduced in the recovery groups (Groups C and E) further establishes that phenytoin reversibly suppresses fertility. In other words, the antifertility effect of phenytoin is reversed upon its withdrawal. This

observation could be directly linked to the ability of phenytoin to reversibly reduce testicular weight (Figure 2), sperm indices (Figure 4), and serum testosterone concentration (Figure 4). It can, therefore, be concluded that Phenytoin has adverse effects on male fertility when taken for long period but these antirfertility effects are reversible once the drug is withdrawn.

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