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# Chronic administration of gabapentin and a gabapentin-carbamazepine combination reversibly suppress testicular function in male Wistar rats (*Rattus norvegicus*)

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ABSTRACT

The effect of chronic administration of gabapentin, carbamazepine or a gabapentin-carbamazepine combination on testicular function in male rats was investigated to determine the effect of combining reduced doses of anti-epileptic drugs on the management of seizures, particularly with respect to the testis sequellae of chronic anti-epileptic administration.

Male rats were randomized into four groups (n = 10). Each group received daily intraperitoneal (i.p.) injections for 28 days as follows: Group I, normal saline 0.1 mL/day; Group II, gabapentin (GBP) 16 mg/kg/day; Group III, carbamazepine (CBZ) 20 mg/kg/day; and Group IV, sub-therapeutic doses of both GBP (8 mg/kg) and CBZ (10 mg/kg)/day. Twenty-four hours after the last treatment, five rats from each group were sacrificed and the remaining rats were allowed to recover for another four weeks. Sperm characteristics, serum testosterone, and histological integrity of the testis was assessed 24 h after treatment and after 28 days of drug withdrawal.

GBP, CBZ, and GBP-CBZ combination significantly reduced the absolute weight of the testis, epididymis, and seminal vesicle ($p < 0.05$). Moreover, epididymal sperm count and morphology were significantly decreased ($p < 0.05$) in GBP, CBZ, and GBP-CBZ groups. Reduction in serum levels of testosterone for all of the treated groups was statistically significant ($p < 0.05$). The cytoarchitecture of the testicular tissue in the testis of CBZ and GBP-CBZ groups showed disorganization. The altered testicular function were almost restored in GBP treated rats. CBZ and GBP-CBZ combination have delayed but reversible antifertility in the rats. Hence, chronic administration of GBP, CBZ, and GBP-CBZ combination reversibly reduced testicular function in male rats.



## 1. Introduction

A seizure occurs when populations of neurons produce a sustained elevated firing during a period of synchronous activity [1]. Epilepsy is a chronic disorder of the central nervous system of various etiologies characterized by recurrent seizures due to excessive discharge of cerebral neurons [2]. The WHO Neuroscience Research Protocol for studying the prevalence of neurological disorders in developing countries defines epilepsy as two or more afebrile seizures unrelated to metabolic disorders or to withdrawal from drugs or alcohol [3]. Epilepsy is the most common non-infectious neurologic disease in developing African countries, including Nigeria [2], and it remains a major medical and social problem [4].

Most of the anti-epileptic drugs available act on the central nervous system by exerting a modulatory effect on bioelectric activity of the cell membrane. The most commonly used anticonvulsants are barbiturates, carbamazepine, phenytoin, pregabalin benzodiazepines, valproic acid, vigabatrin, tiagabine, and gabapentin [5].

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Gabapentin [(1-(aminomethyl) cyclohexane acetic acid)], a novel anti-epileptic agent, was originally developed as a gamma-aminobutyric acid (GABA)-mimetic compound to treat spasticity, and has been shown to have potent anticonvulsant effects [6]. Gabapentin acts at an intercellular site as the maximal anticonvulsant effects are achieved two hours after an intravenous injection of gabapentin in rats. This occurs after plasma and interstitial fluid concentrations have peaked and reflect the additional time required for intra-neural transport [7].

Although carbamazepine (CBZ) was developed as an anti-epileptic drug, typically used for the treatment of seizure disorders and neuropathic pain [8], Okuma [9] also established its use as a mood stabilizer and as a second line treatment for bipolar disorder, as well as its use along with antipsychotic agents in the treatment of schizophrenia [10]. Carbamazepine stabilizes the inactivated state of voltage-gated sodium channels, making fewer of these channels available to subsequently open. This leaves the affected cells less excitable until the drug dissociates. Carbamazepine has also been shown to potentiate GABA receptors made up of alpha$_1$, beta$_2$, and gamma$_2$ subunits [11]. Reduction in sperm parameters has been associated with some anti-epileptic drugs such as carboxiamides [12], phenobarbitone [13], bromide [14], and carbamazepine and sodium valproate [15], thus reducing the levels of male sex hormones and reducing fertility.

Clinical and experimental studies have shown that these centrally acting drugs have adverse effects on male reproductive function [16]. Oral administration of carbamazepine decreased testicular weight, sperm cell concentration, plasma testosterone, follicle stimulating hormone, and luteinizing hormone. Prolonged administration of carbamazepine also increased plasma prolactin levels, which eventually causes degeneration of testicular histology [17].

In a study involving phenytoin, increased metabolism of sex hormones and a decrease in the free androgen index as a consequence of increased serum sex hormone-binding globulin (SHBG) concentrations [18] and/or enzyme induction in the liver [19] was reported. There is no study known to us that investigated and documented the effects of reduced doses of the combination of GBP and CBZ to ascertain its pattern of action and effects on male fertility markers. The present study aims to investigate and document the effects of chronic administration of GBP, CBZ, and combined GBP-CBZ on the markers of fertility in males. It is expected that our results will contribute to knowledge and improve the quality of counseling given to men of reproductive age on these anti-epileptic drugs.

Carbamazepine (CBZ) is the drug of choice for generalized tonic-clonic and complex partial seizures, trigeminal neuralgia, and bipolar disorder [20]. Carbamazepine has a low hepatic extraction ratio (0 < 2) and is 60–80% bound to plasma protein [21].

The four major metabolic pathways of CBZ in humans are the epoxide pathway, aromatic hydroxylation, direct *N*-glucuronidation, and sulfur-containing conjugation; metabolites from these pathways constitute 40%, 25%, 15%, and 5% of urinary excreted drug, respectively [20]. The epoxide pathway is mediated by cytochrome $P_{450}$, (i.e., CPY3A4 and CPY2B), which can be induced by the repeated administration of CBZ to humans and animals [22–24], leading to increased metabolic clearance.

CBZ metabolism and pharmacokinetics can be altered by many drugs, such as phenytoin and phenobarbital, which increase CBZ clearance, and other drugs such as cimetidine, erythromycin, and isoniazid, which inhibit the activity of the cytochrome $P_{450}$ system, decreasing the clearance of CBZ [20]. The active metabolite, CBZ-epoxide, can also induce these enzymes in rats during multiple doses [22–24].

The half-life of CBZ is decreased upon repeated CBZ administration [25]. In rats, the largest portion of CBZ is biotransformed to CBZ-epoxide by the microsomal epoxidation pathway [22]; however, in humans, about 90% of CBZ-epoxide is further metabolized to 10,11-transdiol by epoxide hydrolase [20]. Tateishi et al. [24] suggested that there are more enzymes, such as CYP 2C6 and CYP 2C11, involved in CBZ metabolism in rats than in humans. A decrease in CBZ creatinine clearance rate could reflect altered glomerular filtration, tubular reabsorption, or plasma protein binding [20].

In rats, gabapentin is concentrated in the pancreas and kidneys. Pancreatic and renal tissue concentrations are eight and four times higher than serum concentrations, respectively [26]. The drug does not accumulate in the pancreas in humans since it exists in a highly ionized state at physiological pH and its concentrations in adipose tissue are low [26].

Gabapentin is unique among anticonvulsant drugs in that it lacks hepatic metabolism, exhibits low protein binding, and does not induce or inhibit hepatic microsomal enzymes or inhibit the metabolism of other anti-epileptic drugs [26]. No significant pharmacokinetic interactions have been reported between gabapentin and conventional anti-epileptic drugs (valproic acid, phenobarbital, carbamazepine, or phenytoin) or oral contraceptives [26]. However, cimetidine, which decreases glomerular filtration rate, reduces the clearance of gabapentin by 12% [27]. In addition, the bioavailability of gabapentin is decreased by 20% by antacids when taken simultaneously or within 2 h of gabapentin administration [28].

All of these anti-epileptics are used chronically, leading to adverse effects including decreased or lost fertility. Multiple drugs used for prolonged periods lead to additive untoward effects. In the past few decades, there has been a growing appreciation that not all seizures can be controlled by monotherapy, and this has led to combination therapy for epilepsy. The introduction of over 14 new anti-epileptic drugs (AEDs), including gabapentin, for the adjunct treatment of refractory epilepsy. These new developments triggered clinicians' interest in combination therapy. However, despite the promise of the effectiveness of combination therapy, concerns were raised about various side effects attributed, which include but not limited to unfavorable interactions, difficulty in evaluating individual pharmacological effects, and neurotoxicity [29–31]. Olaibi et al. [32] reported the anticonvulsant synergistic effect of the gabapentin-carbamazepine combination in male rats, which was accompanied by attendant side effects on the histomorphology of the hippocampus and striatum. The effects of chronic administration of GBP, CBZ and GBP-CBZ combination on testicular functions were therefore investigated to see whether combining reduced doses of anti-epileptics in the management of seizure would reduce side effects, particularly the antifertility sequaleae of chronic antiepileptic administration and to elucidate the probable mode of action.

## 2. Materials and methods

### 2.1. Animals

A total of 40 male Wistar rats (150–200 g) were obtained from the animal holding of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun state Nigeria and allowed to acclimate over a period of one week. The rats were housed in plastic cages; food (produced by Grand Cereals Jos, a subsidiary of UAC Nigeria limited) and water were provided *ad libitum*. All procedures concerning animal handling were undertaken according to the International Guidelines for Proper Experimental Animal Care (natural light/dark cycle, room temperature, and humidity) also, approval concerning the schedule of animal care and treatment was obtained from Health Research and Ethics committee of