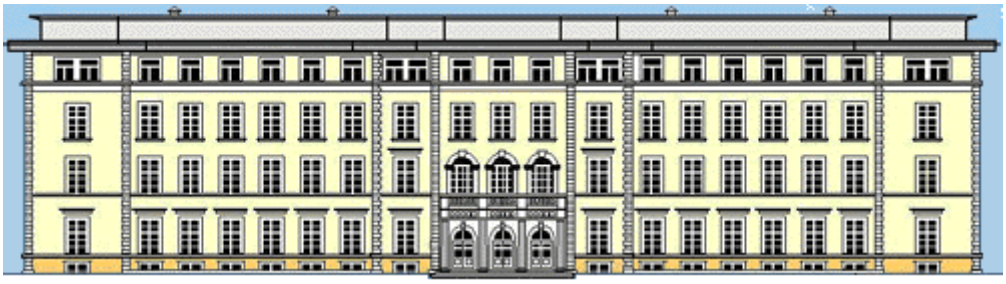
MEDICAL UNIVERSITY – SOFIA

FACULTY OF PHARMACY

DEPARTMENT OF PHARMACOLOGY, PHARMACOTHERAPY AND TOXICOLOGY



RESEARCH PROJECT REPORT

"EVALUATION OF THE TESTOSTERONE-BOOSTING ACTIVITY OF TAXADROL® IN WISTAR RATS"

Sofia

July, 9, 2010

Research team:

Senior researcher

Asst. Prof. Georgi Tsvetanov Momekov, MSci(Pharm), PhD

Laboratory of Exp. Chemotherapy,

Department of Pharmacology, Pharmacotherapy and Toxicology,

Faculty of Pharmacy, Medical University-Sofia

Team

Ventzislav Atanassov, D.V.M., PhD

"VisVitalis" Vet Ambulatory, Sofia, Bulgaria

Technical assistant Theodora Atanassova, BSci

Laboratory of Exp. Chemotherapy,

Department of Pharmacology, Pharmacotherapy and Toxicology,

Faculty of Pharmacy, Medical University-Sofia

Study Supervisor

Kamen Stoychev, MD

MATERIALS AND METHODS; EXPERIMENTAL DESIGN

Experimental animals

160 male Wistar rats were supplied from the National Breeding Center at the Bulgarian Academy of Sciences (Slivnitsa, Bulgaria) and were housed in a controlled environment: temperature 20-22°C, free access to food (Vitaprot-Ltd., Kostinbrod, Bulgaria) and water, 12 h alternating light and dark cycles, at the Animal Care facility of the Faculty of Pharmacy, MU-Sofia.

The animals were randomly distributed in sixteen treatment groups, as follows:

1. Untreated control (sacrificed on the 10th day) (10 animals);
2. Untreated control (sacrificed on the 17th day) (10 animals);
3. Untreated control (sacrificed on the 24th day) (10 animals);
4. Untreated control (sacrificed on the 31th day) (10 animals);
5. Taxadrol-treated (4 mg/kg, p.o., twice daily) (sacrificed on the 10th day) (10 animals);
6. Taxadrol-treated (4 mg/kg, p.o., twice daily) (sacrificed on the 17th day) (10 animals);
7. Taxadrol-treated (4 mg/kg, p.o., twice daily) (sacrificed on the 24th day) (10 animals);
8. Taxadrol-treated (4 mg/kg, p.o., twice daily) (sacrificed on the 31th day) (10 animals);
9. Taxadrol-treated (8 mg/kg, p.o., twice daily) (sacrificed on the 10th day) (10 animals);
10. Taxadrol-treated (8 mg/kg, p.o., twice daily) (sacrificed on the 17th day) (10 animals);
11. Taxadrol-treated (8 mg/kg, p.o., twice daily) (sacrificed on the 24th day) (10 animals);
12. Taxadrol-treated (8 mg/kg, p.o., twice daily) (sacrificed on the 31th day) (10 animals);
13. Taxadrol-treated (16 mg/kg, p.o., twice daily) (sacrificed on the 10th day) (10 animals);
14. Taxadrol-treated (16 mg/kg, p.o., twice daily) (sacrificed on the 17th day) (10 animals);
15. Taxadrol-treated (16 mg/kg, p.o., twice daily) (sacrificed on the 24th day) (10 animals);
16. Taxadrol-treated (16 mg/kg, p.o., , twice daily) (sacrificed on the 31th day) (10 animals);

Treatment

The experiments were carried out in accordance to the requirements of the European Convention for Protection of Vertebrate Animals used for Experimental and other Specific Purposes (1991). Healthy, pathogen free male Wistar rats were used in this study, whereby every experimental group consisted of 10 animals. The oral treatment was carried out using a gastric tube. The animals were treated over 10, 17, or 24 days, and sacrificed accordingly. Groups 8, 12 and 16 were sacrificed 7 days after the last treatment (together with control group 4) in order to assess the reversibility of the investigated pharmacological activity.

Testosterone level monitoring

Animals were sacrificed and thereafter blood samples were collected via cardial punction and thereafter the serum fractions were isolated. The determination of testosterone levels was carried out in the Higher Institute of Veterinary Medicine, using commercially available kits. The blood samples from the animals sacrificed on the 10th day of the treatment period were assayed for total testosterone levels, whereas in all other treatment groups and controls the levels of both total and free testosterone were determined.

Post-mortal evaluation

After collecting the blood samples the carcasses were necropsied by a qualified vet surgeon, and the visceral organs (liver, spleen, stomach, intestines) were examined for signs of toxicity. Moreover the animal body mass was monitored on regular basis as a non-specific marker of general toxicity.

Data processing and statistics

The results from hormonal level investigations were statistically evaluated using a paired Student's t-test and post hoc Dunnet test, using BMD P4V, BMD P3D and BMD P7D software.

EXPERIMENTAL RESULTS

The results from the investigation are located in tables 1,2 and in figures 1,2. The encountered qualitative changes in the testosterone levels, after exposure to the tested product TAXADROL®, which allowed some definitive conclusions regarding the clastogenic effect to be drawn out.

As evident from the presented data, TAXADROL® slightly increased testosterone levels after the first observation period (10 days post treatment), while very positive trends in this respect were evident.

Following 17 days treatment there was a prominent, statistically significant increase in the levels total testosterone in the animals treated with the lower doses (4 and 8 mg/kg, twice daily). The levels of free testosterone at this data point were significantly elevated, however only in the 8 mg/kg twice daily –treated animals. At the end of the treatment period (24 days) these doses again proved to cause efficient increase in the total and free testosterone content, whereas the highest dose caused some decrease of the hormone concentration (though insignificant).

In addition we evaluated whether the established effects were sustained, measuring the testosterone levels 7 days after the treatment cessation. In the animals treated at a dose of 8 mg/kg/twice daily both free and total testosterone concentrations remained higher *vs.* the control group.

Throughout the study period there was neither mortality nor alteration in the feeding behavior of treated animals as compared to the untreated controls. The post mortem examination of the visceral organs failed to reveal any signs of toxic deleterious effects in the treatment groups, as compared to the controls. Moreover the exposure of animas to TAXADROL® caused no alterations in the weight gain rates of treated vs. untreated animals.

Taken together these findings indicate that TAXADROL® exerts modulating effects on testosterone levels in the rat and is devoid of general toxic effects within the tested dose range.

Table 1. Total testosterone levels in Taxadrol® - treated vs. untreated male Wistar rats.

Dose	10 days treatment		17 days treatment		24 days treatment		7 days post treatment	
	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%
Untr. control	0,95	100.0	1.12	100.0	1.06	100.0	1.18	100.0
4 mg/kg bid	1.05	110.3	1.05	93.4 N.S.	0.78	73.5 N.S.	1.31	110.9
8 mg/kg bid	1.14	199.9	1.49	132.2*	1.56	147.9*	1.46	123.8*
16 mg/kg bid	1.14	120,4	1.30	115.6*	1.43	135.6*	1.43	121.8 N.S.

*Each data point represents the arithmetic mean of values for 10 experimental animals; * p<0.05 vs. the untreated control (Student's t-test); N.S. not significant vs. the untreated control (p> 0.05);*

Table 2. Free testosterone levels in Taxadrol®-treated *vs*. untreated male Wistar rats.

Dose	17 days treatment		24 days treatment		7 days post treatment	
	pg/ml	%	pg/ml	%	pg/ml	%
Untr. control	8.67	100	10.55	100	12.40	100
4 mg/kg bid	8.55	98.6 N.S.	9.40	89.1 N.S.	10.42	84.0 N.S.
8 mg/kg bid	12.76	147.1*	18.05	171.1*	15.86	127.9*
16 mg/kg bid	10.24	118.0 N.S.	13.58	128.7*	13.96	112.6 N.S.

*Each data point represents the arithmetic mean of values for 10 experimental animals; * p<0.05 vs. the untreated control (Student's t-test); N.S. not significant vs. the untreated control (p> 0.05);*

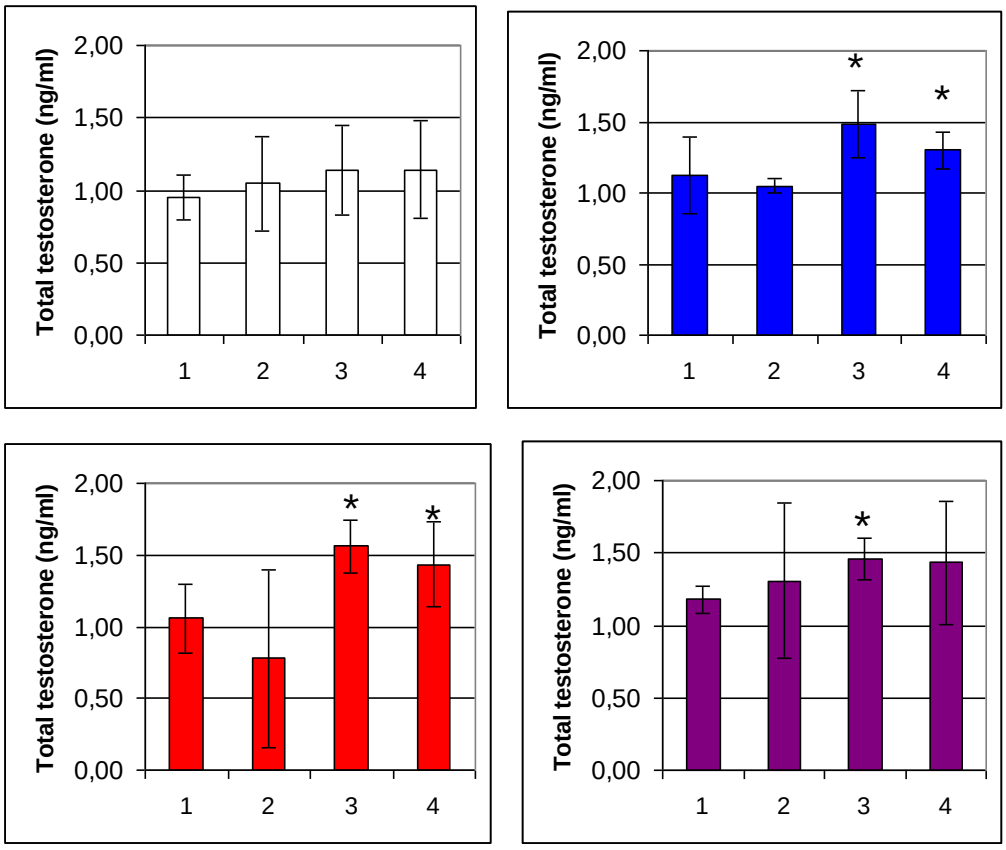


Fig. 1. Total testosterone levels in Taxadrol®-treated vs untreated male Wistar rats after 10 (□), 17 (■) and 24 (■) days of treatment or 7 days post treatment (■). 1 – untreated control; 2 – Taxadrol® (16 mg/kg bid); 3 -Taxadrol® (8 mg/kg bid); 4 - Taxadrol® (4 mg/kg bid);

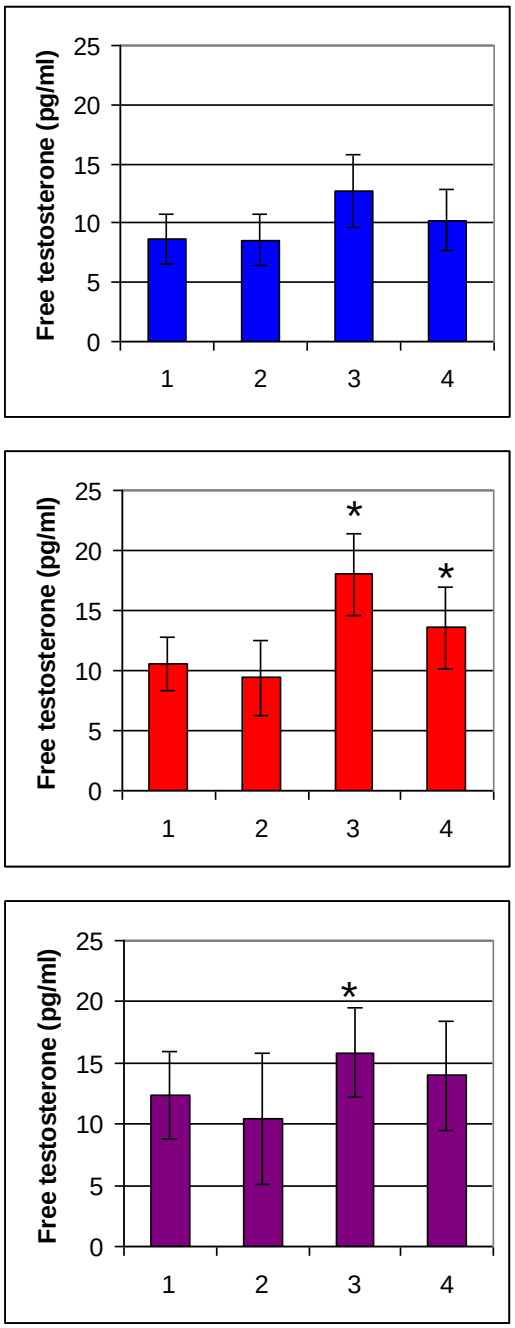


Fig. 2. Free testosterone levels in Taxadrol®-treated vs. untreated male Wistar rats after 17 ( ) and 24 ( ) days of treatment or 7 days post treatment ( ). 1 – untreated control; 2 – Taxadrol® (16 mg/kg bid); 3 -Taxadrol® (8 mg/kg bid); 4 - Taxadrol® (4 mg/kg bid);

CONCLUSION:

This study has proved that Taxadrol® is completely safe, even at levels higher than what will be recommended to humans.

This study also proves that Taxadrol® significantly increases free and total testosterone levels.