Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 28, Issue 1, 2017
DOI: http://doi.org/10.23851/mjs.v28i1.312

Research Article

The Protective Effect of *Panax Ginseng* Root Extract against the Toxicity of Carbon Tetrachloride that Induces Infertility to Male Rabbits

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ArticleInfo

Received
17/10/2016

Accepted
12/12/2016

Abstract

The present study used 20 male rabbits that divided randomly to four groups (each group consist 5 rabbits), control group received only normal diet, the group received carbon tetrachloride (CCL_4) for forty days, the group treated (orally) with carbon tetrachloride and (100mg/per day) root extract for forty days, the fourth treated with carbon tetrachloride and (200mg/per day) root extract for forty days. the rabbits that treated with carbon tetrachloride showed decreased in the counts, motility, number of living sperms and increased the deformity of sperms with decreased the levels of LH, FSH and testosterone show high significant changes ($P < 0.05$) compare with control. In contrast, these parameters still normal when the groups that administrated with Panax ginseng root extract. It was concluded that the root extract play important role against heavy metals that causes fertility of male rabbits.

Keywords: *Panax ginseng*; carbon tetrachloride (CCL_4); sex hormones; sperm analysis.

الخلاصة

استخدمت الدراسة الحالية 20 ذكر ارنب سليم والتي وزعت عشوائيا إلى أربع مجاميع (كل مجموعة تتكون 5 ذكور)، المجموعة الأولى مجموعة السيطرة والتي اعطيت الماء والغذاء فقط، المجموعة الثانية والتي جرعت رباعي كلوريد الكاربون لمدة اربعين يوم، المجموعة الثالثة والتي جرعت رباعي كلوريد الكاربون و 100غم من مستخلص جذور نبات الجيسينك لمدة اربعين يوم، المجموعة الرابعة والتي جرعت رباعي كلوريد الكاربون و 200غم من مستخلص جذور نبات الجيسينك لمدة اربعين يوم. الارانب التي جرعت رباعي كلوريد الكاربون اظهرت انخفاض في الاعداد الكلية للنطف، حركة النطف، واعداد النطف الحية وزيادة في اعداد النطف المشوهة مع انخفاض في مستويات هرمون التيستوستيرون، الهرمون المحفز للجريبات (FSH) والهرمون اللوتيني (LH)، حيث اظهرت هذه المجموعة فروقات معنوية عالية ($P < 0.05$) مقارنه مع مجموعة السيطرة. ولكن الاعداد الكلية للنطف، حركة النطف، اعداد النطف الحية واعداد النطف المشوهة ومستويات هرمون التيستوستيرون، الهرمون المحفز للجريبات (FSH) والهرمون اللوتيني (LH) قد كانت ضمن المستويات الطبيعية عند تجريع الارانب مستخلص جذور نبات الجيسينك. يستنتج من هذه الدراسة أن مستخلص جذور الجيسينك له تأثير جيد في الوقاية من التاثيرات الضارة لرباعي كلوريد الكاربون على خصوبة ذكور الارانب.

Introduction

Panax ginseng was first cultivated around 11 before Christ (BC) and has a medical history of more than five thousand years [1]. *Panax ginseng* is mainly grown in China, Korea, and North America [2]. Ginseng can be divided into red ginseng, which is steam-dried ginseng and white ginseng, which is naturally dried ginseng [3]. According to Chinese Medicine Ginseng promotes and improves young energy, circulation, and increases blood supply and aids recovery from weakness after illness [4]. *Panax ginseng* may enhance male fertility by acting on the pituitary gland as it reduces prolactin production or on the central nervous system increasing dopaminergic actions [5]. Panax is known to affect various tissues including cardiovascular, endocrine, and immune and nervous system tissues; its major physiologically active ingredients include amino acids, ginsenosides, polyacetylenes, polysaccharides, alkaloids, and phenolic compounds [6]. In experimental injuries, CCl4 has been commonly used because it initiates oxidative damage, generates toxic free radicals and decreases the activities of antioxidant enzymes [7]. So, the aim

of this study is to show the role of Panax ginseng root extract against toxicty of carbon tetrachloride on fertility of male rabbits.

Materials and Methods

Animal Model

Twenty adult male rabbits, (wt 1.5-2 kg with age 8-12months) collected from Kirkuk city markers, and kept on standard pellet diet and water for two month to be sure all animals without any diseases.

Preparation of the Extract

The roots of *Panax ginseng* were collected from Kirkuk market, cut into small pieces. The dried roots (by oven) were then grinded to obtain a fine powder. The powder was again dried by using oven and was ready for use. The grinded powder was then extracted with 1000ml double distilled water containing 3-4 drops of chloroform for 48h. The extract was then concentrated at temperature less than 45°C.The residue was then dried and refrigerated [8-9]. The extract was orally used at a two concentrations (100mg & 200mg) as a single dose per day.

Chemicals

Carbon tetrachloride (CCL_4) was obtained from the Dept. of biology at Kirkuk University. The rabbits received a dose of 1ml/kg of CCL4 that was suspended in olive oil (1: 1v/v) by oral administration method [10].

Experimental Design

Twenty adult male rabbits were used and divided to four groups (each group consist five male rabbits) as follow:

1. Control group received normal saline only for forty days, then killed all were euthanized at forty one day.
2. Rabbits received carbon tetrachloride (orally as a single dose per day) for forty days, and then killed.
3. Rabbits received carbon tetrachloride and 100mg root extract (orally as a single dose per day) for forty days, and then euthanized at forty one day.
4. Rabbits received carbon tetrachloride and 200mg root extract (orally as a single dose per day) for forty days, and then euthanized at forty one day.

Blood Samples

Fiveml of blood is collected by cardiac puncture under anesthesia and put in test tubs. Then, the tubes (after clotting) were centerfigation 5000 cycle/min for 10 min to obtain sera. The sera were taken with 1ml distal water added to it.

Semen Collection

The testes were removed with the epididymides. The epididymides divided to tow parts. The caudal part was separated from the testes. The semen collected dilution with normal saline and input in tubes. After that, using centrifuge to obtain semen plasma to studied certain parameters in the semen [11].

Sperm Analysis

The epididymis was removed immediately, the content of the caudal part of each epididymis has been disccharged in glass, diluted with sodium citrate (1.9%) at (37° C). after that, one drop of this solution was mixed with one drop of eosin - nigrosin stain, then smeared on slide. This technique was used to determining the percentage of live/dead and to abnormal/normal sperm forms [12]. The content of the upper part of epididymis was put in clean glass contained 0.1ml. Eosin 5%, this technique was used for counting the sperm concentration [13].

Statistical Analysis

Data were analyzed using a statistical Minitab program. Means of data were compared using Duncan's Multiple Range test. Probability levels of more than 0.05 were regarded as statistically non-significant, whereas values less than 0.05 were considered as significant.

Results and Discussion

Sperm Analysis

The counts, motility and number of live sperm and deformity of sperm showed significant changes ($P<0.05$) between groups of the present study. As shown in Table 1, carbon tetrachloride group showed significant decrease in number of counts and motility of sperm (52 ± 8.2 and 51.3 ± 3.22 respectively) compared with control (91.3 ± 1.5 and 91.67 ± 2.31 respectively). The counts and motility of sperm in group 100mg extract show significant decreased, but better than the carbon tetrachloride group. The counts and

motility of sperm in 200mg extract group showed non-significant changes in the counts and motility of sperm. On the other hand, the number of live and deformity of sperm (50.33 ± 3.79 and 10.97 ± 2.68 respectively) in carbon tetrachloride group showed significant decrease. The number of live and deformity of sperm in 100mg extract group showed significant decreased compare with control, but better than the carbon tetrachloride group. The percent of live and deformity of sperm in 200mg extract group showed non-significant changes as showed in Table 1.

Table 1: Measurements of seminal analysis for all groups.

Groups	Count (10^6)	Motility (%)	Live (%)	Deformity (%)
Control	91.3 ± 1.5 a	91.67 ± 2.31 a	90.67 ± 0.21 a	3.43 ± 0.9 a
CCL_4	52 ± 8.2 c	51.3 ± 3.22 c	50.33 ± 3.79 c	10.97 ± 2.68 c
CCL_4 + 100mg extract	75 ± 4.4 b	70 ± 8.88 b	72.3 ± 8.1 b	6.53 ± 1.17 b
CCL_4 + 200mg extract	93.7 ± 1.53 a	92 ± 2 a	93.3 ± 1.53 a	3.13 ± 0.67 a

*same letters mean non-significant changes and different letters mean significant changes.

Hormonal Tests

The levels of testosterone, FSH and LH showed significant changes (P<0.05) in between groups. As shown in Table 2, carbon tetrachloride group showed significant decreased in the levels of testosterone, FSH and LH (In serum, 1.4 ± 0.3, 1 ± 0.24, 0.5 ± 0.1. In semen, 0.77 ± 0.15, 0.57 ± 0.12 and 0.27 ± 0.15 respectively) compare with control. The levels of testosterone, FSH and LH in 100mg extract group showed significant decreased compared with control group, but better than the carbon tetrachloride group. Levels of testosterone, FSH and LH 200mg extract group showed non-significant changes compare with control group.

Table 2: The levels of Testosterone, FSH and LH in serum and semen

Groups	Serum			Semen		
	T (ng/ml)	FSH (mlU/ml)	LH (mlU/ml)	T (ng/ml)	FSH (mlU/ml)	LH (mlU/ml)
Control	2.8 ± 0.21 a	2.2 ± 0.21 a	1.36 ± 0.12 a	1.5 ± 2.5 a	1.4 ± 0.15 a	1.03 ± 0.25 a
CCL_4	1.4 ± 0.3 c	1 ± 0.24 c	0.5 ± 0.1 c	0.77 ± 0.15 b	0.57 ± 0.12 c	0.27 ± 0.15 b
CCL_4 + 100mg extract	2.3 ± 0.2 b	1.4 ± 0.4 b	0.87 ± 0.15 b	1.03 ± 0.3 ab	0.83 ± 0.06 b	0.57 ± 0.21 ab
CCL_4 + 200mg extract	2.9 ± 0.25 a	2.3 ± 0.32 a	1.4 ± 0.36 a	1.7 ± 0.31 a	1.3 ± 0.25 a	1.07 ± 0.31 a

The pharmacological effects of Panax have been showed in cardiovascular system, central nervous system as well as the endocrine and immune systems. Ginseng has been shown to exhibit antioxidant [14]. Most studies have shown that the ginsenosides play important roles in pharmacological effects of Panax ginseng [15]. *Rajesh and Latha 2004,* referred that carbon tetrachloride (CCl_4) induced oxidative stress by altering the levels of antioxidant enzymes and increased lipid peroxidation [16]. The results of this study show that the rabbits administrated with carbon tetrachloride showed high significant decreased (P<0.05) in the counts, motility, number of living sperms and increasing the deformity of sperms, as well as decreasing the

levels of LH, FSH and testosterone, but these parameters were back to their normal levels when using *Panax ginseng* root extract.

In study carried by *Khan 2012* referred that the CCl_4 lead to induce testis damage. They found, mice that received CCl_4, decreased in the counts, motility and number of living sperms and with increase in dead and abnormal sperm count as compared to control group. They suggested that the CCl_4 caused reduction in the activity of antioxidant enzymes and accumulation of free radicals in testicular tissue [17]. *Sönmez et al. 2011* showed that the CCl_4 can induce significant decreasing in sperm counts and motility and increase in dead and abnormal sperm rate Testosterone levels in serum of rat [18]. *Abou EL – Ghait & Omyma 2004* referred that the CCl_4 lead to induce significant decreased in the Testosterone levels in serum of rat [19]. Also, the agreement results with Abdel Moneim 2014, who repoted that the CCl_4 lead to induce significant decreased in the testosterone, follicle stimulating hormone and luteinizing hormone levels in serum of the rat [20].

Choi et al. 2004 demonstrated that Panax Ginseng have been good effect on the sperm characters. Where, they found the Panax Ginseng lead to increased sperm counts, motility and normal percent. They suggest the mechanism of Ginseng on improving sperm quality is antioxidant property [21]. Also, *Park et al. 2014* referred that *Panax Ginseng* have been good effect on the sperm motility, they found the Panax Ginseng lead to increased motility compare with control group [22]. On the other hand, *Omar & Abdalhafid 2016* referred that Panax Ginseng lead to increased sperm counts and Testosterone levels in male rabbits compare with control group, they suggest that Panax ginseng root powder possesses pro-fertility properties in rabbits which might be a product of both its potent androgenic activities and antioxidant properties [23].

In study carried by *Linjawi 2015,* to explain the role of Panax ginseng against the nicotine that induce sperm and sex hormonal damages.The results of their study showed significant decreases sperm counts and in serum free testosterone LH, and follicle stimulating hormone in male rats received nicotine compared with control. While, the levels of free testosterone, follicle stimulating hormone and luteinizing hormone in rats treated with Panax Ginseng increased compared with control or nicotine treated rats [24]. Also, Jung *et al.* 2015 referred that Panax ginseng play important role against the busulfan induced dysfunction of the male reproductive system. They found busulfan alone lead to decreased total numbers of sperm, sperm motility and serum testosterone levels, but when used Panax ginseng in the treatment against the busulfan toxicity, they found the levels of testosterone, total numbers of sperm and sperm motility increased [25]. So, it was concluded that the root extract play important role against heavy metals that causes fertility of male rabbits.

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