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Ameliorative effects of milk thistle alcoholic extract on reproductive function and testicular oxidative damage in potassium dichromate treated male rats

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Milk thistle (silymarin), a well-known antioxidant and cytoprotective compound, possesses therapeutic and protective properties. Numerous scientific studies have documented the use of milk thistle-based preparations for therapeutic purposes (to treat various pathological processes in animals and humans) or for prophylaxis. Based on the above mentioned, this study aimed to investigate the corrective effect of milk thistle ethanol extract on reproductive function and oxidative testicular damage in rats poisoned with potassium dichromate. In the experiment, adult male albino rats (n=6) were used. Group 1 (Control group): The animals were given distilled water orally for 28 days. Accordingly, Group 2 was administered potassium dichromate intraperitoneally daily for two weeks. Animals in Group 3 were given an oral dose of milk thistle alcoholic extract. Animals in Group 4 were given an oral dose of milk thistle alcoholic extract (daily for 28 days) and potassium dichromate (daily for two weeks). The studies established the negative effect of potassium dichromate, which affected the changes in the level of testosterone, FSH, and LH in the blood serum of male rats (group 2); the mentioned indicators tended to significantly decrease (P≤0.05) compared to those in the control group. It was determined that the level of testosterone, FSH, and LH in the blood serum in the group of rats (group 3) that received milk thistle was similar to the analogous levels in rats (group 1) that did not receive potassium dichromate. At the same time, in this group the level of testosterone, FSH and LH tended to increase (P≤0.05) compared to the group that received potassium dichromate. The studies established the presence of a corrective effect in the group of animals that received potassium dichromate + milk thistle. In particular, serum testosterone, FSH, and LH levels were recorded to increase by 1.6, 1.5, and 1.3 times, respectively (P≤0.05), compared to the potassium dichromate-treated rats. It was also found that the administration of potassium dichromate to rats (Group 2) increased the level of nitric oxide (P≤0.05) and decreased the activity of SOD (P≤0.05) compared to the control animals. The administration of milk thistle to rats (Group 3) contributed to the increase in the activity of SOD (P≤0.05), even compared to the indicator in the control group of animals. In Group 4 of rats, the level of nitric oxide tended to decrease (P≤0.05), and the activity of SOD increased (P≤0.05) compared to the animals that received potassium dichromate. Thus, according to the study, milk thistle extract demonstrated a significant protective effect against potassium dichromate.

Keywords: Milk thistle, rats, testicular oxidative damage.

Корегуючий вплив спиртового екстракту розторопші на репродуктивну функцію щурів за оксидативного стресу, викликаного дихроматом калію

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Розторопша – відомий антиоксидант, що має терапевтичні та захисні властивості. Численні наукові дослідження свідчать про можливість використання препаратів на основі розторопші з терапевтичною метою (для лікування різних патологічних процесів у тварин та людей) або для профілактики. Виходячи з вищезазначеного, мета цього дослідження полягала у вивченні коригуючого впливу спиртового екстракту розторопші на репродуктивну функцію щурів інтоксикованих калієм дихромату. У дослідах використовували дорослих самців білих щурів, яких розділили на 4 групи (n=6). Група 1 (контрольна), де тваринам давали дистильовану воду перорально протягом 28 днів. Щурам з 2-ї групи щодня протягом двох тижнів внутрішньоочеревинно вводили дихромат калію. Тваринам з 3-ї групи перорально задавали спиртовий екстракт розторопші. Тваринам з 4-ї групи перорально задавали спиртовий екстракт розторопші (щоденно протягом 28 днів) та дихромат калію (протягом двох тижнів). Дослідженнями встановлено негативний вплив дихромату калію, що проявлявся змінами рівня тестостерону, ФСГ та ЛГ у сироватці крові самців щурів (група 2). Зазначені показники мали тенденцію до значного зниження (P≤0,05) порівняно з контрольною групою. Було визначено, що рівень тестостерону, ФСГ та ЛГ у сироватці крові щурів (група 3), які отримували розторопшу, був подібним до аналогічних рівнів у щурів (група 1), які не отримували дихромат калію. Водночас, у цій групі рівень тестостерону, ФСГ та ЛГ мав тенденцію до підвищення (P≤0,05) порівняно з групою, яка отримувала дихромат калію. Дослідженнями встановлено коригуючий вплив розторопші у групі тварин, які отримували її разом із дихроматом калію. Зокрема, рівні тестостерону, ФСГ та ЛГ у сироватці крові щурів 4-ї групи мали тенденцію до підвищення (P≤0,05) порівняно зі щурами 2 групи. Також було виявлено, що введення дихромату калію щурам підвищувало рівень оксиду азоту (P≤0,05) та знижувало активність СОД (P≤0,05) порівняно з контрольними тваринами. Введення розторопші плямистої щурам (3-тя група) сприяло підвищенню активності СОД (P≤0,05), навіть, порівняно з показником у контрольній групі тварин. У 4-й групі щурів рівень оксиду азоту знижувався (P≤0,05), а активність СОД – зростала (P≤0,05) порівняно з тваринами, які отримували дихромат калію. Таким чином, згідно з результатами дослідження, екстракт розторопші плямистої продемонстрував значний захисний ефект проти дихромату калію.

Ключові слова: розторопша, щури, оксидативне ушкодження сім'яників.

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Introduction

The testicles generate testosterone, the male hormone that is needed for protein synthesis, muscle and bone growth, and reproductive development [1]. When testicular dysfunction occurs, testosterone levels decrease, leading to various pathophysiological changes and clinical symptoms [2]. Achilonu et al. [3] are conducting research on the use of natural products to improve livestock productivity, including growth rate, feed effectiveness, stress tolerance, and overall well-being.

Milk thistle (Silybum marianum) is a plant with a long history in medicine [4]. Its seeds have been used for more than 2,000 years for a variety of purposes [5]. In addition to destroying free radicals that cause lipid peroxidation, silymarin also exerts antioxidant effects by affecting enzyme systems involving glutathione and superoxide dismutase [6]. The presence of silymarin, a flavonoid complex consisting of silybin A and B, isosilybin A and B, silycristin and silydianin, is directly connected with the medicinal benefits of milk thistle [7]. The highest concentrations of silymarin are found in the plant's achenes, which are mistakenly but often called "seeds" [8]. Having antioxidant, anti-inflammatory properties, the compounds play a crucial role in preventing cancer progression, and the important mechanism in inducing apoptosis, promoting cancer cell death and inhibiting tumour growth, were demonstrated by silymarin [9]. It shows immune modulatory activity, increases lactation and increases protein synthesis [10]. Furthermore, in human prostate, breast, and cervical cancer cells, silymarin inhibits DNA synthesis, cell division, and other mitogenic signals [11].

A chemical compound referred to as potassium dichromate, which includes both potassium and chromate, is toxic to the reproductive system. The compound's history emphasizes its importance in chemical processes and a variety of business applications [12]. Men who are exposed to this chemical may develop decreased sperm production and testicular damage. Furthermore, by damaging the ovaries and reducing fertility, it can have an impact on the female reproductive system [13]. Potassium dichromate has also been connected to a higher risk of developing some cancers, such as lung and nasal cavity cancers, which can further impair reproductive health. To decrease exposure risk and potential reproductive system consequences, this substance must be handled with extreme caution and in compliance with safety protocols [14].

The aim of the study

The work was aimed to investigate the corrective effect of an alcoholic extract of milk thistle on reproductive function and oxidative damage to the testicles in rats intoxicated with potassium dichromate.

Materials and methods

Milk thistle seeds extract preparation

The study's materials and methodology included milk thistle extract (silymarin), which was made by steeping 100 g of freshly ground milk thistle seeds from the local Karbala market in 1,200 ml of boiling water at 100 °C for 210 minutes [15], after being sieved and heated to 40 °C, the extract was dried and kept at -20 °C for storage. Doses equal to the therapeutic dose were used to administer the extract [16].

Experimental animals

Twenty-four mature male albino rats were used in this study, which was carried out at the University of Kerbala's College of Veterinary Medicine in Iraq. Their ages ranged from 14 to 16 weeks, and their mean weight was between 200 and 350 grams. Before the experiment started, the rats were allowed to acclimate for two weeks in groups of four per cage in an animal premises, where they had unrestricted access to food and water. The temperature in the animal premises was between 20 and 25°C, and the lighting cycle was natural.

Experimental design

The rats were separated into four groups (6 rat/ group):

Group 1 – Control: the animals were given regular normal saline for 4 weeks orally.

Group 2 – Milk Thistle Extract: rats were given 200 mg/kg/day of milk thistle extract orally for 28 days.

Group 3 – Potassium Dichromate: rats received 2 mg/kg of body weight of potassium dichromate intraperitoneally once a day for two weeks.

Group 4 – Potassium Dichromate and Milk Thistle Extract: rats were given 2 mg of potassium dichromate/kg/day intraperitoneally for 2 weeks, followed by the treatment with 200 mg/kg/day of milk thistle extract orally for 28 days.

Hormones assay

In the current study, the following indicators were measured: the concentration of serum testosterone hormone (T) by enzyme linked immunosorbent assay (ELISA) according to [17]; the level of the concentration of serum follicle-stimulating hormone (FSH) in accordance with [18]; and the concentration of serum luteinizing hormone (LH) in accordance with [19].

The oxidant and antioxidant parameters

The level of serum nitric oxide (NO), was measured by ELISA technique in accordance with [20], and antioxidant enzyme indices, specifically superoxide dismutase (SOD) activity, were measured by ELISA technique, conducted according to reference [21].

Statistical analysis

The Statistical Package for Social Scientists (SPSS version 18.0) and Microsoft Office Excel 2016 were used to study the data. A one-way ANOVA with LSD post hoc test for significance was used to assess if there was a significant difference between the groups' means. The paired t-test was used for mean comparisons, where $P<0.05$ values were considered significant [22].

Results and discussion

Effect of potassium dichromate administration and milk thistle extract on serum hormones concentration in adult male rats

After 28 days, serum testosterone, follicle-stimulating hormone, and luteinizing hormone levels in the potassium dichromate group were significantly lower – 2.3, 2.0, and 1.6 times, respectively (P≤0.05) – than in the control group. In contrast, serum testosterone, FSH, and LH levels in the milk thistle group were within normal limits compared to the control group, while they significantly increased by 2.5, 2.2, and 1.8 times, respectively (P≤0.05), compared to the potassium dichromate group.

It should be noted that, in comparison with the group receiving potassium dichromate, after 28 days, the levels of testosterone, FSH and LH in the blood serum of the group receiving potassium dichromate + milk thistle increased significantly by 1.6, 1.5 and 1.3 times, respectively (P≤0.05), as shown in ***Table 1***.

Table 1

The effect of potassium dichromate administration and milk thistle extract on serum hormones concentration in adult male rats, mean ± SD

Groups	Parameters		
	Testosterone, ng/ml	FSH, mIU/ml	LH, mIU/ml
Control	4.9± 0.6[a]	3.2 ± 0.8[a]	3.6 ± 0.3[a]
Potassium dichromate	2.1 ± 0.4[c]	1.6 ± 0.4[c]	2.2± 0.4[c]
Milk thistle	5.3 ± 0.7[a]	3.5 ± 0.6[a]	3.9 ± 0.9[a]
Potassium dichromate + milk thistle	3.4 ± 0.5[b]	2.4± 0.3[b]	2.8 ± 0.5[b]
LSD	0.44	0.57	0.34

Note: The differing lowercase letters (a) indicate significant differences between groups, with a significance level set at P < 0.05.

One polyphenolic flavonoid that comes from milk thistle is silymarin. The main component of silymarin, silybin, is responsible for most of its phytochemical characteristics. According to a number of studies, silymarin has immune modulatory, anti-inflammatory, anti-cancer, and anti-fibrotic properties [23]. The synthesis of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), and the number of spermatozoa cells is also enhanced by silymarin in rats [24]. On the other hand, the study found that rabbits, which ate milk thistle seeds had higher sperm concentration, viability, motility, testosterone levels and fertility [25]. In the testes of quail fed with a high-energy diet, milk thistle seed supplementation prevented histopathological damage and decreased testosterone production [26].

Male rats are exposed to potassium dichromate, which is used as a poison or pollutant. The effects on fertility and physical parameters were studied [27]. The reproductive system is significantly affected by exposure to potassium dichromate, particularly due to its hexavalent chromium component, affecting both males and females [28].

The main reason of unfavorable effects occurring in males is disrupting the process of spermatogenesis, or sperm production. According to the previous research, exposure to potassium dichromate reduces sperm motility and number [29]. In addition, there is evidence to support the idea that hormonal imbalance can affect the balance between testosterone and other hormones important to male reproductive health [30].

It is important to understand that the reproductive toxicity of potassium dichromate is often dose-dependent, various routes of exposure, with more severe effects occurring at higher concentrations, reproductive health risks can be attributed to, including prolonged environmental exposure and the work environment [31]. The reproductive systems of both sexes are generally exposed to potassium dichromate, which can cause developmental abnormalities, reduced fertility and other reproductive health problems. The protection of reproductive health in the environmental and occupational context requires reducing the risk through safety measures.

The effect of potassium dichromate administration and milk thistle extract on oxidant and antioxidant levels in adult male rats

Table 2 shows the effect of potassium dichromate administration and milk thistle extract intervention on changes in the SOD activity profile.

Table 2

The effect of potassium dichromate administration and milk thistle extract on oxidant and antioxidant levels in adult male rats (mean ± SD)

Groups	Parameters	
	NO, µmol/mg	SOD, U/mg
Control	5.2 ± 0.4[c]	12.8 ± 1.2[b]
Potassium dichromate	9.8± 0.7[a]	6.5 ± 0.8[c]
Milk thistle	4.9 ± 0.3[c]	14.2 ± 1.5[a]
Potassium dichromate + milk thistle	7.1 ± 0.6[b]	10.3 ± 1.1[b]
LSD	1.2	2.3

Note: The differing lowercase letters (a) indicate significant differences between the groups, with a significance level set at P < 0.05.

The administration of potassium dichromate (Group 2) increased nitric oxide levels by 1.9 times (P≤0.05) compared to the control animals. The animals in this group also showed a 2.0-fold decrease in SOD activity (P≤0.05) compared to the control animals.

It should be noted that milk thistle administration (Group 3) had a positive effect. SOD activity was 1.1 times higher than in the control animals (P≤0.05). Despite this, nitric oxide levels, although slightly lower than in the control group, did not show significant changes. In Group 4, nitric oxide levels decreased by 1.4 times (P≤0.05), and SOD activity increased by 1.6 times (P≤0.05) compared to the animals receiving potassium dichromate.

Enzyme activity in all organs increased to various degrees as a result of the milk thistle extract intervention in the normal group (Group 3). Normally, the body develops defense mechanisms against free radical damage, or oxidative stress, primarily based on antioxidant enzymes (GSH-Px, GSH-Rd, CAT, and SOD) that can scavenge ROS [32, 33]. The way the antioxidant defense mechanisms work is that SODs convert $O_2^{\cdot-}$

to H_2O_2, which is subsequently eliminated by a number of enzymes, including CAT and GSH-Px. Increased ROS generation and mitochondrial dysfunction are caused by decreased antioxidant enzyme activity in both in vitro and in vivo settings [34–36].

By combining lignan and flavonoid components, silybum marianum creates flavonolignans. A flavonoid and a phenylpropanoid, usually coniferyl alcohol, undergo oxidative coupling events, which is the cause of this (oxidative coupling occurs between free radicals generated from the flavonol taxifolin and those generated from coniferyl alcohol) [37].

Conclusions

The studies found an adverse effect of potassium dichromate, which caused the changes in the levels of testosterone, FSH and LH in the blood serum of male rats, which tended to decrease ($P \leq 0.05$) compared to the control group. The levels of testosterone, FSH and LH in the blood serum in the group of rats receiving milk thistle tended to increase ($P \leq 0.05$) compared to the group receiving potassium dichromate. The studies revealed a corrective effect in the group receiving potassium dichromate and milk thistle. In particular, the levels of testosterone, FSH and LH in the blood serum increased ($P \leq 0.05$) compared to the rats receiving potassium dichromate. It was found that the administration of potassium dichromate to rats increased the level of nitric oxide ($P \leq 0.05$) and decreased the activity of SOD ($P \leq 0.05$) compared to the control animals. The administration of milk thistle to rats contributed to an increase in the activity of SOD ($P \leq 0.05$), even compared to the indicator in the control group of animals. In the group of animals that received potassium dichromate + milk thistle, the level of nitric oxide tended to decrease ($P \leq 0.05$), and the activity of SOD increased ($P \leq 0.05$) compared to the animals that received potassium dichromate. Thus, the milk thistle extract demonstrated a significant protective effect against potassium dichromate.

DECLARATIONS

Ethical Statement

The authors confirm that the studies were conducted in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986), national ethical norms, and international standards (NIH).

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Conflict of interest

The authors state that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The authors declare that no artificial intelligence or AI-assisted technologies were used in the preparation of this manuscript.

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