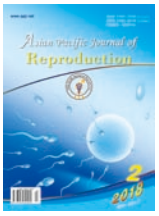

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Effect of voltage-gated sodium channels blockers on motility and viability of human sperm *in vitro*

Hammad Ahmad Gakhar[1], Ishrat Waheed[1], Taseer Ahmad[2]✉, Naeem-ur-rahman[3]

[1]*Riphah Institute of Pharmaceutical Sciences, Riphah International University Islamabad, Pakistan*

[2]*Laboratory of Cardiovascular and Integrative Pharmacology, College of Pharmacy, University of Sargodha, Pakistan*

[3]*Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan*

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ABSTRACT

Objective: To test the effect of voltage-gated sodium channels (VGSCs) blockers on the motility and viability of human sperm *in-vitro* and to evaluate the tested compounds as potential contact spermicidal. **Methods:** Sperm samples were obtained from healthy non-smoking volunteers of age 25-30 years who had not taken any drug 3 months before and during the course of the study. The effect of VGSCs blockers evaluated from two pharmacological classes including antiarrhythmic (amiodarone, procainamide and disopyramide) and antiepileptic (carbamazepine, oxcarbazepine, phenytoin, and lamotrigine) drugs. They were tested on the *in-vitro* motility and viability of human sperm using Computer Assisted Semen Analyzer. **Results:** All tested drugs except oxcarbazepine showed dose dependent inhibition of total motility with significant reduction (P<0.05) at the maximum concentration of 200 μM when compared with the control. The concentrations of drugs that reduced total sperm motility to 50% of control (half maximal inhibitory concentration) were 2.76, 14.16 and 20.29 μM for phenytoin, lamotrigine and carbamazepine, respectively; and 2.53, 5.32 and 0.37 μM for amiodarone, procainamide and disopyramide, respectively. The anti-motility effects were reversible to various degrees. There was statistically insignificant difference in the inhibition of sperm viability among amiodarone, procainamide and disopyramide. Phenytoin demonstrated the most potent spermicidal action. **Conclusions:** VGSCs blockers have significant adverse effects on *in-vitro* motility of human spermatozoa. So *in-vivo* studies are required to determine their potential toxicological effects on human semen quality, which is an important factor regarding fertility. Moreover, these drugs have the potential to be developed into contact spermicidal.

1. Introduction

Motility of ejaculated spermatozoa is an important parameter of sperm function, which has been recognized as an integral part of a semen analysis. Poor motility of sperm results in infertility. Immotile and slow moving sperm are unable to penetrate the cervical mucous and fertilize the ovum. Therefore, sperm motility plays a vital role in fertility[1,2]. Human sperm motility is affected

✉Corresponding author: Taseer Ahmad, Laboratory of Cardiovascular and Integrative Pharmacology, College of Pharmacy, University of Sargodha, Pakistan.
E-mail: drtasir2011@gmail.com
First author: Hammad Ahmad Gakhar, Riphah Institute of Pharmaceutical Sciences, Riphah International University Islamabad, Pakistan.
E-mail: hammad_ahmad_raja@hotmail.com

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by a variety of drugs from different pharmacological classes[3], which exert their impacts via a number of mechanisms including regulation of calcium signaling[4] and action on various receptors like histamine[5], cannabinoid[6], dopamine and neurotransmitter gamma-aminobutyric acid receptors[7]. These interactions of drugs with human sperm suggest that many of the commonly prescribed drugs may have the potential to adversely affect the sperm motility and male fertility. Thus, some of these drugs may also have the potential to be used as contact spermicidal and future contraceptives.

Human sperm membrane contains several ion channels that play an important role in the regulation of sperm intra- and inter-cellular signaling mechanisms[8-10]. There is a movement of various ions via these ion channels which allows transfer of information between a sperm and its surrounding environments and mediums[11]. This communication is vital to correctly guide the sperm through the female reproductive tract and is also necessary for acquiring fertilization ability and interacting with the ovum for fertilization[12]. Over time, various ion channels have been discovered in the sperm cell membrane. Among these, Ca^{2+}, K^+, Na^+ and anion channels like Cl^- are important and are widely distributed on the head and flagellum and account for a vital role in regulating sperm function, which includes motility, viability, capacitation and acrosome reaction (AR)[13]. The difference in ion concentration between the surrounding environment and the cytoplasm of the sperm is essential for normal motility[14]. Ion concentration gradients across cells not only regulates membrane potential (E_m) through selective ion channels, but also the permeable ions can modulate various enzymes that further influences channel activity which leads to additional E_m changes and ion flow. So the rates and direction of ion-flow through different channels and ion-exchangers is ultimately governed by E_m. Fluctuations in E_m modulate intracellular pH ($[pH]_i$), intracellular calcium ($[Ca^{2+}]_i$) and other secondary messengers that influence sperm maturation, sperm motility and AR[15]. The early proof in this regard came from the fact that the composition of the external milieu has significant effects on this process. The uneven trans-membrane distribution of Na^+ and K^+ ions generated by the Na^+/K^+-ATPase is of particular importance[16]. Moreover, the presence of voltage-gated sodium channels (VGSCs) in the human sperm membrane and their role in motility regulation is evident[17].

Therefore, ion channels have the ability to regulate E_m and intracellular ionic environment. It enables them to play a critical role in excitability and action potential characteristics, maintaining pH, cell volume regulation, and epithelial electrolyte transport. Numerous studies have demonstrated that cations like Ca^+, Na^+ and K^+ are primary determinants of sperm cell function, including capacitation, progressive motility, hyper-activated motility, and AR. Drugs that selectively inhibit ion channel function can inhibit sperm function and motility and prevent fertilization[18].

VGSCs blockers are an important class of drugs that have a membrane stabilizing activity. They block ligands and voltages-gated channels and impair conduction of Na^+ through sodium channels. The resulting prolonged inhibition of the influx of Na^+ through the channel produces depolarization of the resting potential of the cell. Overall, there is partial or total inhibition of action potential from being propagated across the cell membrane, thus stabilizing the E_m[19]. Drugs which block VGSCs belong to three distinct pharmacological classes including class-Ⅰ antiarrhythmic agents, some anticonvulsants and local anesthetics[20].

Class-Ⅰ antiarrhythmic drugs are potent Na^+ channel blockers that are further classified into three categories based upon their antiarrhythmic action. Class-Ⅰ includes the drugs quinidine, procainamide and disopyramide[21]. Class-Ⅰ agents are grouped by their effect on the Na^+ channel and cardiac action potentials, which are called Membrane Stabilizing Agents[22].

The anticonvulsants are a diverse group of pharmacological agents used in the treatment of epileptic seizures. Among the antiepileptics, the drugs with Na^+ channel blocking activity as their primary mechanism of action includes phenytoin, carbamazepine, and oxcarbazepine and lamotrigine. These drugs block VGSCs and prolong their inactivated state, which inhibits the rapid and repetitive generation of action potentials across the cell membrane[23].

Drugs with membrane stabilizing potential are potent inhibitors of sperm motility[24], like indormin and propranolol inversely affect *in vitro* human sperm motility and viability[8,25]. This anti-motility effect made these drugs to be evaluated as vaginal spermicidals. In a clinical trial, propranolol showed favorable results as a vaginal spermicidal. Similarly, several antiarrhythmic drugs have shown a sperm immobilizing effect due to their membrane stabilizing activity by blocking voltage dependent sodium channels[26].

It is possible that VGSCs blockers affect sperm motility and male fertility. Therefore, the present study was designed to investigate the effects of Na^+ channel blocking drugs belonging to antiarrhythmic and antiepileptics classes, on the *in-vitro* motility of human sperm. These drugs were evaluated for their toxic effects on sperm motility parameters and their potential used as contact spermicidal.

2. Materials and methods

2.1. Drugs and chemicals

Commercially available brands of amiodarone (Cordarone 100 mg tablets by Sonafi Aventis), procainamide (Pronestyl 250 mg tablets by GlaxoSmithKline), disopyramide (Norpace 100 mg capsules by Searle) lamotrigine (Lamictal 100 mg tablets by GlaxoSmithKline), phenytoin (Dihydin 100 mg tablets by French pharmaceutical group), carbamazepine (Epicar 200 mg tablets by Adamjee pharmaceuticals) and oxcarbazepine (Oxalepsy 300 mg tablets by

S.J. & G.Fazul Ellahie Pvt Ltd) were purchased from the market. Dulbacco (phosphate buffered saline) tablets, sodium pyruvate and Albumin fraction V were obtained from Oxoid limited (UK), Alfa Aesar chemicals (UK) and Merck (UK), respectively.

2.2. Isolation of human sperm

This study was approved by the Ethics Committee of Riphah International University Islamabad, Pakistan and was performed in accordance with national and international guidelines. All subjects were informed with respect to this study and a written consent to participate in the study was obtained. Sperm samples were obtained from five healthy non-smoking volunteers of age 25-30 years who had not taken any drug 3 months before and during the course of the study. Semen samples were obtained in sterile plastic containers by masturbation. Each sample was collected after a minimum of 3 days and a maximum of 7 days of sexual abstinence. Only those samples were used in the study, which met the World Health Organization (WHO)criteria for normal semen parameters, including volume of 1.5-5.0 mL, sperm concentration greater than 20 million/mL, total motility >50%, rapid progressive motility >25%, morphology (percentage of normally shaped sperm) 30% or more normal shapes, and no hyper viscosity (thickening of seminal fluid). All the sperm samples were processed and parameters were assessed in accordance with WHO criteria[27].

2.3. Drug samples preparation

Stock solutions for the drugs were prepared in phosphate-buffered saline (Dulbecco 'A', pH 7.3). For preparing the stock solutions of drugs insoluble in phosphate-buffered saline, the drug was dissolved in 5% ethanol or 1% dimethyl sulfoxide and final volume was adjusted with phosphate-buffered saline (both solvents not affecting sperm parameters at the given strength). Stock solutions were then further diluted with phosphate-buffered saline to make various concentrations of drug samples.

2.4. Motility analysis

Computer Assisted Semen Analyzer (CASA) was used to examine the *in vitro* effect of various VGSCs blockers on sperm motility parameters. Semen samples were allowed to liquefy by incubation for 30 min at 37 ℃. Each semen sample was divided into several 200 μL aliquots, which were then mixed with 200 μL of either drug solution or phosphate-buffered saline (1:1 ratio). Semen-drug or semen-buffer mixture was then loaded into the pre-warmed and maintained at 37 ℃ Makler counting chamber of CASA apparatus. The computer program counted the sperm at 4 different fields of the Makler chamber and expressed the motility parameters as percent motility of sperm. The sperm motility was expressed as rapid progressive%, slow progressive%, non-progressive% and immotile%. Rapid progressive motility (grade A motility) is shown by the sperm cells which are the strongest and can swim fast in a straight line. Slow progressive (grade B motility) includes sperms, which move forward, but tend to travel in a curved or crooked motion. Non-progressive (grade C motility) includes sperm cells that do not move forward although they move their tails. Immotile (grade D motility) are the immotile sperms that fail to move at all. Total motility is the sum of % rapid progressive motility and % slow progressive motility and all parameters were determined in accordance with WHO criteria[27].

2.5. Reversibility study

Motility of treated sperm samples and the sperm samples without a drug (negative control) were first analyzed with the help of CASA. Then these samples were centrifuged at 2 500 r/min for 5 min. The sperm pellets so obtained were washed with phosphate-buffered saline three times and re-suspended in phosphate-buffered saline without any drug. The washed samples were re-suspended in phosphate-buffered saline and incubated for 30 min at 37 ℃ in the incubator and motility parameters were again measured. Motility parameters were compared in the treated and recovery samples.

2.6. Viability study

Viability of sperm was assessed by trypan blue staining. Sperm-drug or sperm-buffer mixture was mixed with 0.4% trypan blue solution in 1:1 ratio. This mixture was then incubated for 30 min at 37 ℃. Microscopic slides were prepared by placing a drop of the stained sample on the slide. Slides were examined under the 40 × magnification of the phase contrast microscope (Nikon, Germany). In this procedure, sperm cells that were not stained by the dye were considered viable. A total of 200 spermatozoa heads was counted, and the percentage of viable (unstained) heads were calculated. The percentage of viable cells was calculated by the formula:

$$\%\ \text{age of viable cells} = \frac{\text{Number of unstained cell}}{\text{Total number of cells counted}} \times 100$$

2.7. Statistical analysis

Data are represented as means ± SD. Half maximal inhibitory concentration (IC_{50}) for each drug was calculated by non-linear regression analysis. The paired *t*-test was used to analyze the significance of the differences between total motility of treated samples and the control sample using GraphPad Prism 6. The data were considered statistically significant at $P<0.05$.

3. Results

3.1. Dose response analysis of VGSCs blockers on human sperm motility

Effects of amiodarone, procainamide and disopyramide on the in-vitro motility of human sperms were estimated by CASA. All these drugs showed the dose dependent inhibition of total motility with significant reduction (P<0.05) at the maximum concentration of 200 μM when compared with the control.

Significant reduction of the total sperm motility treated by amiodarone was observed at concentrations 1.6 μM and above compared to control (P<0.05). The 8 μM concentration of the drug reduced the total motility to 40% that was lower than the normal value according to WHO standards (total motility >50%) (Table 1). On the other hand, procainamide resulted in significant (P<0.05) reduction in sperm motility at concentrations of 8 μM (total motility 20%) and above when compared with control (Table 2).

At the lower concentrations of 0.32 μM and 1.6 μM, the reduction in total motility by procainamide was not significant (P>0.05 compared to control) with values of 75% and 70% respectively. The IC_{50} values for amiodarone and procainamide calculated by non-linear regression analysis were found to be 2.53 μM and 5.32 μM respectively (Figure 1).

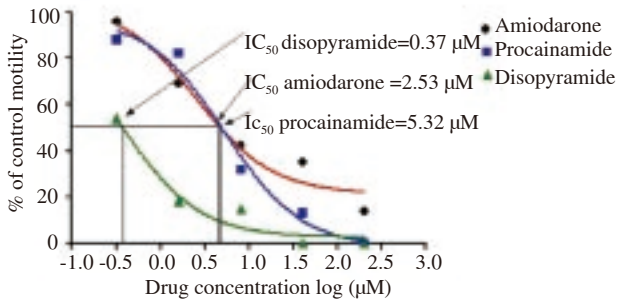

Figure 1. Comparison of IC_{50} by procainamide, amiodarone and disopyramide.

Of all the three VGSCs blocker drugs, disopyramide was the most potent inhibitor of *in vitro* human sperm motility (Table 3). Significant reduction in the total sperm motility was observed even at the lowest concentration of 0.32 μM when compared with control (P<0.05). The total sperm motility at a concentration of 0.32 μM was 35% while it was reduced to 0% at concentration of 40 μM concentration of disopyramide. On the contrary, at the maximum concentration of 200 μM, procainamide reduced the total motility to 0% while amiodarone reduced it to 10% (Table 1, 2). The IC_{50} value for disopyramide was 0.37 μM that was lowest of all three drugs (Figure 1).

Table 1

Effect of various concentrations of amiodarone on motility parameters. Total motility = rapid progressive % + slow progressive % (WHO standard). Mean± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		71.00±3.11	56.00±2.57	15.00±3.09	12.00±1.13	17.00±1.12
Drug concentrations (μM)	0.32	59.00±3.34	43.00±3.05	16.00±1.11	25.00±0.50	16.00±1.27
	1.6	49.00±3.10	29.00±3.10	20.00±3.10	24.00±2.24	27.00±1.39
	8	40.00±3.62	21.00±2.57	19.00±2.27	20.00±0.50	40.00±2.57
	40	25.00±3.65	13.00±3.61	12.00±1.99	22.00±1.11	53.00±0.50
	200	10.00±2.38	4.00±2.10	6.00±1.27	16.00±2.57	74.00±0.50

Table 2

Effect of various concentrations of procainamide on motility parameters. Total motility = rapid progressive%+slow progressive % (WHO standard).

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		85.00±0.25	70.00±0.50	15.00±1.50	7.00±2.00	8.00±0.50
Drug concentrations (μM)	0.32	75.00±3.44	52.00±3.00	23.00±2.50	11.00±2.50	14.00±1.01
	1.6	70.00±2.67	53.00±2.50	17.00±1.00	1.00±1.00	29.00±3.52
	8	28.00±2.27	15.00±1.73	13.00±2.19	11.00±1.50	61.00±2.50
	40	11.00±1.95	3.00±1.12	8.00±0.97	4.00±1.50	85.00±1.00
	200	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00±0.00

Similarly, all these drugs aborted individual motility parameters in a concentration dependent manner. Amiodarone at 200 μM concentration reduced rapid progressive motility and slow progressive motility to 4% and 6%, respectively, with 74% immotile sperms (Table 1). On the other hand, procainamide and disopyramide at 200 μM concentration reduced rapid and slow progressive motility to 0%, with 100% immotile sperms (Table 2 and 3). With disopyramide 0% rapid progressive motility and 100% immotile sperms were achieved at the concentration of 40 μM (Table 1). The concentrations of amiodarone, procainamide and disopyramide that reduced the rapid progressive motility below the normal values given by WHO (rapid progressive >25%) were 8.0, 8.0 and 1.6 μM with rapid progressive motilities of 21%, 15% and 1%, respectively.

3.2. Reversibility of the effects of amiodarone, procainamide, disopyramide on in vitro human sperm motility

CASA analysis was done on the aliquots of spermatozoa treated with a 200 μM concentration of drugs to estimate the reversibility of the effects of each drug. It was observed that inhibitory effects of all drugs on human sperm motility were somewhat reversed upon the removal of drug from the sperm samples after 30 min (Table 4).

There was significant recovery of sperm motility in all samples with maximum reversibility observed in samples treated with disopyramide. Although not completely recovered, total motility increased significantly (P<0.05) in recovery samples when compared to the treated samples, after the removal of each drug. This showed that while disopyramide possessed strong but reversible inhibitory effects on human sperm motility; procainamide exerted a longer lasting and irreversible inhibitory effect (Table 4).

3.3. Effect of amiodarone, procainamide, disopyramide on the viability of human sperm in vitro

The viability of human spermatozoa treated with a 200 μM concentration of blockers of amiodarone, procainamide, disopyramide, was checked using the trypan blue exclusion test. All these drugs demonstrated potent spermicidal effects. There was significant reduction in the viability of human spermatozoa when compared with control (P<0.05). The percentage of viable spermatozoa in control samples and others treated with a 200 μM concentration of amiodarone, procainamide and disopyramide were 87%, 41%, 33% and 30%, respectively. There was no significant difference in inhibition of sperm viability among the three drugs (Table 5).

Table 5

Viability of sperm in the presence of Amiodrane, Procainamide and Disopyramide (Mean ±SD, n = 4).

Sr. No.	Drug	Live sperms (%)	Dead sperms (%)
1	Control	87.000±3.651	13.000±3.651
2	Amiodarone	41.000±6.055	59.000±6.055
3	Procainamide	33.000±7.071	67.000±7.071
4	Disopyramide	30.000±6.000	70.000±6.000

3.4. Dose response analysis of carbamazepine, oxcarbazepine, phenytoin, and lamotrigine on the human sperm motility

Effects of four Anti- Epileptic drugs that block VGSCs including carbamazepine, oxcarbazepine, phenytoin, and lamotrigine, on the *in vitro* motility of human sperm was estimated by CASA. Carbamazepine, phenytoin and lamotrigine showed significant

Table 3

Effect of various concentrations of disopyramide on motility parameters. Total motility = rapid progressive% + slow progressive % (WHO standard). Mean ± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		65.00±2.37	45.00±1.37	20.00±2.98	15.00±0.47	20.00±1.08
Drug concentrations (μM)	0.32	35.00±2.12	25.00±1.70	10.00±2.12	6.00±2.68	59.00±1.93
	1.6	12.00±1.47	1.00±1.41	11.00±1.22	6.00±3.31	82.00±1.49
	8	10.00±1.68	4.00±0.97	6.00±1.17	6.00±2.28	84.00±0.47
	40	0.00±0.00	0.00±0.00	0.00±.0.00	0.00±0.00	100.00±0.00
	200	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00±0.00

Table 4

Reversibility of effects of Amiodarone, Procainamide and Disopyramide on total sperm motility (Mean ± SD, n = 4).

Drugs		Amiodarone	Procainamide	Disopyramide
Parameter		Total motility%	Total motility%	Total motility%
Treated	Control	71.000±2.286	85.000±1.080	65.000±1.354
	200 μM	10.000±2.582	0.000±0.000	0.000±0.000
Recovery	Control	63.000±2.174	70.000±1.258	45.000±1.870
	Drug removed	29.000±4.082	15.000±2.708	28.000±1.826

dose dependent inhibition of total motility compared to control (P<0.05), and no significant effects observed by oxcarbazepine at any concentration (Table 6, 7, 8, 9).

Phenytoin demonstrated potent and significant inhibition of total sperm motility when compared with control (P<0.05). The total motility at the concentrations of 40 and 200 μM was 4% and 0%, respectively (Table 8). Alternatively, lamotrigine reduced total motility to 18% at 200 μM concentration (Table 9).

All drugs at lower concentrations observed similar inhibitory effects on total sperm motility. Carbamazepine initially caused a slight increase in total motility at 0.32 μM with insignificant inhibitions at 1.60 and 8.00 μM concentrations where the total motility was 63% and 64% (P>0.05) (Table 6). However, significant inhibition of total sperm motility was observed at 40 and 200 μM concentrations of carbamazepine when compared with control having values 26% and 14%, respectively (P<0.05) (Table 6).

Phenytoin possessed lowest IC_{50} value for the reduction in total sperm motility compared to lamotrigine and carbamazepine. The concentrations of drugs that reduced total sperm motility to 50% of control were 2.76, 14.16 and 20.29 μM for phenytoin, lamotrigine

Table 6

Effect of various concentrations of carbamazepine on motility parameters. Total motility = rapid progressive%+slow progressive % (WHO standard).

Mean ± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%
Control		65.00±2.62	38.00±2.19	27.00±1.74	15.00±1.93
Drug concentrations (μM)	0.32	77.000±5.715	53.000±4.835	24.000±5.131	9.000±5.049
	1.6	63.00±5.77	48.00±5.17	15.00±4.90	8.00±0.70
	8	60.00±5.77	37.00±5.17	23.00±4.90	11.00±1.25
	40	26.00±5.77	17.00±5.17	9.00±4.90	2.00±2.25
	200	14.00±4.96	5.00±3.93	9.00±4.87	10.00±3.11

Table 7

Effect of various concentrations of oxcarbazepine on motility parameters. Total motility = rapid progressive%+slow progressive % (WHO standard).

Mean ± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		64.000±4.966	52.000±3.291	12.000±4.478	13.000±1.870	23.000±2.254
Drug concentrations (μM)	0.32	68.000±6.683	56.000±5.049	12.000±5.986	13.000±2.780	19.000±0.478
	1.6	64.000±4.966	49.000±4.896	15.000±2.174	15.000±2.943	21.000±0.707
	8	63.000±4.163	54.000±3.119	9.000±1.080	12.000±0.912	25.000±1.936
	40	68.000±8.326	54.000±7.630	14.000±6.896	9.000±1.732	23.000±6.896
	200	55.000±4.966	42.000±4.896	13.000±2.174	9.000±2.041	36.000±5.986

Table 8

Effect of various concentrations of phenytoin on motility parameters. Total motility = rapid progressive%+slow progressive % (WHO standard).

Mean ± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		60.00±4.96	33.00±3.47	27.00±2.34	20.00±6.89	20.00±3.11
Drug concentrations (μM)	0.32	57.00±5.77	35.00±5.04	22.00±2.21	5.00±1.32	38.00±1.73
	1.6	45.00±7.74	26.00±6.89	19.00±5.98	10.00±2.32	45.00±1.93
	8	16.000±4.082	7.000±2.174	9.000±5.202	6.000±2.410	78.000±2.170
	40	4.00±4.69	0.00±0.00	4.00±4.69	5.00±1.93	91.00±5.50
	200	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00±0.00

Table 9

Effect of various concentrations of lamotrigine on motility parameters. Total motility = rapid progressive%+slow progressive % (WHO standard).

Mean ± SD, n = 4.

Parameter		Total motility%	Rapid progressive%	Slow progressive%	Non progressive%	Immotile%
Control		74.00±4.18	62.00±2.41	12.00±3.47	7.00±2.20	19.00±1.08
Drug concentrations (μM)	0.32	58.00±6.27	42.00±6.80	16.00±6.13	19.00±2.78	23.00±3.93
	1.6	48.000±3.915	35.000±5.202	13.000±1.887	17.000±5.200	3.000±3.620
	8	42.00±5.29	31.00±4.08	11.00±2.17	11.00±4.47	47.00±2.32
	40	27.00±4.96	16.00±4.96	11.00±3.91	23.00±3.66	50.00±3.05
	200	18.00±6.05	10.00±5.09	8.00±6.13	12.00±1.93	70.00±2.41

and carbamazepine, respectively (Figure 2).

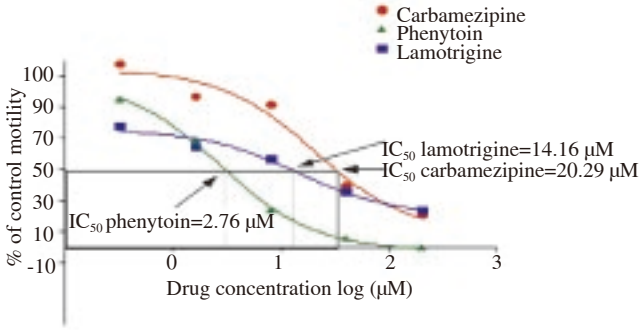

Figure 2. Comparison of IC_{50} by carbamezipine, phenytoin, lamotrigine.

Oxcarbazepine did not show any notable effect upon the individual motility parameters (Table 7). Carbamazepine initially caused a slight rise in rapid progressive motility followed by a concentration dependent inhibition. These results indicated that phenytoin possessed more potent and deleterious effects on human sperm motility as compared to carbamazepine and lamotrigine.

3.5. Reversibility of the effects of carbamazepine, oxcarbazepine, phenytoin, and lamotrigine on in vitro human sperm motility

CASA analysis was done on the aliquots of spermatozoa treated with a 200 µM concentration of drugs to estimate the reversibility of the effects of each drug. It was observed that inhibitory effects of all drugs on human sperm motility were reversed upon the removal of the drug from the sperm samples (Table 10).There was significant recovery of sperm motility in all samples with maximum reversibility observed in samples treated with phenytoin. These results indicated that inhibitory effects of carbamazepine, phenytoin, and lamotrigine on *in vitro* sperm motility were reversible, and the total motility increased upon the removal of these drugs.

3.6. Effects of carbamazepine, oxcarbazepine, phenytoin, and lamotrigine on the viability of human sperm

The viability of human spermatozoa treated with a 200 µM concentration of voltage-dependent VGSCs blockers including carbamazepine, phenytoin, and lamotrigine, was analyzed using the trypan blue exclusion test. The drugs showed a significant spermicidal effect when compared with control (P<0.05). The percentage of viable spermatozoa in control samples and samples treated with a 200 µM concentration of carbamazepine, phenytoin, and lamotrigine were 84%, 45%, 23% and 37%, respectively. Phenytoin was the most potent spermicidal among all drugs (Table 11).

Table 11

Viability of sperm in the presence of carbamazepine, phenytoin and lamotrigine (Mean ± SD, n = 4).

Sr. No.	Drug	Live sperms (%)	Dead sperms (%)
1	Control	84.000±4.690	16.000±4.690
2	Carbamazepine	45.000±8.524	55.000±8.524
3	Phenytoin	23.000±7.745	77.000±7.745
4	Lamotrigine	37.000±9.486	63.000±9.486

4. Discussion

Among the factors that affect male fertility and normal sperm parameters, drugs and chemical compounds play an important role[28]. Many drugs used for other ailments have the potential to adversely affect sperm motility and viability[29]. Presence of VGSCs on the membrane of human sperm and their important role in sperm function including motility, capacitation and AR is evident[17]. Therefore, there is a possibility that blockers of sodium channels may affect the sperm motility and viability. In this study, it has been shown that VGSCs blockers considerably impaired *in-vitro* sperm

Table 10

Reversibility of effects of carbamazepine, phenytoin, and lamotrigine on total sperm motility (Mean ± SD, n = 4).

Drugs		Carbamazepine	Phenytoin	Lamotrigine
Parameter		Total motility%	Total motility%	Total motility%
Treated	Control	65.000±5.202	60.000±3.162	74.000±4.974
	200 µM	14.000±3.915	0.000±0.000	18.000±3.559
Recovery	Control	56.000±2.625	44.000±1.936	65.000±1.732
	Drug removed	29.000±8.679	16.000±3.366	28.000±4.690

motility and viability of ejaculated human spermatozoa. The anti-motility effects were reversible upon the removal of the drugs.

The primary mechanism involved in the anti-motility effects of VGSCs blockers (antiarrhythmic and antiepileptic) on human sperm appears to be the blockade of VGSCs present on the plasma membrane of the sperm. Rapid and transient inward currents produced by VGSCs generate the upstroke of the action potential in excitable cells including neurons and striated muscles[30]. The transmembrane distribution of electrical charges, which results from the uneven distribution of Na^+ and K^+ ions across the cell membrane, contributes to maintain the resting E_m that provides cells with the excitability which is necessary for their movement, contraction, and transmission of impulses[31].

Amiodarone, procainamide, disopyramide block VGSCs in activated and inactivated state and thus inhibit the generation and propagation of action potential. Similarly, lamotrigine, carbamazepine, phenytoin block the VGSCs in an inactivated state, consequently affecting the sodium conductance and inhibiting the rapid firing of action potentials.

VGSCs are complex membrane proteins composed of and one or more auxiliary β -subunits[32,33]. The α -subunit is a large protein and contains an ion-conducting aqueous pore and can function without the β -subunit as a Na^+ channel[34]. Nine different VGSCs subunits have been found in mammals. They are further classified based upon their sensitivity towards a selective blocker tetrodotoxin (TTX). The TTX-sensitive α -subunits includes Nav 1.1, 1.2, 1.3, 1.4, 1.6, and 1.7. The TTX resistant α - subunits include Nav 1.5, 1.8, and 1.9[35]. Excluding only Nav 1.1 and 1.3, all other VGSCs have been detected in mature sperm. The VGSCs (*i.e.*, Nav 1.2, 1.4 and 1.7) are mainly found in the area of connecting piece of the sperm, which plays an important role in sperm signaling[13]. Nav 1.8 is located at the flagellum and around the neck region, and regulates flagellum activity and sperm motility[17].

All the three antiarrhythmic drugs reduced sperm motility in a dose-dependent manner. Disopyramide showed more potent inhibition of *in vitro* human sperm motility than procainamide and amiodarone. Semen plasma concentrations of the drugs are unknown. However, the reported plasma drug concentrations for disopyramide, amiodarone and procainamide were 2.00-4.00 μg/mL[36,37], 0.37 μg/mL[38] and 3.50-4.60 μg/mL[39], respectively. The drugs might not have achieved this much concentration in the semen, but the semen drug concentrations should be determined to rule out the potential hazardous effects of the drug on sperm motility and fertility. Viability of sperm was also affected significantly showing the spermicidal activity in all drugs. Amiodarone abolished ejaculated spermatozoa motility at higher concentrations, and it should be further studied as contact spermicidal.

Structurally amiodarone, procainamide and disopyramide are different, but they follow the backbone structure that is common to many antiarrhythmic and local anesthetics[40,41]. The dissimilarity in the lipophilic and hydrophilic portions among these compounds plays an important role in the pharmacokinetics and pharmacodynamics properties of these drugs[42,43]. It is possible that the variability in the inhibitory effects of these drugs on sperm motility and viability was due to the differences in their lipophilic or hydrophilic moieties.

Antiepileptic drugs including carbamazepine, phenytoin, and lamotrigine inhibited sperm motility in a dose-dependent manner, except oxcarbazepine, which did not show any significant effect on *in-vitro* motility of sperm at any concentration. Phenytoin showed the most potent anti-motility effects as compared to carbamazepine and lamotrigine. All the values were below the normal value of total sperm motility (total motility must be >50%, WHO standard).

The reported semen drug concentration of phenytoin is 0.97 μg/mL[38,43], which shows that enough high levels could have been achieved with phenytoin therapy in human semen that can adversely affect the motility of sperm. The semen drug concentrations of carbamazepine and lamotrigine are unknown. However, the reported plasma drug concentrations of these drugs are 5-12 μg/mL[44] and 1-4 μg/mL[45], respectively. Since both carbamazepine and lamotrigine are highly lipid soluble drugs, they may cross the blood testicular barrier like other psychotropic drugs[35], providing a high possibility to appear in the seminal fluids and interfere with sperm function. Hence, the seminal concentrations of these drugs are needed to be measured, and *in vivo* effects are also recommended to be assessed.

Phenytoin belongs to a class of compounds known as hydantoins. Imidazolidinone ring of phenytoin is believed to be involved in VGSCs blocking activity, as substitution on this group modifies the channel blocking activity of its derivatives[46].

VGSCs blocking drugs in this study influenced all the motility parameters significantly except the percentage of non-progressive motility, which did not changed significantly at lower doses. Since these drugs produce a dose dependent block of the VGSCs and more potent blockade of VGSCs in cells, which undergo rapid Em changes[20,21], non-progressive sperm may not expressed rapid Em changes, making them less vulnerable to the channel blocking effect of these drugs at lower doses, thus showing no significant changes in percentage of non-progressive motility.

Oxcarbazepine is a structural derivative of carbamazepine, with a ketone in place of the carbon-carbon double bond on the dibenzazepine ring. This difference helps reduce the impact on the liver of metabolizing the drug, and also prevents the serious forms of adverse reactions occasionally associated with carbamazepine [47,48]. Moreover, oxcarbazepine is less potent than carbamazepine both in animal models of epilepsy and in epileptic patients; clinical doses of oxcarbazepine may need to be 50% higher than those of carbamazepine to obtain VGSCs blockade and seizure control[49]. These findings may explain the results of this study where at the

maximum concentration of 200 μM, oxcarbazepine did not show any significant effect on total motility of human sperm as compared to carbamazepine which significantly reduced the total motility even at 8 μM concentration.

For sperm motility, an adequate E_m is necessary and prolong cell membrane depolarization has been associated with low sperm motility[50]. Veratridine a VGSCs activator is reported to affect sperm motility which further establishes the functional role of Na^+-channels in mature sperm[17]. It enhanced total motility which was concentration and time dependent. Veratridine acts by inhibiting Na^+-channel inactivation after spontaneous channel opening. These facts demonstrate that VGSCs participate in the regulation of human sperm motility, and primarily it is due to the regulation of sodium ion flux across the membrane, which ultimately affects the E_m.

Regulation of $[pH]_i$ is essential for the function of sperm[14]. An important mechanism that controls proton concentration in somatic cells operates utilizing the transmembrane Na^+ gradient. Changes in $[pH]_i$ have been shown to influence sperm motility[51], which is regulated by voltage-sensitive Na^+/H^+ exchanger[52] and Na^+/K^+-ATPase pump in the cell membrane. Since the VGSCs also regulate the flux of sodium ions across the membrane, their role in the internal pH maintenance cannot be ruled out. Also the VGSCs blockers inhibit the transmembrane conductance of sodium ions, which may affect the internal pH and ultimately affect the sperm motility. Mechanism of action of these drugs in terms of the blockade of VGSCs and membrane stabilizing activity is well documented. It is highly likely that there are also other effects, which are contributing to impairment of sperm motility.

In conclusion, VGSCs blockers including antiarrhythmic and antiepileptic drugs significantly inhibit *in-vitro* human sperm motility. This fact emphasizes that these drugs can adversely affect human sperm function. Thus, it is recommended to perform the *in-vivo* studies to rule out the potential toxicological effects of these drugs on human semen quality and function. The potent spermicidal activity and motility inhibition also suggest that these drugs could be formulated as contact spermicidal in the future.

Conflict of interest statement

The authors declare that they have no competing interests.

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