Effects of Phenobarbital Administration on the Histology of the Liver and Brain, and the Activities of Some Biochemical Parameters of the Liver of Wister Rats

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Abstract

The effects of phenobarbital on the histology of the liver and the brain and on selected biochemical parameters of the liver of wistar rats were studied. Histological examination showed prominent lesions in the liver and brain of the tested groups of rats. Biochemical analysis revealed significant (P < 0.05) increase in the activities of alkaline phosphatase, alanine transaminase and in the level of cholesterol, with increased drug dosage. These correlated with the pathological changes observed in the liver and the brain of the wistar rats. The effects of phenobarbital on the liver and brain cells were found to be dose-dependent. Half the LD_{50} of the drug (8mg/160g) administered on the rats caused reasonable injuries to the tissues of the liver and the brain of the wistar rats.

Keywords: Phenobarbital, Liver, Brain, Alkaline Phosphatase, Alanine Transaminase, Cholesterol**.**

Introduction

There has been a resurgence of clinical interest in the role of phenobarbital (PB) for treating childhood epilepsy in Western countries (Pal, 2007). In 1912, PB became one of the first agents used against epilepsy (Shorvon and Farmer, 1988), and is today the most widely used anti-epileptic drug (AED) in the world (Pal, 2007). In resource-poor countries, an anticonvulsant that controls seizures and prevents recurrence would be ideal (Kokwaro *et al*., 2003). Diazepam is useful in status epilepticus, but it does not offer prophylaxis following single-dose administration (Ogutu *et al*., 2002), whereas multiple doses can cause respiratory depression (Crawley *et al*., 2000). Phenobarbital would be ideal for use in resource-poor countries, since it is cheap, readily available, fast-acting and can be administered *i.m.* at peripheral health facilities with few resources (Kokwaro *et al*., 2003). In western countries, phenobarbital has been in and out of favour as an AED (Pal, 2007). Although widely used as a prophylactic against febrile seizures in the 1960s and 1970s, concerns about its neurobehavioral adverse effect profile led to a decline in its use for all seizure disorders (Shorvon, 1986).

The reasons for renewed interest in phenobarbital arise mainly from doubts about the safety of new licensed pharmaceuticals and public disquiet over the unsatisfactory regulatory framework for preclinical evaluation of new medicines, particularly for children (Pal, 2007). Cost of treatment may also be a consideration: carbamazipine costs around ten times, and lamotrigine almost forty times as much as the equivalent dose of phenobarbital (Pal, 2003).

The effects of phenobarbital on liver physiology are typified by hepatic hypertrophy, hyper proliferation of the smooth endoplasmic reticulum, and induction or repression of numerous genes, especially the genes of cytochrome P_{450} enzymes (Garcia-Allan *et al*., 2000). Hepatic hypertrophy induced by phenobarbital is mediated by a moderate increase in hepatocyte DNA synthesis and dramatic enlargement of individual hepatocytes (Carthew *et al.,* 1998). Hepatic enlargement subsides after phenobarbital withdrawal, and this decrease in liver size is mediated by hepatocyte apoptosis (Bursch *et al.,* 1984). Also, liver regeneration in response to partial hepatectomy is markedly diminished in phenobarbital–treated rat and mouse livers (Aletti *et al.,* 1981). Phenobarbital is also the prototype of liver tumor promoters, dramatically increasing tumor numbers when chronically administered after initial genotoxic carcinogen treatment (Bell and Michalopoulos, 2006).

However, phenobarbital is still being used as a sedative in cases of gastrointestinal and asthmatic functional disorders, as well as to antagonize the adverse central stimulant effects of some drugs such as ephedrine, dextroamphetamine or theophylline. It is also used in cases of withdrawal syndromes of hypnosedative agents (Lopez-Munoz *et al*. 2005). In the field of neurology, phenobarbital is still employed, not only in the treatment of certain types of epilepsy (partial and tonic-clonic generalized seizures), but also in the emergency treatment of some types of convulsions, such as those associated with tetanus, eclampsia, cerebral hemorrhage, status epilepticus, or different forms of poisoning (Lopez-Munoz *et al.,* 2005). Phenobarbital is also capable of improving the hepatic transport of bilirubin in patients with hemolytic jaundice, so that it can be used in newborn babies to treat hyperbilirubinemia and kernicterus (Lopez-Munoz *et al.,* 2005).

The present study is therefore aimed at determining the effects of phenobarbital on the liver and brain of rats, with a view to determining whether it can cause any histological alteration(s) in these organs. The effects of phenobarbital dosage and duration of administration on selected biochemical parameters of the liver were also studied.

Materials and Methods

Drug administration: The LD_{50} of phenobarbital sodium (PHTNa) in rats is 100mg/kg (Ruch *et al.,* 2003). Administration was intraperitoneally, the starting does being about one-third of the LD_{50}. The test groups (groups 1-4, below) received respectively, a corresponding increase in drug dose while the control group (5) received no administration of drug. Drug was administered daily for a period of 3 weeks (Table 1).

Table 1: Dosage of phenobarbital sodium administration

Groups	Dose mg/160g	Drug in mg/kg body wt	ml/l
1 (6 rats)	5.0	31.25	0.25
2 (6 rats)	6.0	37.50	0.30
3 (6 rats)	7.0	43.75	0.35
4 (6 rats)	8.0	50.00	0.40
5 (6 rats)	0.0	-	-

Animal sacrifice: Ten rats, comprising of two rats from each group, were randomly selected and painlessly sacrificed at the end of every week of drug administration by placing them in a container having chloroform-soaked wool. Their post mortem was performed immediately and the organs for study resected and fixed promptly in 10% formal saline. Subsequently they were histological processed, their sera were collected and analyzed biochemically at each sacrifice for alanine transaminase, alkaline phosphatase and cholesterol levels (Mayne, 1994).

Tissue processing: The liver and brain excised from the dissected rats were cleared of the adhering connective tissue, fixed for 24 hours by immersion in equal parts of 10% formal buffered saline. There after, fixed tissues were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in liquid paraffin wax. The tissues were sectioned to a diameter of 3-5 microns using the Heitz 150 rotary microtome (Cambridge model). The sections were then subjected to Erlich's Haematoxylin and Eosin (H and E) staining technique as described by Baker and Silverton (1985). Sections were examined using swift binocular microscope with in-built lighting system and were photographed.

Results

Histopathology: On examination of the photomicrographs from the five different groups of experimental animals, the control showed normal architecture of the tissues under study. The other four groups presented different levels of distortion from the normal cell framework with the greatest level being observed in test group 4 whose animals received the largest drug dose (Figs. 1 - 6).

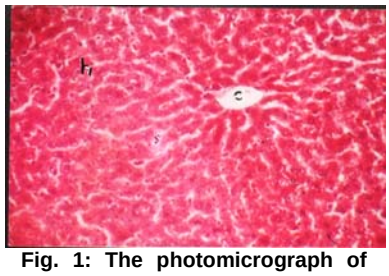

Fig. 1: The photomicrograph of an apparently normal liver tissue of rat (x 200), showing normal hepatocytes (h); clear and normal central canal (c).

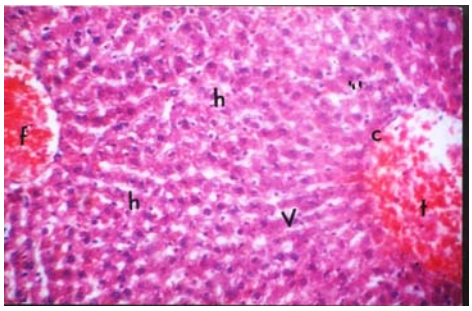

Fig. 2: The photomicrograph of liver tissue of rat (x200) administered with 5mg/160g (phenobarbital/body weight), after 3 weeks of administration, showing normal hepatocytes (h); frank red blood cells (f) in central canal; mild vacuolation (v).

Observations made from the liver microphotographs showed that progressive increase in period of administration of phenobarbital initiated necrosis in the liver of the rats (Fig. 2). The degenerative processes intensified evidenced by a large increase in vacuolation, as the dosage of phenobarbital was increased (Fig. 2).

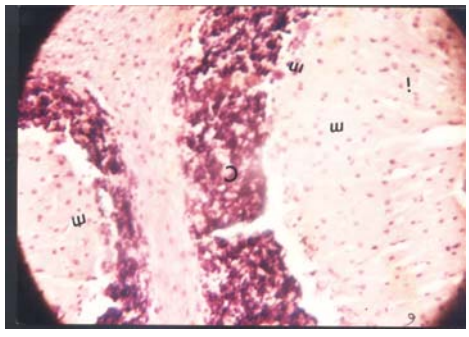

Fig. 3: Photomicrograph of the normal cerebellum of the control group of rats (x200), showing the normal distribution of glial cells: macrocytes (a) in brain cortex (c); microcytes (i) in brain medulla (m).

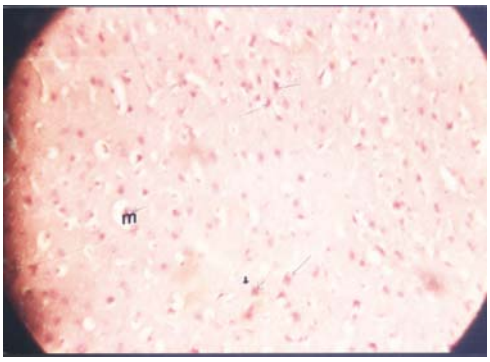

Fig. 4: Photomicrograph of brain tissue cerebellum of rats (x200) from test group 1, administered with 5mg/160g (phenobarbital / body weight) for 3 weeks. Contracted microglial cells adjusting to the initial effects of phenobarbital; macrocytes (m).

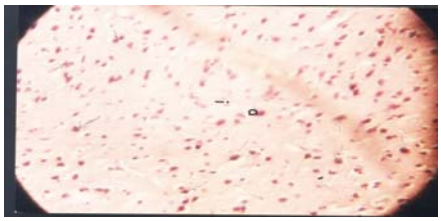

Fig. 5: Photomicrograph of brain tissue cerebellum of rats (x200) from test group 2, administered with 6mg - 7mg phenobarbital /160g body weight for 1 week. Here, there is a reduction in microglial cells (i); macrocytes (a) are fairly constant

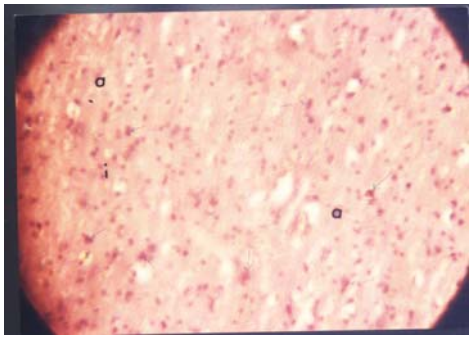

Fig. 6: Photomicrograph of brain tissue cerebellum of rats (x200) from test group, administered with 8mg/160g (phenobarbital / body weight) for 2 weeks. Here, there are cloudy, crowded neuronal cells; there are large pink-stained glial cells. The onset of liquefaction is visible.

Figure 3 was obtained from the control group of the rats, and showed normal brain tissue architecture. Figures 4-6 however, displayed neurons with pyknotic nuclei and necrotic cells. Here, brain cells were largely replaced by pink-staining debris, with phagocytic cells (microcytes) engulfing degenerate materials. Figure 6 represents brain tissues of group 4 rats, which were administered with 8mg of the drug per 160g body weight. They exhibit an extensive neurodegeneration with an onset of gliosis.

Biochemical parameters: The activities of two liver enzymes, alanine transaminase and alkaline phosphatase, together with cholesterol level in the serum of rats were analyzed against drug dose and duration of administration. The effect of the drug was observed to be significantly dose dependent ($p < 0.05$). Using a one-way analysis of variance, the increase in biochemical parameters within the three weeks of drug administration was found to be significant ($p < 0.05$).

Table 2: Cholesterol levels during the period of drug administration

Dose of pheno-barbital (mg)	Cholesterol levels in wk 1 (mmol/l)	Cholesterol levels in wk 2 (mmol/l)	Cholesterol in wk 3 (mmol/l)
0.0(control)	2.8	3.0	3.0
5.0	3.1	3.2	3.6
6.0	3.5	3.9	4.1
7.0	4.3	5.0	5.8
8.0	6.1	7.0	7.6

Table 3: Alanine transaminase (ALT) levels during the period of drug administration

Dose of pheno-barbital (mg)	ALT levels in wk 1 (iu/l)	ALT levels in wk 2 (iu/l)	ALT levels in wk 3 (iu/l)
0.0(control)	10	11	11
5.0	12	12	13
6.0	12	13	13
7.0	13	12	13
8.0	19	21	25

Table 4: Alkaline phosphatase (ALP) levels during the period drug administration

Dose of pheno-barbital (mg)	ALP levels in wk 1 (iu/l)	ALP levels in wk 2 (iu/l)	ALP levels in wk 3 (iu/l)
0.0(control)	33	34	33
5.0	69	76	87
6.0	73	78	82
7.0	88	86	98
8.0	90	95	109

Tables 2, 3 and 4 show the changes in the levels of cholesterol, alanine transaminase and alkaline phosphatase respectively, with increase in phenobarbital dosage. Table 2 shows a progressive increase in cholesterol level with increase in the period of phenobarbital administration, for each test group. There was also an increase in cholesterol level with increase in phenobarbital concentration. Similarly, the levels of both alanine transaminase (table 3) and alkaline phosphatase (table 4) increased with increase in the period of phenobarbital administration, and with increase in phenobarbital concentration.

Using a one-way analysis of variance (ANOVA), the level of significance ($p<0.05$) of these increases with increased administration of phenobarbital was determined (table 5). It was found that increase in the level of either cholesterol, alanine transaminase or alkaline phosphatase was significant ($p<0.05$).

Table 5: One-way analysis of variance for the determination of the level of significance of increases in the biochemical parameters with dosage of phenobarbital

Parameters		Sum of squares	df	Mean square	F	P
Cholesterol level (mmol/l) in weeks	**Between groups**	29.600	4	7.400		0.05
	Within groups	2.773	10			
	Total	**32.373**	**14**	0.277	26.683	
Alkaline phosphatase level (iv/l) in weeks	**Between groups**	7566.933	4	1891.733	39.193	0.05
	Within groups	482.667	10			
	Total	**8049.600**	**14**	48.267		
Alanine transaminase (iv/l) in weeks	**Between groups**	228.667	4	57.167	26.797	0.05
				2.133		

Discussion

A rise in plasma activities of alanine transaminase has been shown to characterize liver-cell damage, while alkaline phosphatase activities are increased in cholestasis (Mayne, 1994). The results obtained from this study showed significant increases ($P < 0.05$) in the plasma activities of alkaline phosphatase and alanine transaminase. Plasma level of cholesterol also increased significantly ($P < 0.05$). This showed that high doses of phenobarbital or discrete doses administered for long periods had adverse effects on the liver. This finding is further supported by the histological results which showed the formation of cellular vacuoles on administration of very mild doses of phenobarbital.

The histological results obtained from the brain tissue showed that phenobarbital causes damage to the brain. Neurodegeneration increases as phenobarbital dosage are increased progressing ultimately to gliosis and then liquefaction. This is in support of the finding by Yazar *et al.,* (2002) that phenobarbital increased the activities of marker enzymes in the brain and the liver of mice. It has also been shown that phenobarbital increased the level of serotonin in the forebrain and cerebellum of mice with a hereditary susceptibility to seizures (Matsumoto *et al.,* 1983). Similarly, Lewin and Bleck (1997) found that phenobarbital inhibits seizures by decreasing the activities of neurons. These findings agree considerably with the histological results obtained in this study which showed features that can indeed impair transmission of impulses and hence the functional ability of the brain.

From all indications however, phenobarbital will continue to be clinically relevant in the treatment of epilepsy and other types of convulsions owing to the fact that it is very cheap compared to other remedies. It is therefore necessary to advice that the drug be taken only on the prescription and under the strict supervision of a qualified medical personnel.

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