



Study The Potential Effects Of Some Plant Parts In Raising The Fertility Level In Male Rats

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Abstract : This study was conducted to investigate the effect of alpinia (*Alpinia officinarum*) and valerian (*Valeriana officinalis*) powder on fertility level in male rats. Thirty mature Sprague dawley male rats were randomly distributed into equal six groups. Group (1) was negative (normal) control and group (2) was injected with a dose of cadmium chloride ($CdCl_2$, 0.1%) at 0.1ml/100g Body weight, and the group was a positive control. Rats of the other three groups were injected with $CdCl_2$ and fed into the powder of the plant alpinia and valerian with 5% and their mixture. At the end of the experiment, blood samples were collected for determination of serum levels of testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The testicles were extracted for a pathological examination and measured the activity of antioxidant enzymes. The results showed that consuming the powder and mixture of alpinia and valerian at 5% for 4 weeks caused a significant ($P \leq 0.05$) increases in serum levels of T, FSH and LH hormones and relative weight of testes as compared to control positive group. There was also increased testis tissue antioxidant activities associated with alleviation of testicular degeneration. In conclusion, the study recommended that using some plant parts such as alpinia and valerian in food, may be useful for male patients with low fertility and oxidative stress.

Key words: Cadmium chloride, luteinizing hormone (LH), fertility level, follicle-stimulating hormone (FSH).

Introduction

Non Fertility is one of the major health problems in life and approximately about 30 percent of this problem is due to male factors (**Isidoriet al., 2006**). Several factors can interfere with the process of spermatogenesis; reduce sperm quantity and quality and decrease male fertility. Not only semen deficiency or abnormality (testicular dysfunction) and anatomical abnormalities in the male reproductive system (obstructive azoospermia) have been discussed in complementary alternative medicine evidences as causes of male infertility, but also decreased libido and erectile dysfunction because of their general effect on the impairment of sexual functions (**Weiss et al., 2011 and Nejatbakhshet al., 2012**).

Many diseases such as coronary heart diseases, diabetes mellitus and chronic liver diseases as well as insufficient vitamins intake have deleterious effect on spermatogenesis and production of normal sperm (**Mosher and Pratt, 1991; Mahgoup and EL-Medany, 2001; Agbajeet al., 2007 and Abdulbariet al., 2009**). However, intake of natural antioxidants with vitamins E and C Protected sperm DNA from oxidative stress in rat testes (**Jedlinskaetal., 2006**).

Plants are considered to have valuable health promoting effects mainly due to the high levels of wide range of antioxidant compounds present in their tissues (**Liu, 2003 and Moselhy and Ali, 2009**). alpinia and valerian are most abundant in potent natural antioxidant polyphenols and flavonoids.

Alpinia belong to the ginger family, cultivated in Southeast Asia. It originated in China, where its name ultimately derives. alpinia used as, remedial properties, pharmacological effects. It is commonly used for its anti-inflammatory, antihyperlipidemic bioactivity, anticancer, dysmenorrhea, osteoblast, anti-influenza virus activity, antibiotic resistance, antimicrobial effect and male infertility. The results from this study was shown that alpinia is a multi-purpose medicinal candidate. While alpinia has been used in traditional medicine for many centuries, further clinical trials are needed to confirm its remedial use. Major chemical constituents of alpinia include volatile oil, diaryheptanoid, sterol and flavonoids. The chemical components of this plant are galangoflavonoid, 1'S-1'-acetoxychavicol acetate, phenylpropanoids and phydroxybenzaldehyde, acetoxycineoles (trans and cis)-2- and 3-

acetoxy-1, 1, 8-cineoles, 1'-acetoxychavicol acetate (galangal acetate), β -Sitosteroldiglucoside (AG-7) and β -sitsterylArabinoside (AG-8) ,The phenylpropanoids. (**Adams, 2011; Miraj, 2016 and Mak, 2013**).

Valerian is a perennial flowering plant, with heads of sweetly scented pink or white flowers that bloom in the summer and can reach a height of 1.5 metres (5 ft). Valerian flower extracts were used as a perfume in the 16th century. Valerian has been used as a medicinal herb since at least the time of ancient Greece and Rome. Hippocrates described its properties, and Galen later prescribed it as a remedy for insomnia. compounds detected in valerian that may contribute to its method of action are Alkaloids: actinidine, chatinine, shyanthine, valerianine, and valerine, Isovaleramide may be created in the extraction process., gamma-aminobutyric acid (GABA), Isovaleric acid, Iridoids, including valepotriates: isovaltrate and valtrate Sesquiterpenes (contained in the volatile oil): valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid, flavanones: hesperidin, 6-methylapigenin, and linarin (Yuan *et al.*, 2004 ; Shohet, 2009 and Van *et al.*, 2015).

The present study was designed to determine the impact of the alpinia and valerian powder plant to increasing fertility in male rats injected with cadmium chloride.

Materials and Methods

Materials

-**Plants** (Alpinia and valerian) were obtained from Agricultural Seed, Spices and Medicinal Plants Co. (Harras), Cairo, Egypt.

- Experimental animals

Thirty adult male albino rats, Sprague Dawley, weighing (210-215 g) were purchased from Medical Insects Research Institute, Dokki, Cairo, Egypt. Rats were housed in environmentally controlled atmosphere and were fed on standard diet according to AIN-93 guidelines (Reeves *et al.*, 1993) in Animal laboratory in Faculty of Home Economics, Minoufiya University.

-**All chemicals**, solvents and buffers in analytical grade, cadmium chloride , vitamin and salt mixtures components used for rats feeding were purchased from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical Instruments Cairo, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt.

Methods

Alpinia and valerian powder preparation: Alpinia and valerian were powdered by electric grinder (Moulinex, France), packed in dusky stoppard glass bottles until use at 4°C.

Basal diet: The basic diet prepared according to the following formula as mentioned by **AIN, (1993)** as follow: protein (10%), corn oil (10%), vitamins mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by **Campbell, (1963)** while the salts mixture used was formulated according to (**Hegstedet al.,1941**).

Induction of rat fertility

Normal healthy male albino rats were injected by cadmium chloride (CdCl₂, 0.1%) at 0.1ml/100g body weight, twice during the experiment (**Rekhaet al.,2009**), Also it is a teratogen which is commonly used in industry. Although it is well known to cause toxicity in testes, kidney, heart and liver. Cadmium, a toxic heavy metal is an effective short- and long-term cadmium toxic heavy metal with short and long term effects particularly showing accumulation in organs as kidney, liver, brain and testis (**Holt and Webb,1987**).

Experimental design

All rats were fed on standard diet for one week for adaptation then rats were randomly divided into two main groups, the first group, negative control group (n=6) fed standard diet, and the second group: fertility groups (n=24) were injected with a dose of Cadmium chloride (CdCl₂, 0.1%) at 0.1ml/100g body weight, twice during the experiment (**Rekhaet al.,2009**) and divided into four sub groups (6 rats per each), as follow: subgroup 1: positive control group fed standard diet and sub group 3, 4 and 5 fed on standard diet containing 5% of alpinia or valerian and their mixture, respectively. At the end of the experiment, rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected into a dry clean centrifuge glass tubes. Serum was separated by centrifugation at 4000 rpm for 15 minutes at room temperature according to **Schermer (1967)** Serum was carefully aspirated and transferred into clean quiet fit plastic tubes and kept frozen at (-20 C⁰) until analysis.

Hormonal assay:

Serum testosterone concentration was measured by enzyme-linked immunosorbant assay (ELISA) according to **McCann and Kirkish (1985)**. In brief, the unknown or standards samples were incubated with radioactive iodine labeled testosterone in antibody-coated tubes. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter. The serum total testosterone levels were then calculated. Serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) hormones were determined by an enzyme-linked immunosorbent assay (ELISA) Using specific commercial kits (Amersham, Buckinghamshire, UK) According the method described by **Ballesteret et al., (2004)**.

Histological procedure

Specimens from liver were collected after fixing in formalin, embedded in paraffin, 4-6 thick sections were prepared and stained with hemetoxlin and eosin according to **Bancroft et al., (1996)**

The fixed specimens of testes were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin (H & E) then examined microscopically according to **Carleton (1978)**.

Statistical analysis

The data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean \pm SD. Differences between treatments at $p \leq 0.05$ were considered significant according to **Snedecor and Cochran (1986)** using computerized SPSS program.

Results and Discussion

Table (1): Effect of alpinia and valerian and their mixture combination on serum levels of total testosterone (T), FSH and LH hormones in rats with testicular damage induced by Cadmium Chloride.

Groups	Serum levels of hormones (Mean±SD, ng/ml)		
	T	FSH	LH
Group(1):negative control	4.70 ^a ±0.03	9.95 ^a ±0.3	1.95 ^a ±0.4
Group(2):positive control	2.15 ^c ±0.01	5.65 ^c ±0.2	0.95 ^d ±0.3
Group(3):5% alpinia	2.65 ^c ±0.02	6.69 ^c ±0.4	1.33 ^c ±0.1
Group(4):5% valerian	3.55 ^a ±0.01	7.75 ^b ±0.02	1.75 ^b ±0.03
Group(5):5% mixture	4.55 ^a ±0.04	9.70 ^a ±0.3	1.90 ^a ±0.3

Means With Different Superscript Letters In The Same Column Are Significant Different at $P \leq 0.05$. Mixture Mean Alpinia Plus Valerian By Equal Parts.

Table (2): Effect of alpinia and valerian and their mixture on weights of Liver and Testis in rats with testicular damage induced by Cadmium Chloride.

Groups	Relative weight of organs (g/100g b.wt)	
	Liver	TESTIS
	Mean ±SD	Mean ±SD
Group(1):negative control	1.634 ^e ±0.073	1.56 ^a ±0.05
Group(2): positive control	2.5 ^a ±0.158	0.90 ^d ±0.03
Group(3): 5% alpinia	2.35 ^b ±0.079	1.25 ^c ±0.06
Group(4): 5% valerian	2.2 ^c ±0.090	1.35 ^b ±0.04
Group(5): 5% mixture	2.05 ^d ±0.079	1.45 ^b ±0.02

Means with different superscript letters in the same column are significant different at $P \leq 0.05$. Mixture Mean Alpinia Plus Valerian By Equal Parts.

Table (3): Effect of alpinia and valerian and their mixture on activity of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

	SOD (u/mg protein)	GPX (nmol/min/mg protein)	CAT (nmol/min/mg protein)
	Mean ±SD	Mean ±SD	Mean ±SD
Group(1):negative control	22.09 ^a ±0.5	244.5 ^a ±8.6	372.9 ^a ±9.5
Group(2):positive control	11.20 ^d ±0.3	136.6 ^d ±8.5	290.5 ^d ±8.2
Group(3):5% alpinia	16.25 ^c ±0.6	180.6 ^c ±6.2	305.6 ^c ±9.2
Group(4):5% valerian	19.25 ^b ±0.9	198.6 ^b ±6.3	325.6 ^b ±8.6
Group(5):5% mixture	1.25 ^a ±0.8	212.6 ^b ±8.8	367.2 ^a ±7.3

Means with different superscript letters in the same column are significant different at $P \leq 0.05$. Mixture Mean Alpinia Plus Valerian By Equal Parts.

Results

Tables (1-3) show the results in concern to the effect of alpinia and valerian and their mixture at 5% level to male rats with testicular damage induced by CdCl₂ significantly ($P \leq 0.05$) increased the weight of liver and testes; normalized serum levels of testosterone, FSH and LH hormones. There were also significant increases ($P \leq 0.05$) of antioxidant enzyme activities in testicular tissue. These findings were similar to those of **Williamson et al., (1996)** who reported that medicinal plants have been reported to possess anti fertility effects by various mechanism of actions. One of the major action is their effect on sex hormones particularly for suppressing fertility, regularizing menstrual cycle, relieving dysmenorrhoea, treating enlarged prostate, menopausal symptoms. Moreover plants with estrogenic property can directly influence pituitary action by peripheral modulation of luteinizing hormone (LH) and follicle stimulating hormone (FSH), decreasing their secretions and blocking ovulation (**Brinker, 1997**). Looking forward to the tradition of using herbal medicines, which have minimum and less side effects (**Jain et al., 2012**). However, in the recent past much interest has been shown to control regulation of fertility by using medicinal plants (**Chowdhury et al., 2001**), fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (**Allagand Rangari, 2002**).

There were also a significant decrease in malondialdehyde (MDA) level and marked increases in reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) activities in rats treated with testicular damage induced by CdCl₂. Alpinia rhizome showed a concentration-dependent radical scavenging activity by inhibiting diphenylpicrylhydrazyl (DDPH)- free radical, also the hydroalcoholic extract prepared by hot maceration process showed better reducing and total antioxidant activity. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were dose dependently enhanced in cells treated with the extract (**Srividya et al., 2010**).

The dry root and rhizome of Alpinia has long been used in traditional Chinese medicine for its antioxidant, anti diabetic, anti cancer, anti diarrhoea, anti inflammatory and anti coagulation effects (**Xie et al., 2013**). Antioxidant activity of the plants is attributed to their

phytochemicals such as flavonoids, phenolic acids and polyphenolic compounds. They play an important role in cancer, emphysema, cirrhosis, atherosclerosis, aging and various other degenerative diseases by neutralizing free radicals naturally occurring in human metabolism, lead reducing the oxidative damage (Soong and Barlow, 2004 and Patricia *et al.*, 2005) Therefore, they can be used as their possible utilization in foods or functional foods and pharmaceutical supplements. Valerian grown in Turkey was studied in this work. Plant material was dried, powdered and extracted with methanol. Antioxidant activity of the methanolic extract of Valerian was determined by different antioxidant activity methods such as DPPH radical scavenging assay, Cupric reducing antioxidant capacity assay and Ferric reducing antioxidant capacity (Apaket *et al.*, 2004 and Soong and Barlow, 2004)

Histopathological examination of Testis :

Histopathological examination of the testis of normal control rats showed normal histological structure. Microscopically, testis of rat from group 1 showed the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis (photos 1 and 2). However, some examined sections from group 2 revealed slight degeneration and desquamation of spermatogoneal cells lining seminiferous tubules (Photo. 3), Testis of rats from groups 3, 4 and 5 revealed no histopathological changes (Photo.4,5 and 6).

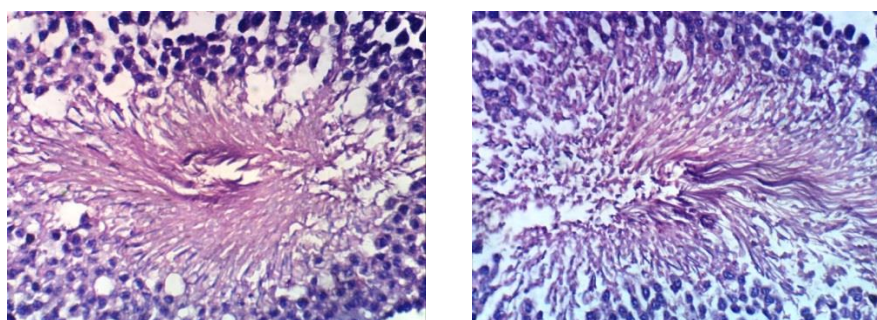


Photo.(1&2): Testis of rat from group 1 showing the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis (H&E X 400).

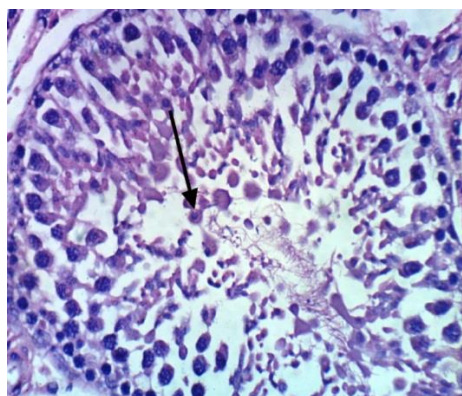


Photo.(3): Testis of rat from group 2 showing slight degeneration of spermatogoneal cells lining seminiferous tubules (H&E X 400).

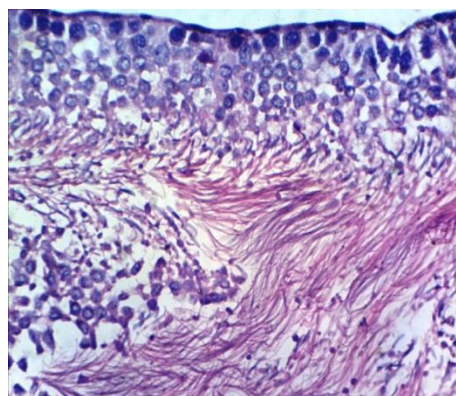
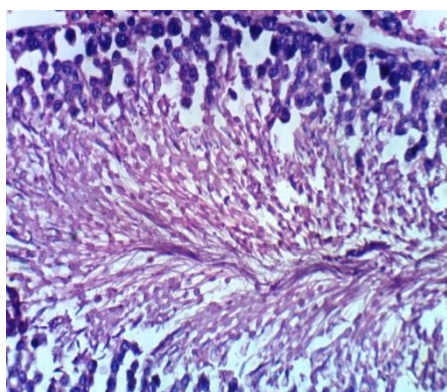
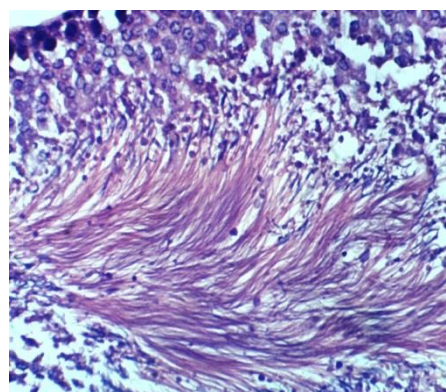


Photo (4): Testis of rat from group 3 showing no histopathological changes (H&E X 400).



Photo(5): Testis of rat from group 4 showing no histopathological changes (H&E X 400).



Photo(6): Testis of rat from group 5 showing no histopathological changes (H&E X 400).

Histopathological examination of liver

Microscopically, liver of rats from group 1 showed the normal histological structure of hepatic lobule (Photos7and8). Meanwhile, liver of rats from group 2 revealed cytoplasmic vacuolation of hepatocytes, portal oedema and inflammatory cells infiltration (Photos9and10). However, liver of rats from group 3 revealed no changes except binucleation of hepatocytes (Photo.11). Examined sections from group 4 showed cytoplasmic vacuolation of hepatocytes (Photo. 12and13), binucleation of hepatocytes (Photo. 14) and Kupffer cells activation (Photo. 15). Moreover, liver of rats from group 5 revealed slight cytoplasmic vacuolation of some hepatocytes (Photos16and17) and congestion of hepatic sinusoids (Photo. 18).

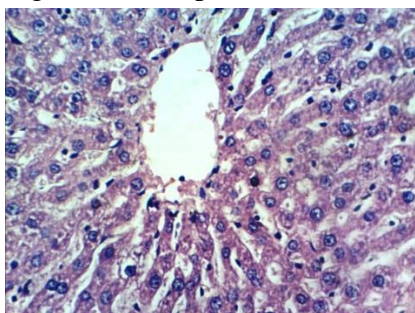


Photo. (7): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

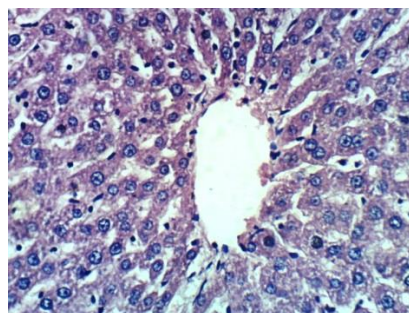


Photo. (8): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

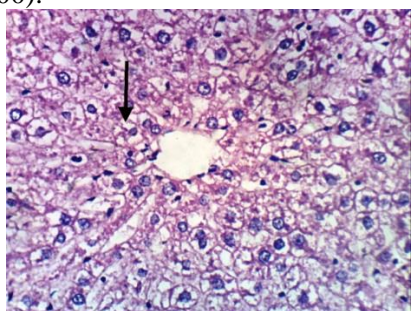


Photo. (9): Liver of rat from group 2 showing cytoplasmic vacuolation of hepatocytes (H & E X 400).

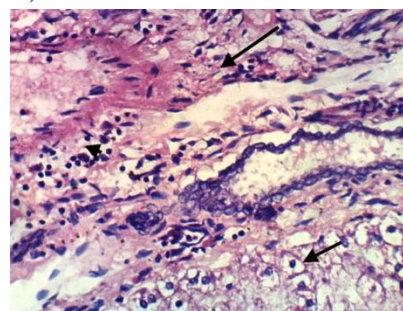


Photo. (10): Liver of rat from group 2 showing cytoplasmic vacuolation of hepatocytes, portal oedema and inflammatory cells infiltration (H & E X 400).

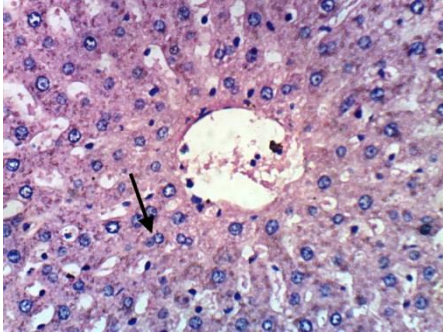


Photo. (11): Liver of rat from group 3 showing binucleation of hepatocytes (H & E X 400).



Photo. (12): Liver of rat from group 4 showing cytoplasmic vacuolation of hepatocytes and binucleation of hepatocytes (H & E X 400).

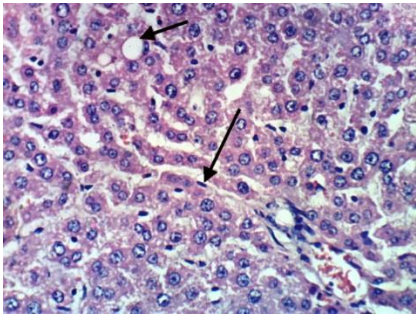


Photo. (13): Liver of rat from group 4 showing cytoplasmic vacuolation of hepatocytes and Kupffer cells activation (H & E X 400).

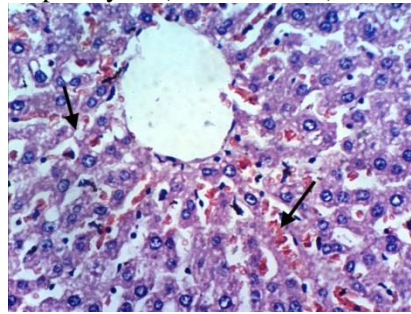


Photo. (14): Liver of rat from group 5 showing slight cytoplasmic vacuolation of some hepatocytes and congestion of hepatic sinusoids (H & E X 400).

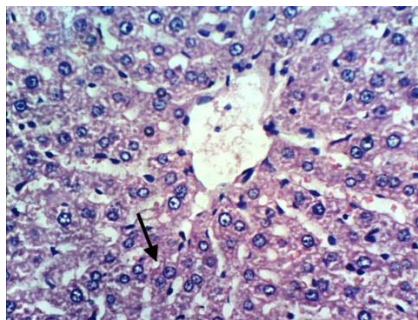


Photo. (15): Liver of rat from group 5 showing slight cytoplasmic vacuolation of some hepatocytes (H & E X 400).

In conclusion, the study therefore recommends that both *Alpinia* and *Valerian* plant be used to improve and raise the level of fertility, while no improvement in hepatic tissue is evident through pathological screening. It is clear that medicinal plants play an important role as anti-fertility agents. Despite of various commercially available oral contraceptives in the market, herbal anti-fertility agents shows promising output by minimizing the number of adverse drug properties. Current research towards traditional medicine is growing rapidly because of its safety and less cost consumption.

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الدراسات المحتملة لبعض الأجزاء النباتية في رفع مستوى الخصوبة في الفئران

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الملخص العربي

صممت هذه الدراسة لمعرفة التأثيرات المحتملة لبعض الأجزاء النباتية لنباتات النارددين والخلونجان على مستوى الخصوبة في ذكور الفئران. تم توزيع ثلاثون من ذكور الفئران بطريقة عشوائية إلى خمس مجموعات متساوية. استخدمت المجموعة الأولى كمجموعة ضابطة سالبة (فئران سليمة)، وتم حقن فئران المجموعة الثانية بجرعة من كلوريد الكاديوم (1 مل/كجم من وزن الجسم) داخل التجويف البريتوني، وكانت مجموعة ضابطة موجبة (فئران مصابة)، وتم حقن فئران المجموعات الثلاثة الأخرى بكلوريد الكاديوم كذلك، والتي تغذت على مسحوق نباتات النارددين والخلونجان ومخلوط منهم بنسبة 5% من الوجبة الأساسية. وفي نهاية التجربة تم تجميع عينات الدم لقياس مستوى كل من هرمونات التستوستيرون الكلي وهرمون

Hormone, Luteinizing Follicle Stimulating Hormone (FSH) ((LH)

كما تم إجراء الفحص الهستوباثولوجي وقياس نشاط الإنزيمات المضادة للأكسدة بأنسجة الخصية. وأظهرت النتائج أن إعطاء مسحوق نباتات النارددين والخلونجان وخليطهما معا بنسبة (5%) لمدة 4 أسابيع أدى إلى زيادة معنوية في مستويات هرمونات التستوستيرون، FSH و LH. وكانت هناك زيادة معنوية في وزن الخصيتين بالمقارنة بالمجموعة الضابطة الموجبة (المصابة)، كما أدى تناول مسحوق النباتات إلى زيادة نشاط الإنزيمات المضادة للأكسدة بأنسجة الخصية، وكذلك تحسن ملحوظ في التغيرات الهستوباثولوجية الضارة التي سببها كلوريد الكاديوم. وتوصي الدراسة أن تناول مسحوق نباتات النارددين والخلونجان في الغذاء قد يكون مفيدا للمرضى الذكور الذين يعانون من ضعف الخصوبة الناتج عن الإجهاد التأكسدي.

الكلمات المفتاحية: الخصوبة، النارددين، الخولنجان، هرمون التستوستيرون، FSH، LH، التغيرات الهستوباثولوجية.