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Gonadal Responses to Antipsychotic Drugs: Chlorpromazine and Thioridazine Reversibly Suppress Testicular Functions in Albino Rats

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Abstract: This work was undertaken to investigate the individual effects and probable mechanism of action of chlorpromazine hydrochloride (largactil) and thioridazine hydrochloride (melleril) on male reproductive functions, in albino rats. A total of 45 adult male albino Wistar-strain rats were used. Five rats served as the control while the remaining forty rats were divided into four groups, of 10 rats each. Rats in group I were treated with 2.3 mg kg^{-1} BW, while those of group II received 5.7 mg kg^{-1} BW of chlorpromazine. Rats in group III, were treated with 1.7 mg kg^{-1} BW, while those of group IV received 2.3 mg kg^{-1} BW of thioridazine. Control rats received vehicle of the drugs (i.e. distilled water). Drugs and vehicle were administered orally on a daily basis. Five rats, in each of the four drug-treated groups served as the recovery rats. Sperm characteristics evaluation, serum levels of testosterone and histopathological alterations in the testis were assessed both after four weeks of continuous drug administrations and four weeks of drug withdrawal. Chlorpromazine and thioridazine significantly caused a reduction in the absolute weights of the testis, epididymis and seminal vesicles ($p<0.01$) at high and low doses. Weight of the prostate gland was also reduced significantly ($p<0.05$) at the high dose. The epididymal sperm motility, viability (life/death ratio) and counts were significantly reduced ($p<0.01$) at high dose of chlorpromazine and thioridazine. Moreover, sperm morphological abnormalities were significantly increased ($p<0.01$) at both doses of the drugs. Reduction in serum levels of testosterone for both drugs was statistically significant ($p<0.01$). The histopathological alterations observed in the testis includes moderate to severe degeneration of seminiferous tubular epithelium. Fertility and other associated changes were restored within four weeks of cessation of treatment. Chlorpromazine and thioridazine appear to have reversible antifertility actions in male albino rats. These actions were probably mediated within the testis and epididymis.

Key words: Antipsychotic, reproduction, sperm, testosterone, fertility

INTRODUCTION

Antipsychotic drugs are central nervous system (CNS)-influencing drugs that are used in most psychiatric hospitals to treat people with mental disorders. Chlorpromazine and thioridazine, of the class phenothiazines are the commonly used ones. Phenothiazines have been used mainly for the treatment of schizophrenia but are also effective in some other psychoses[1].

Chlorpromazine and thioridazine were originally developed as antihistamine and antihypertensive agents[1]. However, fortuitous observations of their calming properties led to their trial in psychiatric patients. The antipsychotic drugs generally have the antiemetic, antinausea, analgesic, sedatives and general anaesthetic effects.

Several studies have shown that the central nervous system influencing drugs do have adverse effects on male reproductive functions[2,3]. Phenothiazines are known to cause hyperprolactinaemia leading to amenorrhea, ceasation of normal cyclic ovarian function, loss of libido, occasional hirsutism and long-term risk of osteoporosis in women. The effects of hyperprolactinemia induced by antipsychotic drugs in men are impotence, loss of libido and hypospermatogenesis[4]. The main mechanism of action of these drugs on reproduction is by blocking dopamine receptors and also by increasing the conversion of androgens to estrogens thereby elevating the plasma levels of the latter hormone. It is however not known whether these effects can lead to alterations in male reproductive efficiency sufficient to cause male infertility. The present study was therefore designed to investigate the individual effects of chlorpromazine

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hydrochloride and thioridazine hydrochloride (largactil and melleril, respectively) and their probable mechanism of action on male reproductive functions in albino rats.

MATERIALS AND METHODS

Animals: Adult male Wistar-strain albino rats (180-200 g) obtained from the central animal house, College of Medicine, University of Ibadan, were used for the study. These animals were maintained and housed under standard laboratory conditions (temperature, 27°C and lighting, about 12 h/day) and they were fed with standard rat diet (Ladokun Feeds Nig. Ltd-Ibadan) and water *ad libitum*. A total of 45 adult male rats were used. 5 rats served as the control group and the remaining rats were divided into four equal groups of ten rats each and treated as indicated in the study protocol.

Drug and study protocol: Chlorpromazine hydrochloride (Hawgreen Ltd, Dublin) and thioridazine hydrochloride (Norvatis Pharmaceuticals, U.K) were used in this study. Different concentrations of these drugs were prepared by dissolving known milligrams in a known volume of distilled water, based on comparison between the human dosage and body weights of the animals. Group I rats received 2.3 mg kg^{-1} BW of chlorpromazine, while group II rats received 5.7 mg kg^{-1} BW of chlorpromazine. Rats in group III received 1.7 mg kg^{-1} BW of thioridazine, while those in group IV were treated with 2.3 mg kg^{-1} BW of thioridazine. Control rats received distilled water, which was the vehicle for the drugs. Daily administration of drugs and vehicle was done intragastrically for four weeks using oral cannula. 5 rats from each drug-treated group were kept for additional four weeks to recover after the last treatment was given. All rats were weighed weekly.

Autopsy: Animals were weighed and autopsied under ether anesthesia 24 h after last dosing. Blood was collected from each animal via cardiac puncture from which serum used for testosterone assay was obtained. The remains of the rats were cut open and the liver, heart, kidneys, testes, seminal vesicles, prostate gland and epididymis were removed and weighed separately.

Epididymal sperm motility and sperm counts: Epididymal sperm motility was assessed immediately the rats were autopsied. Sperm progressive motility was determined by conventional methods[5-7] and the number of motile spermatozoa were calculated per unit area and expressed as percentage motility. Sperm counts were done using a haemocytometer and the results were expressed as million/ml of semen suspension[6].

Viability and morphological studies: Viability study was done by preparing a uniform smear of the spermatozoa on slides with Eosin/Nigrosin stain. Hundred sperm cells were counted per slide, in order to obtain the percentage of live/death ratio[5]. Morphological aberrations of abnormal spermatozoa were determined from a total count of 400 spermatozoa, in smears obtained with Eosin/Nigrosin stain and smear made with Wall and Ewas stain. Abnormal spermatozoa were observed under the microscope, using X 100 objective under immersion oil. Sperm abnormalities were classified, as described by Bloom[8,9].

Analysis of serum levels of testosterone: Serum testosterone concentrations were determined with the tube-based enzyme immunoassay (EIA) method. The EIA testosterone kit was obtained from the Nzemat (Nig) Limited, Akoka-Yaba, Lagos. The kit was manufactured in United Kingdom by Immunometrics (UK) Ltd. This method meets the WHO standards and is part of its programme for research in human reproduction. The within assay variation was 8.1% and the sensitivity was 0.3 ng mL^{-1}. The optical density was read by using a spectrophotometer that was sensitive at wavelengths of between 492 and 550 nm.

Testicular histology: This was carried out as earlier described[7]. Briefly, the testes were fixed in 10% formal saline immediately after weighing. The preserved testes were then passed through the routine laboratory histological procedures and slides were stained with haematoxylin and eosin.

Statistical analysis: Data in text and tables are presented as mean±standard error of the mean (SEM). Statistical significance between groups was determined using student "t" test and ANOVA where appropriate[10].

RESULTS

Body and organ weights of rats treated with chlorpromazine and thioridazine hydrochloride: There was a significant increase in the absolute body weights of drug treated rats ($p<0.05$) when compared with their control counterparts (Table 1 and 2). There was also a significant decline in the weights of the testis, epididymis and seminal vesicles ($p<0.01$) when compared with their control counterparts (Table 1 and 2). The reduction, in weights of prostate gland was significant ($p<0.05$) at the high dose of the drugs. During the recovery period, there was a gradual increase in weight of the testis and epididymis towards the normal control values.

Table 1: Absolute Organ weight and Final % difference in body weight of Chlorpromazine-treated male rats Values are expressed as mean±s.e.m for n=5

Treatement group	Final % difference in body weight	Testis (g)	Adrenal gland	Epididymis	Seminal vesicle (g)	Prostate gland (G)
Control	+144.9±2.8	1.44±0.02	0.027±0.09	0.45±0.01	0.99±0.01	0.32±0.02
	(+144.9±2.7)	(1.46±0.02)	(0.027±0.09)	(0.45±0.01)	(0.99±0.01)	(0.32±0.02)
2.3mg kg^{-1}BW	+155.3±3.9	1.13±0.04*	0.020±0.01	0.33±0.009*	0.77±0.02	0.26±0.02
Chlorpromazine	(+160.5±3.3)	(1.24±0.02)	(0.022±0.01)	(0.038±0.03)*	(0.97±0.01)	(0.33±0.02)
5.7mg kg^{-1}BW	+154.7±2.5	0.93±0.06*	0.010±0.03	0.19±0.02*	0.75±0.03	0.24±0.02
Chlorpromazine	(+160.4±2.8)	(1.01±0.11)*	(0.023±0.01)	(0.23±0.02)	(0.91±0.02)	(0.30±0.006)

Table 2: Absolute Organ weight and Final % difference in body weight of Thioridazine-treated male rats. Values are expressed as mean±s.e.m for n=5

Treatement group	Final % difference in body weight	Testis (g)	Adrenal gland	Epididymis	Seminal vesicle (g)	Prostate gland (G)
Control	+144.8±2.8	1.44±002	0.027±0.0009	0.45±0.01	0.99±0.01	0.32+0.02
	(+144.7±2.8)	(1.44±0.02)	(0.027±0.09)	(0.045±0.001)	(0.99±0.01)	(0.32+0.02)
1.7mg kg^{-1}BW	+156.3±4.1	0.96±002**	0.020±0.001	0.25±0.002**	0.76±0.02**	0.26+0.08
Thioridazine	(+155.4±3.8)	(1.14±0.02)*	(0.025±0.001)	(0.33±0.01)**	(0.96±0.02)	(0.31+0.02)
2.3mg kg^{-1}BW	+161.4±2.5	0.59±0.05**	0.022±0.001	0.16±001**	0.73+001	0.22+0.009
Thioridazine	(+160.1±2.3)	(0.83±0.03)*	(0.024±0.001)	(0.25*±0.01)*	(0.96±0.01)	(0.29+0.005)

*Significantly different from the control ($p<0.01$). + = Gain in body weight;-=Loss in body weight. Values presented in parenthesis show recovery experiment value

Table 3: Effect of chlorpromazine and thioridazine on sperm characteristics

Treatment group	Control	Chlorpromazine (2.3mg/kgBW)	Chlorpromazine (5.7mg/kgBW)	Thioridazine (1.7mg/kgBW)	Thioridazine (2.3mg kg^{-1}BW)
% Motility	97.80±0.20	76.00±1.88*	38.00±3.74*	77.00±2.00*	53.00±1.22*
	(95.80±0.20)	(93.00±1.22)*	(84.00±2.45)*	(91.00±1.87)*	(87.00±1.26)**
% Viability	98.00±0.10	84.80±1.16*	56.60±3.61*	89.40±0.87*	70.20±1.39*
(Life/ death)	(96.00±0.20)	(96.20±0.73)*	(94.20±0.97)*	(95.20±1.46)	(94.00±1.00)**
Epididymal sperm	6.96±0.50	5.020±0.14*	4.52±0.15*	4.93±0.07**	3.50±0.08*
count (10^6/m/)	(6.86±0.40)	(6.50±0.54)	(4.54±0.09)*	(5.90±0.85)	(5.22±0.53)**

Results are presented as Mean±SEM (n=5) Statistically significant *($p<0.01$) **($p<0.05$)Values presented in parenthesis show recovery experiment value

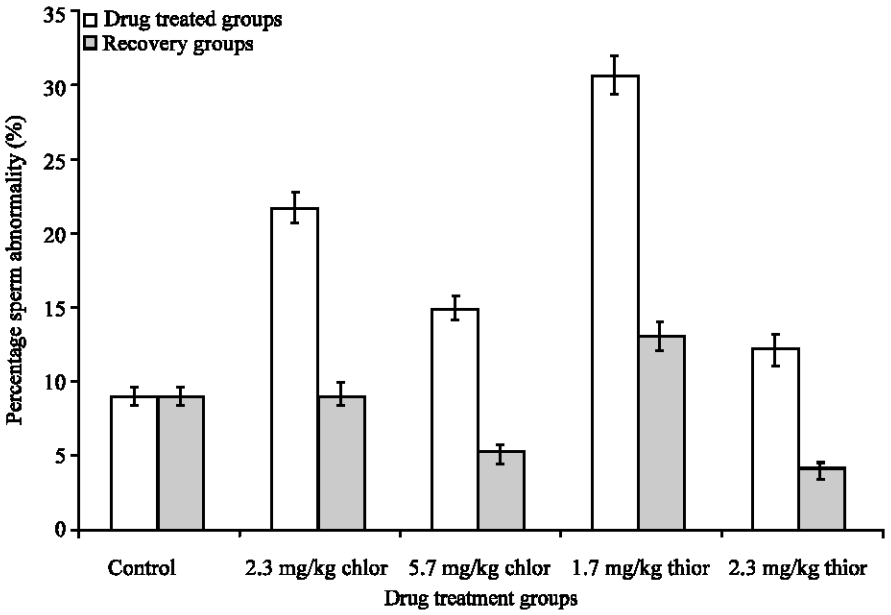

Fig. 1: Effect of chlorpromazine and thioridazine on morphological characteristics of sperm in albino rats

Effects of chlorpromazine and thioridazine hydrochloride on sperm functions in rats: Table 3 and 4show the effects of chlorpromazine and thioridazine on sperm functions in rats, after 4 weeks of treatment and subsequent four weeks of recovery. The sperm motility was significantly reduced ($p<0.01$) when compared with the controls, in both doses of the drugs. The qualitative motility was gradually affected which led to complete regression of motility after 4 weeks of treatments. The reduction in sperm motility was accompanied by significant ($p<0.01$) dose-dependent increase in morphological defects (Fig. 1). The most common abnormality encountered for

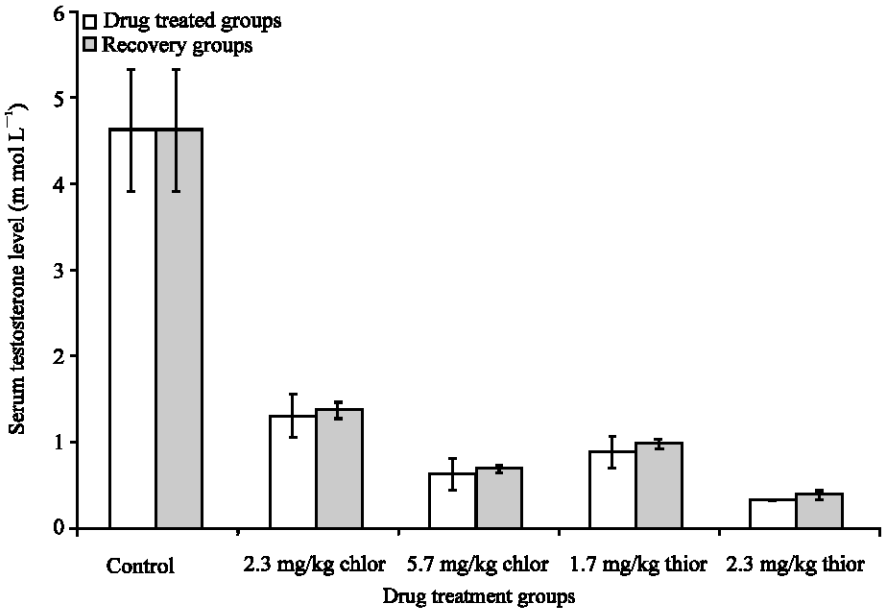

Fig. 2: Effect of chlorpromazine and thioridazine on serum levels of testosterone in male albino rats

Table 4: Histologic scores of the testes Chlorpromazine and Thioridazine hydrochloride treated rats

Treatment	Seminiferous tubule degeneration	Oedema/vascular congestion	Germ cell necrosis
Control	-	-	-
(Recovery)	-	-	-
Chlorpromazine			
2.3 mg kg^{-1} b.w	++	+	++
(Recovery)	+	+	+
5.7 mg kg^{-1} b.w	+++	++	++
(Recovery)	+	+	+
Thioridazine			
1.7 mg kg^{-1} b.w	++	++	+
(Recovery)	+	+	+
2.3 mg kg^{-1} b.w	++	++	+++
(Recovery)	+	+	+

Key to scores: - = Absence of abnormal histologic features, + =Mild abnormal histologic features, ++ =Moderately severe histologic abnormality; +++ =Severe histologic abnormality

epididymal sperm was simple bent tail (about 60%). However, the sperm motility gradually increased during the recovery period. There was also a decrease in sperm abnormalities in the recovery groups.

The decrease in epididymal sperm counts in response to each of the drugs was significantly ($p<0.01$) different from the control (Table 3). In the recovery group, the epididymal sperm counts for both drugs were significantly increased ($p<0.05$) only at the high dose group. However there was no significant change in this parameter at a low dose (Table 3). Similarly, the viability of spermatozoa (i.e. percentage live) was significantly reduced in both drugs. The reduction in sperm viability in both doses was also significantly different from controls. In the recovery groups the percentage live spermatozoa was significantly increased ($p<0.001$) only at high dose of the drugs, when compared with the controls

Effects of chlorpromazine and thioridazine hydrochloride on serum testosterone levels: There were dose-dependent decreases in serum testosterone levels in chlorpromazine and thioridazine treated rats. This reduction was highest for the high dose ($p<0.01$) and lowest for the low dose ($p<0.01$) of each of the two drugs (Fig. 2).

Histology of rat testis treated with chlorpromazine and thioridazine hydrochloride: As shown in Table 4, the control group exhibited normal testicular histology, with evidence of spermatogenesis, whereas there were germ cell necrosis, oedema (vascular congestions) and dose-dependent increase in seminiferous tubular degeneration in groups that were treated with each of the drugs. There was however some evidence of regenerative changes with some disorganization and a few tubules (containing mature spermatozoa) still visible in the recovery groups.

DISCUSSION

The results suggest that chlorpromazine and thioridazine could cause reversible reproductive impairment in male albino rats. Chlorpromazine and thioridazine are antipsychotic drugs that are known to have anaesthetic effects; they produce a loss of consciousness and a tendency to sleep[11]. In humans they may increase weight by stimulating appetite.

It has been reported that a change in either absolute or relative weight of an organ after administering a chemical is an indication of the toxic effect of the chemical[12]. The observed change in relative weight of the testes and other accessory reproductive organs in our study indicate that chlorpromazine and thioridazine might be toxic to these organs at least during the period of treatments. Consequently the reductions in the weights of the testis, epididymis, seminal vesicle and prostate gland could not be unconnected with the sensitivity of these organs to these drugs *in vivo*. These dugs have been reported to have the ability to permeate the biomembrane barriers of most reproductive organs[11]. However, the weights of the kidney, heart, liver and adrenal glands were not affected, both during the drug administration and recovery periods suggesting that they are not toxic to these organs.

The decrease in weights of the testis, epididymis, seminal vesicle and prostate gland, were accompanied by a decline in sperm motility, sperm counts and viability, in chlorpromazine and thioridazine-treated rats. The decline in percentage viability of the sperm could be correlated with the reduction in sperm motility, since non-motile spermatozoa were usually considered dead and could not be counted as live spermatozoa. The viability was significantly increased in the recovery group. Moreover, the decline in epidydimal sperm counts in response to the drugs, might be due to a direct effect of these drugs on the epididymal site probably by acting as a spermatoxic agent. This finding agrees with earlier reports that the maturational events of spermatozoa taking place in the epididymis are vulnerable to chemical interference[13].

It is important to note that a significant decline in serum levels of testosterone was observed in chlorpromazine and thioridazine treated rats when compared with the controls in this study. The observed effects of these drugs on reproductive organs appear therefore to be partly mediated by alteration in circulating androgen levels. This alteration in the hormonal *milieu* of male albino rats was dose-dependent and consistent with the report that antipsychotic drugs produce striking adverse effects on reproductive system[14]. The drugs are known to decrease libido and cause gynecomastia in males while ammenorrhea, galactorrhea and false-positive pregnancy tests and increase libido are common side effects in females. Phenothiazines (chlorpromazine and thioridazine) block dopamine receptors resulting in hyperprolactinemia[4,15]. These actions appear to be non-specific for phenothiazines since there is high prevalence of sexual dysfunction in men and women treated with neuroleptic drugs[14,16]. They have also been reported to increase peripheral conversion of androgens to estrogens[1]. This is in agreement with the increased concentration of estrogen earlier reported[17]. Women undergoing antipsychotic drug treatment displayed significantly lower levels of estradiol and progesterone, whereas in men, the levels of free testosterone and DHEA-S were significantly lower than in controls[17]. This mechanism of action of phenothiazines supports the significant reduction in serum levels of testosterone observed in the present study.

It is also possible that the normal negative feedback control of secretions of gonadotrophin releasing hormone from the hypothalamus and /or gonadotrophins especially luteinizing hormone from the adenohypophysis was adversely affected in view of their adverse effect on testosterone secretion. The hypothalamic nervous pathways that control the secretion of gonadotrophins are inhibited by CNS-influencing drugs such as marijuana, the narcotics, barbiturates and tranquilizers, making these pathway a major mechanism for the effects of drugs on reproductive hormones[18,19]. Moreover Soliman *et al.* [3] reported male antifertility effects of antiepileptic drugs, carbamazepine and sodium valproate in rats. These authors reported significant decrease in plasma testosterone, FSH and LH and an increase in prolactin levels in rats treated with the drugs.

After four weeks of recovery period, the serum testosterone remained at low levels that were similar to those of the rats, which were not, allowed a recovery period. This is consistent with the report of Daniel *et al.*[20] on the pharmacokinetics of thioridazine in rats. According to their study, chronic treatment of male rats with thioridazine produced significant increases in the plasma concentrations of the parent compound and its metabolites, which were accompanied, with prolongation of their plasma half-lives. Similar effects are likely with other phenothiazines including chlorpromazine. This probably indicates that the effect of these drugs on testosterone secreting cells (Leydig cells) in rats persisted beyond this period. Thus, a more prolonged recovery period would be required for full restoration of normal testosterone secretion in the treated rats. The reduction in sperm counts, motility and viability and significant increase in sperm abnormalities were accompanied with changes in testicular histology in this study which could be induced by alterations in steroid synthetic capacity of Leydig cells. There were visible lesions in the testis of the treated rats when compared to the control (Table 4). Congestions of blood vessels and degeneration of the seminiferous tubules, which could lead to inhibition of spermatogenesis, were also observed. At higher concentrations of the drugs, the seminiferous tubular degeneration appeared more severe indicating that the drugs produced deleterious effects on the testis. Although prolactin concentration was not measured in

the present study, it is possible that hyperprolactinemia was induced by the significant increase in estrogens secretion[4]. The increased estrogens secretion could form the basis for the disorganization of the cytoarchitecture of the testes[21]. Measurement of FSH, LH, GnRH and prolactin levels and possibly enzymes involved in the conversion of androgens to estrogens might throw more light into the probable mode of antireproductive effects of these drugs and could facilitate efforts at reducing the reproductive side effects of these drugs.

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