

Effect of aqueous green tea extract on male Wistar rats reproductive hormones level

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Abstract

The current study was carried to investigate the effect of green tea extract in improvement of male rats reproductive efficiency after exposed to oxidative stress by streptozotocin. 40 male rats at 60 days old with 135 ± 11 gm in weight were randomly divided into four equal groups, the first drenched with distilled water for 60 days as control group (C). the second (T1) was given aqueous extract of green tea (100mg/kg/bw) for 60 days, while the third group (T2 injected (i.p) single dose of streptozotocin (60mg/kg/bw) for induction of oxidative stress, the fourth group injected with single dose of streptozotocin (60mg/kg/bw) and after 30 days drenched with green tea extract (100mg/kg/bw) for 30 days. at day 60 of experiment all animals were sacrificed, blood samples were collected from ventral vein and serum samples were isolated for measurement of male reproductive hormones (LH, FSH, and Testosterone) by ELISA test. the testes samples were taken for histological study and dimensions of seminiferous tubules, samples of epididymis for study of seminiferous tubules dimensions. The results of study was revealed significant increase ($P \geq 0.05$) in testosterone and FSH in (T1) group compared with other groups while there is non-significant changes in LH concentration compared with other groups.

Key words: aqueous extract, green tea, Wistar rats, reproductive hormones.

تأثير المستخلص المائي للشاي الأخضر على مستوى الهرمونات التكاثرية لذكور جرذان الوستر

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الخلاصة

اجريت الدراسة الحالية بهدف الكشف عن تأثير مستخلص الشاي الأخضر في رفع الكفاءة التناسلية لذكور الجرذان البيض المعرضة للإجهاد التأكسدي بمادة الستربتوزوتوسين. تم توزيع 40 جرذا ذكرا بعمر 60 يوما ومعدل وزن 135 ± 11 غم عشوائيا على 4 مجاميع متساوية جرعت الاولى بالماء المقطر بوصفها مجموعة سيطرة (C) وجرعت المجموعة الثانية (T1) بالمستخلص المائي للشاي الأخضر (100 ملغم/كغم من وزن الجسم) لمدة 60 يوم. بينما حقنت المجموعة الثالثة (T2) في البريتون (i.p) بمادة الستربتوزوتوسين (60 ملغم/كغم من وزن الجسم) لمرة واحدة لإحداث الإجهاد التأكسدي. وحقنت المجموعة الرابعة (T3) بمادة الستربتوزوتوسين (60 ملغم/كغم من وزن الجسم) لمرة واحدة لإحداث الإجهاد التأكسدي وبعد 30 يوم تم تجريعها بالمستخلص المائي للشاي الأخضر (100 ملغم/كغم من وزن الجسم) يوميا لمدة 30 يوم. تمت التضحية بجميع الحيوانات في اليوم الـ 60 من التجربة. اخذت نماذج دم من الوريد البطني وعزل المصل لتقدير تركيز الهرمونات الذكرية: الهرمون محفز الجريب (FSH)، والهرمون اللوتيني (LH)، وهرمون الشحمون الخصوي (Testosterone) باستخدام تقانة الاليزا. بينت نتائج مستوى الهرمونات في مصل ذكور الجرذان لهذه الدراسة ارتفاعا معنويا في هورمون التستوستيرون وهورمون محفز الجريب في المجموعة الثانية (T1) مقارنة مع المجاميع الأخرى في حين اظهر الهرمون اللوتيني انخفاض معنوي في المجموعة الثالثة (T2) مقارنة مع المجاميع الأخرى.

الكلمات المفتاحية: المستخلص المائي، الشاي الأخضر، الهرمونات التكاثرية، الجرذان.

Introduction

Green tea a beverage so healthy that it is full of antioxidants, leading to the health claims about promoting health and prolonging life, recall that testosterone production is dependent upon oxidizing reactions. Green tea has been shown in the

lab to inhibit certain effects of testosterone apparently by inhibiting the conversion of testosterone to the more potent androgen. green tea, specifically (EGCG) may also affect aromatase the enzyme that converts testosterone to estrogen; in some studies aromatase is suppressed, in others it is

increased animal studies and epidemiologic studies have shown that green tea consumption is associated with lower androgen and estrogen levels in Asians. Green tea appears to be protective against cancers that respond to sex hormones (prostate, breast) (1). epigallocatechin 3-gallate (EGCG) inhibits type I 5 α reductase activity in vitro, which is partially responsible for conversion of testosterone to dihydrotestosterone green tea was shown to be an aromatase inhibitor in rat ;a causative factor for an increase in testosterone level.(2,3).

Materials and methods

Experimental Design

In this experiment we used (40)adult male Wistar rats (average weight was 135 \pm 11g)were obtained from animal house in veterinary college of Al- Qadisiya university .these animals reared under controlled conditions(12L:12D at 22C $^{\circ}$) and fed standard laboratory food (19%protein ratio and 3000kilocalories energy)and drinking distilled water. Adult male Wistar rats divided randomly into(4 groups) each group consist of (10males) and we recorded the primary weights of animals after 5 days acclimatization at the animal facilities, streptozotocin injected in 20 male rats at dose (60mg/kg,b.w.,i.p.)(4) oxidative stress has been confirmed by plasma concentration of glucose (to be more than 200 mg /dl). after that all groups treated as following:

1-Control Group(C): Given the standard food and distilled water.

2-The First Treated Group(T1) Given green tea extract in a dose of 100mg/kg b.w./day. for 60 consecutive days

3-The Second Treated Group (T2) Injected streptozotocine(STZ) in a dose of 60mg/kg /b.w by single intra peritoneal injection.

4-The Third Treated Group (T3) Injected streptozotocine in a dose 60mg/kg b.w. by single intra peritoneal injection then after first month were given green tea extract in a dose of 100mg/kg b.w./day. for 30consecutive days. Twenty four hours after last administration all animals were anesthetized by doses of ketamine and xylazine at (90mg/kg/b.w,10mg/kg/b.w) respectively administrated ip. and then sacrificed and blood

samples were collected from abdominal vein to obtained the serum of animals for assessment of reproductive hormones (FSH,LH and Testosterone).

Chemical Preparation

Preparation of Citrate Buffer

1 molar citrate buffer (2.1014g) was dissolved in 50ml and measured the pH must be 4.5 by using (Na OH) and added by pasture pipette then complete the volume to the (100ml).

Preparation Of Streptozotocin for intraperitoneal injection:

1-prepare it according to weight of animals where the dose is (60mg/kg).2-The suitable amount of (stz) was dissolved in citrate buffer (sodium citrate) freshly prepared (20 minute before injection).3-The container was covered with aluminum foil to protect the solution from direct exposure to light.

Induction Of Oxidative Stress:

1-for induced oxidative stress (O.S) the rats were fasted overnight then injected a single intraperitoneal injection of (stz) at dose (60mg/kg) body weight hyperglycemic which indicate occurrence of oxidative stress. 2-Hyperglycemia fallowed up for (72 hours) by using urine glucose dipstick.

3-male rats with blood glucose concentration more than 200mg/dl were considered as under oxidative stress.

Preparation Aqueous Extract Of Green Tea

Aqueous extract of green tea was prepared by soxhlet apparatus according to (5).

Hormonal assays in blood serum by using ELISA technique

According to the manufacturer instructions, (6) LH, FSH and Testosterone hormones were measured.

Results and discussion

Reproductive hormones:-

The current study revealed that the male rats in group(T1)which received green tea extract have significant increase ($P\leq 0.05$) in serum testosterone concentration in comparison with (T2,T3) and control(Table4-1),which indicate stimulation in testosterone synthesis in testes by components of green tea extract which increase the activity of enzymes which are responsible for synthesis and metabolism of lydig's cells steroids by

stimulation of interstitial cells stimulating hormone (ICSH) which convert cholesterol to pregnonolone and then testosterone (7). Also spermatogenic stimulating hormone (FSH) or (SSH) play a role in testosterone synthesis by increasing receptors sensitivity to (LH) or (ICSH) which found on the surfaces of Leydig's cells and then increase steroidogenesis and testosterone release in the seminiferous tubules (8) also the results revealed significant increase in FSH in (T1) which received green tea extract compared with other groups because of ability of green tea to stimulate synthesis of pituitary hormones since FSH play important physiological role in stimulation of sperms production (9) also play synergistic role with testosterone in development of spermatogonia. (10). While LH in (T1) which received green tea extract significantly decrease ($P \leq 0.05$) compared with T2 and non-significant compared with (T3 and control), this decrease may be due to testosterone increase through the negative feedback

mechanism on the level of LH in pituitary gland and GnRH in hypothalamus, when testosterone concentration reach above normal the GnRH secretion will be inhibited, which lead to LH decrease and then testosterone (11). While the (T2) showed significant decrease in testosterone under the effect of streptozotocin on the testis is specially Leydig's cells. since the histological section of testis showed necrosis and degenerative in Leydig's cells with decrease in their number due to the effect of streptozotocin also atrophy of Sertoli cells (12). which lead to decrease testosterone production. The (T3) which received STZ and then treated with green tea extract, show positive change and increase in hormone levels which are decreased in (T2) which received STZ only and this may be due to that green tea extract contents of flavonoids which stimulate LH and FSH release from pituitary gland and then testosterone from testis (13).

Table (1) effect of green tea extract on reproductive hormones level of adult male Wistar rats

Groups	C	T1	T2	T3
Testosterone (nmol/L)	b 15.7 ± 0.66	a 32.5 ± 0.73	b 14.9 ± 0.82	b 16.1 ± 0.95
LH (ng/L)	ab 8.91 ± 0.33	b 8.40 ± 0.42	a 9.30 ± 0.56	ab 8.90 ± 0.72
FSH (mIU)	b 1.95 ± 0.9	a 2.75 ± 0.05	c 1.13 ± 0.07	b 1.90 ± 0.08

C=Control group T1=Drenched Green Tea Extract (100mg/kg/b.w) for (60) days T2=Injected STZ (60 mg/kg/b.w) once T3=Injected STZ (60mg/kg/b.w) after 30 days drenched green tea extract (100mg/kg/b.w) for 30 days. Numbers=average ±S.E. (n=10) Different letters was significant ($P \geq 0.05$). S.E=Standard Error

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