Effect of Green tea *Camellia sinensis* (L.) on Sperm Criteria in Testes and Epidydimis of Rats.

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Abstract

This study aimed to evaluate the probable effect of hot aqueous extract of green tea *Camellia sinensis L.* leaves on sperm characteristics of adult albino rats.Animals administrated orally of (0, 0.25, 0.5, 0.75 mg /ml) of plant extract for 30 days. The result indicated that there was significant ($p<0.05$) decrease body weight in 0.75 mg /ml, increased weight of testes in 0.5, 0.75 mg /ml group, and the weight of epidydimis was increased significantly ($p<0.05$) in 0.25 mg/ml only compared with control group .Sperm concentration in testes and epidydimis decreased significantly ($p<0.05$) in 0.75 mg /ml only concentration in compared with control group. Grade degree of sperm decreased significantly ($p<0.05$) in concentration of 0.5 and 0.75 mg /ml group only ,while sperm abnormality increased in 0.75 mg /ml significantly ($p<0.05$).Sperm viability not changed when compared with control group.

الخلاصة

هدفت الدراسة الحالية إلى تقييم التأثير المحتمل للمستخلص المائي الحار لأوراق نبات الشاي الأخضر *Camellia sinensis* L. في صفات النطف في الجرذان البيض البالغة .جرعت الجرذان فمويا" بالتراكيز (0،0.25،0.5،0.75 ملغم/مل) من المستخلص النباتي لمدة ٣٠ يوما".دلت النتائج على وجود وانخفاض معنوي.($p\leq0.05$) في وزن الجسم في التركيز 0.75 ملغم/مل وارتفاع وزن الخصى معنويا"($p\leq0.05$) في التراكيز 0.5 و 0.75 ملغم /مل وانخفاضا" معنويا".($p\leq0.05$) في وزن البربخ في التركيز 0.25 ملغم/ مل مقارنة مع مجموعة السيطرة. انخفضت درجة نشاط النطف وعيوشيتها في التركيزين 0.5 و 0.75 ملغم/مل معنويا"($p\leq0.05$)،وازدادت التشوهات النطفية معنويا" في التركيز 0.75 ملغم / مل مقارنة مع مجموعة السيطرة.

Introduction

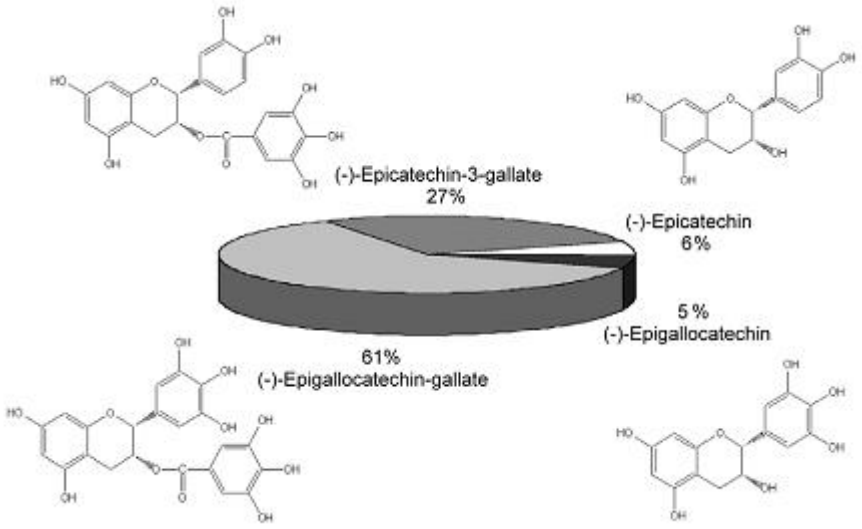
Green tea ,*Camellia sinensis* (L.) family Theaceae .Tea, beside water, is the second consumed beverages in the world. According to the different manufacturing processes, teas can be classified into three types: green tea (non Fermented tea), black tea (fermented tea), and oolong tea (semi-fermented tea). The raw material of all of these teas are the leaves of the tea plant *Camellia sinensis* and its varieties. During fermentation, a series of complex chemical reactions takes place; the most important one representing the oxidation of polyphenols. This results in the formation of teaflavins thearubigins, and other oxidized-polymerised compounds, which are responsible for the characteristic colour and flavor of black tea (Rietveld and Wiseman ,2003; Willson,1999).In the past, green tea had an official state of medicine in several European Pharmacopoeias. Recently, cases of hepatic attack after the use of hydroalcoholic extracts of green tea in complement of reducing diets (Seddik *et al*., 2001).

The composition of constituents of a green tea infusion is highly dependent on the amount of used tea leaves, on the extraction time, and on the quality of water used for extraction. Therefore, the composition can be subject to a strong variance within a certain range (Fig. 1).

Component	Dry weight
Soluable in water	
Flavlnls	18-32
(-)-EGCG	9-14
(-)-EGC	4-7
(-)-ECG	2-4
(-)-EC	1-3
(-)-GC	1-2
(-)-C	0.5-1
minor catechins	0.4-1
Flavonol glucosides	3-4
Proanthocyanidins	2-3
Caffein	3-4
Amino acids	2-4
Carbohydrates	3-5
Organic acids	0.5-2
Saponins	0.04-0.07
Pigments	0.5-0.8
Vitamins	0.6-1
Solube minerals	2-4
Insoluble or slightly soluble in water	
Celullose	6-8
Lignin	4-6
Polysaccharides	4-10
Lipids	2-4
Insoluble pigments	0.5
Insoluble minerals	1.5-3
Volatiles	0.01-0.02

Figure (1) Composition of constituents in green tea leaves (Zhen, 2002)

The traditional preparation of green tea as an infusion contains a broad spectrum of components of the drug. In general, green tea contains about 30% (w/w) of catechins in the dry leaves (Graham, 1992). The major catechins are epigallocatechin-3-gallate (EGCG) epigallocatechin (EGC), (-)-epicatechin (EC), and (-)-epicatechin-3-gallate (ECG), which comprise more than 60% of the total catechins (Fig. 2) (Yang and Koo, 1997).



Fig(2): Structure and composition of the major catechins in green tea

Other green tea constituents are the flavonols (quercetin, kaempferol, and rutin), caffeine, phenolic acids, the green tea specific amino acid theanine, and flavour compounds such as (Z)-3 hexenols and its esters (Graham, 1992).

After oral administration green tea catechins are well absorbed (Yang *et al*., 1998). Catechins are then biotransformed in the liver, and presumably already in the intestine (Zhang *et al*., 2004 ;Vaidyanathan *et al*. 2002), to conjugated metabolites such as glucuronidated, methylated sulfated derivatives. While EGC and EC are predominantly conjugated, EGCG is usually present in the free form in human plasma (Lee *et al.*, 2002 ; Chow *et al.*, 2001). Conjugates pass into the bile through enterohepatic circulation and might therefore reach the colon as glucuronides or other metabolites via this route (Scalbert *et al*., 2000). In the colon deconjugation may occur due to tissue -glucuronidases and microflora (Kroon *et al.*, 2004 ;Aura *et al*., 2002). After absorption the catechins are widely distributed to the different tissues with concentrations presumably not exceeding the lower micromolar to nanomolar range (0.1 M) (Lee *et al*., 2002 ; Yang, 1997).

For medicinal use is available in other there was some traditional indications of green tea: For oral use, diuresis, mild diarrhea, recovery from fatigue, and dietary supplement for weight reduction and for external use, calm for itching of skin ailment and treatment of cracks, grazes, and insect bites, etc. (Varnam and Sutherland,1994). Wu and Wei (2002) referred that Green tea has been considered a medicine and a healthful beverage since ancient times. The traditional Chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and, in general, to prolong life, beneficial effects in oral diseases such as protection against dental caries, periodontal disease, and tooth loss (which may significantly affect a person's overall health) .Numerous studies have demonstrated that the aqueous extract of green tea possesses antimutagenic, antidiabetic, antibacterial, anti-

inflammatory, and hypocholesterolemic properties (Pan *et.al*.,2003 ; Amantona *et al*.,2002 ; Feng *et al*.,2001).

Material and methods:

Plant material:-leaves of green tea *Camellia sinensis* (L.) were obtained from local market, and it crushed into coarse powder, which was used for extraction with hot distilled water according to (Al- Mansoor,1995),the extract was kept in refrigerator until use .

Animals:-Male albino rats(*Ruttus ruttus*) aged 8-10weeks with weight 318-360 gm obtained from the Animal House of Babylon University. Animals were housed in standard cages and 12-12h light /dark period with free access to food and water. The animals were divided into four experimental groups of 3 rats per each group. Group 1 considered as controlled treatment, while the other groups were daily administrated orally with (0, 0.25, 0.50, 0.75 mg/ ml) of leave extract for 30 day. One day after the last treatment all the animals were sacrificed.

Body and genital organs weight: the body weight were estimated before the treated and 24h after the last dosing of the respective treatment , all animals were sacrificed, the testes and epididymis were removed, cleaned from adherent tissues, drying by filter paper and weight immediately.

Sperm viability, density(counts),morphology and grade degree:

The testes from each rat were carefully exposed and one of them was removed together with its epididymis. The spermatozoa were obtained from caudal epididymis by mixed the epididymal contents with 1 ml of physiological saline.Within 5 min. of sacrificed, one drop of evenly mixed sample was applied to a glass slide under a cover glass .Sperm motility and Grade degree of sperm classified immediately .After this, caudal epidydimis sperm density (count) was estimated by using the haemocytometer and was expressed as million/ml of suspension.(WHO,1983).the sperm abnormality determined as described by Siegmund (1979).

Statistical analysis

Statistical analysis of the obtained data was performed according to Snedecor and Cochran (1980).

Results

The result showed significant decrease ($p<0.05$) in percent increasing of body weight by using 0.75 mg/ml of green tea extraction compared to control and animals treated with 0.25 mg/ml. Also there is a significant decrease ($p<0.05$) in epididymis weight in treated groups with 0.25 compared to control ,while there is a significant increase ($p<0.05$)in testis weight of treated animals with 0.5 and 0.75 mg/ml compared to control (table-1).

The result showed significant decrease ($p<0.05$) in sperm concentration in testes by using 0.75 mg\ ml green tea extraction compared to control and treated animals 0.25 mg\ml and 0.5 mg\ml . also there is significant decrease ($p<0.05$) in sperm concentration in epididymis and number of sperm in mg of teastes in 0.75 mg\ml compared with control treated animal groups (table 2) .

In table(3) the grade degree of sperm significant decrease ($p<0.05$) in groups of 0.5 and 0.75 mg\ml compared to control and 0.25 mg\ml of green tea extract , but the percentage of sperm viability did not significant changes ($p>0.05$) in control and all treated groups ,

while the sperm abnormality increased significant ($p<0.05$) in animal treated with 0.75 mg \ml of extract compared with animal groups that treated with 0.25 and 0.5 mg\ml green tea extract .

Table(1):Effect of different concentration of hot aqueous extract of green tea on mean of body ,testes and epididymis in adult male albino rats after treatment for 30 days.

concentration / parameters	control	0.25 mg/ml	0. 5 mg/ml	0.75 mg/ml	Significant level
	$\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE	
increasing in body weight(gm)%	a 40.66±4.85	a 34.25±0.9	ab 29.23±5.59	b 23.09±3.8	0.05
Weight of testes (gm)/100 gm body weight	a 0.315±0.012	ab 0.336±0.081	b 0.438±0.024	b 0.453±0.016	0.05
Weight of epididymis (gm)/100 gm body weight	a 0.183±0.05	b 0.169±0.03	ab 0.171±0.03	ab 0.176±0.03	0.05

X ± SE: mean ± standard error.

Different symbols mean significant effect .

Table(2):Effect of different concentration of hot aqueous extract of green tea on mean of sperm concentration in testes and epididymis and mean of sperm concentration in mg of testes (million) in adult male albino rats after treatment for 30 days.

concentration / parameters	control	0.25 mg/ml	0.5 mg/ml	0.75 mg/ml	Significant level
	$\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE	
Sperm concentration in testes million/ ml	a 30.596±1.099	a 37.868±2.392	a 31.53±4.340	b 9.936±2.439	0.05
Sperm concentration in epididymis million/ ml	a 35.8±3.752	a 40.033±2.184	a 34.666±4.765	b 13.6±1.093	0.05
Number of sperm in mg of testes	a 28.434±2.18	a 31.141±3.191	a 22.078±1.093	b 7.215±0.573	0.05

X ± SE: mean ± standard error.

Different symbols mean significant.

Table(3):Effect of different concentration of hot aqueous extract of green tea on grade of sperm , percentage of sperm viability and percentage of sperm abnormality in adult male albino rats after treatment for 30 days.

concentration / parameters	control $\bar{X}$ ± SE	0.25 mg/ml $\bar{X}$ ± SE	0.5 mg/ml $\bar{X}$ ± SE	0.75 mg/ml $\bar{X}$ ± SE	Significant level
Grade degree of sperm	a 2.083±0.381	a 2.416±0.381	b 1.416±0.144	b 1.166±0.166	0.05
Sperm viability %	a 89±1.732	a 93±2.027	a 85±4.041	a 78±1.4371	N.S
sperm abnormality	ab 11±1.154	a 7.666±1.201	a 6.000±1.154	b 20.333±5.364	N.S

X ± SE: mean ± standard error.
Different symbols mean significant

Discussion:-

Tea is the most consumed drink in the world after water. Green tea is a 'non-fermented' tea, and contains more catechins, than black tea or oolong tea . Catechins are *in vitro* and *in vivo* strong antioxidants. In addition, its content of certain minerals and vitamins increases the antioxidant potential of this type of tea (Cabrera,2006) .

Result in table (1) showed that the body weight decreased significantly in 0.75 mg/ml, this result may be due to that green tea suppressing the appetite, decreasing fat absorption, acting on neurotransmitters that modulate feelings of hunger and satiety, or increasing thermogenesis (production of body heat, which burns calories(Chantre and Lairon,2002). In addition, caffeine and theanine have been found to strengthen polyphenol effects on body weight control and fat accumulation in mice(Zheng *et al*.,2004). In vitro studies have also shown that green tea extracts interfere in the fat emulsification process, which occurs before enzymes act, and is indispensable for lipid intestinal absorption (Chantre and Lairon,2002) Green tea also exhibits a fatty acid synthesis inhibitor activity ,in addition, green tea may have thermogenic properties not only attributable to its caffeine content, but to the joint-effect of caffeine and catechins (Tian *et al*,2004).

Weight of testes increased significantly ($p \leq 0.05$) in 0.5 and 0.75 mg /ml group in contrast with control group,theis chang may be due to the increased in protein contents in these organ which may be due to protein(15-20 %) and amino acids (1-4%) content of green tea extract (Costa *et al*.2002 ; Zhen,2002).

Table (2) referred to that there was no significant change($p \geq 0.05$) in(0.25,0.5 mg/ml) group, while there was significant ($p \leq 0.05$) decrease in 0.75 mg/ml in compared with control treatment in sperm concentration in testes and epidydimis and number of sperm in mg of testes. this result may be attributed decrease in serum testosterone and

estradiol, may explain why the sperm count reduced by aqueous extract of green tea in 0.75 mg /ml ,estrogen regulate the reabsorbtion of the luminal fluid in the head of epididymis therefore, the semen is diluted rather than concentrated (Mei *et al*.,2001).Testosterone is important in spermatogenesis as well. In this way ,the reduction in hormonal levels can influence sperm count (Shahin *et al.*,2005).

Table (3) illustrated that the green tea extract decrease significantly ($p \leq 0.05$) the grade degree and viability of sperm in treated rats in 0.5 and 0.75 mg/ml ,while sperm abnormality not changed in all treatments.

The result in table 2 ,3 may be due to the negative effect of plant extract on androgen levels, which produced in testes especially testosterone which reflected on sperm parameter (Gupta *et al.* 1999),or the plant extract have the anti androgenic action when drinking by blockimg the development of the production of androgens and decreasing the conversion of androgens by inhibiting the 5- alpha reductase type I ,or block the androgen receptor it self (Julie and Harper ,2002). Satoh *et a*l .(2002) found that orall administration of green tea extract catechins, in the diet (0, 1.25 and 5%) to male rats for 2, 4 and 8 weeks initiated at 5 weeks old. It was found that a 5 % dose to male rats for 2-8 weeks induced goiters .Endocrinologically, elevating luteinizing hormone (LH) and testosterone levels. In this respect the results agree with Raji (2004) who found that the extract of *Alstonia boonei* stem bark reduced significantly sperm viability, motility, and counts in male rats treated for 2 and 4 weeks. Gupta and Sharma (2003) found that *Tinospora cordifolia* stem extract reduced significantly sperm density which resulted in reduction of male fertility by 100 %.

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