EVALUATION OF 90-DAY REPEATED DOSE ORAL TOXICIY AN ANDROGENIC ACTIVITY OF THE TRUONG XUAN CB CAPSULES IN RATS

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SUMMARY

Objectives: To evaluate 90-day repeated dose oral toxicity and androgenic activity of the Truong Xuan CB capsule in rats. Subject and method: 90-day repeated dose oral toxicity were evaluated on rats follow the OECD 408 Guideline; androgenic activity were evaluated on castrate-peripubertal male rats follow the OECD 441 Guideline. Results: The Truong Xuan CB capsule at doses 0.42 g/kg/day and 1.26 g/kg/day for 90 days continuously has no effect on the general condition of the rats, the body weight increase, hematological and biochemical indicators; histopathology of spleen, kidney and liver were normal. The Truong Xuan CB capsule at doses 0.42 g/kg/day and 0.84 g/kg/day showed an androgenic properties when evaluated in the Hershberger assay, increases the weight of the secondary sexual organs including the seminal vesicles, the ventral prostate, Cowper's gland, glans penis and the levator ani-bulbocavernosus muscle in the experimental rats. Conclusion: Truong Xuan CB capsules were safe and had androgenic activity when evaluated in experimental rats.

** Keywords: 90-day repeated dose oral toxicity; Androgenic activity; Truong Xuan CB.*

INTRODUCTION

The growing use of the modern production from traditional medicine in the present age has necessitated the thorough evaluation of safety and efficacy of the modern production from traditional medicine. Truong Xuan CB capsule (TCBC) is a research product from traditional medicine. Hence, the current study targeted at a 90-day repeated dose oral toxicity and androgenic activity of TCBC were performed.

MATERIALS AND METHODS

1. Study material.

TCBC were obtained from the provincial research project, meeting the house standard.

2. Animals.

Wistar rats were purchased from the Board provides experimental animals - Vietnam Military Medical University. These were housed in a controlled environment of temperature and relative humidity, with photo periods of 12/12h light-dark cycles. Provided with pelleted diet, filtered water *ad libitum*.

3. Study design.

For 90-day repeated dose oral toxicity study healthy adult Wistar rats of both sexes,body weights were within the range of 160 - 180 g. OECD Guideline 408 [3] was followed for the study.All animals of

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each sex were randomly divided into 3 groups: Group 1 was treated as control group; groups 2 and 3 were treated with 0.42 g/kg/day (equivalent therapeutic dose) and 1.26 g/kg/day (3 times the therapeutic dose) of TCBC, respectively. Distilled water (group 1) and TCBC (group 2 and 3) were administered orally for 90 days. General health condition, body weight, signs of toxicity and mortality were monitored throughout the study period. The blood samples were collected on day 0, 30, 60 and 90 for all groups. The blood samples were analyzed for hematological (red blood cell [RBC], hematocrit [HCT], hemoglobin concentration [HBG], mean corpuscular volume [MCV], total white blood cell [WBC] and platelets [PLT]) using Humancout 30TS (Human, Germany) hematology analyzer and biochemical (cholesterol, alanine aminotransferase [ALT], aspartate transaminase [AST], creatinine and albumin) parameters using 3000 Evolution (Biochemical Systems International Srl, Italia) chemistry analyzer.

After blood collection on day 90, animals were sacrificed and subjected to gross necropsy. Once gross necropsy is done the organs like liver, kidney, spleen were surgically removed and stored for histopathological studies.

For androgenic activity study: Immature male Wistar rats (42 days of age) were castrated by method described by Ottani et al [5] and were divided into 4 groups of ten rats each. The vehicle control (negative control) group received daily oral administration of either distilled water; two treatment groups received daily oral administration TCBC dose 0.42 and 0.84 g/kg body weight (TCBC 0.42 g/kg and TCBC 0.84 g/kg groups) and the positive control group received daily subcutaneous injection testosterone propionate 0.2 mg/kg (TP 0.2 mg/kg group). The treatment was given for 10 days and necropsy studies were carried out on 11th day of treatment.

After 10 days of treatment, animals were sacrificed and the seminal vesicles, the ventral prostate, Cowper's gland, glans penis and levator ani-bulbocavernosus (LABC) muscle were carefully dissected and weighed. OECD Guideline 441 [2] was followed for the study.

** Statistical analysis:*

The results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and post hoc least-significant differences (LSD) test were used to determine statistical difference between groups. $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using SPSS 16.0 software.

RESULTS

1. 90-day repeated dose oral toxicity.

** General health condition, signs of toxicity and mortality:*

There were no treatment-related changes in general clinical signs or unscheduled deaths in any group.

** Effect on the body weights:*

There were no significant differences in body weight between treated and control groups during the study.

* *Hematology:*

Table 1: Effects of 90-day oral administration of TCBC on hematological parameters in rats.

	Parameters	Control	TCBC 0.42 g/kg	TCBC 1.26 g/kg
Day 0	RBC (10^{12}/L)	7.78 ± 0.49	8.16 ± 1.44	7.81 ± 0.75
	HGB (g/dL)	130.30 ± 8.19	135.40 ± 27.43	136.60 ± 19.09
	HCT (%)	40.61 ± 2.38	41.26 ± 6.81	41.50 ± 4.21
	MCV (fL)	52.30 ± 2.11	50.10 ± 2.18	52.80 ± 1.40
	WBC (10^9/L)	11.62 ± 2.06	11.14 ± 1.99	11.59 ± 4.55
	PLT (10^9/L)	715.30 ± 120.95	561.90 ± 180.47	649.20 ± 103.09
Day 30	RBC (10^{12}/L)	8.74 ± 0.68	9.00 ± 1.26	8.55 ± 0.77
	HGB (g/L)	146.00 ± 9.96	143.80 ± 14.51	145.10 ± 6.90
	HCT (%)	43.26 ± 2.70	43.19 ± 5.33	42.22 ± 2.05
	MCV (fL)	49.60 ± 2.17	48.30 ± 3.43	49.10 ± 1.97
	WBC (10^9/L)	12.51 ± 2.87	12.63 ± 4.55	12.12 ± 2.88
	PLT (10^9/L)	575.40 ± 74.71	625.90 ± 136.33	609.00 ± 119.57
Day 60	RBC (10^{12}/L)	7.99 ± 0.71	8.20 ± 1.21	8.12 ± 1.33
	HGB (g/L)	137.60 ± 15.13	141.40 ± 21.04	144.80 ± 9.89
	HCT (%)	41.96 ± 4.51	42.08 ± 6.72	44.30 ± 3.58
	MCV (fL)	50.90 ± 2.38	51.30 ± 1.89	50.80 ± 1.32
	WBC (10^9/L)	11.23 ± 3.92	11.77 ± 3.41	11.40 ± 3.47
	PLT (10^9/L)	593.10 ± 139.33	637.40 ± 124.89	702.60 ± 100.25
Day 90	RBC (10^{12}/L)	8.83 ± 0.54	9.03 ± 0.96	8.37 ± 0.43
	HGB (g/L)	153.00 ± 9.23	159.40 ± 11.55	148.20 ± 5.98
	HCT (%)	44.51 ± 2.45	43.16 ± 2.56	43.09 ± 2.25
	MCV (fL)	50.40 ± 1.78	48.40 ± 3.17	50.11 ± 1.10
	WBC (10^9/L)	12.74 ± 4.17	12.45 ± 2.48	11.89 ± 3.90
	PLT (10^9/L)	588.60 ± 39.59	609.30 ± 110.42	635.70 ± 150.94

(Each value represents the mean ± standard deviation (n = 10))

In treatment groups, there were no significant changes in any parameters ($p < 0.05$).

** Blood biochemistry:*

Table 2: Effects of 90-day oral administration of TCBC on biochemical parameters in rats.

	Parameters	Control	TCBC 0.42 g/kg	TCBC 1.26 g/kg
Day 0	AST (U/L)	95.30 ± 29.45	96.20 ± 36.64	90.50 ± 25.82
	ALT (U/L)	73.10 ± 20.37	67.30 ± 14.43	71.40 ± 25.29
	Creatinine (mmol/L)	55.80 ± 10.81	56.00 ± 17.74	56.90 ± 13.39
	Albumin (g/L)	33.30 ± 1.34	31.20 ± 2.62	32.10 ± 1.60
	Cholesterol (mmol/L)	1.27 ± 0.25	1.28 ± 0.23	1.22 ± 0.20
Day 30	AST (U/L)	89.70 ± 19.45	88.80 ± 43.30	85.40 ± 8.82
	ALT (U/L)	64.60 ± 16.82	55.90 ± 9.77	61.70 ± 11.82
	Creatinine (mmol/L)	49.20 ± 4.59	46.50 ± 4.35	50.30 ± 4.22
	Albumin (g/L)	32.80 ± 1.99	30.40 ± 3.50	31.70 ± 1.64
	Cholesterol (mmol/L)	1.02 ± 0.41	1.05 ± 0.42	1.10 ± 0.51
Day 60	AST (U/L)	101.20 ± 34.15	104.60 ± 39.92	104.20 ± 42.71
	ALT (U/L)	72.50 ± 23.21	64.80 ± 19.83	77.10 ± 26.76
	Creatinine (mmol/L)	45.80 ± 10.96	45.00 ± 20.79	47.70 ± 5.95
	Albumin (g/L)	31.10 ± 0.74	30.60 ± 3.69	33.00 ± 1.70
	Cholesterol (mmol/L)	1.08 ± 0.42	1.31 ± 0.16	1.20 ± 0.16
Day 90	AST (U/L)	87.20 ± 25.59	85.20 ± 14.14	82.20 ± 10.81
	ALT (U/L)	66.80 ± 17.90	63.50 ± 18.11	66.50 ± 10.41
	Creatinine (mmol/L)	46.00 ± 11.69	48.70 ± 9.29	47.60 ± 5.56
	Albumin (g/L)	30.70 ± 3.68	31.90 ± 1.52	32.30 ± 2.41
	Cholesterol (mmol/L)	1.14 ± 0.26	1.24 ± 0.16	1.23 ± 0.18

(Each value represents the mean ± standard deviation) (n = 10)

In treatment groups, there were no significant changes in any parameters ($p < 0.05$).

** Histopathology:*

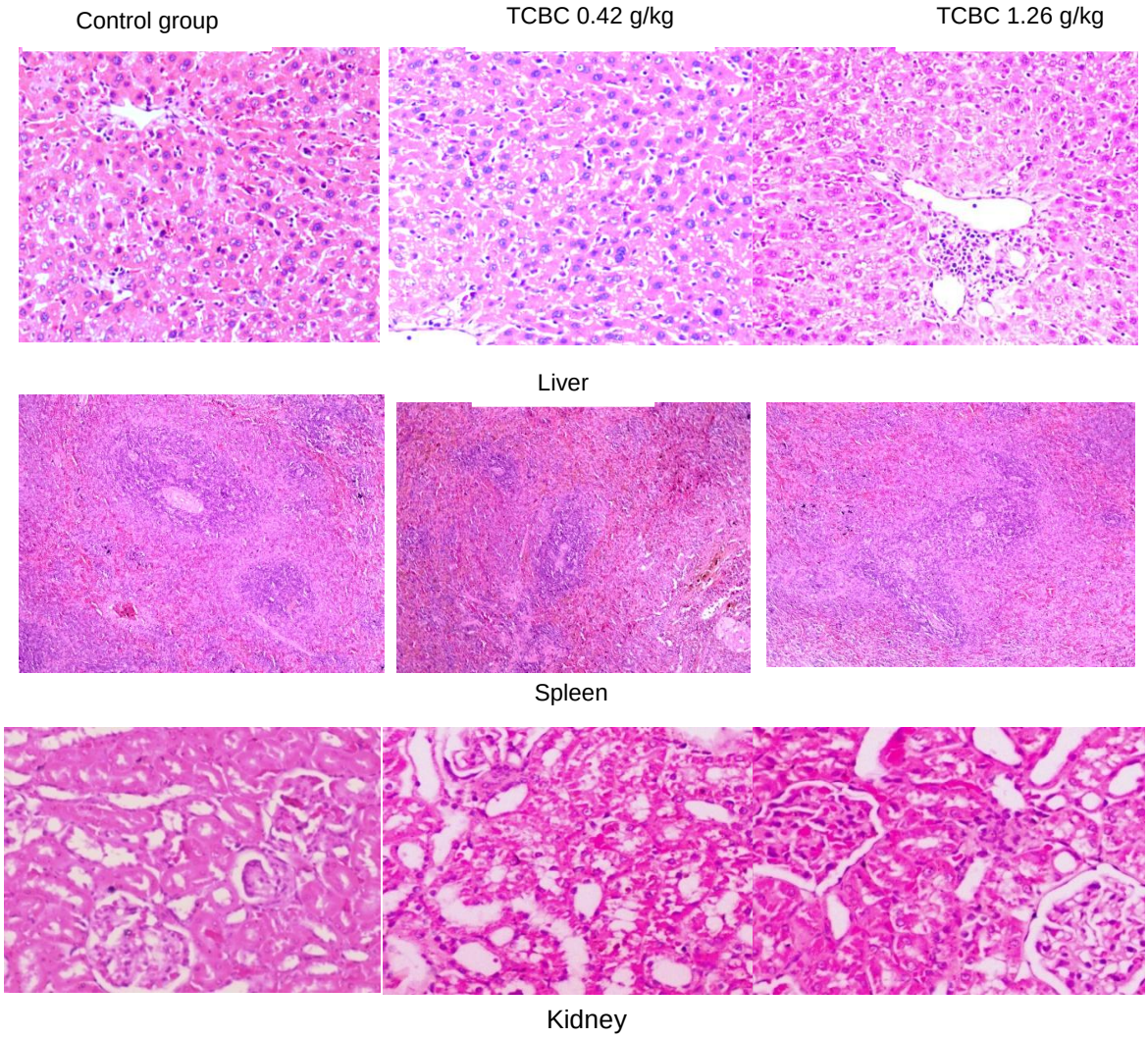

Fig. 1: Histopathological examination of liver, spleen and kidney (HE x400).

In all groups, there were no treatment-related histological changes in any organs or tissues.

2. Androgenic activity.

Table 3: Effect of TCBC on the relative weights of androgen-dependent accessory sex organs in castrated rats.

Organs	Group			
	Vehicle control	TP 0.2 mg/kg	TCBC 0.42 g/kg	TCBC 0.84 g/kg
Seminal vesicles (g/100 g bw)	0.273 ± 0.047	0.403 ± 0.104*	0.354 ± 0.089*	0.368 ± 0.082*
Ventral prostate (g/100 g bw)	0.098 ± 0.022	0.123 ± 0.023*	0.116 ± 0.021	0.114 ± 0.017

Cowper's glands (g/100 g bw)	0.019 ± 0.011	0.031 ± 0.009*	0.028 ± 0.006*	0.029 ± 0.005*
Glans penis (g/100g bw)	0.031 ± 0.013	0.033 ± 0.010	0.035 ± 0.007	0.034 ± 0.010
LABC (g/100 g bw)	0.350 ± 0.030	0.398 ± 0.050*	0.392 ± 0.043*	0.394 ± 0.043*

*(Each value represents the mean ± standard deviation (n = 10); *: Significantly different from vehicle control at p < 0.05; bw: Body weight)*

TCBC induced a significant increase ($p < 0.05$) in the relative weights of seminal vesicles, Cowper's glands and LABC.

DISCUSSION

The growing use of the modern production from traditional medicine in the present age has necessitated the thorough evaluation of safety and efficacy of the modern production from traditional medicine. Keeping in view the necessity of evaluation of safety and efficacy of the modern production from traditional medicine, the present chronic toxicity study and the androgenic activity were carried out for the TCBC. The present 90-day chronic toxicity study, the androgenic activity study was done to evaluate the possible health hazard likely from repeat exposure over a relatively long period of time and efficacy of the TCBC.

None of the rats belonging to different treatment groups showed any signs and symptoms of toxicity during cage side observation (results not shown). There were no changes in their skin, fur, eyes when compared to the control group. No signs of toxicity and mortality were observed on any the administered doses when compared with the control group. All the treated groups gained weight during the study period and absence of significant changes in body weight suggested that the TCBC was fairly nontoxic. Gross necropsy finding also did not reveal any adverse effect on organs and hence TCBC is said to be safe.

Blood is an important physiological and pathological status in animals and human. Normally, the parameters measured are RBC, PLT, WBC, HGB, HCT, MCV. Toxicity induced by drugs can alter the normal range of these hematological parameters [1]. The hematological parameters of rats treated with TCBC 0.42 g/kg and 1.26 g/kg were comparable to that of the control group.

Analysis of serum biochemical parameters is relevant for risk evaluation, as any changes in the biochemical system have higher predictive value for human toxicity when data are transferred from animal studies to human. The biochemical parameters of rats treated with different doses of TCBC were comparable to that of the control group.

The results of the study suggested that chronic administration of the TCBC produced no significant toxic effects on Wistar rats.

About androgenic activity: This study is an attempt to investigate the effect of TCBC on the castrated rat, which is a convenient *in vivo* model for studying the

androgenic activity. In the treatment groups, there were significant increases in the relative weights of seminal vesicles, Cowper's glands and LABC when compared to that of the vehicle control group. Hershberger assay was designed to evaluate the endocrine disruption activity of chemical by focusing on single mechanism of androgen. Increase in the weights of these androgen-dependent organs could be due to the increased in androgen biosynthesis and the antioxidant effect of the components of the product [4, 6].

CONCLUSION

Truong Xuan CB capsule 0.42 g/kg and 1.26 g/kg body weight oral administration are safe in Wistar rat. TCBC 0.42 g/kg and 0.84 g/kg body weight have an androgenic activity.

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