

EFFECT OF CLOMIPRAMINE HYDROCHLORIDE ON SEMEN QUALITY AND BIOCHEMICAL PARAMETERS OF RABBITS

***Hedia El-Said Ahmed, M.E. El-Sabeiy, Rawia, S. Hamed,
M.M. Zeitouny**

*Animal Production Research Institute, Dokki, Giza, Egypt.

**Department of Animal Production, Faculty of Alexandria,
University of Alexandria, Egypt.

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ABSTRACT: *The objective of this study was to determine the effects of different levels of clomipramine hydrochloride supplementation on sperm characteristics, seminal plasma lipid peroxidation and enzyme activities of mature rabbits. Twenty-one male New Zealand White rabbits (7 months old) were divided into three equal groups. The first group served as control, while groups 2 and 3 were orally treated with low (1.5 mg/kg of body weight), and high doses (3 mg/kg of body weight), respectively of clomipramine hydrochloride every other day for 12 weeks.*

Results exhibited that administration of clomipramine hydrochloride did not affect body weight and body weight gain ($P > 0.01$). Likewise, no significant effect was found due to treatment on feed intake (184.4, 179.3 and 174.8 g feedstuffs/animal/day for control, low and high doses clomipramine, respectively).

Treatment with clomipramine hydrochloride significantly ($p < 0.05$) decreased ejaculate volume (EV), and dead sperm. Reaction time, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate, total functional sperm fraction (TFSF) were significantly increased as compared to the control. Live sperm was found to be significantly ($P < 0.05$) increased due to treatments with low or high doses of clomipramine hydrochloride compared to the control.

Lactate dehydrogenase (LDH), the activities of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were significantly ($p < 0.05$) decreased due to administration of clomipramine hydrochloride.

Clomipramine hydrochloride at the low dose significantly ($P < 0.01$) increased testosterone in blood serum, however no significant difference

was obtained between the bucks given the high dose of clomipramine and the control. Blood plasma total protein, globulin and AST were significantly increased as results of clomipramine treatment. On the other hand, plasma triglyceride was significantly decreased compared with control. No significant difference was found between the bucks given the clomipramine and the control in plasma glucose, urea and AST activity. These results showed that treatments with clomipramine hydrochloride caused an improvement of some semen characteristics in rabbits especially with high dose without any harmful or negative effects on male fertility.

INTRODUCTION

Clomipramine hydrochloride (Anafranil) is a tricyclic psychotropic agent with both antidepressant and antiobsessional properties. Clomipramine hydrochloride inhibits norepinephrine and serotonin uptake into central nerve terminals, possibly by blocking the membrane-pump of neurons, thereby increasing the concentration of transmitter monoamines at receptor site. McDougle et al. (1998) reported that potent serotonin reuptake inhibitor (SRI) drugs as clomipramine is used as treatment for obsessive compulsive disorder as it act on the serotonin transporter protein. Laboratory assessment indicated that men with primary premature ejaculation were better able to control ejaculatory response with clomipramine therapy (Haensel et al., 1996). It has recently shown that these dopamine and serotonin inhibitors have specific effect on the gonadal steroid hormones (Rehavi et al., 2000). The dopamine transported blockers suppressed testosterone serum levels in the rat males. It seems that the effect of these agents on ovarian and testicular hormones is related to the impact of the monoamine reuptake inhibitors on the hypothalamic-pituitary-gonadal axis.

MATERIALS AND METHODS

In this study, the effects of clomipramine hydrochloride on the reproductive performance and seminal plasma biochemistry of male rabbits were investigated. Twenty one male New Zealand White rabbits 7 months old and initial body weight of 2.840 ± 0.25 kg were used. Animals individually housed in stainless steel wired cages and were provided feed and water ad libitum. Rabbits were fed on a commercial ration pellets with following constitution (Table 1):

Table (1): Proximate analysis of pelleted basal diet.(% on a dry matter basis).

Ingredients (%)	%	Calculated chemical analysis**	%
Berseem hay	30.0	Crude protein (%)	17.5
Yellow corn	25.0	Crude fiber (%)	14.0
Wheat bran	26.2	Ether extract (%)	2.7
Soybean meal	14.0	Nitrogen free extract (%)	56.4
Molasses	3.0		
CaCl ₂	1.0		
NaCl	0.4		
Vit.&Min. mix.*	0.3		
DL-Methionine	0.1		

*The vitamin and mineral premix/kg contained the following IU/gm for vitamins or minerals: A-4,000,000, D3-5000,000, E-16,7 g, K-0.67 g, B1-0.67 g, B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.).

**** Based on NRC (1994)**

Rabbits were randomly divided into three equal groups. Group 1 served as control, however, groups 2 and 3 were orally supplemented with low (1.5 mg/kg of body weight), and high doses (3 mg/kg of body weight), respectively of clomipramine hydrochloride every other day for 12 weeks. The doses of clomipramine hydrochloride were calculated according to the animal's body weight on the week before dosing. clomipramine hydrochloride was supplied from Novartispharma S.A.E- Cario, Egypt.

Semen collection was carried out weekly from all animals throughout the 12 weeks of the experimental period. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (using a graduated collection tube) after removal of the gel mass.

A weak eosin solution (Smith and Mayer, 1955) was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide. Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. Assessments of live, dead, and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture. The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using a phase-

contrast microscope with heated stage (Blom, 1950). Total number of motile sperm was calculated by multiplying percentage of motile sperm and total sperm output. Reaction time was recorded from the moment of subjecting a doe to the buck and completion of erection; it was measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universal indikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility by normal morphology (Correa and Zavos, 1996).

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C, and was stored at -20 °C until later analysis. Seminal plasma samples were analyzed for the activity of lactate dehydrogenase (LDH) by the method of Cabaud and Wroblewski (1958). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957).

Heparinized tubes were used to collect the blood samples which obtained from the ear vein of each buck every week. Blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma, and stored at -20°C. Blood plasma of glucose, albumin, total protein, AST, ALT activity, triglyceride and urea were measured according to commercial kits.

Testosterone levels were determined by the Radio immunoassay method (Wise, 1987) in the laboratory.

Data were analyzed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model procedure of SAS (1986). Means were compared by Least Significant Difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Body weight and feed intake:

Initial body weight (IBW), final body weight (FBW), body weight gain (BWG) and feed intake during the 12-wks experimental period of the rabbits are presented in Table 2. Results exhibited that administration of clomipramine hydrochloride did not affect body weight and body weight gain ($P>0.01$). Likewise, there were insignificant decreased due to treatment on feed intake (184.4, 185.3 and 174.8 g feedstuffs/animal/day for control, low and high doses of clomipramine, respectively).

Clomipramine may affect appetite by blocking the reuptake of serotonin and noradrenaline. Serotonin is recognized to have an influence on food intake (Mok, et al. 2000) Noradrenaline has also been shown to have an effect on food intake that opposes the effects of serotonin, but its effects on macronutrient selection are not clear (Currie, et al. 1993) TCA may also affect appetite by blocking histaminergic (H1) pathways (Ookuma, et al. 1990).

Semen Characteristics:

Data of ejaculate volume (EV), initial hydrogen ion concentration (pH), reaction time (RT), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), normal and dead sperm and total functional sperm fraction (TFSF) of rabbits treated with low (1.5 mg/kg of body weight) and high doses (3 mg/kg of body weight) of clomipramine hydrochloride every other day for 12 weeks are presented in Table 3. Results show that treatment of male rabbits with low and high doses of clomipramine hydrochloride increased ($P < 0.05$) the reaction time, and decreased ($P < 0.05$) ejaculate volume (EV) especially low dose group. In spite of this, sperm motility was significantly higher in high dose of clomipramine compared with low dose and control, even though sperm concentration was the highest ($P < 0.05$) in low dose of clomipramine, consequently total motile sperm per ejaculate (TMS), total functional sperm fraction (TFSF) were significantly higher in low dose compared with high dose and control groups. However, dead and abnormal sperm were decreased ($P < 0.05$), while initial hydrogen ion concentration (pH) was increased ($P < 0.05$) in low and high doses groups compared with control group. It is clear from the present results that administration of clomipramine hydrochloride improved semen characteristics and has a positive effect on semen quality but not quantity.

It has been well established that clomipramine hydrochloride inhibits norepinephrine and serotonin uptake into central nerve terminals, possibly by blocking the membrane-pump of neurons, thereby increasing the concentration of transmitter monoamines at receptor sites (Hendrick et al., 1999). Hansel et al. (1996) indicated that men with primary premature ejaculation were better able to control ejaculatory response with clomipramine therapy. Also, Rehavi et al. (2000) found that increases in testosterone levels in male rats given serotonin reuptake inhibitors such as clomipramine. There must exist some kind of stimulation for P-450 enzymes which might be involved in spermatogenesis and steroidogenesis.

It has also been found that antidepressants cause reciprocal changes in dopamine and 5-Hydroxytestosterone release in different areas of the brain may promote copulation and sexual satiety (Hull et al., 1999).

In the present study, the low dose resulted in higher testosterone in peripheral blood and enhanced semen characteristics. This could explain that the low dose represented a physiological state, but the high dose represented a pharmacological dose. Recently, Barrett et al. (2006) stated that there is clear evidence in the clinical literature for an association between serotonergic function and male potential.

Seminal plasma characteristics:

In the present study, treatment of rabbits with clomipramine hydrochloride decreased ($p < 0.01$) in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) in seminal plasma (Table 4). The decrease in the activities of these enzymes coincided with the increase of semen quality of treated rabbits with clomipramine hydrochloride (Table 3). The LDH activity of seminal plasma was negatively correlated with each of ejaculate volume, sperm motility, concentration and percent live cells (Roussal and Stallcup, 1965).

Blood plasma:

The changes in plasma glucose, total protein (TP), albumin (A), globulin (G), TG, urea and activities plasma AST and ALT of rabbits treated with clomipramine hydrochloride are summarized in Table 5. No significant changes of plasma glucose, ALT and urea were detected in clomipramine groups compared to the control group. On the other hand, treated bucks with clomipramine hydrochloride caused significant increases in plasma total protein and globulin, where plasma albumin and AST activity showed significant decreases compared to control group.

In conclusion, antidepressants could be used with caution to enhance the fertility status of rabbits. However, a multidiscipline integrated study involving pharmacologist, biochemist and physiologist must be conducted to monitor the side effects of the drug on other body organs and on the biochemical attributes of the animal blood.

Table 2. Effect of low and high doses of clomipramine hydrochloride on initial live body weight (IBW; kg), final live body weight (FBW, kg), body weight gain (BWG; g/kg) and feed intake (g feedstuffs/head/day) of male rabbits (mean ± SE)

Parameters	Control	Low Dose	High Dose
IBW	2.845±0.127	2.830±0.107	2.850±0.100
FBW	3.352±0.103	3.450±0.208	3.407±0.113
BWG	6.18±0.627	7.042±1.17	6.160±0.510
Feed Intake	184.4±6.21	179.3±7.87	174.8±8.02

Table 3. Effect of low and high doses of domipramine hydrochloride on semen characteristics and serum testosterone of male rabbits (means ± SE).

Parameters	Control	Low Dose	High Dose
Ejaculate volume(ml)	1.22 ± 0.14A	0.99 ± 0.12C	1.07 ± 0.13B
pH	7.42 ± 0.87C	7.79 ± 0.92B	8.00 ± 0.94A
Reaction time (sec.)	10.40 ± 1.46B	17.55 ± 1.89A	19.44 ± 1.88A
Sperm concentrate (×10 ⁶ /ml)	469.0 ± 55.27C	658.2 ± 77.57A	492.2 ± 58.00B
Total sperm output (×10 ⁶)	571.7 ± 67.37B	654.7 ± 77.16A	527.6 ± 62.18C
Sperm motility (%)	64.31 ± 7.58C	68.06 ± 8.02B	74.50 ± 8.78A
Total motile sperm (×10 ⁶)	367.8 ± 43.35B	450.7 ± 53.11A	395.2 ± 46.58B
TFSF*	293.5 ± 34.59C	397.0 ± 46.79A	349.4 ± 41.17B
Live sperm (%)	70.43 ± 8.30B	84.30 ± 9.93A	83.38 ± 9.83A
Dead sperm (%)	29.57 ± 3.49A	15.70 ± 1.85B	16.62 ± 1.96B
Abnormal sperm (%)	20.74 ± 2.44A	11.76 ± 1.39B	11.41 ± 1.34B
Serum testosterone (ng/ml)	1.76 ± 0.47B	3.64 ± 0.41A	2.07 ± 0.52B

^{ABC} Within row, means with different superscript letters differ significantly (p < 0.05).

*TFSF: Total functional sperm fraction

Table 4. Effect of low and high doses of clomipramine hydrochloride on lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of seminal plasma of male rabbits .

Parameters	Control	Low Dose	High Dose
LDH (U/L)	2807.1±39.5 ^A	2253.3±62.1 ^B	2147.1±68.3 ^C
AST (U/L)	38.20±0.6 ^A	31.93±0.28 ^B	31.88±0.54 ^B
ALT (U/L)	30.20±0.43 ^A	23.13±0.42 ^B	22.66±0.45 ^B

^{ABC} Within rows, means with different superscript letters differ significantly (p<0.05).

Table 5. Effect of low and high doses of clomipramine hydrochloride on plasma glucose (mg/dl), total protein (TP; mg/dl), albumin (A; mg/dl), globulin (G; mg/dl), spartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), triglisride (TG, mg/dl) and urea (mg/dl) of male rabbits (mean \pm SE).

Parameters	Control	Low Dose	High Dose
Glucose	125.8 \pm 0.48	125.9 \pm 0.42	125.4 \pm 0.51
TP	5.80 \pm 0.08C	6.08 \pm 0.03B	6.25 \pm 0.01A
Alb	4.20 \pm 0.01A	3.15 \pm 0.03C	3.85 \pm 0.01B
Glo	1.60 \pm 0.07C	2.94 \pm 0.02A	2.40 \pm 0.02B
AST	36.87 \pm 0.13A	35.88 \pm 0.17A	22.51 \pm 1.18B
ALT	21.60 \pm 0.60	22.46 \pm 0.21	21.93 \pm 0.20
TG	54.51 \pm 0.63A	37.33 \pm 0.31B	26.56 \pm 0.23C
Urea	42.57 \pm 0.20	42.15 \pm 0.84	42.73 \pm 0.41

^{ABC} Within rows, means with different superscript letters differ significantly ($p < 0.05$).

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الملخص العربي

تأثير مادة هيدروكلوريد الكلومبرامين علي جودة والخصائص البيوكيماوية للسائل المنوي بالارانب

هدية السعيد احمد* - محمد السيد السبيعي*- روية صادق حامد* - مصطفى زيتون**

* معهد بحوث الانتاج الحيواني

** قسم الانتاج الحيواني-كلية الزراعة-جامعة الاسكندرية

هدفت هذه الدراسة لمعرفة تأثير اضافة مستويات مختلفة من هيدروكلوريد الكلومبرامين علي خصائص السائل المنوي وجودته.

تمت التجربة علي عدد 21 من ذكور الارانب النيوزيلاندي البالغة (عمر 7 شهور) وقسمت الي ثلاث مجاميع متسوية ، المجموعة الاولى كانت المجموعة المقارنة (الكنترول) والمجموعتين الثانية والثالثة اعطيت عن طريق الفم يوم بعد يوم الجرعة المنخفضة (1.5 ملجرام /كيلوجرام وزن جسم) والجرعة المرتفعة (3 ملجرام /كيلوجرام وزن جسم) من مادة هيدروكلوريد الكلومبرامين لمدة 12 اسبوع.

اظهرت النتائج ان المعاملة بهيدروكلوريد الكلومبرامين لم يؤثر معنويا علي وزن الجسم و وزن الجسم المكتسب و المأكول. المعاملة بهيدروكلوريد الكلومبرامين خفضت معنويا حجم القذفة، نسبة الحيوانات المنوية الميتة، الرغبة الجنسية، تركيز الحيوانات المنوية، العدد الكلي للحيوانات المنوية، حيوية الاسبرمات، العدد الكلي للحيوانات المنوية الحية، العدد الكلي للحيوانات المنوية الحية لكل قذفة زادت معنويا نتيجة المعاملة بهيدروكلوريد الكلومبرامين.

انزيمات البلازما المنوية اظهرت تحسن واضح نتيجة المعاملة بهيدروكلوريد الكلومبراميين.

المستوي المنخفض من هيدروكلوريد الكلومبراميين ادي الي زيادة مستوي التستستيرون في الدم بينما لم يكن هناك فرق بين الجرعة العالية والكنترول. المعاملة بهيدروكلوريد الكلومبراميين ادت الي زيادة مستوي البروتين الكلي والجلوبيولين ونشاط انزيم السبرتيبت ترانزامينيز. بينما الثراي جلسريد واليوريا انخفضت معنويا مقارنا بالكنترول.

تظهر النتائج ان المعاملة بهيدروكلوريد الكلومبراميين حسنت بعض خصائص السائل المنوي في ذكور الارانب خاصا الجرعة العالية.