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EFFECT OF HYDROALCOHOLIC EXTRACT OF *CITRUS AURANTIFOLIA* PEEL ON SERUM LEVEL OF TESTOSTERONE, FSH, LH AND TESTIS TISSUE IN ADULT MALE RATS

HEMAYATKHAH JAHROMI V [1*], FARAJMAND M [1], AZHDARI S [2], GHAEDI SH[1] FARZAM M[2] AND KARGAR JAHROMY H[3]

1: Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

2: Department of Anatomy and Embryology, International Branch, Shiraz University, Shiraz, Iran

3: Young researchers club, Jahrom Branch, Islamic Azad University, Jahrom, Iran

***Corresponding Author: E Mail: Dr.hemayatkhah@yahoo.com**

ABSTRACT

Among citrus, *Citrus aurantifolia* are popular for their attractive color, distinctive flavor and vitamin C content. They have a great variety of uses, culinary, pickles and medicinal. Therefore, the effects of hydroalcoholic extract of *Citrus aurantifolia* peel were investigated on serum level of testosterone, FSH, LH and testis tissue in adult male rats. Male rats were divided into four groups (10 rats in each group). Treatment 1, 2 and 3 were treated intraperitoneal with doses of 50,100 and150 mg/kg/day hydroalcoholic extract of lemone peel in 1ml solvent (water), and group 4 was control, the same volum of drinking water was used. The treatment group was separated into two periods: treatment (2 weeks injection) and post-treatment (1week without injection). After injections, the blood from each rat was taken and concentration of FSH, LH and testosterone hormones was measured by ELISA kit. Furthermore, the sample tissue of testis from each rat was collected. Leydig and sertoli cells number were calculated by light microscop. The results of the study indicated that the concentration of LH in treatment groups reduced in comparison with control group (p-value <0.05). Also the number of leydig and sertoli cells indicate significant decrease ($p< 0.05$) in comparison with control group, but significant difference had not been observed in the average of testosterone hormones and FSH in comparison with control group ($p< 0.05$).

According to our findings, it concluded peel of *Citrus aurantifolia* cause to reduce the concenteration of LH hormones and the number of leydig cells in rats.Therefore, proliferation of sex cells is decreased.

Key words: Lemon Peel Extract, Rat, Testosterone, FSH, LH

INTRODUCTION

Many current studies have been conducted to evaluate the actual efficacy and adverse effects of plants on reproductive organs and functions **[1]**. The genus Citrus, belongs to Rutaceae family consist of about 140 genera and 1300 species. Some important fruits species include such as: *C.sinensis (orange), C.paradisi (Grapefruit), C.medica (Citron) and C.aurantifolia (lemone)*.Citrus are recognized as one of the world's major fruit crops **(2)**. Among citrus, C.lemone are popular for their attractive color, distinctive flavor and vitamin C contant. They have a great variety of uses, culinary, pickles and medicinal **[3, 4]**. It is rich of monoterpene **[5, 6]**, flavonoids compound **[7]**, limonoids **[8]**, hydrocarbons, erioctrin, hesperidin, pinenes α & β and coumarins **[6, 9, 10]**.

The major class of phytoestrogen is flavonoids **[7]** phytoestrogens are naturally derived plant of compounds that are functionally and structurally similar to estrogen such as endogenous estradiol **[11]**. Various studies have demonstrated the health benefits of phytoestrogens for various conditions including vasomotor symptoms **[11]** postmenopausal health risk **[12, 13, 14],** as well as their promising anticarcinogenic, neuroprotective and cardioprotective activities **[15]** and prostatic health, and bone promoting properties [**16, 17].** In males, estrogen is present in low concentration in blood, but it can be found in high concentration in seminal fluid. It is well proven that male reproductive tissue express estrogen receptors **[18]**. Many potentially health promoting effects have been ascribed to the citrus flavonoids for example: analegesic, anti-inflammatory and anti oxidant properties **[19].** Many biological activities of limonoids have been reported, such as cell differentiation **[20, 21].** Effect of lemone peel has not been investigated on male reproductive system. In the present study we aimed to examine the effects of hydroalcoholic extract of *Citrus aurantifolia* peel on serum level of testosterone, FSH and LH hormones and testis tissue in adult male rats.

MATERIAL AND METHODS

Plant Material

Lemon fruits were harvested directly from a garden in October of 2009 from Jahrom that is one of the most important horticultural center in the south of Iran **[22]**.

Preparation of Hydroalcoholic Extract

Before being used, fruits were thoroughly washed with tap water, peeled and dried in room temperature. They were powdered in an electrical blender and the hydroalcoholic extract was obtained by the percolation method **[23]**. One hundred grams of powder was put into a percolator and 1000 ml of 50% ethanol was added to the powder during three days. The extract solution was collected and the solvent was evaporated. Twenty –three grams of a semisolid extract was obtained frome 100 g of powder peel of lemon. The extract was mixed with normal saline to obtain different concentrations (50,100 and 150).

Animal

An adult male rats were age 80-90 days, weighing 200-250 g obtained from the animal house of Jahrom university. They were 5 animal in each cage in a room constant humidity (50-55%) and temperature (22-24°C) and a period of 12 hours of light and 12 hours of darkness. Rats fed with pellet and drinking water.

Experimental Design

The animals were acclimated to the animal house in Islamic azad university of Jahrom for two weeks prior to beginning the treatment. The rats were divided randomly into four groups (10 rats in each group). The control group received water and each of the rats in experimental group was treated intraperitoneal injection with 50, 100 and 150 mg/kg dose of lemon peel extract during two weeks. The treatment animals was separated into two periods: treatment and post-treatment. On the last day of the treatment, the animals were weighted (5 of 10 rat in each group were randomly selected at the end of treatment or post-treatment). Then they were anaesthetized and blood samples were obtained from heart **[1]**.

Hormonal Analysis

Blood samples were obtained and the serum were separated by centrifugation and stored at -20°C for hormone assays **[24]**. Serum testosterone level were measured by ELISA kits (DRG company, Germany.lot:29ko30-2) and concentration of FSH and LH hormone were measured by ELISA kits (PISHTAZ TEB Zaman, Tehran, Iran. LOT: FSH (89001), LH (88005)).

Histological Examination

After the overnight fixation of reproductive organs in 10% formalin buffer, tissues were dehydrated in a series of ethanol gradient and clearing in xylene. Tissues were then embedded and blocked in paraffin, cut into 5-µm sections, and stained with hematoxylin and eosin. Permanent preparations of all tissues were histologically examined and photographed using a camera (Nikon) mounted on the microscope (Olympus) **[1]**.

7-Statistical Analysis

The results were expressed as Mean±SE. Statistical Package for the Social Sciences (SPSS) program version 18. T-test, one-way analyses of variance (ANOVA) and Duncan test were submitted for the comparison of all parameters between treatment groups than control group. ($P< 0.05$) were considered to be statistically significant **[1]**.

RESULT

Body Weight

The results revealed no significant different in the average weight of the body in the treatment groups compared with the control group **(Table 2)**.

Serum Level of LH, FSH and Testosterone Hormones

Hormonal evaluation indicated that the concentration of LH hormones in the treatment groups 50, 100 and 150 mg/kg have significant decrease in comparison with control group ($p<0.05$) (**Table 1**) but significant changes in serum testosterone and FSH was'nt observed. Animals that received 50 mg/kg and 150 mg/kg in treatment and post-treatment periods respectively were decreased in serum testosterone level. Also animal that recieved 100 mg/kg in treatment period was decreased in serum FSH level in comparison with control group ($p<0.05$).

Leydig and Sertoli Cells Count

These cells calculated by light microscop **[1]**. The results showed the leydig and sertoli celles count were decreased significantly ($p<0.05$) in the treatment groups compared with the control group **(Table 2)**.

Table 1: The Means of Concentrations of Serum of LH, FSH and testosterone in Control and Treatment Groups (X ± SE)

Group	Testosterone levels (ng/ml)		FSH level (ng/ml)		LH level (ng/ml)		N	P-value
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment		
Control	1.72 ± 0.11^{ab}	1.72 ± 0.11^{ab}	0.41 ± 0.01^{b}	0.41 ± 0.01^{b}	0.37 ± 0.03^{c}	0.37 ± 0.03^{c}	5	0.05
50 mg/kg	$4.80 \pm 0.23^{*d}$	2.02 ± 0.12^{ab}	0.43 ± 0.03^{b}	0.37 ± 0.04^{ab}	0.35 ± 0.01^{c}	$0.21 \pm 0.01^{*ab}$	5	0.05
100 mg/kg	2.24 ± 0.47^{b}	2.02 ± 0.31^{ab}	$0.30 \pm 0.03^{*a}$	0.38 ± 0.03^{ab}	$0.25 \pm 0.02^{*b}$	$0.18 \pm 0.01^{*a}$	5	0.05
150 mg/kg	1.04 ± 0.02^{a}	$3.40 \pm 0.60^{*c}$	0.36 ± 0.02^{ab}	0.35 ± 0.01^{ab}	$0.24 \pm 0.02^{*b}$	$0.15 \pm 0.01^{*a}$	5	0.05

Adult male rats treated with *Citrus aurantifolia* (50, 100 and 150 mg/kg BW/day); *NOTE: Each value represents the mean ± SE of 5 animals. *p<0.05; Duncan^{a,b}

Table 2: The means of Body weight and Sertoli and Leydig cells count in control and treatment groups(X ± SE)

Group	Body weight		Sertoli cells count		Leydig cells count		N
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment	
Control	254.30 ± 4.28[b]	254.30 ± 4.28[b]	36.60 ± 2.56[b]	36.60 ± 2.56[b]	33.40 ± 1.20[c]	33.40 ± 1.20[c]	5
50 mg/kg	235.20 ± 20.01[ab]	249.20± 12.17[ab]	23.58 ± 1.08[*a]	22.09 ± 1.35[*a]	14.74 ± 0.58[*a]	23.83 ± 2.50[*b]	5
100 mg/kg	234.60 ± 14.76[ab]	234.60 ± 14.76[ab]	24.10 ± 1.12[*a]	23.27 ± 0.61[*a]	17.99 ± 1.33[*a]	17.88 ± 1.06[*a]	5
150 mg/kg	227.20 ± 27.08[ab]	232.40 ± 5.70[a]	23.94 ± 0.29[*a]	24.82 ± 1.30[*a]	13.71 ± 0.91[*a]	18.66 ± 2.50[*b]	5

Each value is express as the mean ± SE of 5 animals. *p<0.05; Adult male rats treated with *Citrus aurantifolia* (50, 100 and 150 mg/kg BW/day); Duncan[a,b]

DISCUSSION AND CONCLUSION

We investigated the effects of hydroalcoholic extract of *Citrus aurantifolia* peel on the serum levels of LH, FSH and testosterone hormones and testis tissue. Lemon is rich of vitamin C, flavonoids, monoterpens and coumarins. Flavonoids are a kind of phytoestrogen **[7, 9, 10, 25]**. The influence of phytoestrogen on sperm characteristics is controversial **[8]. Qin** *et al.*, **2000, [26]** showed that flavonoids increased secration of testosterone and LH hormones in vitro and in vivo in rats **[26**]. Also, some studies showed that sperm numbers and their motility are decreased(in a dose – depended manner) by phytoestroges **[8]**.

Azarneoshan in 2009 [27] showed that some compounds such as phytoestrogens are very effective on the hypothalamus pituitary gonad axis**.** On the other hand, some evidence indicates that the effects of dietary phytoestrogen are independent from changes in the pituitary-gonadal axis **[8]**. Therefore, it may have a direct effect on the gonad to change the hormonal level). It should be noted that in the study of herbal extracts, we cannot attribute the observed biological effects to a particular constituent, because many other compounds are present in the plant extracts **[24]**.

Hormonal evaluation indicated that the concentration of LH hormone in treatment groups (50, 100 and 150 mg/kg) reduced in the treatment period in comparison with control group ($p<0.05$), and in the post-treatment period in comparison with tereatmaent period increased. So extract of lemon peels may had inhibitory effect on serum level of Tarrago *et al*., in 2006, observed that the phytoestrogen had an inhibitory effect on spermatogenesis **[28]**. One of the component peels of lemone is coumarins **[29]**. Coumarins have antiandrogenic properties **[27].** Hence, it has the capability reduce the concenteration of LH hormones. On the other hand, decreasing leydig cells may explain why serum LH was also reduced by of lemon peels extract. The results revealed no

differences in the average concentration of FSH hormone in the treatment group compared with the control. It can be effected of inhibin hormone that releas of sertoli cells. Finally, it should not be forgotten that, although the testis is under overall control by pituitary gonadotropin (the successful initiation of testicular function is dependent on the hypothalamic secretion of GnRH which in turn stimulates FSH and LH to act on the testis. These actions initiate spermatogenesis and testosterone production), intratesticular control mechanisms are important 1- because of the unique structural organization of the testis, and 2- because of the organization and local requirements of spermatogenesis. Testosterone works via the Sertoli cell and the testosterone is produced locally by the Leydig cells in response to luteinzing hormone (LH), it is suggested that local control of intratesticular levels of testosterone is likely to be effected by a factor (or factors) produced by the Sertoli cell wich acts on the Leydig cell, and which interacts with LH to modulate the levels of testosterone in testicular interstitial fluid **[29, 30]**.

However, it is better to do further research works in this field.

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REFERENCES

[1] Jaroenporn S, Malaivijitnond S, Wattanasirmkit K, Trisomboon H, Watanabe G, Taya K and Cherdshewasart W, Effects of *Pueraria mirifica* a herb containing phytoestrogenes on reproductive organs and fertility of adult mice. Endocrine, 30 (1), 2006, 93-101.

[2] Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN and Aladedunye FA, Physico-chemical characteristics of Citrus seeds and seed oils from Pakistan, J. Am. oil Chem. Soc., 85, 2008, 321-330.

[3] Verma P and Dashora LK and Nair A, Oil-enhances shelf life of crops, Farmers & Parliament, 34, 1999, 16-17.

[4] Verma P and Dashora LK, Post harvest physiconutritional changes in Kagzi limes (*Citrus aurantifolia swingle*) treated with selected oil emulsions and diphenyl, Plant Foods For Human Nutrition, 55, 2000, 279-284.

[5] Katz E, Fon M, Lee YJ, Phinney BS, Sadka A and Blumwald E, The citrus fruit proteome: insights into citrus fruit metabolism, Planta, 226, 2007, 989-1005.

[6] Kirbaslar FG, Tavman A, Dulger B and Turker G, Antimicrobial activity

of *Turkish citrus* peel oil, Pak. J. Bot., 41 (6), 2009, 3207-3212.

[7] Miyake Y, Yamamoto K, Tsujihara N and Osawa T, Protective effects of lemon flavonoids on oxidative stress in diabetic rats. Lipids, 33, 1998, 689-695.

[8] Panjeshahin M, Dehghani F, Tahei T and Panahi Z, The effects of hydroalcoholic extract of *Actindia chinesis* on sperm count and motility, and on the blood levels of estradiol and testosterone in male rats, Arch. of Iranian Med., 8 (3), 2005, 211-216.

[9] Andrews AC, Acclimatization of citrus fruit in the Mediterranean reigon, Agricul. History, 35 (1), 1961, 35-46.

[10] Kuroyangi M, Ishii H, Kawahara N, Sugimoto H, Yamada H, Okihara K and Shirota O, Flavonoid glycosides and limonoid from *Citrus molasses*, J. Nat. Med. Pharmacog., 62, 2008, 107-111.

[11] Bagchi D, Das DK, Tosaki A, Bagchi M and Kothari SC, Benefits of resveratrol in womens health, Drugs Exp. Clin. Res., 27, 2001, 233-248.

[12] Chiechi LM, Secreto G and Damore M, Efficacy of a soy rich in preventing postmenopausal osteoporosis: the menfits randomizeal trial, Maturitas, 42, 2002, 295-300.

[13] Coxam V, Prevention of osteopaenia by phyto-oestrogens: animal studies, Br. J. Nutri., 89, 2003, 75-85.

[14] Mei J, Yeung SS and Kung AW, High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal women but not premenopausal, J. Clin. Endocrinol. Metab., 86, 2001, 5217-5221.

[15] Brzezinski A and Debi A, Phytoestrogens: the "natural" selective estrogen receptor modulators?, Eur. J. Obstet. Gynaecol. Biol., 85, 1999, 47-51.

[16] Arjmandi BH, Birubaum RS, Juma S, Barengolts E and Kukreja SC, The synthetic phytoestrogen, ipriflavone and estrogen prevent bone loss by different mechanisms, Clacif. Tissue Int., 66, 2000, 61-65.

[17] Viereck V, Grundker G, Blaschke S, Siggelkow H, Emons G, and Hofbauer LC, Phytoestrogen genistein stimulates the production of osteoprotegrin by human trabecular osteoblast, J cell Biochem., 84, 2002, 725-735.

[18] Hess RD, Bunick D and Lee KH, A role for oestrogens in the male reproductive system, Nature, 390, 1997, 509-512.

[19] Chunmei LI, Haifeng GU, Hongliang DO and Liming Z, Identification of flavanones from peel of *Citrus chengshan-huyou* Y.B. chang, by HPLC-MS and NMR, Eur. Food Res. Technol., 225, 2007, 777-782.

[20] Silalahi J, Anticancer and health protective properties of citrus fruit components, Asia Pac. J. Clini. Nutri., 11 (1), 2002, 79-84.

[21] Sugiyama S, Umehara K, Kuroyanagi M, Ueno A and Taki T, Studies on the differentiation inducers of myeloid leukemic cells from *Citrus species*, Chem. Pharm. Bull., 41, 1993, 714-719.

[22] Rafiee S, Keramat M, Jafari A, Shrifi M, Mirasheh R and Mobli H, Determining some physical properties of bergamot (*Citrus medica*), Int. Agrophy., 21, 2007, 293-297.

[23] Dehghani F, Azizi M and Panjehshahin MR, Toxic effects of hydroalcoholic extract of *Citrullus colocynthis* on pregnant mice, Iranian J. Vet. Res., Shiraz University, 9, 2008, 42-5.

[24] Karbalaydoust S, Noorafshan A, Dehghani F, Panjeshahin MR and Monabati, A, Effect of hydroalcoholic extract of *Matricaria chamomilla* on serum testosterone and estradiol levels, spermatozoon quality and tail length in rat, Iranian J. Med. Sci., 35 (2), 2010, 122-128.

[25] Tsai BY, Effect of peels of lemon, orange and grapefruit against *Meloidogyne incognita*, Plant Pathol. Bull., 17, 2008, 195-201.

[26] Qin DN, She BR, She YC and Wang JH, Effects of flavonoids from Semen cuscutae on the reproductive system in male rats, Asian J. Androl., 2, 2000, 99-102.

[27] Azarneoshan F, Khatamsaz S and Sadegh H, The effects of hydroalcoholic extract of *Dorema aucheri* on blood concentration of gonadotropin and androgen hormones in adult rats, Armaghan Danesh, 14 (3), 2009, 65-70.

[28] Tarrago CR, Diaz V and Velazquez J, Gonadotropin levels and morphological testicular features in rats after different doses of the phytoestrogen coumestrol, Neuro. Endocrinol. Lett., 27, 2006, 489-92.

[29] David M and Dekretser MD, Endocrinology of the male

reproductiv system, 15 July, Ph.D., Chapter 1, 2007.

[30] Sharp RM, Intratesticular factors controlling testicular function, Biology of Reproduction, Edinburgh, Scotland, 30, 1984, 29-49.