

The Spermatogenic Effect of Yacon Extract and Its Constituents and Their Inhibition Effect of Testosterone Metabolism

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Abstract

We screened the pharmacological effects of a 50% ethanol extract of Yacon tubers and leaves on spermatogenesis in rats. As a result, we found that Yacon tuber extracts increased sperm number and serum testosterone level in rats. It has been reported that the crude extract of Yacon tubers and leaves contain phenolic acids, such as, chlorogenic acid, ferulic acid and caffeic acid by HPLC/MS analysis. We were interested in the contributions made by phenolic acid, particularly chlorogenic acid of Yacon tuber extract to the spermatogenic activity. After administering Yacon tuber extract or chlorogenic acid to rats for 5 weeks, numbers of sperm in epididymis were increased by 34% and 20%, respectively. We also administered ferulic acid, which has been reported to be a metabolite of chlorogenic acid and a constituent of Yacon tuber extract to investigate its spermatogenic activity in rats. Yacon tuber extract and ferulic acid increased sperm numbers by 43% and 37%, respectively. And, Yacon tuber extract, and chlorogenic acid showed significantly inhibition effect of testosterone degradation in rat liver homogenate. We considered that the spermatogenic effect of Yacon tuber extract might be related to phenolic compounds and their inhibitory effect of testosterone degradation. Yacon showed the possibility as ameliorable agents of infertility by sperm deficiency and late onset hypogonadism syndrome with low level of testosterone.

Key Words: *Smilax sonchifolius*, Sperm, Spermatogenesis, Chlorogenic acid, Testosterone

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 counts from $113 \times 10^6/\text{ml}$ in 1940 to $66 \times 10^6/\text{ml}$ in 1990; a fall of $0.94 \times 10^6/\text{ml}$ 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).

On the other hand, biologically active free testosterone levels are known to decline with age by 2% to 3% annually in men which may result in andropause symptoms (Harman *et al.*, 2001). The clinical condition associated with low levels of serum testosterone with specific symptoms is called late-onset hypogonadism (LOH) syndrome. The widely recognized clinical signs of LOH syndrome are decreases in libido and sexual desire, decreases in muscle mass and strength, a decrease in bone mineral density, an increase in visceral

fat, loss of memory, anemia, and deterioration of insulin resistance (Comhaire, 2000; Bassil *et al.*, 2009; Zitzmann, 2009). Furthermore, some studies indicated that low testosterone levels are associated with increased risk for the development of type 2 diabetes mellitus or cardiovascular disease (Ding *et al.*, 2006; Khaw *et al.*, 2007; Bassil *et al.*, 2009).

The use of testosterone therapy has generated increased attention in recent years, primarily as treatment for men with LOH. The benefits of testosterone therapy include improvements in low libido, erectile dysfunction, fatigue, decreased muscle strength, bone mineral density, increased body fat, anemia, hot flashes, and mood changes. It is balanced by potential adverse effects of BPH, sleep apnea, gynecomastia, erythrocytosis, testicular atrophy, fluid retention and local reaction at injection sites (Shabsigh *et al.*, 2009). Natural therapy in the form of natural herbal supplementation has been the acceptable form of therapy for men with LOH syndrome.

A number of plants, e.g., *Withania somnifera*, *Cynomorium coccineum*, and *Macuna pruriens* have been reported to increase the production of sperm (Abdel-Magied *et al.*, 2001;

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Ahmad *et al.*, 2008). These plants usually also have other pharmacologic effects, such as, anti-stress, adaptogenic, and cardioprotective effects, and have histories of use in folk medicines as, for example, aphrodisiacs and geriatric tonics.

Smallanthus sonchifolius (Yacon, Asteraceae) was originally cultivated in the Andean highlands of South America, and has been used as a food and a medicine by the local population. Its tubers are used as a source of natural sweeteners and syrups, which are used to treat digestive problems, and contain fructose, glucose, saccharose and β -(2-1)-fructo oligosaccharide (inulin type oligofructans) (Ohyama *et al.*, 1990; Goto *et al.*, 1995). Aybar *et al.* (2001) reported that tuber extracts have hypoglycemic effects, and much information is available on the beneficial effects of Yacon roots, in diabetes or as a means of weight reduction. Recently, the antioxidant activity of Yacon tuber extract was studied, and tryptophan and chlorogenic acid were identified as major components (Yan *et al.*, 1999). Both the leaves and tubers of Yacon are rich in phenolic acids (Valentova *et al.*, 2003). Barcellona *et al.* (2012) reported that aqueous extracts of Yacon leaf did not show any acute and subchronic toxicity in rat.

This study aimed at investigating the usefulness of a treatment of hypogonadic men suffering from infertility and LOH. We reported that the extracts of Yacon tuber and leaf have spermatogenic activity in rat (Park *et al.*, 2008) and spermatogenic activity and testosterone level increasing effect in men (Park *et al.*, 2009). Herein, we firstly report on the spermatogenic effect of Yacon constituent, that is, chlorogenic acid and ferulic acid. Furthermore, we found that the spermatogenic effect of Yacon and its constituents attributed to the inhibition effect of testosterone degradation. Yacon showed the possibility as safe ameliorable agents of infertility by sperm deficiency and LOH syndrome with low level of testosterone.

MATERIALS AND METHODS

Preparation of Yacon extract

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

HPLC analysis of phenolic acids of Yacon tuber extract

Yacon tuber extracts (YTE) was analyzed by HPLC (Hitachi L-6000 pump, L-6200 intelligent pump, L-4200 UV-VIS

Detector and D-2500 Chromato-integrator) on an Kromasil C₁₈ column (250x4.6 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 from the column was performed using the linear gradient detailed in Table 1. The injection volume was 25 μ l and the elution of phenolic acids was monitored at 236 nm.

Animal

Six-week-old Sprague-Dawley albino male rats (Samtako, Kyungkido, Korea), weighing 200 g, were used to examine levels of spermatogenesis. The animals were maintained and handled in accordance with a protocol approved by the Institutional Animal Care and Use Committee of Laboratory Animal Research Center at Chungbuk National University.

Administration of YTE

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

Administration of YTE or chlorogenic acid

YTE and chlorogenic acid were dispersed in purified water. Rats were dosed once daily, by oral gavage at 0 mg/kg (control), 200 mg/kg of YTE or 5 mg/kg of chlorogenic acid, 8.85 times amount of chlorogenic acid in 200 mg of YTE, for 5 weeks. The control and chlorogenic acid treated groups were sacrificed at 5 weeks for sperm counting.

Administration of YTE or ferulic acid

YTE or ferulic acid (a metabolite of chlorogenic acid) were dispersed in 0.5% Na-CMC. The rats were dosed once daily by oral gavage at 0 mg/kg (control), 200 mg/kg of YTE, or 5 mg/kg of ferulic acid.

Sperm counts

Male rats were weighed and anesthetized with ether at the next day after final dosing. Right testes were weighed and used for sperm analysis. The number of sperm heads in the testis was determined by counting using a slight modification of the procedure reported by Toth *et al.* (1989). The tunica albuginea of testes were removed and the remaining tissues from each animal were placed in 10 ml of 0.9% saline. Tissues were homogenized twice in a homogenizer for 30 seconds and then ultrasonicated for 3 minutes. Homogenates were then diluted to a suitable concentration. 10 μ l aliquots of these mixtures were then placed on a hemocytometer, covered with a cover glass, and sperm numbers were determined by counting under 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 μ m, stained with Harris' hematoxylin and eosin and observed under a microscope.

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

Time (min)	Phase A	Phase B	Flow rate
0	100	0	0.5
39	0	100	0.5
40	0	100	1.0
41	100	0	1.0
46	100	0	1.0
47	100	0	0.5

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.

Total phenolic content analysis

Total phenolic content in YTE was determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). 50 μ l of YTE in distilled water was mixed with 1ml of Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and maintained at room temperature for 5 min; 1 ml of sodium bicarbonate (75 mg/ml) was added to the mixture. After 90 min at 30°C, absorbance was measured at 725 nm. Results were expressed as chlorogenic acid equivalents.

DPPH assay

DPPH assay was carried according to the method of Brand-Williams *et al.* (1995) with some modification. DPPH solution was prepared at a concentration of 2×10^{-4} mol/L in methanol. During the test, 2 ml of sample was mixed with 2 ml of DPPH solution. The test tube was capped after nitrogen bubbling and placed in the dark. After standing for 60 min at 20°C, absorbance was measured at 517 nm by a UV/VIS spectrophotometer. All samples were made in triplicate, with vitamine E as a positive control.

Cell culture

RAW 264.7 cells were purchased from American Type Culture Collection (Manassas, VA, USA). They were grown in Dulbecco's modified Eagle's media supplemented with 10% FBS, benzylpenicillin potassium (143 U/ml) and streptomycin sulfate (100 μ g/ml) under 37°C and 5% CO₂ atmosphere.

NO assay

RAW 264.7 cells were treated with YTE or chlorogenic acid for 2 hrs and then stimulated with LPS (1 μ g/ml) for 24 hrs. Amounts of nitrite were measured as described previously (Archer, 1993). In brief, aliquots (100 μ l) of the culture media were reacted with 1:1 mixture (100 μ l) of 1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine, and the absorbance values were measured at 540 nm.

Preparation of rat microsomes

Rat microsomal suspensions were prepared by the method reported by Liu *et al.* (2006), with some modifications. Male SD rats were sacrificed and livers were removed and rinsed with cold normal saline solution. Specimens were then minced with scissors and homogenized in a solution composed of 0.32 M sucrose and 1mM dithiothreitol in 0.02 M phosphate buffer (pH 6.5). The homogenate was then centrifuged at 5,000xg, 0°C for 15 min. The supernatants containing microsomal particles were tested for soluble protein by the Lowry method (Lowry *et al.*, 1951) and kept at -70°C until use.

Measurement of enzyme inhibitory activity of Yacon leaf extracts (YLE) on testosterone degradation

Enzyme inhibitory activity of YLE on testosterone degradation was measured according to the method of Matsuda *et al.* (2001) with some modifications. The 3.0 ml reaction solutions each contained 0.2 ml of YLE in 50% ethanol solution, 1.0 ml of 0.02 mM phosphate buffer (pH 6.5), 0.3 ml of freshly

prepared 500 ppm testosterone solution in 50% ethanol solution, and 1.0 ml of microsomal suspension. Reactions were then initiated by the addition of 0.5 ml of 0.77 mg/ml NADPH in phosphate buffer; samples were then incubated at 37°C. 0.5 ml of samples were taken at 0 min, 5 min and 10 min for the measurement of residual testosterone. The reactions were then stopped by adding 2.0 ml dichloromethane, followed by adding 0.1 ml of ibuprofen (250 μ g/ml) in 50% ethanol (as an internal standard for HPLC). Samples were shaken for 10 min, and then centrifuged at 5,000xg for 15 min. Two ml of organic phase was decanted and evaporated to dryness. The residue was redissolved in 0.5 ml methanol and injected into an analytical Vydac C₁₈ column (250x4.6 mm i.d.). The mobile phase used was 45% CH₃CN with a flow rate of 0.8 ml/min and detected by UV absorbance at 242 nm.

Statistics

The data was analyzed using one-way ANOVA followed by Dunnett test as a post hoc test with SigmaStat®. Differences were considered statistically significant when **p* was <0.05 and ***p*<0.01.

RESULTS

HPLC analysis of phenolic acids in YTE

Simonovska *et al.* (2003) reported that the crude extract of Yacon leaves contains phenolic acids, i.e., chlorogenic acid, ferulic acid, and caffeic acid, by HPLC/MS analysis. In the present study, chlorogenic acid was found to be major component of YTE (Fig. 1). 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 of YTE

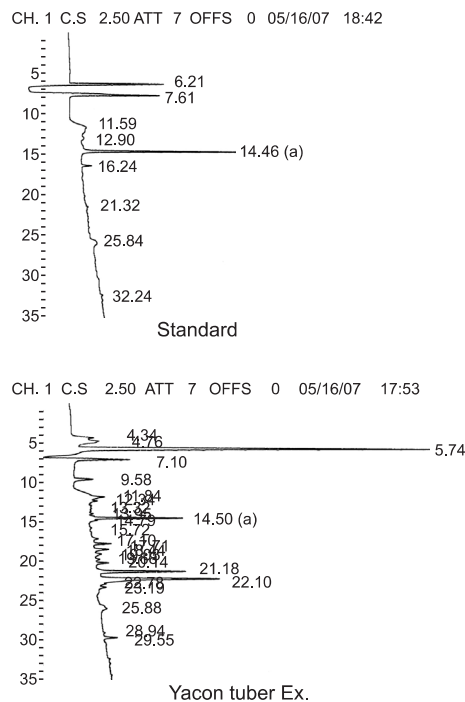


Fig. 1. HPLC chromatogram of standard solution and YTE. (a), chlorogenic acid.

was 28.26 µg.

Sperm counts after YTE administration

As shown in Fig. 2, the animals administered YTE showed significant dose dependant increases in sperm counts in testis. Numbers of sperm heads in the 50 mg/kg, 100 mg/kg and 200 mg/kg dosing groups were 1.51, 1.61 and 1.78 times higher, respectively, than the control group.

Histopathology of the testes of YTE treated rats

In the control and the test group, no histopathologic alterations were observed in testes. Leydig cells and Sertoli cells were normal in appearance. The seminiferous tubules of rats treated with YTE were more closely arranged and larger than those of the control (Fig. 3). Some tubules in the YTE treated group showed clear signs of spermatogenesis. The presence of dense spermatid nuclei and sperm heads within seminiferous epithelium was most apparent in the YTE treated group.

The effect of YTE on serum testosterone levels

Mean serum testosterone level was 3.0 times higher in the group treated with 200 mg/kg of YTE than the control group (5.09 ± 2.53 ng/ml vs. 1.66 ± 1.08 ng/ml, respectively).

The effects of YTE or chlorogenic acid on body, testis, and epididymis weights

Rats administered YTE or chlorogenic acid showed no significant difference in body weight versus control (Fig. 4). Final mean weights of testis and epididymis in all groups were similar to those of the control (Table 2).

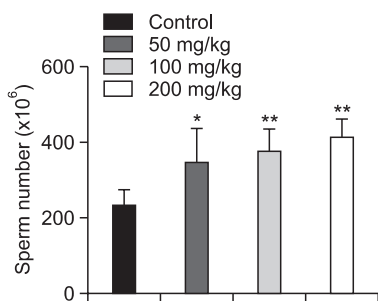


Fig. 2. Numbers of sperm in testes according to the amount of YTE administered to rats for 6 weeks. Data are presented as means ± SD (n=9). *Significantly different from the control value (ANOVA test, **p*<0.05, ***p*<0.01).

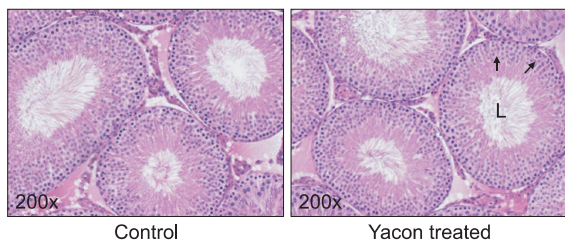


Fig. 3. H & E histology of rats testes treated with YTE. Magnification approximately x 200. In the seminiferous epithelium of rats treated with YTE, many more elongated sperm heads (arrows) were found compared with control. The lumen (L) contained sperm tails.

The effect of YTE or ferulic acid on body, testis and epididymis weights

Animals administered YTE or ferulic acid showed no significant difference in body weight compared with the control (data not shown). Final weights of testes and epididymides in all groups were similar to those of the control (data not shown).

Sperm counts after chlorogenic acid administration

As shown in Tables 3 and 4, the animals administered chlorogenic acid or YTE showed increase in the number of sperms in testis and epididymis. In testes, the numbers of sperm heads in the chlorogenic acid and YTE treated group were approximately 20% and 34% higher than in the control group, respectively. In epididymis, sperm head counts were approximately 9% and 19% higher than in the control group, respectively.

Sperm counts after ferulic acid administration

As shown in Table 5 and Fig. 5, when ferulic acid and YTE were administered to rats for 5 weeks, significant increases in sperm counts in epididymis were observed compared with the control group; sperm counts were 38% and 43% higher, respectively. No significant difference was observed between the ferulic acid and YTE administered group. Mean numbers of sperm/g of epididymis after administering ferulic acid or YTE were 674 ± 51x10⁶ and 660 ± 93x10⁶, respectively. The spermatogenic effect of 200 mg/kg of YTE had the same effect as 5 mg/kg of ferulic acid.

Total phenolic content analysis

Total phenolic compound content of YTE was 3.60%. The

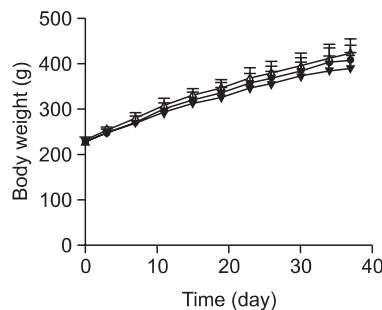


Fig. 4. Effect of chlorogenic acid or YTE on the body weights of male rats. Rat were administered 5 mg/kg of chlorogenic acid or 200 mg/kg of YTE when 7 weeks old. Data are presented as means ± SD (n=9). Key: (●): Control, (Δ): chlorogenic acid 5 mg/kg, (▼): YTE 200 mg/kg.

Table 2. Body, testes, and epididymides weights after administering chlorogenic acid or YTE to rats for 5 weeks

	Body weight (g)	Testis (g)	Epididymis (g)
Control	407.78 ± 31.34	3.144 ± 0.378	0.966 ± 0.193
Chlorogenic acid 5 mg/kg	414.44 ± 37.03	3.157 ± 0.217	0.931 ± 0.111
YTE 200 mg/kg	390.00 ± 31.22	2.870 ± 0.388	0.976 ± 0.085

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

Table 3. Numbers of sperms in testis after administering chlorogenic acid or YTE to rats for 5 weeks

	Testis (g)	Sperm/Ratx10 ^{6a}	Sperm/gx10 ⁶	Sperm/dayx10 ^{6b}
Control	3.144 ± 0.378	379.18 ± 34.01	120.60 ± 10.82	62.16 ± 5.58
Chlorogenic acid 5 mg/kg	3.157 ± 0.217	456.26 ± 34.25*	144.52 ± 10.85	74.47 ± 5.98
YTE 200 mg	2.870 ± 0.388	509.94 ± 61.74*	177.68 ± 21.51	82.30 ± 10.19

^{a,b}6.1, 6.1 is the period of spermatid development from stage I to X IV.

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

*Significantly different from the control value (ANOVA test, **p*<0.05).

Table 4. Numbers of sperms in epididymis after administering chlorogenic acid or YTE to rats for 5 weeks

	Epididymis (g)	Sperm/Ratx10 ^{6a}	Sperm/gx10 ⁶	Sperm/dayx10 ^{6b}
Control	0.966 ± 0.193	519.29 ± 57.43	537.57 ± 59.45	85.13 ± 9.41
Chlorogenic acid 5 mg/kg	0.931 ± 0.111	563.69 ± 84.01	613.35 ± 94.45	92.41 ± 1.38
YTE 200 mg/kg	0.976 ± 0.085	618.28 ± 67.92*	615.42 ± 51.02	10.14 ± 1.11

^{a,b}6.1, 6.1 is the period of spermatid development from stage I to X IV.

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

*Significantly different from the control value (ANOVA test, **p*<0.05).

Table 5. Numbers of sperms in epididymis after administering ferulic acid or YTE to rats for 5 weeks

	Epididymis (g)	Sperm/Ratx10 ^{6a}	Sperm/gx10 ⁶	Sperm/dayx10 ^{6b}
Control	1.078 ± 0.047	505.54 ± 44.79	468.83 ± 41.54	82.88 ± 7.34
Ferulic acid 5 mg/kg	1.033 ± 0.064	696.97* ± 53.30	674.96 ± 51.62	114.26 ± 8.74
YTE 200 mg/kg	1.096 ± 0.049	724.57* ± 102.21	660.96 ± 93.24	118.78 ± 16.76

^{a,b}6.1, 6.1 is the period of spermatid development from stage I to X IV.

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

*Significantly different from the control value (ANOVA test, **p*<0.05).

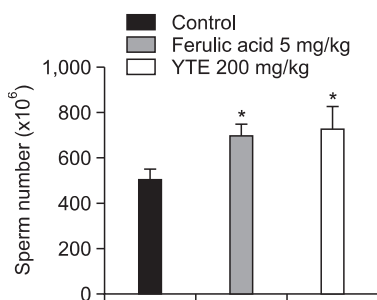


Fig. 5. Numbers of sperms in epididymis after administering ferulic acid for 5 weeks. Data are presented as means ± SD (n=9). *Significantly different from the control value (ANOVA test, **p*<0.05).

presence of chlorogenic acid, caffeic acid and dicaffeoylquinic acid were proven by comparison with standards and LC-MASS (data not shown).

DPPH scavenging activity of YTE

Table 6 showed DPPH scavenging activity of YTE, chlorogenic acid, ferulic acid and vitamin E. Chlorogenic acid and ferulic acid showed DPPH scavenging activity compared to vitamin E, while YTE showed DPPH scavenging activity in proportion to their total phenolic compounds.

Effect of YTE on nitric oxide production in LPS-stimulated RAW 264.7

We examined the effect of YTE on LPS-induced NO release from RAW 264.7 cells by detecting changes of nitrite concentration in cell culture media. The cells were incubated with YTE for 2 hrs and then stimulated with LPS (1 µg/ml) for 24 hrs. The culture media were collected and assayed for nitrite production. As shown in Fig. 6, YTE dose dependently inhibited LPS-induced NO release in RAW 264.7 cells.

HPLC analysis of testosterone

We used that the rat microsomal suspensions contained 0.125 mg/ml soluble protein as assessed by the Lowry method (Lowry *et al.*, 1951). With given HPLC conditions, ibuprofen (an internal standard) and testosterone showed retention times of around 11 min and 17 min, respectively. HPLC chromatograms are shown in Fig. 7.

Enzyme inhibitory activity on testosterone degradation of YLE and chlorogenic acid

The inhibitory activities of YLE and chlorogenic acid against testosterone degradation enzyme were shown in Table 7. YLE contains a high amount of chlorogenic acid, a phenolic compound. We evaluated the enzyme inhibitory activity on testosterone degradation of chlorogenic acid which is one of

Table 6. Antioxidant activity of YTE, chlorogenic acid and ferulic acid compared with vitamin E, as positive control

	EC ₅₀ (µg/ml)
YTE	658.28
Chlorogenic acid	15.01
Ferulic acid	26.34
Vitamene E	29.17

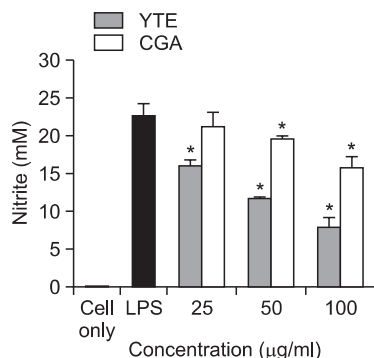


Fig. 6. The effects of YTE and chlorogenic acid on LPS-induced NO production in RAW 264.7 cells. Cells were pretreated with different concentrations (25,50,100 µ/ml) of YTE or chlorogenic acid for 2 hrs, LPS (1 µ/ml) was then added and cells were incubated for 24 hrs. Data are presented as means ± SD (n=3). *Significantly different from the control value (ANOVA test, *p<0.05).

the component of YLE. YLE and chlorogenic acid significantly inhibited the degradation of testosterone in a dose dependent manner.

DISCUSSION

Spermatogenesis is affected by a wide variety of nutrients and enzymes, such as vitamins (C, E, B12) (Sandler and Faragher, 1984; Rolf *et al.*, 1999), minerals (zinc, copper, selenium, and calcium) (Scott *et al.*, 1998), amino acids (Schachter *et al.*, 1973) and Coenzyme Q10 (Lewin and Lavon, 1997). However, no reports have been issued on the spermatogenic activity of polyphenols, like chlorogenic acid, caffeic acid and ferulic acid.

Valentova *et al.* (2003) and Simonovska *et al.* (2003) reported on the presence of large amounts of phenolic compounds in extracts from Yacon leaves and tubers, these were primarily chlorogenic, protocatechuic, ferulic, rosmarinic, gallic, genisic, and caffeic acid and their derivatives. We also confirmed the presence of chlorogenic acid and caffeic acid in YTE by HPLC analysis. In this study, YTE induced a significant dose dependant increase in numbers of sperm in testis. Furthermore, after administering chlorogenic acid and ferulic acid to rats, numbers of sperm in epididymis increased by 9% and 38%, respectively. This is the first report on spermatogenesis of polyphenols.

Polyphenols have antioxidant activities that are similar to those of vitamin C and E, which can enhance fertility by decreasing free-radical damage to sperm cells (Fraga *et al.*, 1991; Geva *et al.*, 1996). Valentova *et al.* (2005) also provided

Table 7. Enzyme inhibition activity of YLE on Testosterone degradation

	Conc.	Inhibition activity (%)
YLE	20 mg/ml	19.34 ± 0.42
	50 mg/ml	41.53* ± 0.01
	100 mg/ml	44.96* ± 4.74
Chlorogenic acid	2 mg/ml	4.62 ± 1.86
	4 mg/ml	58.90* ± 7.93
	10 mg/ml	98.67** ± 1.15

Data are presented as means ± SD (n=3).

***Significantly different from the control value (ANOVA test, *p<0.05, **p<0.01).

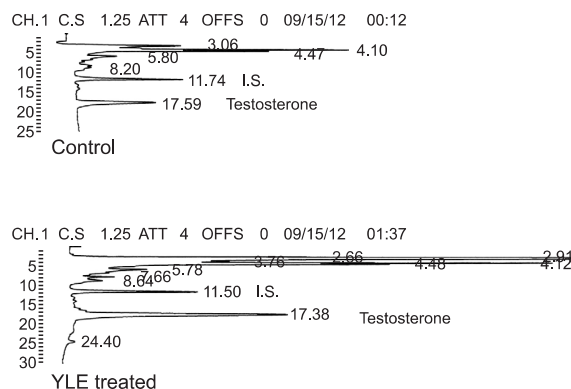


Fig. 7. HPLC chromatogram of contol and YLE treated.

evidence for the radical scavenging and anti-lipoperoxidative activity of Yacon extract in relation to phenolic content. In this study, chlorogenic acid and ferulic acid exhibited similar radical scavenging activity when compared with vitamin E, as positive control. And YTE showed DPPH scavenging activity in proportion to their total phenolic compounds. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of the most potent environmental contaminants, which has been shown to induce oxidative stress in testis and epididymal sperm of rats. Latchoumycandane *et al.* (2003) reported that administration of TCDD increased the production of reactive oxygen species such as hydrogen peroxide while the activities of antioxidant enzymes superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were found to be decreased in the epididymal sperm. They (2002) also reported that treatment with vitamine E protected rat testis from oxidative stress induced by TCDD. We (Park *et al.*, 2008) reported that TCDD/YTE treated rats showed lower weight losses of body, testis, and epididymides versus TCDD treated rats. And YTE treatment also increased seminiferous tubule numbers and diameters and significantly increased sperm numbers in testis. We thought that YTE might repair the oxidative damage of testis and epididymis induced by TCDD.

Nitric oxide (NO), a freely diffusible radical, has recently been recognized as an intra- and intercellular messenger for the control of several cellular functions (Moncada *et al.*, 1991; Lowenstein *et al.*, 1994). Excess NO generated in cells may inhibit mitochondrial metabolism, protein modification and DNA cleavage, any one of which could lead to testosterone

secretion impairment from Leydig cells (Stadler *et al.*, 1991; Kaneto *et al.*, 1995). Testosterone secretion by Leydig cells is critical for normal spermatogenesis (Sharpe, 1994). Thus normal spermatogenesis may be derailed by insufficient testosterone during conditions of stress when NO levels are pathologically elevated. In this study, YTE dose dependently inhibited LPS-induced NO release in RAW 264.7 cells.

Furthermore, serum testosterone level was higher than the control group in rat treated with YTE. Also, we found the enzyme inhibition effect of YTE and chlorogenic acid, a constituent of YTE on testosterone degradation in liver homogenate of rat. We thought that the increasing effect of sperm production of YTE might be related to the increased testosterone level by inhibition of testosterone degradation enzyme. We suggest that this could be a part of direct mechanism about increasing effect of testosterone level and spermatogenesis of Yacon extracts.

Previously described, LOH associated with low levels of serum testosterone. Some studies indicated that low testosterone levels are associated with increased risk for the development of type 2 diabetes mellitus or cardiovascular disease. There are many beneficial reports on diabetes, lipid metabolism and weight reduction of Yacon.

Conclusively, Yacon showed possibility to be a suitable herbal supplement in treating male infertility and alleviating chronic low testosterone levels such as LOH syndrome.

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