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·*Complementary Medicine*·

Reproductive, cytological and biochemical toxicity of Yohimbe in male swiss albino mice

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Abstract

Aim: To study the effect of *Corynanthe Yohimbe* (Yohimbe) on germ cells in Swiss albino mice. **Methods:** Adult male mice were orally (gavage) treated with different doses (188, 375 and 750 mg/[kg·day]) of aqueous suspension of Yohimbe for 90 days. The following parameters were evaluated: (i) reproductive organ weight, (ii) motility and count of sperm, (iii) study on rate of pregnancy and mean implants, (iv) spermatozoa morphology, (v) cytology of the testes chromosomes, and (vi) biochemical study on estimation of proteins, RNA, DNA, malondialdehyde, nonprotein sulfhydryl (NP-SH) and hormones. **Results:** The treatment caused significant increase in the weight of seminal vesicles, motility and count of spermatozoa, pre- and post-implants. Male fertility was decreased. These results are confirmed by our data on spermatozoa abnormalities and chromosomal aberrations. The data on biochemical parameters showed increase of malondialdehyde and depletion of NP-SH, proteins, RNA and DNA in the testicular cells. **Conclusion:** Our results elucidated the role of free radical species in cytological and reproductive changes, possibly, under the influence of yohimbine (principal constituent of Yohimbe) on neurotransmitters, including norephinephrine. These data warrant careful use of Yohimbe. *(Asian J Androl 2006 Jul; 8: 469–476)*

Keywords: Yohimbe; cytological; reproduction; mice; germ cell; sperm

1 Introduction

Erectile dysfunction or impotence is a widespread, age-related problem, which affects 52% of men between 40 and 70 years of age. There are several well-known therapeutic and surgical measures to promote sexual function. The natural products however dominate the arena, under the belief that they are safe. Some of these products include the bark of *Corynanthe Yohimbe* (Yohimbe) and *Pausinystalia johimbe* (Rubiaceae), which are used to improve fertility, bodybuilding and performance of athletes in human, and in captive breeding programs of wild animals. In addition to their use to enhance sexual prowess and virility, the bark of these trees is traditionally used to treat cardiac diseases [1, 2]. The principal constituent of Yohimbe is yohimbine. Yohimbine possesses α-2 antagonist activity, which leads to increase in synaptic norepinephrine. Due to this activity, it is successfully used in the treatment of narcoleptic cataplexy in humans [3, 4]. Clinical evaluation on yohimbine has shown that it can cause dizziness, nausea, insomnia, nervousness, anxiety, panic, headache, sweating, hypertension, tachycardia, tremors, increased urinary frequency, diarrhea, dyspepsia, and rash and erythrodermic skin eruption [3]. In addition, there are isolated

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cases of manic-like symptoms, agranulocytosis, angina pectoris, bronchospasm, renal failure and lupus-like syndrome [5].

There are several published papers on the pharmacology and the toxicity of yohimbine [3, 4]. However, little is known on the effect of Yohimbe on reproductive function, and its cytological and biochemical toxicity, except for an earlier report [6] showing that it causes stimulation of the mitotic activity of spermatogonia in mature male rats and increases spermatozoa count. The present study was undertaken in view of the immense use of Yohimbe and a paucity of published literature on its reproductive function and toxicity.

2 Materials and methods

2.1 Test of herbal product

Commercially available Yohimbe was used as the tested herbal product in the present study. It is manufactured by Good 'N Natural Manufacturing Corporation (Holbrook, NY, USA) and is marketed in the form of tablets by the General Nutrition Corporation of USA in Saudi Arabia. Each tablet contains 1 500 mg (Yohimbe bark powder of *Corynanthe Yohimbe*). The other ingredients of the tablet included silica, croscarmettose, vegetable stearic acid, vegetable magnesium and stearate cellulose coating. One tablet per day was recommended for human use.

2.2 Dose selection and route of administration

The dose of Yohimbe was determined by: (i) maximum tolerated dose (MTD); (ii) human therapeutic dose with reference to the surface area rule; and (iii) preliminary experiments conducted in our laboratory. The different doses selected for Yohimbe were 188, 375 and 750 mg/[kg·day], corresponding to 1/64, 1/32 and 1/16, respectively, of the evaluated MTD (12 g/kg), [7]. The daily human therapeutic dose of Yohimbe is 1 500 mg per adult human weighing 60 kg. According to the rule of surface area ratio of mice (20 g) and man (60 kg), the calculated ratio is 0.0026 and the dose of Yohimbe on mice would be (0.0026 × 1500 mg × 5 = 195.0 mg/kg). The dose used experimentally is generally six times more than the calculated value (195.0 × 6 = 1 170.0 mg/[kg·day]). This is because the metabolic rate is more in mouse as compared to human being [7]. The dose selected on the basis of MTD is much less than the dose calculated on the basis of human therapeutic dose according to the rule of surface area. Aqueous suspension of Yohimbe was administered by gastric intubation (oral) per kg per day for 90 days (sub-chronic treatment) [7]. Because commercially available Yohimbe tablets (as a whole) were used as the herbal drug for treatment, the mice in the control group were given tap water as the vehicle.

2.3 Animal stocks

Male Swiss albino mice aged 6–8 weeks and weighing 25–28 g were obtained from the Experimental Animal Care Center, King Saud University (Riyadh, Saudi Arabia). The animals were fed on Purina chow diet and water *ad libitum* and were maintained under standard conditions of humidity, temperature and light (Light : Dark cycle, 12 h : 12 h). The Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia) approved the conduct of experiments and the procedure used to kill the mice (using ether).

2.4 Experimental groups

The experimental groups of mice consisted of the following four groups: group 1, control (0.3 mL/mouse, tap water); group 2, Yohimbe (188 mg/[kg·day]); group 3, Yohimbe (375 mg/[kg·day]); and group 4, Yohimbe (750 mg/[kg·day]). Each of the following parameters were evaluated in the above four groups (The total number of mice used are mentioned between the parenthesis against each parameter):

- Reproductive organ weight, motility and count of sperm (20 mice);
- Spermatozoa morphology (20 mice);
- The rate of pregnancy and mean implants (40 male mice and 120 female mice in each week of the mating);
- Cytology of the testes chromosomes (20 mice);
- Biochemical study on estimation of proteins, nucleic acids, malondialdehyde (MDA) and nonprotein sulfhydryl (NP-SH) in testicular cells (20 mice).

2.5 Analysis of reproductive organ weight

The mice were killed after the last day of sub-chronic treatment and weighed for essential reproductive organs, such as testes, caudae epididymis, seminal vesicles and prostate gland [8, 9].

2.6 Evaluation of spermatozoa motility, count and abnormalities

The spermatozoa were obtained by making small cuts

in caudae epididymis and vas deferens placed in 1 mL of modified Krebs Ringer-bicarbonate buffer (pH = 7.4). With no loss of time, the suspension was evaluated for percentage motility of sperm. The motility was determined by the progressive and non-progressive movement of sperm observed under a compound microscope (Laborlux 11, Leitz, Germany) [9, 10]. The sperm count was determined under a Neubauer haemocytometer (Superior, Marienfeld, Germany) [8]. To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin, smears were made on slides, air-dried and made permanent. The slides were examined by bright-field microscope with an oil immersion lens. The different spermatozoa abnormalities screened included amorphous, banana shaped, swollen acrosome, triangular head, macrocephali and rotated head [8, 10].

2.7 Studies on rate of pregnancy and mean implants per female mouse

The methods described in a male anti-fertility study [8] and dominant lethal assay [11] were followed to evaluate: (i) the rate of fertility in male mice; (ii) induction of pregnancy; (iii) total and pre-implantation loss; and (iv) embryo-toxicity. After the treatment, each male mouse in the treated and control groups was caged with three female mice for each week of mating. The female mice were necropsied 13 days following the mid-week of their caging and presumptive mating and the number of pregnant mice was recorded to determine percentage fertility [8]. The pre-implantation loss was calculated by comparing the number of implant per pregnant female mouse in the treated and control groups. The dead implants per pregnant female mouse were determined to obtain the post-implantation embryonic loss [8, 11].

2.8 Cytological analysis of germ cells

The protocol described in a study of chromosomal aberrations in the testis [8] was followed. The testes were removed in an isotonic tri-sodium citrate dihydrate (2.2% in distilled water, pH 7.8) and the seminiferous tubules were teased to form a cell suspension. The suspension was centrifuged at 250 × *g* for 5 min under 23ºC, and the pellet was re-suspended in the hypotonic tri-sodium citrate dihydrate (1.1% in distilled water, pH 7.8). After the second centrifugation the supernatant was discarded and the pellet suspended in a fixative (methanol : acetic acid = 3:1). The air drying technique was used for chromosomal preparations. The slides were stained in Giemsa solution and screened for aberrations, including aneuploids, autosomal univalents, sex-univalents and polyploids.

2.9 Biochemical evaluation

Twenty-four hours following the 90-day treatment, the mice were killed. The testes were immediately excised and frozen at –70ºC. The frozen samples of testes were used to estimate proteins, RNA, DNA, MDA and NP-SH levels.

2.9.1 Estimation of total proteins and nucleic acids

Total proteins were estimated using the method described by Al-Majed *et al.* [12]. Bovine serum (Merck, Darmstadt, Germany) albumin was used as standard. To determine the levels of nucleic acids, testes were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation (500 × *g* for 3 min at 23ºC), the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

2.9.2 Determination of malondialdehyde concentrations

Testes were homogenized in TCA solution and the homogenate suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard [12].

2.9.3 Quantification of the nonprotein sulfhydryl levels

The testes were homogenized in ice-cold 0.02 mmol/L ethylene diamine tetra acetic acid disodium (EDTA) and mixed with TCA. The homogenate was centrifuged at 3 000 × *g*. The supernatant was suspended in Tris buffer (pH 8.9), 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB) and read at 412 nm against reagent blank with no homogenate [12].

2.9.4 Estimation of hormones in the serum

The plasma samples were analyzed to determine the concentration of different hormones (human chorionic gonadotropin, progesterone, leuteinizing hormone, follicle stimulating hormone, estradiol, prolactin and testoste-

rone). The analysis was carried by direct immunoenzymatic calorimetric method based on ELISA. The protocol used for each hormone was according to the methods described for the particular kit (DIA, METRA, Italy).

2.10 Statistical analysis

The means of the four groups were compared using analysis of variance (ANOVA). When the ANOVA $P < 0.05$, a Tukey–Kramer's post hoc comparison test was used to locate specific group differences. Data are expressed as mean ± SD. The percentage of fertility and dead embryos of four groups were compared using the χ^2-test. The two-tailed 0.05 level of significance was used for all data analysis. Data were analyzed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA) software.

3 Results

The sub-chronic treatment with Yohimbe caused a significant ($P < 0.05$) increase in the weight of seminal vesicles at the high dose (750 mg/[kg·day]) (Figure 1). The sperm count and the percentage sperm motility were significantly decreased ($P < 0.05$) at the high dose, compared to the values observed in the control (Table 1).

The sub-chronic treatment with Yohimbe significantly increased ($P < 0.05$) the amorphous- and banana- shaped sperm abnormalities (375 and 750 mg/[kg·day]) and swollen acrosome, triangular head and total sperm abnormalities (750 mg/[kg·day]) (Figure 2).

The frequency of aneuploids, sex-univalents and total chromosomal aberrations was significantly ($P < 0.05$) increased at the high dose (750 mg/[kg·day]) after prolonged treatment of Yohimbe (Figure 3).

In mating week 1, the prolonged treatment of male mice with Yohimbe resulted in a reduction in the percentage of pregnant female mice to 50%, which was statistically insignificant ($P > 0.05$) as compared to the control. There were no significant changes in total, live and dead implants per pregnant female mice and the percentage of dead embryos at any of the doses of Yohimbe

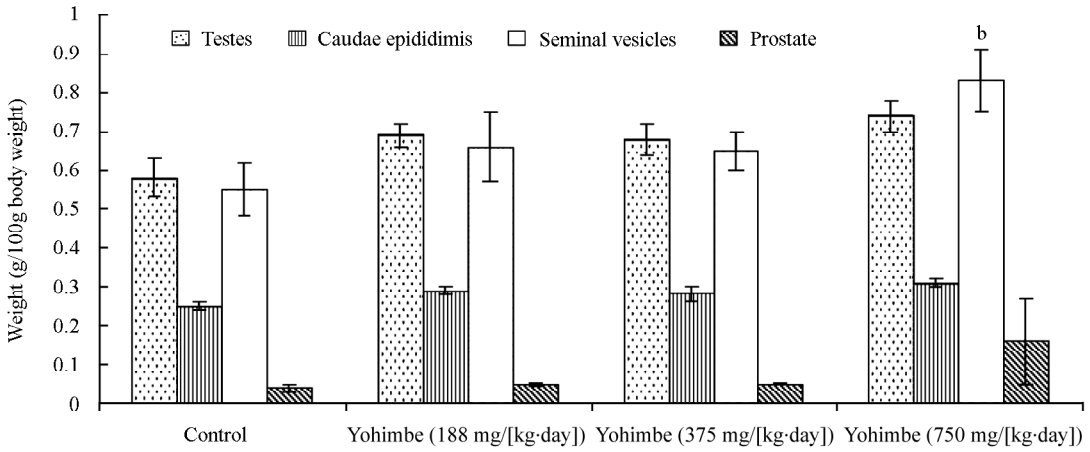

Figure 1. Effect of chronic treatment with Yohimbe on the reproductive organ weight in male Swiss albino mice. Five mice were used in each group. $^bP < 0.05$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test), compared with the control.

Table 1. Effect of Yohimbe on sperm motility and count in Swiss albino mice after sub-chronic treatment in Swiss albino mice. Five mice were used in each group. $^bP < 0.05$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test individually for different parameters), compared with the control.

Group	Treatment	Dose (mg/[kg·day])	Percent sperm motility (mean ± SD)	Sperm count (log N/mm^2) (mean ± SD)
1	Control	0.3 mL tap water/mouse	90.00 ± 6.13	4.76 ± 0.13
2	Yohimbe	188	70.25 ± 28.93	4.88 ± 0.09
3	Yohimbe	375	66.25 ± 34.56	4.79 ± 0.16
4	Yohimbe	750	50.00 ± 34.75^b	4.51 ± 0.20^b

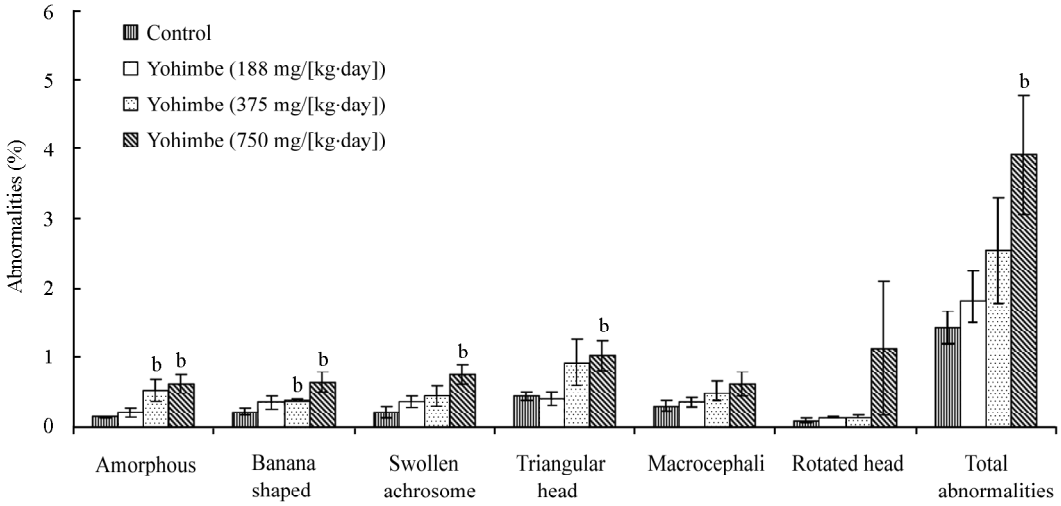

Figure 2. Effect of Yohimbe on epididymal spermatozoa in Swiss albino mice after sub-chronic treatment. Five mice were used in each group. $^{b}P < 0.05$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test) , compared with the control.

Table 2. Effect of Yohimbe on the induction of dominant lethal mutations after sub-chronic treatment in male Swiss albino mice. Figures between parentheses denote percent. A total of 10 male and 30 female mice were used in each group. $^{b}P < 0.05$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test), $^{c}P < 0.01$ (χ^2-test), compared with the control.

Treatment and dose (mg/[kg·day])	Mating week 1					Mating week 2				
	Pregnant female mice (%)	Implants/pregnant female mice (mean ± SD)			Percentage dead embryos (%)	Percentage female mice (%)	Implants/pregnant female mice (mean ± SD)			Percentage dead embryos (%)
		Total	Live	Dead			Total	Live	Dead	
Control (tap water)	23/30 (76.67)	11.69 ± 1.14	11.13 ± 1.30	0.56 ± 0.40	13/269 (4.83)	20/30 (66.67)	11.70 ± 1.01	11.20 ± 1.70	0.50 ± 0.29	10/234 (4.27)
Yohimbe (188)	16/30 (53.33)	11.87 ± 0.83	11.06 ± 0.87	0.81 ± 0.42	13/190 (6.84)	21/30 (70.00)	11.05 ± 0.67	10.43 ± 0.76	0.62 ± 0.36	13/232 (6.03)
Yohimbe (375)	18/30 (60.00)	11.56 ± 1.54	10.78 ± 1.59	0.78 ± 0.38	14/208 (6.73)	18/30 (60.00)	11.72 ± 0.80	10.72 ± 0.96	1.00 ± 0.56	18/211 (8.53)
Yohimbe (750)	15/30 (50.00)	12.53 ± 1.23	11.93 ± 1.25	0.60 ± 0.36	9/188 (4.78)	14/30 (46.67)	10.36 ± 0.85^{b}	8.67 ± 1.01^{b}	1.71 ± 1.25^{b}	24/159 (15.09)^{c}

(Table 2). In mating week 2 the pregnancy rate of mated female mice was reduced to 46% (statistically insignificant as compared to the control). There was a statistically significant ($P < 0.05$) decrease in total and live implants per pregnant female mouse and increase of dead implants per pregnant female mouse ($P < 0.05$) and percentage dead embryos ($P < 0.01$) at the high dose (750 mg/[kg·day]) of Yohimbe (Table 2).

Treatment with Yohimbe significantly ($P < 0.05$) decreased the testicular concentrations of proteins at the high dose (750 mg/[kg·day]). The testicular levels of RNA and DNA were also decreased significantly at the medium dose (375 mg/[kg·day]) ($P < 0.05$) and high dose (750 mg/[kg·day]) ($P < 0.01$) of Yohimbe (Table 3). Yohimbe treatment also increased and decreased the testicular concentrations of MDA and NP-SH, respectively, at the medium dose ($P < 0.05$) and the high dose ($P < 0.01$) of Yohimbe (Table 3).

The prolonged treatment with Yohimbe in male mice significantly increased the plasma levels of estradiol ($P < 0.05$), prolactin ($P < 0.001$) and testosterone ($P < 0.05$) at the high dose (Table 4).

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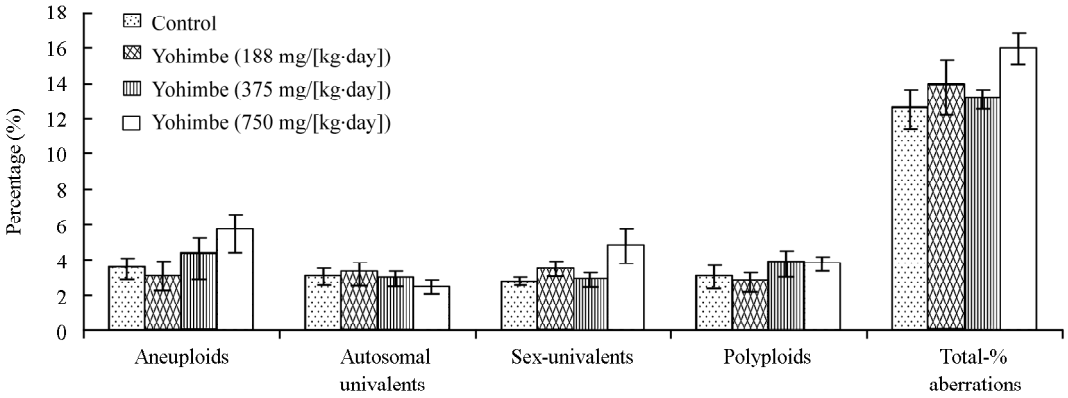

Figure 3. Effect of Yohimbe on testis chromosomes in Swiss albino mice after sub-chronic treatment. Five mice were used in each group. $^{b}P < 0.05$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test) , compared with the control.

Table 3. Effect of Yohimbe on certain biochemical markers (proteins, nucleic acids, MDA and NP-SH) in testicular tissue of mice after sub-chronic treatment. Five mice were used in each group. mean ± SD, $^{b}P < 0.05$, $^{c}P < 0.01$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test were done), compared with the control. MDA, malondialdehyde; NP-SH, nonprotein sulfhydryl.

Treatment and dose (mg/[kg·day])	Proteins (mg/100 mg wet tissue)	RNA (μg/100 mg wet tissue)	DNA (μg/100 mg wet tissue)	MDA (nmol/g wet tissue)	NP-SH (nmol/100 mg wet tissue)
Control (tap water)	11.16 ± 0.65	346.60 ± 36.02	235.40 ± 14.94	193.40 ± 16.50	110.60 ± 11.43
Yohimbe (188)	10.52 ± 0.69	304.20 ± 48.72	224.60 ± 14.94	216.00 ± 17.87	95.40 ± 13.17
Yohimbe (375)	9.82 ± 1.50	274.60 ± 15.05^{b}	207.00 ± 14.80^{b}	229.60 ± 10.42^{b}	88.80 ± 8.76^{b}
Yohimbe (750)	8.76 ± 2.03^{b}	252.14 ± 39.67^{c}	199.60 ± 9.26^{c}	246.20 ± 17.15^{c}	82.00 ± 5.57^{c}

Table 4. Effect of Yohimbe on certain pituiary-gonadal hormones in plasma of male Swiss albino mice after sub-chronic treatment. Five mice were used in each group. mean ± SD, $^{c}P < 0.01$ (one way analysis of variance [ANOVA] and post hoc Tukey–Kramer multiple comparison test) , compared with the control.

Pituitary-gonadal hormones in plasma	Control (tap water)	Yohimbe (188 mg/[kg·day])	Yohimbe (375 mg/[kg·day])	Yohimbe (750 mg/[kg·day])
Human-chorionic gonadotropin	0.97 ± 0.11	0.98 ± 0.13	0.95 ± 0.04	0.93 ± 0.07
Leutenizing hormone	1.70 ± 0.27	1.80 ± 0.18	1.90 ± 0.25	1.62 ± 0.20
Follicle-stimulating hormone	1.50 ± 0.18	1.60 ± 0.13	1.45 ± 0.07	1.37 ± 0.11
Estradiol	0.28 ± 0.02	0.27 ± 0.02	0.30 ± 0.04	0.36 ± 0.04^{c}
Prolactin	0.46 ± 0.04	0.48 ± 0.07	0.45 ± 0.07	0.62 ± 0.04^{c}
Testosterone	18.70 ± 2.53	19.20 ± 2.91	20.10 ± 2.71	25.00 ± 1.99^{c}

4 Discussion

Yohimbe is a commercially available herbal product widely used in folk medicine to improve erectile function for fertility, and to increase the performance of athletes. In the present study, sub-chronic treatment with Yohimbe causes a decrease in the fertility of male mice. These data are confirmed by our observation on the reduction of percentage motility and spermatozoa count in caudal epididymis. Nevertheless, these data are not in agree-

ment with an earlier report [6], which shows johimbe to cause stimulation of mitotic activity in spermatogonia and increase of the spermatozoa count in rats. The discordance might be attributed to the use of a different plant (*Pausinystalia johimbe*) and strain of experimental rodent by Kuncheva *et al.* [6]. Lahdetie *et al.* [13] also found that tissue, species and strain-specific differences influence the exposure to drugs. The observed reduction in the rate of pregnancy of female mice shows that the treatment with Yohimbe incapacitates the male mice to cause successful mating. In addition to several pharmacological and physiological etiologies, reduction in motility and count of spermatozoa might also be responsible for the Yohimbe-related reduction of fertility in male mice. Our study on hormonal profile showed an increase of prolactin, estradiol and testosterone, after treatment with Yohimbe. These changes might have disrupted the balance of hormonal profile resulting in sexual dysfunction. Previous studies show that the release of prolactin results in inhibition of the enzyme activity of 20-alpha-hydroxysteroid dehydrogenase at the luteal level [14]. There is no published scientific literature to confirm the role of Yohimbe plants in the reproductive function. Nevertheless, the rampant use of Yohimbe plants is ongoing to promote erectile function. This is primarily because of the yohimbine content of these plants and also perhaps the belief that natural drugs are free of toxicity.

The treatment with Yohimbe also caused pre- and post-implantation loss in the female mice mated during the second week after the treatment. The embryonic loss, before implantation, observed in the present study is difficult to distinguish between the fertilized and unfertilized eggs. However, the causal factors for both the events are described as chromosomal anomalies [8, 11]. The induction of pre-implantation loss and changes in the rate of pregnancy corroborate with our data on chromosomal aberrations in the male germ cells. More specifically, the loss of aneuploids might have arrested the early embryogenesis and caused the death of embryos before implantation. The implant loss and reduction of fertility observed are also confirmed by our data on sperm abnormalities, which is described as contributing factor [8].

The results obtained in the present study show clearly that treatment with Yohimbe induces chromosomal aberrations, spermatozoa abnormalities, reduction in male fertility and pre- and post-implantation losses in Swiss albino mice. Our results confirm published literature reporting that depletion of antioxidants cause chromosomal aberrations in the testis [8, 15]. Furthermore, decrease in activity of antioxidant enzymes in the epididymis increase reactive oxygen that could damage the epididymal environment and sperm function [15, 16].

There are no parallel studies to demonstrate the oxidant activity of Yohimbe. However, the genesis of free radicals is attributed to its principal constituent (yohimbine). Although, there is no direct evidence to show the oxidant potentials of yohimbine, it is found to enhance the ischemia-induced analgesia [17]. The exact mechanism of action of yohimbine-induced oxidant activity is not known, but might be related to its influence on neurotransmitters, including norephinephrine. There are published reports suggesting that yohimbine increases the release of norephinephrine by blocking alpha-2-noradrenergic receptors [3, 4]. The increase of norephinephrine is shown to enhance the superoxide anion production in adult mice [18]. Fu *et al.* [19] reported norephinephrine to cause apoptosis in cardiac cells of neonatal rats. Furthermore, in a study on the influence of norephinephrine on cytokines, Miksa *et al.* [20] showed it can cause organ dysfunction by increasing pro-inflammatory cytokine release from Kupffer cells. Therefore, it is quite likely that yohimbine-induced genesis of free radicals and the related changes might be mediated through norepinephrine. In conclusion, our findings demonstrate reproductive, cytological and biochemical toxicity of Yohimbe after sub-chronic treatment. In view of these observations, we suggest consideration on therapeutic relevance and toxicity of Yohimbe before it is registered for use as a herbal drug.

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