

The Spermatogenic Effect of 50% Ethanol Extracts of Yacon and Its Ameliorative Effect Against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin Induced Testicular Toxicity in the Rat

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Abstract – The authors screened the pharmacological effects of 50% ethanol extracts of Yacon on spermatogenesis in rats. Numbers of sperm in animals treated with 25, 50, or 100 mg/kg/day for 6 weeks of Yacon tuber extracts (YTE) were approximately 1.51, 1.61 and 1.78 times higher, respectively, than in the untreated control group. Moreover, the spermatogenic effect of Yacon leaf extract was found to be 1.03~1.38 times higher than that of YTE. The ameliorative effect of Yacon tuber extracts on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced toxicities in the rat were also investigated. Rats were assigned to three groups (6 rats/group), a control group, a TCDD exposed group, and a group treated with Yacon tuber extract (YTE) after TCDD exposure (TCDD/YTE group). 40 µg/kg of TCDD was injected i.p., and 200 mg/kg/day of YTE was also administered for 4 weeks by oral gavage. The TCDD/YTE group showed a significant increase in sperm number as compared with the TCDD exposed group. In conclusion, TCDD induced testicular toxicity was significantly ameliorated by YTE. The results of the present study suggest that Yacon extract is a possible therapeutic for the treatment of spermatogenic disorder.

Keywords – *Smallanthus sonchifolius*; Yacon; spermatogenesis; sperm; TCDD; testicular toxicity

Introduction

Male reproductive function seems to have deteriorated considerably in the past 4 to 5 decades. Carlsen *et al.* (1992) observed a significant decline in mean sperm concentrations from $113 \times 10^6/\text{ml}$ in 1940 to $66 \times 10^6/\text{ml}$ in 1990; a fall of $0.94 \times 10^6/\text{ml}/\text{year}$. The World Health Organization defines infertility as the inability of a couple to conceive after 1 year of regular, unprotected intercourse (WHO, 1995). Moreover, approximately 15% of all couples who attempt to conceive fail to do so within the first year (WHO, 1991).

A number of plants, e.g., *Withania somnifera*, *Cynomorium coccineum*, *Macuna pruriens* have been reported to increase the production of sperm (Abdel-Magied *et al.*, 2001; Ahmed *et al.*, 2007). These plants usually have several pharmacologic effects, such as anti-stress, adaptogenic, and cardioprotective effect.

Smallanthus sonchifolius (Yacon, Asteraceae) was

originally cultivated in South America and has gradually received more attention due high contents of fructooligosaccharide and phenolic compounds. The tubers of Yacon contain fructose, glucose, saccharose and β -(2-1)-fructooligosaccharide (inulin type oligofructans) (Ohyama *et al.*, 1990; Goto *et al.*, 1995). Valentova *et al.* (2003) reported on the presence of large amounts of phenolic compounds in extracts of Yacon leaves and tubers, and these were primarily found to contain chlorogenic, protocatechuic, ferulic, rosmarinic, gallic, gentisic, and caffeic acids and their derivatives (Simonovska *et al.*, 2003; Valentova *et al.*, 2003). Moreover, five caffeic acid derivatives were isolated from Yacon tubers (Takenaka *et al.*, 2003). With regard to its biological activity, Yacon has been reported to have a hypoglycemic effect (Manual *et al.*, 2001), antioxidant activity (Valentova *et al.*, 2003; Yan *et al.*, 1999), and an anti-fungal effect (Inoue *et al.*, 1995). In this study, we screened the pharmacological effects of Yacon extracts on spermatogenesis in rats.

Furthermore, we investigated the ability of Yacon extracts to treat 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

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induced spermatogenic disorder. TCDD and its related congeners have been shown to act as developmental and reproductive toxicants (Peterson *et al.*, 1993; Sommer *et al.*, 1996; Chaffin *et al.*, 1996), which reduce testicular and accessory sex organ weights, alter testicular morphology, and decrease sperm production (Mably *et al.*, 1992). The present study was conducted to evaluate the possibility of using Yacon extracts as a therapeutic agent to treat spermatogenic disorders caused by endocrine disruptors, like dioxin.

Material and Method

Preparation of Yacon extracts – The Yacon tubers and leaves were purchased from Bongwha (Kyungbuk, Korea) and authenticated by Dr. Hwang a botany Professor at the College of Pharmacy, Chungbuk National University (Cheongju, Korea). The dried, pulverized tubers or leaves were extracted with 50% absolute alcohol at 50 °C for 5 hr with sonication and then filtered through filter paper (Whatman No. 1). Extracts were evaporated and dried under vacuum at –70 °C, to yield dark brown residues.

HPLC analysis of the phenolic acids of Yacon extracts – Yacon tuber extract (YTE) was analyzed by HPLC (Hitachi L-6000 pump, L-6200 intelligent pump, L-4200 UV-VIS Detector and D-2500 Chromato-integrator) using a Kromasil C₁₈ column (250 × 4.6 mm). The mobile phase consisted of phase A (10% (v/v) CH₃CN in water, 0.05% (v/v) of acetic acid) and phase B (90% (v/v) CH₃CN in water, 0.05% (v/v) of acetic acid). Elution was performed using the linear gradient detailed in Table 1. The injection volume was 25 µL and phenolic acid elution was monitored at 236 nm.

Animal model – Six-week-old Sprague-Dawley albino male rats (Samtako, Kyungkido, Korea), weighing 200 g, were used to examine levels of spermatogenesis. Animals were maintained under controlled conditions [temperature (23 ± 1 °C), relative humidity (55 ± 10%), and 12-h/day lighting]. Standard laboratory chow and tap water were provided *ad libitum*.

Administration of Yacon extracts – The Yacon tuber or leaf extracts were dispersed in purified water. Rats were assigned randomly to weight matched groups (9 rats/group) and extract was administered after allowing them a week to acclimatize. The rats were dosed once daily, by oral gavage at 0 (control), 50, 100 and 200 mg/kg/day for 6 weeks. The volume administered was 2 ml/kg of body weight. Body weights were recorded before starting this administration and then at every 3 or 4 days,

Table 1. Gradient conditions of mobile phase used to analyze phenolic acids

Time	Phase A	Phase B	Flow Rate
0 min	100	0	0.5
39 min	0	100	0.5
40 min	0	100	1.0
41 min	100	0	1.0
46 min	100	0	1.0
47 min	100	0	0.5

and on the day of necropsy.

Induction of oligospermia by TCDD in rats, and the ameliorative effect of YTE – YTE (Yacon tuber extract) was also administered to rats in order to determine the ameliorative effect of this extract on TCDD induced oligospermia. Initially, rats were injected with 40 µg TCDD/kg i.p. At one week after TCDD exposure, YTE was administered to rats once daily, by oral gavage at 200 mg/kg/day for 4 weeks. Body weights and daily food intakes were measured every 3 or 4 days. Control, TCDD exposed, and YTE treated after TCDD exposure (Yacon treated) groups were sacrificed at 5 weeks after TCDD exposure.

Sperm counts – Male rats were weighed and anesthetized with ether on day following final dosing. Right testes were weighed and used for sperm analysis. Numbers of sperm heads in testes were counted using a slight modification of the procedure reported by Toth *et al.* (1989). Briefly, tunica albuginea of testes were removed and remaining tissues were placed in 10 mL of 0.9% saline, and homogenized twice for 30 seconds, and then ultrasonicated for 3 minutes. Homogenates were then diluted to a suitable concentration. 10 µL aliquots of these mixtures were placed in a hemocytometer and covered with a cover glass. Sperm numbers were counted using an optical microscope (Nikon, YS 100).

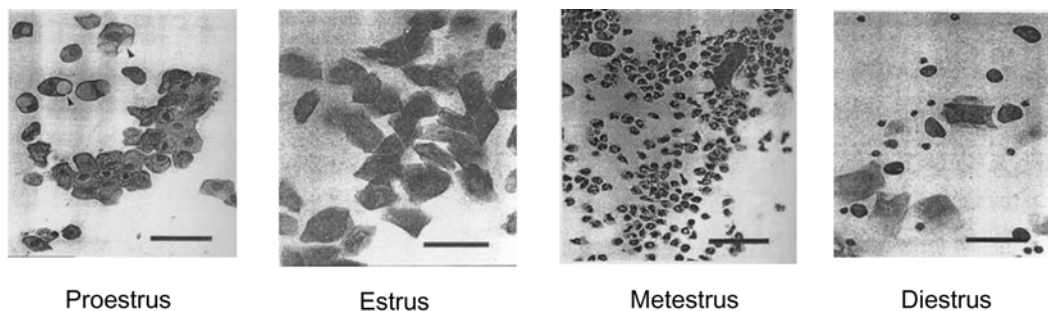
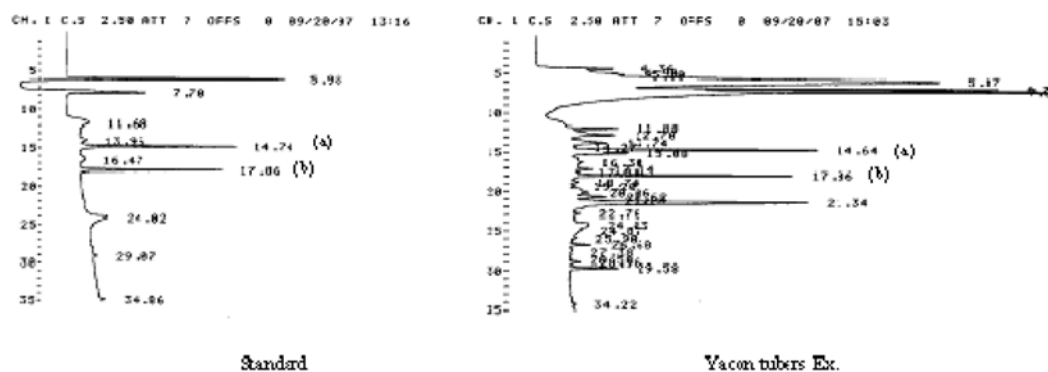
Testicular histology – Left testes were fixed in Bouin's fluid, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin wax. The sections were cut at 5 µm, stained with Harris' hematoxylin and eosin, and observed under a microscope.

Serum testosterone measurements – Serum total testosterone levels were estimated using solid phase (antibody-coated tube) RIA, using materials purchased from the Diagnostic Products Corporation (CA, USA) according to the manufacturer's instructions.

Influence of YTE on the estrous cycle – The influence of YTE on the estrous cycle was investigated by administering YTE at dose of 0 (control) or 200 mg/kg/

Table 2. Microscopic characteristics of vaginal smears as a function of the sexual cycle

Phase	Duration days	Microscopic characteristics of vaginal smears
Diestrus	1-3	Exclusively leukocytes
Proestrus	~ 1	Leukocytes and nucleated epithelial cell
Early estrus	0.5~1	Epithelial cells, may have some cornified cell
Estrus	0.5~1	Exclusively cornified cells
Metestrus	~ 1	Leukocytes and cornified cell

**Fig. 1.** Vaginal smears, day of proestrus, estrus, metestrus and diestrus respectively, in rat. Bar = 50 μ m (Ref. Fundamentals of Toxicologic Pathology, Academic Press, pp 491-494).**Fig. 2.** HPLC chromatograms of standard solutions and YTE. (a); chlorogenic acid, (b); caffeic acid.

day. After 8 weeks, the estrous cycle of each animal was checked using the method described by Yamazaki *et al.* (1977). Vaginal smears of rats were obtained before 9:00 AM, applied to a slide glass, and observed under an optical microscope. Three main cell types were used to classify the estrous cycle, i.e., leukocytes, epithelial cells and cornified epithelial cells. Fig. 1 and Table 2 show the characteristic of the estrous cycle.

Statistics – Data was analyzed using one-way ANOVA followed by Dunnett's test as a post hoc test using SigmaStat[®]. Differences were considered statistically significant at the $*p < 0.05$ and $** < 0.01$ levels, as indicated.

Results

HPLC analysis of phenolic acids in YTE – Simonovska *et al.* (2003) reported that crude extracts of Yacon leaves contain phenolic acids, i.e., chlorogenic acid, ferulic acid, and caffeic acid, by HPLC/MS analysis. In the present study, chlorogenic acid and caffeic acid were found to be major components of YTE (Fig. 2). The peak shown in the figure at a retention time of 21.34 min was presumed to be a dicaffeoylquinic acid by LC-MASS (not shown data). The position of caffeoyl group is going on study by NMR. The amount of crude extract obtained from Yacon tubers (10 g) using 50% ethanol was 7.31 g, and the amount of chlorogenic acid obtained from 10 mg

Table 3. Body, testes and epididymides weights after administering YTE to rats for 6 weeks

	Body Weight (g)	Testis(g)	Epididymis (g)
Control	404.44 ± 63.86	3.200 ± 0.169	1.144 ± 0.058
YTE 50mg/kg	415.56 ± 27.44	3.145 ± 0.252	1.111 ± 0.070
YTE 100mg/kg	378.89 ± 31.00	3.185 ± 0.303	1.103 ± 0.119
YTE 200mg/kg	401.25 ± 29.49	3.175 ± 0.177	1.080 ± 0.095

Data are presented as means ± SD (n = 9).

Table 4. Sperm numbers in testis after administering YTE to rats for 6 weeks

	Testis (g)	Sperm/Rat ($\times 10^6$) ^{a)}	Sperm/g ($\times 10^6$)	Sperm/day ($\times 10^6$) ^{b)}
Control	3.200 ± 0.169	234.15 ± 40.07	73.17 ± 12.52	37.70 ± 6.67
YTE 50mg/kg	3.145 ± 0.252	347.13 ± 88.95*	110.38 ± 28.28	59.48 ± 13.24
YTE 100mg/kg	3.185 ± 0.303	375.59 ± 57.65**	117.92 ± 18.10	61.57 ± 9.45
YTE 200mg/kg	3.175 ± 0.177	409.77 ± 47.24**	130.03 ± 15.44	66.70 ± 8.00

b) a)/6.1, 6.1 is the period required for spermatids to develop from stage I to X IV. Data are presented as means ± SD (n = 9).

*, ** significantly different from the controls (ANOVA test, * p < 0.05, ** p < 0.01).

Table 5. Sperm numbers in testis and seminiferous tubule (ST) numbers and diameters after administering YLE to rats for 6 weeks

	Control	YLE 50 mg/kg	YLE 100 mg/kg	YLE 200 mg/kg
Testis Weight (g)	3.633 ± 0.327	3.793 ± 0.468	3.732 ± 0.427	3.671 ± 0.359
Sperm No/g ($\times 10^6$)	72.75 ± 18.31	113.42 ± 8.99**	135.11 ± 16.61**	179.30 ± 16.62**
ST Number	74.00 ± 6.13	78.00 ± 3.39	81.67 ± 3.01	84.40 ± 4.22
ST Diameter (μ m)	28.73 ± 0.44	27.40 ± 0.24	26.65 ± 0.44	27.79 ± 0.36

Data are presented as means ± S.D.(n = 9).

** significantly different from the controls (ANOVA test, ** p < 0.01).

of YTE was 56.52 mg.

The effects of YTE on body, testis and epididymis weights – Body, testes, and epididymides weights in the three groups at the end of the treatment period were similar to those of non-treated controls, suggesting that YTE induced a dose dependent increase in spermatogenesis without altering testes and epididymides weights (Table 3).

Sperm counts after Yacon extract administration – As shown in Table 4, animals administered YTE showed significant dose dependant increases in testes sperm counts. Numbers of sperm heads in the 50, 100, and 200 mg/kg/day groups were approximately 1.51, 1.61 and 1.78 times higher, respectively than those of the control group. Moreover, those treated with Yacon leaf extract (YLE) were approximately 1.56, 1.86 and 2.46 times higher, respectively, than those of the control group (Table 5). In addition, seminiferous tubule numbers dose-dependently increased, but tubule sizes were unchanged versus the control. Thus, the spermatogenic effect of YLE was 1.03~1.38 times higher than that of YTE (Fig. 3). In

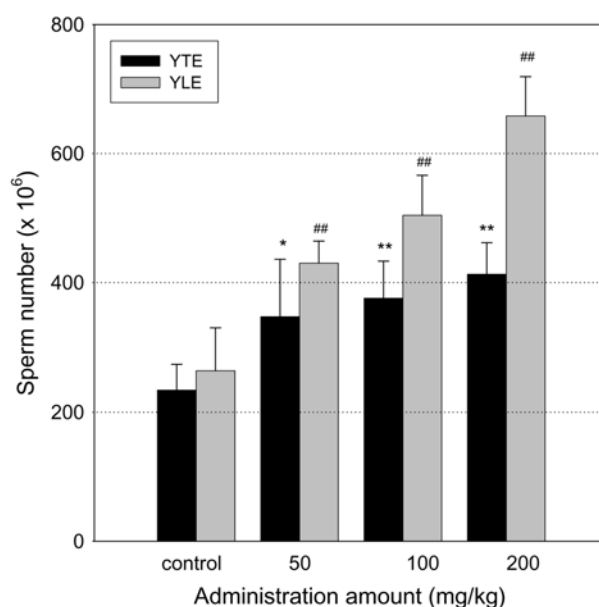


Fig. 3. Sperm numbers in testes after administering YTE or YLE to rats for 6 weeks. Data are presented as means ± SD (n = 9). *, ** significantly different from the controls (ANOVA test, * p < 0.05, ** p < 0.01), #, ## p < 0.05, ** p < 0.01).

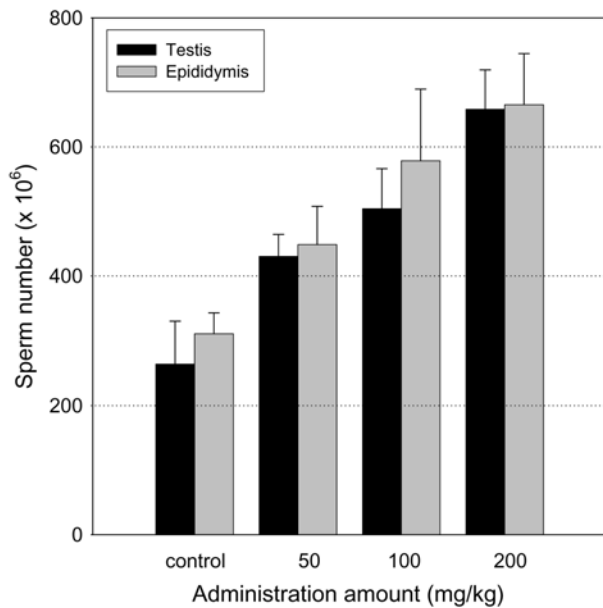


Fig. 4. Sperm numbers in testes and epididymides after administering YLE to rats for 6 weeks. Data are presented as means \pm SD (n=9). No significant difference was observed between sperm numbers in testis and epididymis.

YLE administered animals, numbers of sperm heads in epididymes tended to be higher than in testis, but this was without significance (Fig. 4).

The effect of YTE on serum testosterone levels – Mean serum testosterone levels were significantly higher in animals treated with 200 mg/kg/day of YTE than in controls (5.09 ± 2.53 ng/ml vs. 1.66 ± 1.08 ng/mL, respectively).

Influence of YTE on the estrous cycle of female rats – In male rats, serum testosterone levels in the YTE treated groups were significantly higher than in the control group. Accordingly, we examined the estrous cycle administering YTE orally at 200 mg/kg for 8 weeks to estimate the effect of YTE in female rats. YTE was found to have no significant difference on the estrous cycle versus untreated controls, suggesting that YTE did not affect the estrous cycle.

Sperm counts in rats administered YTE after TCDD exposure – As shown in Fig. 5, TCDD exposed rats showed body weight reductions at one week after TCDD exposure compared with the control group. However, this weight loss was reduced by treating rats with TCDD/YTE, i.e., with YTE after TCDD exposure. The TCDD exposed group also showed significant testis and epididymides weight loss versus untreated controls (Table 6). On the other hand, TCDD/YTE treated rats showed lower weight losses body, testis, and epididymides versus TCDD treated rats, and YTE treatment also

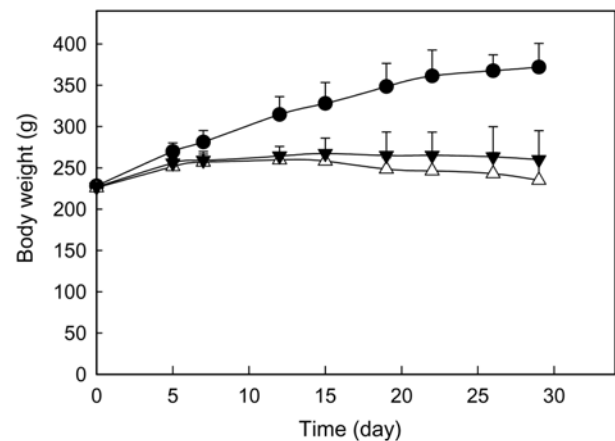


Fig. 5. Effect of YTE on male rat body weights. Rats were exposed at age 7 weeks to 40 μ g/kg TCDD and body weights were recorded over the following 4 weeks. Data are presented as means \pm SD (n=6).

Key : (●); Control, (▼); TCDD + YTE (200 mg/kg/day), (△); TCDD alone

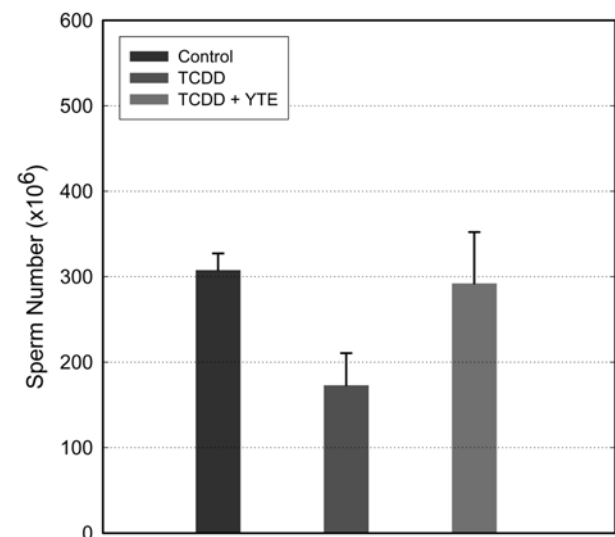


Fig. 6. Sperm numbers in epididymis. 200 mg/kg/day of YTE was administered for 4 weeks after exposure to 40 μ g/kg of TCDD. Data are presented as means \pm SD (n=6). #, * significantly different from the untreated controls (ANOVA test, #, * p < 0.05).

increased seminiferous tubule numbers and diameters (Table 6), and significantly increased sperm numbers in testes (Table 7 & Fig. 6). These findings indicate that YTE has an ameliorative effect on TCDD-induced; weight loss, reduced sperm production, and testicular atrophy.

Testes Histopathology in TCDD/YTE treated rats – In the control group, histopathologic examinations of seminiferous tubules showed the usual arrangement of Leydig's cell, Sertoli cells, and intracellular spaces (Fig. 7, a). However, in TCDD-treated rats, cell differentiation, including that of spermatogonia, tended to be lower than

Table 6. Organ weights and seminiferous tubule numbers and diameters (ST) after administering YTE to TCDD exposed rats

	Control	TCDD alone	TCDD + YTE
Body weight (g)	353.33 ± 28.75	220.00 ± 24.49*	235.00 ± 35.00
Testis (g)	3.48 ± 0.28	2.82 ± 0.17*	3.00 ± 0.42
Epididymis (g)	1.02 ± 0.13	0.82 ± 0.08*	0.91 ± 0.06
Number of ST	85.67 ± 2.94	79.2 ± 6.83	81.6 ± 2.70
Diameter of ST (µm)	28.3 ± 0.37	26.53 ± 0.29	27.26 ± 0.21

Data are presented as means ± SD (n = 6).

* significantly different from the controls (ANOVA test, * p < 0.05).

Table 7. Sperm numbers in epididymides after administering YTE to TCDD exposed rats

	Epididymis (g)	Sperm/Rat (× 10 ⁶) ^{a)}	Sperm/g (× 10 ⁶)	Sperm/day (× 10 ⁶) ^{b)}
Control	1.02 ± 0.13	307.62 ± 19.52 [#]	301.58 ± 19.14	50.43 ± 3.20
TCDD alone	0.82 ± 0.08	172.93 ± 37.56 ^{#,*}	210.89 ± 45.80	28.35 ± 6.16
TCDD + YTE	0.91 ± 0.06	292.19 ± 59.95*	321.09 ± 65.88	47.90 ± 9.83

b) a)/6.1, 6.1 is the period required for spermatids I to X IV develop from stage to.

Data are presented as means ± SD (n = 6).

[#], * significantly difference from the controls (ANOVA test, [#],* p < 0.05)

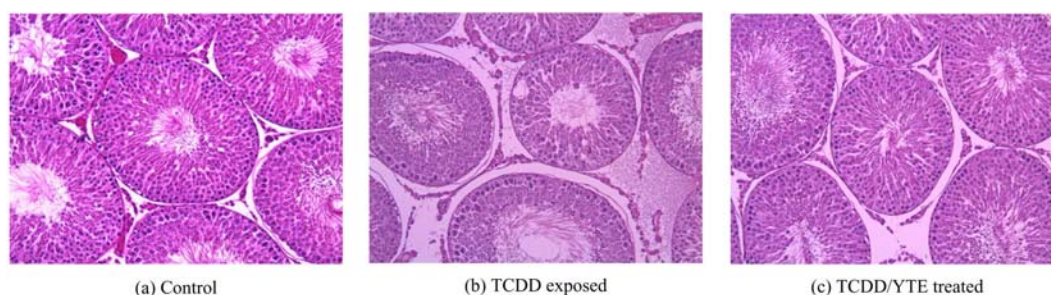


Fig. 7. Effects of YTE on testis histology after exposure to TCDD. H & E. Original magnification approximately × 200. In TCDD treated rats (b), a decrease in cell differentiation was observed in most cells, including spermatogonia, as compared with untreated controls. In TCDD/YTE treated rats (c), sperm development was higher than in untreated rats after TCDD exposure. (a) Control, (b) TCDD exposed, (c) TCDD/YTE treated rats.

in controls (Fig. 7, b), maturation levels of spermatocytes and spermatids were also lower. In TCDD/YTE treated animals (Fig. 7, c), all sperm developmental stages had almost completely returned to the control level (Fig. 7, b), and sperm development was higher than in TCDD treated animals.

Discussion and Conclusions

Decreases in human sperm number and quality caused by endocrine disrupter, particularly dioxin, are of considerable concern. TCDD, a derivative of dioxin, is known to be one of the most potent toxic environmental pollutants (Poland and Knuston, 1982). It is highly lipophilic and extremely resistant to biodegradation, which leads to TCDD accumulating in adipose tissue and

being recycled via the food chain. It seems to impossible substantial protection from the environmental pollutants. Many scientists have attempted to screen the agent that can protect the toxic damage induced by endocrine disruptor. We reported on the protective effect of tissue cultured *Panax ginseng* against TCDD-toxicity (Park *et al.*, 2006).

In the present study, Yacon extract was administered orally to rats over a 6-week period, and it was found that sperm numbers in testis were significantly higher than in the controls. The final body, testis and epididymis weights of all groups were similar to those of the controls. Our findings suggest that YTE dose-dependently affects spermatogenesis without altering testis and epididymis weights

Spermatogenesis is affected by a wide variety of

nutrients and enzymes, such as Vitamins (C, E, B12) (Rolf *et al.*, 1999; Sandler and Faragher, 1984), minerals (Zinc, Copper, Selenium, Calcium) (Scott *et al.*, 1998), amino acids (Schacter *et al.*, 1973), Coenzyme Q10 (Lewin and Lavon, 1997). However, no reports have been issued on the spermatogenic activities of polyphenols, like chlorogenic acid and caffeic acid.

Polyphenols have antioxidant activities that are similar to those of Vitamins C and E, which can enhance fertility by decreasing free-radical damage to sperm cells (Fraga *et al.*, 1991; Geva *et al.*, 1996). In the present study, chlorogenic and caffeic acids were found to be the major components of YTE. Accordingly, we estimated the spermatogenic effect of chlorogenic acid, but found that it had a substantially lower effect than expected (unpublished data). Thus, the spermatogenic effects of Yacon extract are possibly due to the combined effects of chlorogenic acid and other phenolic components.

Yacon tubers also contain fructose, glucose, saccharose and β -(2-1)-fructo oligosaccharide (Ohyama *et al.*, 1990; Goto *et al.*, 1995). Seminal plasma also contains high concentrations of fructose, which is essential for normal sperm metabolism and also serves as a nutrient for spermatozoa during their journey in the female genital tract (Schoenfeld *et al.*, 1979). In the present study, YTE administration to male rats increased serum testosterone levels and numbers of sperm in the testis. Testosterone is synthesized by the Leydig cells of the testes, and promotes the growth and function of the epididymis, vas deferens, prostate, seminal vesicles and penis, whereas estrogens play a central role in female reproduction, but also affect the male reproductive system (Hess *et al.*, 1997). We also found that Yacon tuber extract increased spermatozoa count, serum testosterone and estradiol levels in men (unpublished data).

It has been reported that male rats exposed to TCDD show adverse effects such as weight losses of the testicles, seminal vesicles and prostate glands, and changes in the form of the testes (Johnson *et al.*, 1992; Rune *et al.*, 1991). In the present study, we investigated the ameliorative effect of YTE on the toxicity of TCDD in rats. TCDD (40 μ g/kg) caused a significant decrease the weights of testes and epididymis (Table 6). On the other hands, testicular weight in YTE treated rats was greater than in non-treated controls (Table 6) and significant increased daily sperm production (Table 7, Fig. 6). These results suggest that YTE has promotes testicular development, and thus, affects sperm production. We propose that YTE might ameliorate the testicular damage cause by environmental toxins.

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